"Synthesis And Evaluation Of Some Five Member Heterocyclic Compounds"

Thesis submitted for the degree of

Doctor of Philosophy (Ph.D.) in

Pharmaceutical Sciences

BY

Mr. BEENKUMAR R. PRAJAPATI

(M. Pharm.)

Department of Pharmacy

SUMANDEEP VIDYAPEETH

JULY 2011

SUMANDEEP VIDYAPEETH, PIPARIYA, GUJARAT

Certificate

I certify that the thesis entitled **"SYNTHESIS AND EVALUATION OF SOME FIVE MEMBER HETEROCYCLIC COMPOUNDS"** submitted for the Degree of Doctor of Philosophy by **Mr. BEENKUMAR R. PRAJAPATI** is the record of research work carried out by him under my guidance and supervision and that this research work has not formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles in this University or any other University or institution.

Dr. A.K. Seth
Dean
Dept. of Pharmacy,
Sumandeep Vidyapeeth,
Piparia.

Date:
Place: Pipariya.

SUMANDEEP VIDYAPEETH, PIPARIYA, GUJARAT

Certificate

I declare that the thesis entitled **"SYNTHESIS AND EVALUATION OF SOME FIVE MEMBER HETEROCYCLIC COMPOUNDS"** submitted by me for the Degree of Doctor of Philosophy (Ph.D.) is the record of research work carried out by me under the guidance of **Dr. A. K. SETH** and has not formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles in this University or any other similar institution of higher learning.

Mr. BEENKUMAR R. PRAJAPATI
Research Scholar
Dept. of Pharmacy
Sumandeep Vidyapeeth,
Pipariya.

Date:
Place: Pipariya.

Acknowledgement

Research has always been an ongoing process, a journey and not the destination. It has widened our vision, opened newer avenues and lightened the dark obscure facets of mysterious universe. The work depicted in this thesis is a bucketful of contribution to the large ocean of research occurring globally. Success never comes alone. As one flower makes no garland, this presentation would not have taken shape without wholehearted encouragement & live involvement of some generous souls. I would like to thanks to all; those who have contributed to the successful completion of this work.

A successful outcome in any research endeavor attributes itself to the selfless guidance of the mentor. With a feeling of profound pleasure I can say that the credit of this work goes to a giant personality in himself, who has brought about a "better me" in myself, my mentor and esteemed guide ***Dr. A.K.Seth,*** *M. Pharm, PhD, Director and Dean, Department of Pharmacy Sumandeep Vidyapeeth University. I would like to thank Seth sir for his erudite guidance, overwhelming enthusiasm, untiring cooperativeness, constant encouragement, critical remarks, precise discussions, timely suggestions and the nourishment of knowledge conferred upon me. He constantly motivated me to step towards success, without being dissipated by frolics & failures.*

I wish to express my sincere thanks, with a deep sense of gratitude, to ***Dr. K.I. Molvi****, M. Pharm, Ph.D., Principal, Ali-Allana College of Pharmacy, Department of Pharmaceutical Medicinal Chemistry, akkalkuawa, Nandurbar, India, for initiating and suggesting the theme of work, for his valuable guidance, supervision, creative suggestions and meticulous attention, sustained interest, immense guidance, dedicated support he has bestowed upon me for the timely completion of this work. I am extremely indebted to Molvi sir for his motivational inspiration, kind expertise*

during the writing up of my thesis and the scientific attitude he has nurtured in me which will definitely stand in all my future endeavours.

I would like to express my cordial thanks to ***Dr. B.N. Suhagia,*** *M.Pharm., Ph.D., F.I.C., L.L.B., Professor and Head, Department of pharmaceutical chemistry, L. M. College of Pharmacy, Ahmedabad, for his timely help, moral support and useful guidance which helped me, to achieve my target. Whose fatherly blessings have always been bestowed upon me throughout my educational journey.*

It gives me profound pleasure to express my deep gratitude towards ***Mr. I.S. Rathod****, Assistant Professor, Department of Pharmaceutical Chemistry L. M. College of Pharmacy, Ahmedabad. I would like to thank Rathod sir for his keen interest and valuable support in technical matter. He was always available to give me the environment and suggestions. He trained me to be self-reliant to face the hard realities of life and in traveling any unknown path that I may encounter in future.*

I am also thankful to ***Dr. M.T.Chhabria*** *Assistant Professor, Department of Pharmaceutical Chemistry L. M. College of Pharmacy, Ahmedabad, who was my M.Pharm. Guide. Chhabria sir is path-setters for my journey towards synthetic chemistry. I am proud to begin my journey with such great personalities.*

With profound gratitude and regards, I give my sincere thanks to ***Dr. Mukesh C.. Gohel,*** *Principal, L. M. College of Pharmacy, for providing the facility to work out this project.*

I am thankful to ***Dr, V. Sudarsanam****, PERD center Ahmedabad, for spectra interpretation.* ***Dr. Shukla****, Head of Microbiology Department, M.G.Science Ahmedabad &* ***Dr. Dilip N. Zaveri****, Director Bio-care Research, Paladi, Ahmedabad for providing antianaerobic activity.*

Friendship is a treasured gift and true friends are very few. I express my deep sense of gratitude to my friends ***Dr. Pathik Bhramkshatriya, Dr. Diveysh Doshi, Mustakim Mansuri, Desai sir, Dharmesh Darji & Pragnesh Patni*** *who helped me to complete successfully this research work in one or the other way.*

Educational institutions have always been the pillar of an individual's personality and they always bring the real gem out of an individual and luckily, I am not an exception to this. I pay my reverence to, ***L. M. College of Pharmacy, Ahmedabad & PLM Kansara Pharmacy College.*** *I am undeniably proud to be associated with this college and express my deepest indebted thanks to my esteemed, renowned and prestigious institution.*

Finally, this thesis would not have been possible without the confidence, endurance and support of my family. Words are an inadequate medium to express my deep sense of gratitude to my ***Parents, my wife, my daughter Ruchi and my brother Chandresh and his wife*** *for moral support, encouragement and their prayer to God has made me achieve milestones in my carrier. Their trust has always inspired me to do my best. I am highly indebted to them forever.*

Finally, I am thankful to Almighty for always being with me and for blessing me with good family, teachers, friends and well wishers.

July 2011 ***Beenkumar R Prajapati***

CONTENTS

CONTENTS

CONTENTS

CONTENTS

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

CB1	Cannabinoid receptor 1
DNA	Deoxyribo nucleic acid
DMSO	Dimethyl sulfoxide
HIV	Human Immuno Virus
HWPs	Half wave potentials
INH	Isoniazide
IR	Infrared
LFER	Linear free energy relation ship
M. tb	*Mycobacterium tuberculosis*
MAP	Mitogen anti protein
MDR-TB	Multi drug resistant tuberculosis
MIC	Minimum inhibitory concentration
MR	Molar refraction
MTZ	Metronidazole
mV	Mili Volt
MV	Molar volume
MW	Molecular weight
NMR	Nuclear magnetic resonance
NNRTIs	Non-nucleoside reverse transciptase inhibitors
NO	Nitric oxide
OERPs	One electron reduction potentials
RT	Reverse transciptase
PAM	Pralidoxime
PCR	Polymerase chain reaction
PFOR	Pyruvate ferredoxin oxido reductase
PTK	Protein tyrosine kinase
QSAR	Quantitative structure activity relationship
TNZ	Tinidazole
VIF	Variance inflation factor

ABSTRACT

The nitroheterocyclic compounds have proved to be very effective antimicrobial agents and very valuable member of limited armamentarium of antiprotozoal agents. The mechanism of action of nitroheterocyclic agents depend solely upon the reduction of the nitro group to toxic radicals and binding to the protein and DNA. The two families of nitroheterocyclic; the nitroimidazole and nitrofurans are very different in their reduction characteristics. Drug resistance to the nitroheterocyclic drugs and cross resistance leads to the development of new derivatives. A number of 5-nitoimidazole with substitution at 2nd position of 5-nitroimidazole with various substituents like beta lactam ring, dioxane, 2-hexahyropyrimidine, 1-formyl and 2-formyl ring, pyridinium, tetrahyropyridine derivatives and nitroimidazole with a trisubstituted ethylenic double bond are reported recently. Such studies indicate the thrust for the future development of new nitroimidazole drugs. In the present study the 5- nitroimidazoles like tinidazole and dimetridazole molecule is thought to be modified at 2nd position.

Three series of 2-substituted, 5-nitroimidazole compounds were designed and synthesized i.e., nitroimidazole-oxime derivative, nitroimidazole with various 2-substituted heterocyclic rings and nitroimidazole-enamine derivatives. All the synthesized compounds were characterized by IR, ^{1}HNMR and MASS spectra. Twenty compounds from all three series of synthesized nitroimidazole were selected on the basis of their one electron reduction potential and their antianaerobic activity were carried out against *Clostridium sporogenus* and *Bacteriodes fragilis* using metronidazole as control drug. All the compounds sensitivity was found same as the metronidazole (control standard) at 10 µg/mL concentration against the Gram negative bacteria, *Bacteriodes fragilis.*Some of the synthesized compounds were found were found more potent then metronidazole at 20 µg/mL concentrations against Gram positive bacteria i.e., *C. sporogenus*, the compound number were (59-d, 67-c, 74-k, 74-d, 74-I, 74-h, 74-j). Compound 59-d belongs to the nitroimidazole-oxime derivative; 1-(2-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanone while compound number 67-c is 6-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-5-(furan-2-yl)-4H-1,3,4-thiadiazin-2-amine. Compounds 74-k, 74-d, 74-I, 74-h and 74-j were belonging to 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinylsubstitutedamine series.

Thus most potent series against Gram positive anaerobe; *C. sporogenus* was found to be nitroimidazole-enamine series i.e., 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinylsubstitutedamine which were more active as compared to the metronidazole. The compound 74-k i.e., 1-ethyl-6-fluoro-8-(4-(1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)vinyl)piperazin-1-yl)-4-oxo-1, 4-dihydroquinoline-3-carboxylicacid) were dimetridazole-norfloxacin enamine derivatives and 74-j i.e.,1-ethyl-8-(4-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoli ne-3-carboxylicacid, belongs to tinidazole-norfloxacin enamine derivatives, thus it indicates the increase in the potency of activity against gram positive anaerobe by fusion of quinolone moiety with nitroimidazole drugs, which acts as hybrid antibacterial to show dual activity against both anaerobic and aerobic bacteria due to nitroimidazole and quinolone moiety in a single molecule. Surprisingly it was found that isoniazide-tinidazole enamine compound, 74-i; *N*-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)isonicotinohydrazide were inactive against Gram positive bacteria while active against Gram negative bacteria.

QSAR study showed significant correlation between antibacterial activity and Extended Huckel partial atomic charges on oxygen of nitro group present in nitroimidazole against *Bacteriode fragilis* for the reported nitroimidazole drugs. The correlation was found reverse to those obtained for cytotoxicities, mutagenicity and DNA damage activity because the more electron affinity of the drugs were found in between -0.80 to -0.84 (Extended Huckel partial atomic charges on oxygen of nitro group present in nitroimidazole) have greatest potency against the test organism and also it's range of reduction for nitroimidazole in anaerobic condition. The correlation was also exactly reverse for the toxicity of aerobic bacteria which concludes that most electron affinic drug were reduced in the aerobic condition while compounds having less electron affinity and having low reduction potential were showing activity against anaerobes.

The QSAR studies along with physicochemical parameter like lipophilic, steric and electronic were carried out. It was found that the addition of partition coefficient i.e., lipophilic parameter values produced positive correlation, imparting better regressed line and increasing the correlation. However the molar volume and other steric factor

were not influencing the activity of nitroimidazoles. The electronic parameters such as LUMO, HOMO was found to be producing no significant change in the R value. It was thus concluded that nitroimidazole falling under -0.80 to -0.84 (Extended Huckel Partial atomic charges on oxygen of nitro group present in nitroimidazole) were considered to be safest drug for anaerobic infections.

As Extended Huckel Partial atomic Charges on oxygen of nitro group present in nitroimidazole less than -0.84 is not reduced under anaerobic condition and greater than -0.80 probably cytotoxicity in nature and produce DNA damage. All the 2-nitroimidazole taken in study shows value of Extended Huckel partial atomic charges on oxygen of nitro group present in nitroimidazole greater than -0.80, imparting cytotoxicity that was shown in Fig 6.

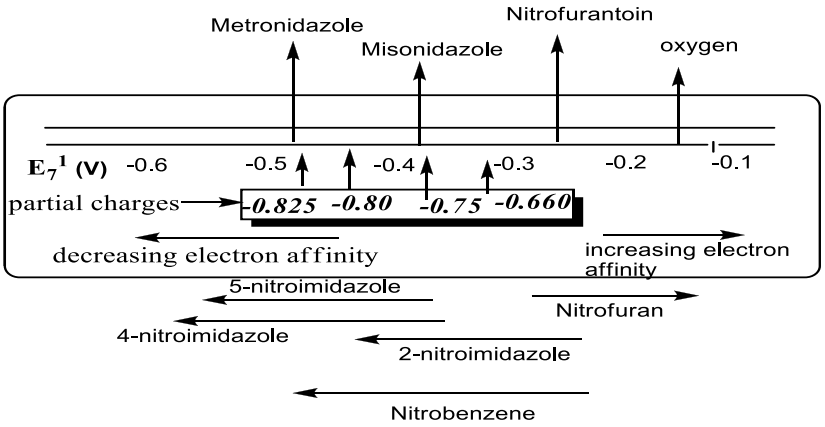


Fig 6 (modified): The redox spectrum showing the elctron affinity (E_7^1) of nitroaromatic drugs and calculated Extended Huckel partial atomic charges on oxygen showing an active range as (-0.80 to -0.825) for various biological activities of nitroimidazole drugs.

Thus it was concluded that as the one electron reduction potential can governs the DNA damage capacity, cytoxicity, mutagenicity, good anaerobic activity and aerobic toxicity, computed Extended Huckel partial atomic charges on oxygen of nitro group present in nitroimidazole could be utilize to correlates all biological activity significantly relevant to nitroimidazole drugs before its synthesis and with out taking any polarographic half wave reduction potential or one electron reduction potential practically.

INTRODUCTION

1.1 Five member heterocyclic compounds - Biological importance

Five-member nitrogen-containing heterocyclic occurs in a diversity of natural products and drugs and is of great importance in a wide variety of applications. Aromatic nitrogen-containing five-member heterocycles include pyrroles, pyrazoles, imidazoles, 1,2,3-triazoles, 1,2,4-triazoles and tetrazoles with one to four nitrogen atoms in the ring. Additionally, aromatic nitrogen heterocycles may contain another heteroatom, such as the oxygen in isoxazoles, oxazoles, 1,3,4-oxadiazoles and 1,2,4-oxadiazoles; or the sulfur in isothiazoles and thiazoles. Non-aromatic nitrogen-containing heterocycles include partially saturated "-olines" (preferably named with the prefix dihydro-) and completely saturated "-olidines". Partially or totally reduced heterocycles are of less interest in drug discovery because of their probable instability and chirality.

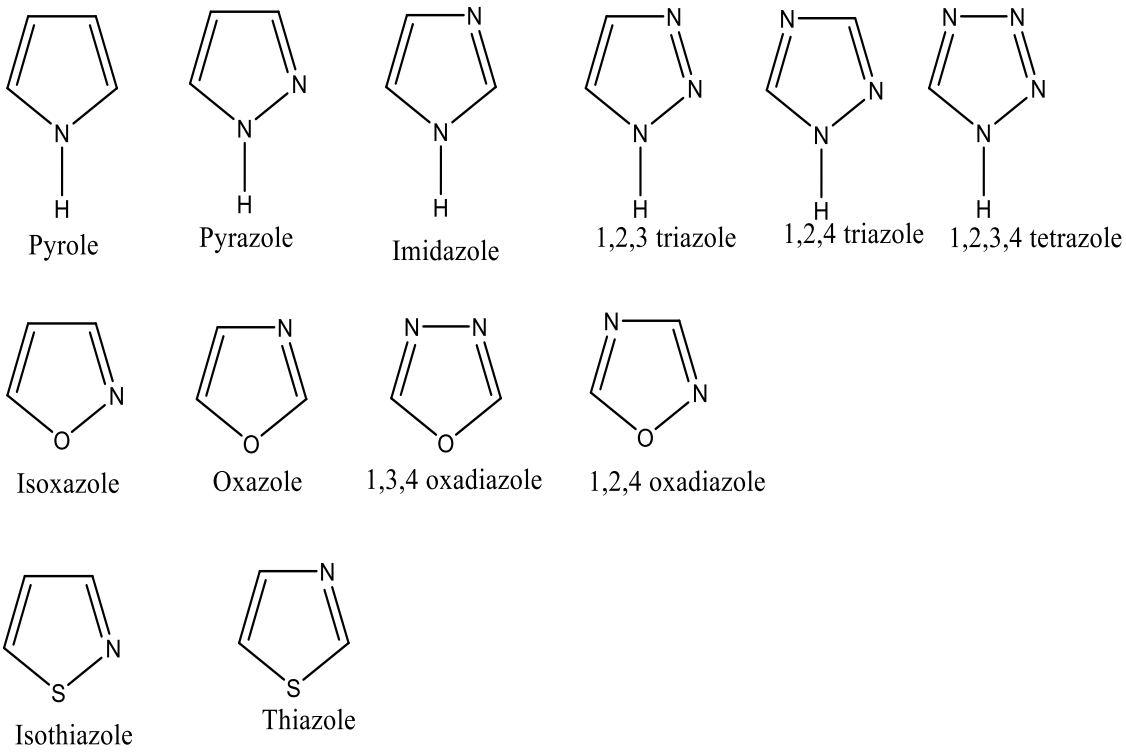
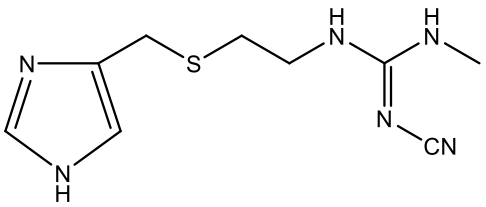


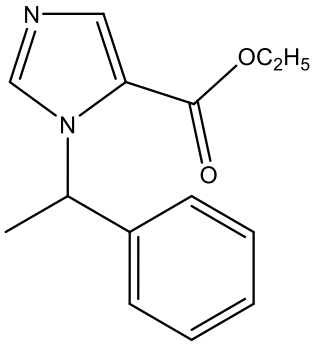
Fig 1: structure of some nitrogen containing five member heterocycles

Among five member nitrogen containing heterocycles represent a class of compounds of great importance in biological chemistry. For instance, five member nitrogen containing derivatives posses the biological activities like antidepressant, anticonvulsant, antimicrobial, analgesic, antitumour, human acyl-CoA: cholesterol acyltransferase inhibitors, anti-inflammatory drug (Fabio *et al*., 2007).

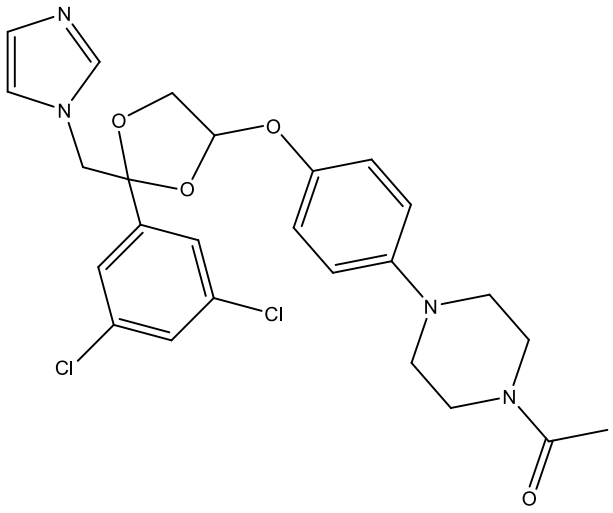
Among the diazole derivatives, imidazoles are common scaffolds in highly significant biomolecules, including biotin, the essential amino acid histidine, histamine, the pilocarpine alkaloids, and other alkaloids, which have been shown to exhibit interesting biological activity such as antimicrobial, anticryptococcal, inhibition of nitric oxide synthase, and cytotoxicity activity. Imidazole derivatives have also been found to possess many pharmacological properties and are widely implicated in biochemical processes. Members of this class of diazoles are known to possess NO synthase inhibition, antibiotic, antifungal, and antiulcerative activities and include compounds, which are inhibitors of 5-lipoxygenase and substances with CB_1 receptor, VEGF receptor I and II, and neuropeptide Y antagonistic activities. In addition, these heterocycles include several inhibitors of p38 MAP kinases, subgroup of mitogen-activated protein kinases, which are thought to be involved in a variety of inflammatory and immunological disorders and some derivatives such as cimetidine **(1),** etomidate **(2)** and ketoconazole **(3)** which have found application in drug therapy (Fabio *et al*., 2007).



(1)



(2)



(3)

1.2 Five member heterocyclic-nitroimidazole

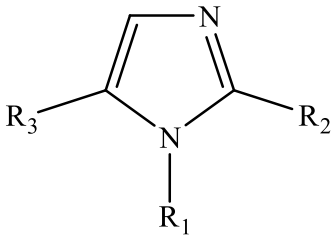
1.2.1 Introduction

The introduction of nitrohetercyclic drugs in the late 1950's and 1960's heralded a new era in the treatment of gram negative and gram positive bacteria and a wide range of pathogenic protozoan parasite and anaerobe. Azomycin (a 2-nitroimidazole) an antibiotic isolated in Japan from a streptomycete was the first compound of the nitroimidazole to be discovered (Maeda *et al*., 1953) and acted as the main impectus for the systematic search for drugs with activity against anaerobic protozoa. This led to the synthesis of 5-nitroimidazole, metronidazole and the demonstration of activity against *Trichomonas* (Cosar and Julou, 1959). Subsequently metronidazole was demonstrated to cure, giardiasis (Scheider, 1961), amoebiasis (Powell *et al*., 1966) and *Balantidium* infections. The first indication of activity of metronidazole against bacteria came from studies of patient with acute ulcerative gingivitis who was treated for concurrent trichomoniasis with metronidazole.

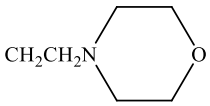
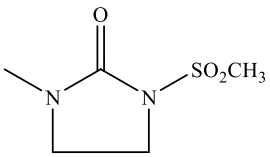
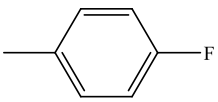
The 5-nitroimidazole are now widely used as prophylaxis against post-operative sepsis and in the treatment of gastric ulcers caused by *Helicobacter pylori* (Edwards, 1993). Metronidazole is the most extensively used 5-nitroimidazole and is currently recommended treatment for protozoan infection of *Giardia intestinalis* (Boreham, 1931) *Trichomonas vaginalis* (Lossik, 1990), *Entamoeba histolytica* (Knight, 1980), *Blastocycstic hominis* (Boreham and Stenzel, 1993) and also used to treat *Leishmania* (Arora *et al*., 1991). As an antibacterial agent it has been successfully used to treat *Helicobacter pylori*, *Bacteroides* spp. *Eubacterium* spp, *Preptococcus* spp., *Peptostereptococcus* spp., *Clostridium* spp., *Fusobacterium* spp., and *Gardnerella vaginalis* (Appelbaum *et al*., 1978; Hager and Rapp, 1992). Some of the nitroimidazole as shown in Table 1, such as tinidazole, nimorazole, ornidazole, satranidazole, secnidazole, nimorazole and carnidazole (Edward, 1983) have been used clinically or experimentally to treat the anaerobic pathogenic protozoa and bacterium in human and veterinary medicine.

The 2-nitroimidazole have an improtant place in medicine with benznidazole effective in the treatment of *Trypanosoma cruzi* infection and misonidazole, used for the treatment of selective chemotherapy of radio-resistant hypoxic tumors.

Table 1: Structure of nitroimidazole



(4)

Generic name	R_1	R_2	R_3
5-nitroimidazoles			
Metronidazole	$-CH_2CH_2OH$	$-CH_3$	$-NO_2$
Nimorazole		-H	$-NO_2$
Ornidazole	$-CH_2CH(OH)CH_2Cl$	$-CH_3$	$-NO_2$
Tinidazole	$-CH_2CH_2SO_2C_2H_5$	$-CH_3$	$-NO_2$
Secnidazole	$-CH_2CH(OH)CH_3$	$-CH_3$	$-NO_2$
Satranidazole	$-CH_3$		$-NO_2$
Flunidazole	$-CH_2CH_2OH$		$-NO_2$
Ronidazole	$-CH_3$	$-CH_2OCONH_2$	$-NO_2$
Dimetridazole	$-CH_3$	$-CH_3$	$-NO_2$
2-nitroimidazoles			
Azomycin	-H	$-NO_2$	-H
Misonidazole	$-CH_2CH(OH)CH_2OCH_3$	$-NO_2$	-H

1.2.2 Mode of action nitroimidazole in anaerobic bacteria

The 5-nitroimidazole is reduced to cytotoxic nitro-radicals by components of electron transport system in anaerobic and microaerophillic bacteria. The resultant short-lived radicals are thought to act by non-specific binding to and inactivation of the organism's DNA and enzymes. The electron transport components, unlike mitochondrial system depend upon ferredoxins and flavodoxin reduction via hydrogenase or pyruvate ferrodxin oxidoreductase (PFOR) as shown in Fig 2.

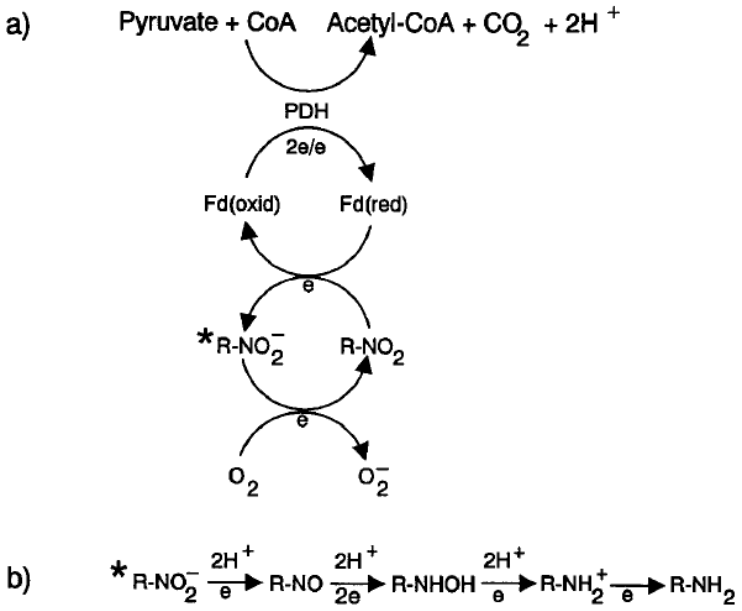


Fig 2: Proposed pathway of meteronidazole reduction in anaerobic protozoa and bacteria

a. Ferrodoxin is reduced by pyruvate: ferrodoxin oxidoreductase (PFOR) which accepts electrons from pyruvate. Meteronidazole is subsequently reduced in a one electron step to the unstable oxygen reactive intermediate ($*R\text{-}NO_2^-$) by reduced ferrodoxin. Oxygen may in turn oxidize reduced meteronidazole leading to futile cycling of the drug and the formation of superoxide. Hydrogenase may replace PFOR in the reduction of ferrodoxin. Ferrodoxin may be reduced by one or two electrons (2e/e) depending on the number of iron-sulphur cluster in the protein. Flavadoxin can serve as an electron acceptor from hydrogenase or PFOR and replace ferrodoxin in the reduction of metronidazole.

b. A two electron reduction of metronidazole leads to the formation of the highly toxic nitroso intermediate. Further reduction results in the formation of hydroxylamine radical ion and finally the amine.

The selective toxicity of 5-nitroimidazole for anaerobic or microarerophillic organism is due to the redox potential of their electron transport component, which sufficiently

negative to reduce the nitro group of these drugs. Aerobic organisms are capable of reducing nitroimidazole with higher redox potential such as misonidazole.

An alternative mechanism for nitroimidazole toxicity involves the conversion in to a thiamine analogue by thiaminase with the resultant analogue acting as a vitamin B1 antagonist (Alston *et al*., 1987). Thiamine metabolism of anaerobic bacteria and parasitic protozoa is poorly understood. Xanthine oxidase is capable of reducing nitroheterocyclic compounds including metronidazole (Morita *et al*., 1971) while the presence of xanthine oxidase in the parasitic protozoa is uncertain; it is likely that most anaerobic bacteria synthesize this enzyme. Thus enzymatic reduction *in vivo* may be an alternative mode of nitroimidazole activation. Xanthine oxidase in mammalian tissues may also reduce metronidazole which is likely that futile cycling by oxygen and cause toxic effect of this reaction (Goldman *et al*., 1986). The proposed mechanism of DNA damage is shown in Fig 3 (Horiato *et al*., 2005).

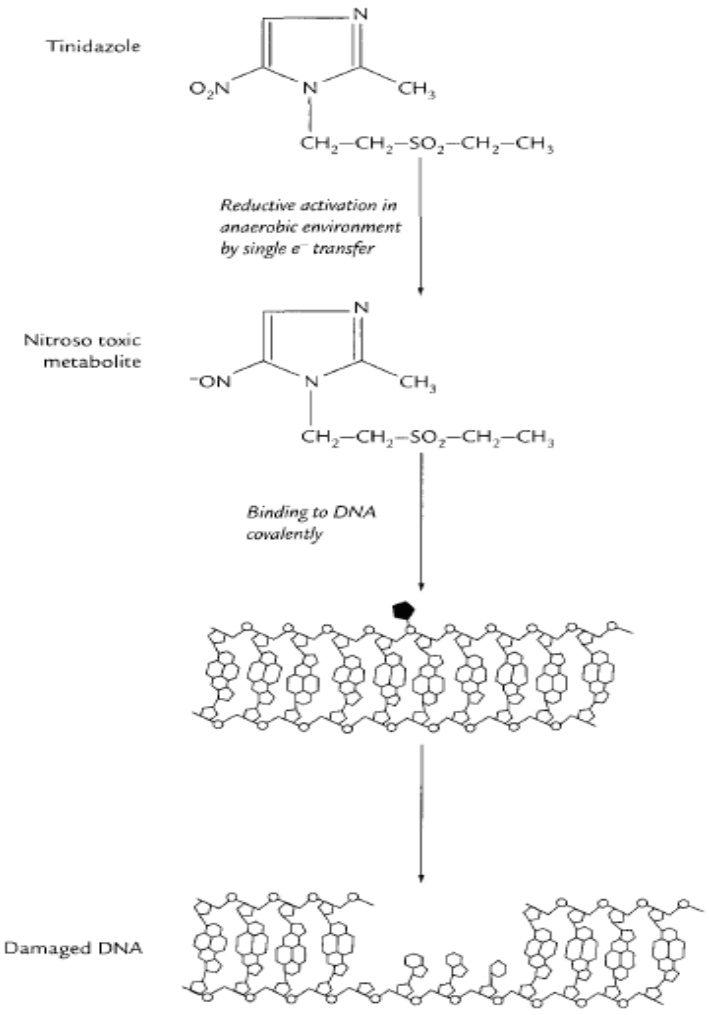


Fig 3: Proposed mechanism for tinidazole in anaerobic bacteria and protozoa

1.2.3 Mode of action in protozoa

In *Trichomonas vaginalis* and *Trichomonas foetus* the PFOR and to lesser extend hydrogenase reduce ferredoxins which in turn can be oxidized by nitroimidazole in this organisms, nitroimidazole is reduced to toxic radical in a specialized carbohydrate metabolizing organelle, the hydrogenosome, which contain hydrogenase, PFOR and ferredoxins (Muller *et al*., 1988).

1.2.4 Nitroimidazole resistance in bacteria

Nitroimidazole resistance in bacteria is associated with the inability of the organism to reduce the drug to its toxic radical. A decrease in the PFOR activity is one mechanism of resistance in *Bacteriodes*. A number of other mechanisms has been identified that is decreased drug uptake and increased lactate dehydrogenase activity. The later enable the bacteria to utilize an alternative energy pathway to pyruvate reduction by PFOR. Alternative mechanisms of resistance have been demonstrated in *Bacteriodes* spp., where nitroimidazole resistance has been associated with conjugative plasmids or chromosomally encoded resistance genes (Breuil *et al*., 1989).

1.2.5 Nitroimidazole resistance in protozoa

Metronidazole resistance was first reported in *Trichomonas vaginalis* in 1962 and subsequently has been extensively studied in this human parasite. Reduced PFOR, hydrogenase acitivites and altered hydrogenosome morphology correlated with degree of resistance. Loss of PFOR and hydrogenase activities resulted in compensatory metabolic changes in the metronidazole-resistance organisms, these change included increased rate of glycolysis and enhanced activities of pyruvate fermentation pathway in *Trichomonas foetus* this enhancement resulted in fermentation to ethanol and in *Trichomonas vaginalis* to acetaldehyde (Cerkasovova *et al*., 1988).

1.2.6 Cross-resistance among the nitroimidazoles

Cross-resistance to drugs of the same family is important in the management of resistance organism. Amongst 5-nitroimidazole, cross-resistance have been demonstrated in bacteria and protozoa. A total of eleven 5-nitroimidazole compounds shows the reduction in sensitivity varied between 2- and 6-fold, thus it indicates that it is inappropriate to use other nitroimidazole. In *Bacteroides* the cross resistance among

metronidazole, ornidazole and tinidazole has been reported (Sindar *et al*., 1982 and Towson *et al* 1984).

1.2.7 Toxicity of 5-nitroimidazoles

Metronidazole is rapidly and almost completely absorbed from the intestine with peak serum levels of around 14µg/mL following administration of 500mg to adults, the half life is around 7 hr. Adverse effect of nitroimidazole has been including nausea, vomiting, metallic taste, anorexia and diarrhoea. The most serious reactions are those which affect the central nervous system and include peripheral neuropathy, ataxia, vertigo, headache, confusion and convulsions. Available evidence suggests that there is no carcinogenic or mutagenic risk to human taking normal dose of metronidazole, for prescribed periods of time. However, unstable intermediate products resulting from metronidazole reduction appear in the urine of some test animals in sufficient quantities to give rise to Ames salmonella tests. DeMeo and colleagues evaluated the mutagenic and genotoxic potencies of forty eight nitroimidazole and imidazole derivatives using modified versions of SOS Chromotest and Ames test. They found that various derivatives could be mutagenic and genotoxic in bacteria, and in most case this depended upon the bacterial and mammalian nitroreductase activites on the nitro group at the 5^{th} position. Substitutions at 1^{st} and 2^{nd} position were found to modulate this activity.

1.3 Anaerobic infection

1.3.1 Introduction

Leeuwenhoek in 1680 was the first to observe anaerobic bacteria, their study dates from Pasteur's discovery in 1861 of the 'vibrione butyrique'. 'These infusory animalculae', he said, 'live and multiply indefinitely without requiring the least quantity of air and not only do they live without air, but air actually kills them'. How such obligate anaerobes contrive to generate energy and synthesize their substance without recourse to molecular oxygen, and why they are so adversely affected by oxygen, have remained the two questions which have most intrigued subsequent generations of microbiologists and biochemists (Morris, 1975).

Substantial protection against oxygen toxicity is afforded to aerobic and facultative anaerobic organisms by their possession of superoxide dismutase (Finegold, 1994). This enzyme was reportedly not present in obligatory anaerobic bacteria, i.e. species which exhibit sensitivity to oxygen at 0.2 atm or less (Finegold, 1994).

Anaerobes are the predominant components of the normal human skin and mucous membranes, bacterial flora (Summanen *et al.*, 1997 and Finegold, 1977) and are therefore, a common cause of bacterial infections of endogenous origin. Because of their fastidious nature, these organisms are difficult to isolate from infectious sites, and are often overlooked. Their exact frequency is difficult to ascertain because of the inconsistent use of adequate methods for their isolation and identification. The lack of directing adequate therapy against these organisms may lead to clinical failures. Their isolation requires appropriate methods of collection, transportation and cultivation of specimens (Brook *et al.*, 2004). Treatment of anaerobic infection is complicated by the slow growth of these organisms, by their polymicrobial nature and by the growing resistance of anaerobic bacteria to antimicrobials.

The major anaerobes encountered clinically are shown in the Table 2, in which the most common species of gram positive and gram negative anaerobic bacteria were included. Table 3 indicates the, types of infection involving anaerobes respectively where location of organ most commonly infected by the anaerobic bacteria.

Table 2: Major anaerobes encountered clinically

Anaerobe	Major anaerobes encountered clinically
Gram-negative bacilli	*Bacteroides fragilis* group Especially *B. fragilis, B. thetaiotaomicron, B. distasonis, B.ovatus, B. vulgatus*
Gram-positive cocci	Peptostreptococcus Especially P. *anaerobius, P. intermedius P. micros, P. magnus, P. asaccharolyticus, P. prevotii*
Microaerophilic streptococci	**Gram-positive sporeforming bacilli** *Clostridium perfringens, C. ramosum, C. septicum, C. novyi, C. histolyticum,C. sporogenes, C. sordeUii, C. bifermentans, C. fallax, C. difficile, C. innocuum, C. botulinum, C. tetani* **Gram-positive non-sporeforming bacilli** *Actinomyces (israelii, meyerii, naeslundii, odontolyticus, viscosus) Propionibacterium propionicum, P. acnes, Eubacterium lentum, E. nodatum, Bifidobacterium dentium*
Other Bacteroides	*B. gracilis, B. ureolyticus, B. splanchnicus, Porphyromonas* spp. Especially *P. asaccharolytica,* Pigmented *Prevotella* spp. (P. *corporis, P. denticola, P.intermedia, P. Ioescheii, P.elaninogenica, P. nigrescens)* Other *Prevotella* spp. *R oris, P. buccae, P. oralis* group *P. bivia, P. disiens, Fusobacterium* spp. *F. nucleatum, F. necrophorum, F. mortiferum, F. varium, Bilophila wadsworth*

Table 3: Types of infection involving anaerobes

Location	Type of infection
Upper respiratory tract	Dental infections , pulpitis, gingivitis, periapical or dental abscess, perimandibular space, chronic sinusitis , recurrent tonsillitis, chronic otitis media, mastoiditis, peritonsillar abscess
Lower respiratory tract	Aspiration pneumonia, necrotizing pneumonia, lung abscess, empyema
Gastrointestinal tract	Peritonitis, intraabdominal abscess, liver abscess
Female genital tract	Tubo-ovarian abscess, salpingitis Septic abortion and endometritis, bartholin's gland abscess, bacterial vaginosis
Skin and soft tissue	Crepitant cellulites, necrotizing fasciitis, myonecrosis (Gas gangrene), decubitus ulcer, diabetic foot ulcer, bite wound
Central nervous system	Cerebral abscess, epidural abscess, subdural empyema

1.3.2 Diagnosis

PCR-based detection of pathogen genomic DNA

Immediately after collection, all samples were washed in 1 ml of sterile DNAase-free water, and stored at -20 ^{0}C until analyzed. Total genomic DNA was isolated by a DNA isolation kit (Roche Diagnostics GmbH, Mannheim, Germany) and used as a template for PCR. The pathogen concentration was determined with the commercially available semi quantitative diagnostic kit micro Dent (Hain Life science GmbH, Nehren, Germany).PCR-amplified DNA fragments were hybridized with pathogen-specific sequences linked to the membrane stripes. The amount of hybridized DNA was measured by an enzyme-mediated colorimetric reaction (Smola *et al*., 2003).

1.3.3 Management of anaerobic infection

The two key approaches to treatment are surgery and antimicrobial therapy. Debridement and drainage are typically essential. Failure to carry out necessary surgical therapy, promptly and thoroughly, may lead to failure of response to appropriate antimicrobial agents. Some abscesses are amenable to percutaneous drainage under guidance of ultrasound or computed tomography.

Antimicrobial selection is simplified when reliable culture results are available. Because of the problems in obtaining appropriate specimens in anaerobic infections, many patients are treated empirically. Fortunately, the types of anaerobes involved in many anaerobic infections and their antimicrobial susceptibility patterns tend to be predictable, although they may vary in a particular hospital. Some anaerobes, however, have become resistant to antimicrobial agents or may become so while a patient is receiving therapy.

Rational empirical therapy of infections in which anaerobes are involved should in general be based on the following principles:

1. Modification of the environment to make if difficult for anaerobic bacteria to proliferate by performing debridement of dead tissues, drainage of collections of pus, elimination of any obstruction, decompression of swollen tissues under tight fascia and release of trapped gas in order to improve blood circulation and tissue oxygenation.
2. The spread of anaerobic bacteria into healthy tissues should be monitored. Several antimicrobial agents have been proved important in limiting the spread of microorganisms.
3. Clinical assessment of the nature of the infectious process
4. Knowledge of the administrations of prior antibiotics
5. Patterns of resistance of antimicrobial agents in each hospital
6. Careful analysis of Gram stain(s)
7. The required pharmacokinetic and pharmacodynamic properties at the infection site
8. Duration of pre-infection hospitalization
9. Potential toxicity
10. Effectiveness

1.3.3.1 Hyper baric oxygen therapy

Hyperbaric oxygen could have an application in surgical infections because:

(1) It brings O_2 levels back to normal or even produces hyperoxygenation. As a result the bactericidal effect of neutrophils and perhaps of macrophages on anaerobic bacteria increases.

(2) Hyperbaric oxygen therapy has a direct toxic effect on anaerobic bacteria because it induces an increased concentration of free radicals (peroxide and superoxide) from the increased O_2 levels in the previously hypoxic tissues.

(3) Hyperbaric oxygen therapy stimulates fibroblast activity and angiogenesis; consequently an increase rate of collagen disposition occurs thus decreasing the time necessary for wound healing. Eleven male patients (mean age 59.5 years) with Fournier's gangrene of the scrotum was treated with 6 to 24 cycles of hyperbaric oxygen plus tissue debridement in six and reconstructive surgery in three patients. There was a 0% death rate (Pizzaorono *et al*., 1997).

1.3.3.2 Surgical therapy

In many cases, surgical therapy is the most important and sometimes the only form of treatment required, whereas in others it is an adjunct to a pharmacological approach. It includes draining abscesses, debriding necrotic tissues, decompressing closed-space infections and relieving obstructions. Without drainage the infection may persist and serious complications can develop (Brook, 2004).

1.3.3.3 Antimicrobial therapy

Appropriate management of mixed aerobic and anaerobic infection requires the administration of antimicrobial effective against both components. A number of factors should be considered when choosing appropriate antimicrobial agents. They should have efficacy against the entire target organism, achieve sufficient levels in the infected site and have minimal toxicity and maximum stability.

Table 4: Antimicrobial drugs of choice for anaerobic bacteria

Microbe	First	Alternate
Peptostreptococcus spp.	Peniclillin	Clindamycin, chloramphenicol, cephalospoirns
Clostridium spp.	Penicillin	Metronidazole, chloramphenicol, cefoxitin, clindamycin
C. difficile	Vancomycin	Metronidazole, bacitracin
Gram-negative rods (Non β lactamase)	Penicillin	Metronidazole, clindamycin, chloramphenicol
Gram-negative rods (β lactamase)	Metronidazole, imipenem, penicillin and β-lactamase inhibitor, clindamycin	Cefoxitin, chloramphenicol, piperacillin

Table 5: Parenteral antibiotics used to cover the aerobic and anaerobic components of the human colonic microflora

Combination therapy	Single-drug for aerobic and anaerobic
Aerobic Aminoglycosides Aztreonam Cephalosporins Quinolones	Amoxycillin:clavulanate , ampicilin:sulbactam Ticarcillin:clavulanate, piperacillin:tazobactam Cefoxitin Cefotetan Ceftizoxime Imipenem: cilastatin
Anaerobic Metronidazole Ornidazole Tinidazole Clindamycin Chloramphenicol	Meropenem Third generation quinolone (Trovafloxacin, sparfloxacin, clinafloxacin, gatifloxacin, and moxifloxacin)

1.3.3.4 New drugs to treat anaerobes

The newest agents approved by the FDA to treat anaerobic infections, including intra-abdominal, skin and soft tissue infections are ertapenem, doripenem, tigecycline and moxifloxacin. The organisms covered by these compounds vary but generally include some *B. fragilis* species, *clostridia*, and *peptostreptococci* (Goldstein *et al*., 2008).

Doripenem

Doripenem (common name doripenem monohydrate) is an ultra-broad spectrum injectable antibiotic. It is a beta-lactam and belongs to the subgroup of carbapenems. It was launched by Shionogi Co. of Japan under the brand name Finibax in 2005 and is being marketed outside Japan by Johnson & Johnson. *In vitro*, doripenem has similar anaerobic activity to the other approved carbapenems. Doripenem can be used for bacterial infections such as: complex abdominal infections, pneumonia within the setting of a hospital, and complicated infections of the urinary tract including kidney infections with septicemia. When doripenem is absorbed into the body, the drug takes effect by eliminating the initial bacteria causing the infection. Primarily, doripenem decreases the process of cell wall growth, which eventually leads to elimination of the infectious cell bacteria altogether. Doripenem was approved by the United States Food and Drug Administration on October 12, 2007, to be sold under the trade name Doribax (US FDA, 2007).

Tigecycline

This antibiotic is the first clinically-available drug in a new class of antibiotics called the glycylcyclines (Christine, 2009). It is structurally similar to the tetracyclines in that it contains a central four-ring carbocyclic skeleton and is actually a derivative of minocycline. Tigecycline has a substitution at the D-9 position which is believed to confer broad spectrum activity (Rose *et al*., 2006 & Kasbekar, 2006). Tigecycline is bacteriostatic and is a protein synthesis inhibitor by binding to the 30s ribosomal subunit of bacteria and thereby blocking entry of Aminoacyl-tRNA into the A site of the ribosome during prokaryotic translation (Keith *et al*., 2008). It is broadly active *in vitro* against usual and unusual anaerobes; in one tigecycline study, it was against all gram-positive strains and 228 of 232 gram-negative anaerobes at ≤1 ug/mL with one strain of *Prevotella oralis* that was non susceptible at 8 ug/mL. *B. fragilis* strains with MICs of 8 ug/mL are also reported (Goldstein *et al*., 2008).

Moxifloxacin

Moxifloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell division. Moxifloxacin susceptibilities have been variable between different B. fragilis group isolates, according to studies published amongst different laboratories and according to the clinical source of the isolate (e.g., pelvic, intra-abdominal, skin and soft tissue) and the control strain performance readings (Goldstein *et al*., 2008).

Faropenem

Faropenem remains in development with MIC of 1 mg/L for many Gram-negative and Gram-positive anaerobes. One study reported that only 5 strains of the *B. fragilis* group (1.1% of all anaerobes tested) were resistant to faropenem, and another noted 2/176 strains of *B. fragilis* had faropenem MICs of 64 mg/mL and imipenem MICs of >32 ug/mL (Mustaq *et al*., 2007).

Dalbavancin

Dalbavancin is a novel second-generation lipoglycopeptide antibiotic. It belongs to the same class as vancomycin (Chen *et al*., 2008), Dalbavancin's *in vitro* activity against 120 anaerobic isolates from pretreatment diabetic foot infections showed an MIC_{90} of ≤0.125 mg/mL against *C. perfringens*, other *clostridia, Peptoniphilus asaccharolyticus, Finegoldia magna*, and *Anaerococcus prevotii.*

Fidaxomicin

It is also known as OPT-80 and PAR-101.

It is the first in a new class of narrow spectrum macrocyclic antibiotic drugs (Revill *et al*., 2008). It is non-systemic, meaning it is minimally absorbed into the bloodstream, it is bactericidal and it has demonstrated selective eradication of pathogenic *Clostridium difficile* with minimal disruption to the multiple species of bacteria that make up the normal, healthy intestinal flora. The maintenance of normal physiological conditions in the colon can reduce the probability of *Clostridium difficile* infection recurrence (Louie *et al*., 2009; Johnson *et al*., 2009). It is being developed by Optimer Pharmaceuticals for treatment of *Clostridium difficile*

infection. It is administered orally. It works by inhibiting the bacterial enzyme RNA polymerase, resulting in the death of *Clostridium difficile*. It is active against gram positive bacteria especially *Clostridia*.

1.4 References

Alston TA, Abeles RH. Enzymatic conversion of the antibiotic metronidazole to an analog: of thiamine. Arch Biochem Biophys 1987;257:357-362.

Appelbaum PC, Chatterton SA. Suspetitbilty of anaerobic bacteria to ten antimicrobial agents. Antimicrob Agents chemeother 1978;14:371-376.

Arora SK, Sinha R, Sehgal S. Use of invitro method to assess different brand of antileshmanial drug. Med Microbial Immunol 1991;180:21-27.

Boreham PFL, Stenzel DJ. Blastocystis in humans and animals; morphology, biology and epizootiology. Adv Parasitol 1993;32:1-70.

Breui J, Dublanchet A, Truffaut N, Sebald M. Transferable 5-nitroimidazole resistance in *Bacteroides fragilis* group. Plasmid 1989;21:151-154.

Brook I. Management of anaerobic infection Exp Rev Anti-infective. Ther 2004;2(1): 153-158.

Cerkasovova A, Novak J, Cerkasov J, Tachezy J. Metabolic properties of Trichonmonas vaginalis resistant to metronidazole under anaerobic condition. Acta Univ Carol Biologica 1988b;30:505-512.

Chen AY, Zervos MJ, Vazquez JA. Dalbavancin: a novel antimicrobial. Int J Clin Pract 2007;61:853–863.

Christine MS, Keith A, Rodvold, Larry H, Danziger. Tigecycline: A Novel Broad-Spectrum Antimicrobial: Pharmacology and Mechanism of Action, Infectious Diseases Fellow, Department of Pharmacy Practice, University of Illinois at Chicago.

Cosar C, Julou L. Activity of 9 (hydroxy-2-ethyl)-1-methyl-2-nitro-5 imidazole against *Trichomonas vaginalis*. Ann Inst Pasteur 1959;96:238-241.

Edward DI. Nitroimidazole drugs-action and resistance mechanism I. J Antimicrob Chemother 1993;31:9-20.

Fabio B, Silvia C, Rossi R. Synthesis and biological activity of vicinal diaryl-substituted 1*H*-imidazoles. Tetrahedron 2007;63:4571–4624.

Finegold SM. Review of early research on anaerobes. Clin Infect Dis 1994;18:248-249.

Goldman P, Koch RL, Yeung TC, Chrystal EJT, Beulieu BB, Mclafferty MA, Sudlow G. Comparing the reduction of nitroimidazoles in bacteria and mammalian tissues and relating it to biological activity. Biochem Pharmacol 1986;35:43-51.

Goldstein EJC, Citron DM. New Antibiotics to Treat Anaerobic Infections , Anaerobe 2008, The 9th Biennial congress of the Anaerobe society of the Americas Renaissance Hotel Long Beach, California USA. 2008; June 24-27.

Hager WD, Rapp RP. Meterondiazole. Obstet Gynecol Clin North Am 1992; 19:497-510.

Horatio BF, Doan T. Tinidazole: A Nitroimidazole Antiprotozoal Agent. Clinical Therapeutics 2005;27:1859-1884.

Johnson S. Recurrent Clostridium difficile infection: a review of risk factors, treatments, and outcomes. J of Inf 2009;58 (6):403–410.

Kasbekar N. Tigecycline: a new glycylcycline antimicrobial agent. Am J Health Syst Pharm 2006;63:1235–1243.

Knignt R. The chemotherapy of amoebiasis. J Antimicrob Chemother 1980;6:577-593.

Lossick JG. Therapy of urogenital trichomoniasis. Springer Newyork 1990;324-341.

Louie T, Judy E, Walter K, Brendan B, Manuel M. OPT-80 Eliminates Clostridium difficile and is Sparing of Bacteroides. Antimicrob Agents and Chemother 2009;535:261–263.

Maeda K, Osata T, Umezawa H. A new antibiotic, Azomycin. J Antibiot A 6, 182 1953.

Morita M, Feller DR, Gillete JR. Reduction of nitridazole by rat liver xanthine oxidase. Biochem Pharmacol 1971;20:217-226.

Morris JG. Nitroimidazoles II. Microb Physiol 1975;12:169-246.

Muller M. Mode of action of metronidazole on anerobic bacteria and protozoa. Surgery 983;93:165-171.

Muller M, Lossick JG, Gorrell TE. In vitro susceptibility of Trichomonas vaginalis to metronidazole and treatment outcome in vaginal trichomoniasis. Sex Transm Dis 1988;15:17-24.

Mushtaq S, Hope R, Warner M, Livermore DM. Activity of faropenem against cephalosporin-resistant Enterobacteriaceae. J Antimicrob Chemother 2007;59:1025–1030.

Pizzorno R, Bonini F, Donelli A, Stubinski R, Medica M, Carmignani G. Hyperbaric oxygen therapy in the treatment of Fournier's disease in 11 male patients. J Urol 1997;158:837-840.

Powell SJ, Macleod I, Wilmit AJ, Elsdon-Dew R. Metronidazole in amoebic dysentery and amoebic liver abscess. Lancet 1966;2:1329-1331.

Revill P, Serradell N, Bolos J. Tiacumicin B: macrolide antibiotic treatment of C. difficile-associated diarrhea. Drugs of the Future 2006;31:494-497.

Rose W, Rybak M. Tigecycline: first of a new class of antimicrobial agents. Pharmacotherapy 2006;26:1099-1110.

Scheider J. Traitment de la giardiase (lambliase) par la metronidazole. Bull Soc Path Exot 1961;54:84-93.

Sindar P, Britz M, Wilkinson RG. Isolation and properties of metronidazole-resistants of Clostridium perfringes. J Med Microbiol 1982;15:503-509.

Smola SF, Rettenberger G, Simmet T, Burysek L. Comparison of sample collection methods for the PCR detection of oral anaerobic pathogens. Letters in Applied Microbiology 2003;36:101–105.

Townson SM, Boreham PFL, Upcroft, Upcroft JA. Resistance to the nitroheterocyclic drugs. Acta Tropica 1994;56:173-194.

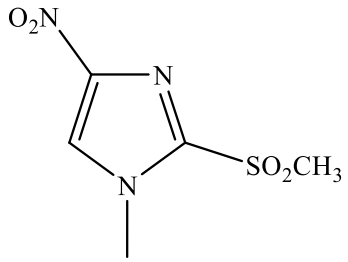
U.S. Food and Drug Administration. FDA Approves New rug to Treat Complicated Urinary Tract and Intra-Abdominal Infections. Press release 2007. http://www.fda.gov/bbs/topics/NEWS/2007/NEW01728.html.

REVIEW OF LITERATURE

2.1 Biological importance of nitroimidazoles

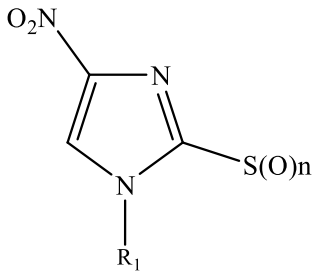
2.1.1 Nitroimidazole as antibacterial agents

The nitroimidazole compound (**5)**, 1-methyl-2-methylsulfonyl-4-nitroimidazole is an antiprotozoal and bactericidal compound with the unique and surprising property of being totally non-mutagenic and thus of a much higher degree of safety than is found with other nitroimidazoles (Nagrajan *et al.,* 1982; Bagan *et al.,* 1988).



(5)

The invention of the nonmutagenic 1,2-disubstituted-4-nitroimidazole compounds with structure **(6)** useful as antiprotozoal agents (Miwa *et al.,* 1987).

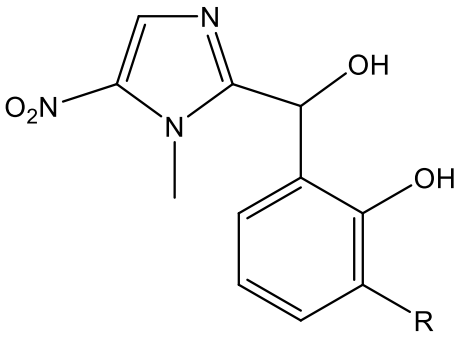


(6)

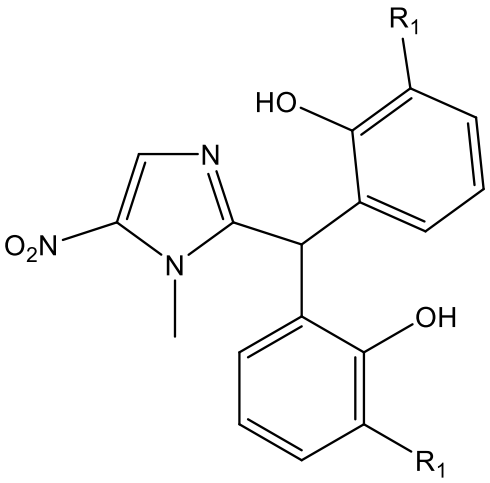
The compounds are 1,4-dimethyl-4-nitro-1*H*-imidazol- 2-yl methylcarbamate, 1,4-dimethyl-2-(2-hydroxyethyl)-4-nitroimidazole, 1,2-dimethyl-4-(2-hydroxyethyl)-4-nitroimidazole and pivaloyl esters. These novel compounds are not mutagenic in Ames strain TA100 and are highly potent against protozoal diseases. They also demonstrated that 1,2,4-substituted,5-nitroimidazoles are potent antiprotozoal and/or antibacterial agents with little or no mutagenic and drug residue level problems. There was an attempt to differentiate among the structural elements contributing to mutagenicity and antitrichomonal activities as a basis for the rational design of safer nitroimidazoles (Scalesiani *et al.,* 1983).

Walsh *et al.,* presented two approaches for developing nonmutagenic 5-nitroimidazoles based on knowledge of the likely mechanisms by which reactive intermediates are metabolically formed. These approaches are the incorporation of a substituent at the C-4 position of the imidazole ring and the addition of readily oxidizable functionalities (gallate ester derivatives) into the molecule. These structural features revealed that the addition of a 4-substituent significantly reduced or eliminated the mutagenicity, and always reduced the *in vitro* antitrichomonal activity.

Differently substituted 2-hydroxyaryl-(1-methyl-5-nitro-1*H*-imidazol-2-yl)methanols, **(7), (8)** with substituted phenolic rings in the ortho and/or para positions and containing bulky and strong lipophilic groups such as a t-butyl group in the carbocyclic ring have been reported in the patents (Tessitore, 1983 and Fudan, 1992) for their antimicrobial activity and lack of mutagenicity (Arredondo *et al.,* 1996).

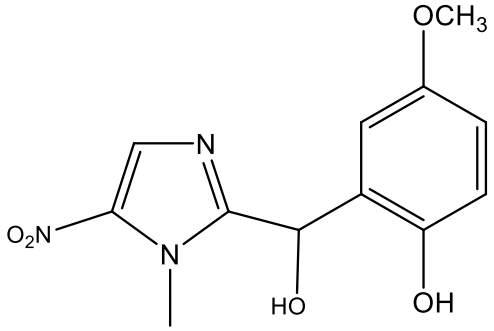


(7)



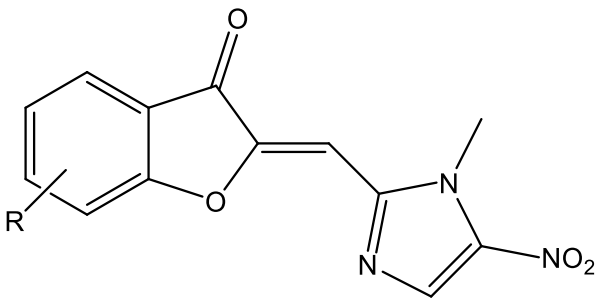
(8)

The antimicrobial profile of a new nitroimidazole derivative EU 11100 (5-nitro-1-methylimidazol-2-yl-(2-hydroxy-3-*tert*-butylphenyl)carbinol) has been studied Dubini *et al.,*1992). The *in vitro* activity has been evaluated against both aerobic and anaerobic bacteria, *T. vaginalis*, and mycetes, under suitable experimental conditions. The compound was compared with ampicillin against aerobic bacteria; with metronidazole against anaerobic bacteria lactobacilli and *T. vaginalis;* with nistatin and econazole against candida and with econazole and bifonazole against filamentous fungi. This derivative has been shown to be moderately active against some anaerobic bacteria belonging to both the Gram-positive and Gram-negative groups. Its inhibitory activity against *T. vaginalis* was similar to that of metronidazole (Dubini *et al.,*1992).

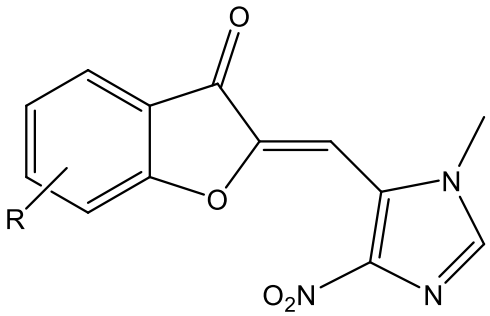


(EU 11100)

A new series of substituted (*Z*)-2-arylidene-3(2*H*)-benzofuranones bearing 1-methyl-5-nitroimidazole or 4-nitroimidazole **(9) & (10)** were synthesized and assayed for their antibacterial activity against Gram-positive and Gram-negative bacteria (Crozet *et al.,* 2009 and Hadij *et al.,* 2007). Most of the 5-nitroimidazole analogues showed a remarkable inhibition of a wide spectrum of Gram-positive bacteria (*Streptococcus aureus*, *S. epidermidis*, *MRSA*, and *Bacillus subtilis*) and Gram-negative *Klebsiella pneumonia*e, whereas 4-nitoimidazole analogues exhibited no effect against selected bacteria. The quantitative structure–activity relationship investigation was applied to find a correlation between different physicochemical parameters of the compounds studied and their biological activity. Their antibacterial activity, suggests that possibly negative charge of $–NO_2$ group on C-5 of imidazole and partial charge on carbonyl oxygen in benzofuran are necessary for action.

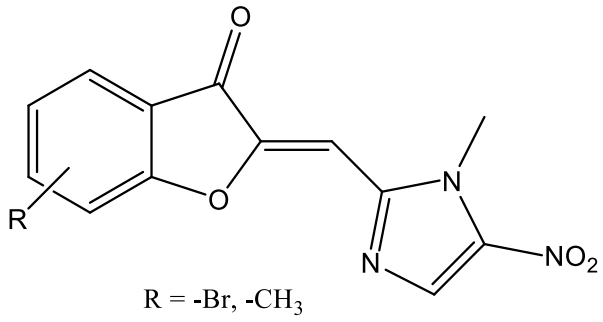


(9)



(10)

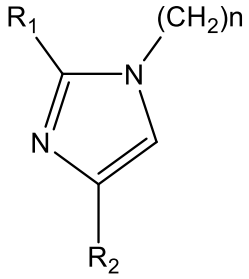
Moreover, there is a limited space available for this moiety of benzofuran system, thus the bulkier substituted analogues **(11)** (R = -Br, $-CH_3$) were inactive. Changing the position of the substituent on the benzofuran appears to have little influence on the antibacterial activity.



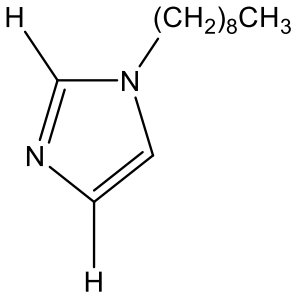
R = -Br, -CH_3

(11)

The series of alkylimidazole are investigated the relationship between the length of the alkyl chain and its antibacterial activity (Khabnadideh *et al.*, 2003). The compound **(12)** was investigated for antibacterial activity against *Staphylococcus aureus, Pseudomanas aeruginosa* and *Escherichia coli*. Several compounds showed significant *in vitro* activity against *E. coli, S. aureus* and *P. aeruginosa.* Antibacterial activity of 1-alkylimidazoles increases as the number of carbons in the alkyl chain rises to nine and then decreases. So 1-nonylimidazole **(13)** is the most effective compound, as it has the lowest MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) (MIC *S. aureus* = 10 μg/mL; MBC *S. aureus* = 19 μg/mL; MIC *P. aeruginosa* = 39 μg/mL; MBC *P. aeruginosa* = 78 μg/mL; MIC *E. coli* = 19 μg/mL; MBC *E. coli* = 39 μg/mL).



R_1=H, CH_3, R_2 = H, NO_2



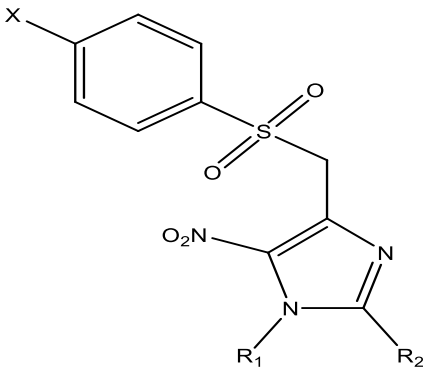
(12) **(13)**

The antiparasitic activity of nitroimidazoles **(14)** bearing an arylsulfonylmethyl group was assessed against *T. vaginalis*, and the *in vitro* cytotoxicity was evaluated on human monocytes and the mutagenicity was determined by the salmonella mutagenicity assay (Crozet *et al.,* 2009). Molecules, bearing an additional methyl group on the 2nd position, showed a lower mutagenicity than metronidazole. All the tested molecules were found to be mutagenic in the salmonella mutagenicity assay

using the most sensitive strain TA100 (Herelia *et al.*, 1998 and Purohit *et al.*, 2000). Moreover, three derivatives were characterized by a low mutagenicity and an efficient antitrichomonal activity. The methyl group at the 2nd position and the arylsulfonylmethyl group at the 4th position of imidazole ring modulated the nitro reduction at the 5th position as in **(14)**. They have shown that the replacement of the methyl group at the 2-position by a lactam group could double the mutagenicity of metronidazole (De Meo *et al.*, 1992) In this study, the methyl group at the 2-position combined with the arylsulfonylmethyl group at the 4th position lowered the MPTA100 of dimetridazole, metronidazole and secnidazole series (Herelia *et al.*, 1998).

A good correlation was demonstrated between the antiprotozoal activity and the mutagenicity for these molecules which reflects their ability to damage DNA through covalent binding and induction of DNA breaks (Dobias *et al.*, 1994). They were characterized by a low mutagenicity and high antiparasitic activity. Although the mechanism of action explaining their biological activity remains to be elucidated, these newly synthesized compounds allow the disconnection between mutagenicity and biological activity for the first time.

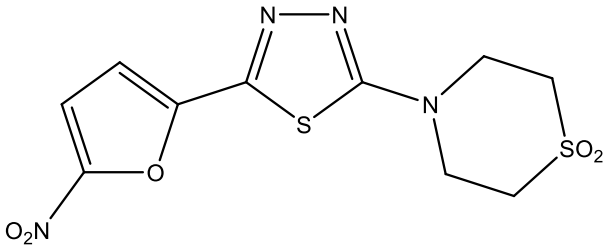
This approach offers the possibility of synthesizing new and potentially safer 5-nitroimidazoles. A series of *N*-[5-(5-nitro-2-heteroaryl)-1,3,4-thiadiazol-2-yl] thiomorpholine derivatives were synthesized and evaluated for *in vitro* anti-*Helicobacter pylori* activity (Mirzae *et al.*, 2008).



X= -H, -CH_3, -Cl, -Br, -F
R_1,R_2 = -H, -CH_3, -$(CH_2)_2OH$

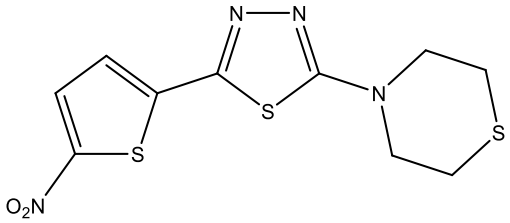
(14)

Nitrofuran analogue **(15)** containing thiomorpholine S,S-dioxide moiety was the most potent compound displaying very strong activity at 8 mg/disc (inhibition zone diameter >40 mm) against both metronidazole-sensitive and resistant strains.

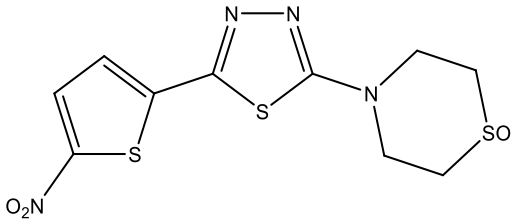


(15)

Among nitrothiophenes, compound **(16)** showed moderate activity and compound **(17)** showed strong activity against *H. pylori* at three different concentrations.



(16)



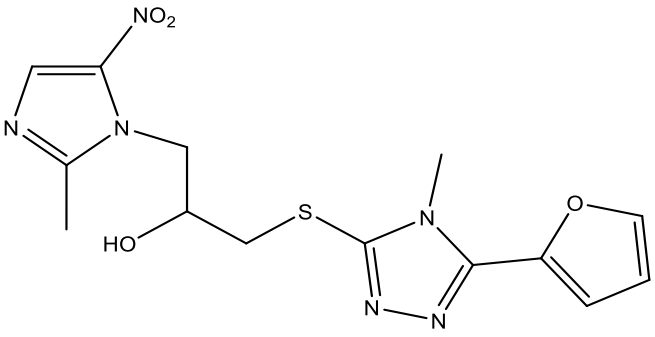
(17)

2.1.2 Antifungal nitroimidazoles

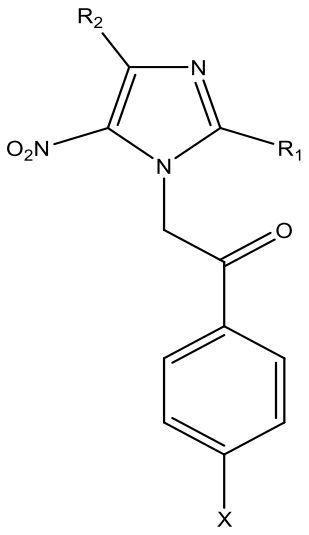
The imidazoles are a class of antifungal azole derivatives having a broad spectrum of activities both *in vitro* and *in vivo*. Four 5-nitroimidazoles derivatives, satranidazole, S750400 A, flunidazole and ronidazole were tested and found to be more active than metronidazole, the drug commonly used to treat infections caused by *Blastocystis hominis* in humans (Dunn *et al.*, 1991). Ketoconazole and iodoquinol have been reported to have therapeutic activity in infections caused by *B. hominis*, and were found to be less active than metronidazole. Some new nitroimidazole derivatives bearing a 1,2,4-triazolylthioethyl, 1,2,4-triazolylthiopropyl or *N*,*N*-disubstituted dithiocarbamoyl-propyl residue at N-1 were synthesized and evaluated for *in vitro* antibacterial and antifungal activity (Gunay *et al.,* 1999). Although not as active as the standard ampicillin, compound **(18)** were found to be active against *S.aureus* ATCC 6538 and/or *S. epidermidis* ATCC 12228 where ornidazole was devoid of activity. The most active compound was **(19)** (antifungal activity against *Trichophyton tonsurans* NCPF 245; MIC = 3 μg/mL) exerting about one half the activity of

ornidazole. A panel of antifungal and antihelmintic drugs was tested for activity against *Mycobacterium tuberculosis in vitro*.

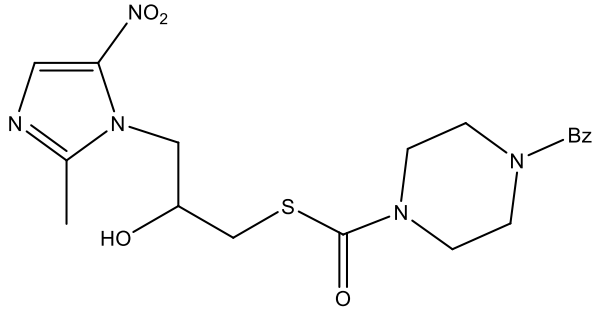
Antifungal drugs, miconazole, 2-nitroimidazole, clotrimazole, and the antihelmintic drug niclosamide were found to have significant antituberculosis activity, with MICs between 1 and 10 μg/mL (Sun *et al.,* 1999). Niclosamide and 2-nitroimidazole also had activity against stationary phase tubercle bacilli. Some derivatives have been synthesized by treating 4,5-dinitro- and 2-methyl- 4,5-dinitroimidazoles with epoxypropane, epichlorohydrin or phenacyl bromide and tested for their antioxidant and antifungal properties against fungi species *Sclerophoma pityophila* (Olender *et al.,* 2009). The nitro group in N-substituted 4,5-dinitro- and 2-methyl-4,5-dinitroimidazoles has been replaced with primary and secondary amines to afford 4-amino-5-nitroimidazole derivatives. Nearly all of them have shown significant antioxidant activity in comparison with that of tocopherol, which is used as a reference substance. Amongst the most active derivatives **(20),** bearing chlorine atom in 2-hydroxypropyl and 2-oxopropyl chains or phenacyl group at N-1 position of the imidazole ring, two compounds have very strong fungistatic activity against *S. pityophila*. High effectiveness was induced by the displacement of nitro group at 4-position on the imidazole ring to morpholine or piperidine and by the presence of a chlorine atom at 4th position on the phenyl ring.



(18)



(19)



(20)

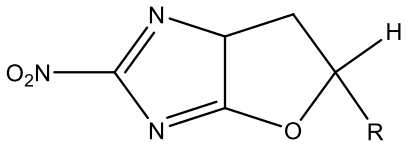
2.1.3 Antimycobacterial nitroimidazoles

One of the first reports to note the antimycobacterial activity of 2-nitroimidazoles compounds were a series of compounds synthesized with a variety of substituents at the 1st and 5th positions (Cavelleri *et al.,* 1973). They were tested for activity against a panel of Gram-negative and Gram-positive organisms, as well as fungi. The majority of compounds with alkyl, amide or alcohol substituents were found to be inactive against all organisms tested. There was improvement in activity, however, when the 5-substituent was replaced with a vinyl group. A four-fold increase in *Mycobacterium tuberculosis* activity occurred when the N-1 substituent was changed from methyl to ethyl. Activity was the highest against *Mycobacterium tuberculosis* when the N-1 substituent was ethyl and the 5-vinyl group was left unsubstituted. These vinyl substituted 2-nitroimidazoles were explored further in a subsequent report by the same group (Cavelleri *et al.,* 1977) and the best activity against this strain (2 μg/mL) was for 1-methyl-2-nitro-5-(2-nitrohex-1-enyl)-1*H*-imidazole, a compound with a vinyl group substituted with both an *n*-butyl group and a second nitro group.

Several series of 2-nitro and 2-aminoimidazoles with hydrophilic substitutions at the 5th positions such as oximes and hydrazones were synthesized and tested for antibacterial activity (Cavelleri *et al.,* 1977). Most of these compounds had moderate activity against *Mycobacterium tuberculosis* (10–200μg/mL), but they displayed enhanced activity against many other organisms. The most potent compound against *Mycobacterium tuberculosis* (5 μg/mL) was bearing an *n*-decyl substituted oxime at the 5th position of the imidazole ring. The authors noted that lipophilic substituents at the 5th position increased activity against Gram positive organisms, including *Mycobacterium tuberculosis*. 4- and 5-nitroimidazoles have been the subject of significant efforts to optimize antitubercular activity. One particular class of

nitroimidazoles, containing an oxazole (or oxazine) ring structure fused to the imidazole, has shown particular promise. These compounds were originally discovered to create novel dinitroimidazoles that might have improved characteristics as radiosensitizers.

Dinitroimidazoles, nitroimidazo[2,1-*b*]oxazoles were synthesized by reaction of the unsubstituted dinitro precursor with oxiranes, spontaneous cyclization, accompanied by loss of the 2-nitro group (Shaegal *et al*., 1979). These compounds were later found to have potent antitubercular activity and analogued by chemists from Hindustan Ciba-Geigy, India for antitubercular optimization (Nagargan *et al.*,1982 and Astekar *et al.,* 1993). Three discrete series of structural analogs were explored: 4-nitroimidazo [2,1-*b*]oxazoles, 5-nitroimidazo[2,1-*b*] oxazoles, and 4-nitroimidazo-1-ethanols. The uncyclized nitroimidazo-1-ethanols were not as active as the corresponding imidazo[2,1-*b*]oxazoles. The lead compound was **(21a)**, with a methyl group on the 2-position of the imidazo-oxazole ring, and a MIC of 1.95 μg/mL against *Mycobacterium tuberculosis* H37Rv. Variations in the imidazo[2,1-*b*]oxazole series with a single substitution at the 2-position, increasing chain length slightly (to ethyl to give compound CGI 17341, **(21b)** or adding a single halogen atom **(22c)** increased *in vitro* activity dramatically (30 and 16-fold, respectively). Replacement of the methyl group with a benzene ring **(21d)** increased activity only slightly (2-fold) while replacement with long, straight-chain alkyl groups decreased *in vitro* activity.



21 a, R = CH_3

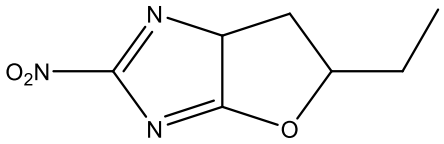
21 b, R = C_2H_5

21 c, R = CH_2Cl

21 d, R = Ph

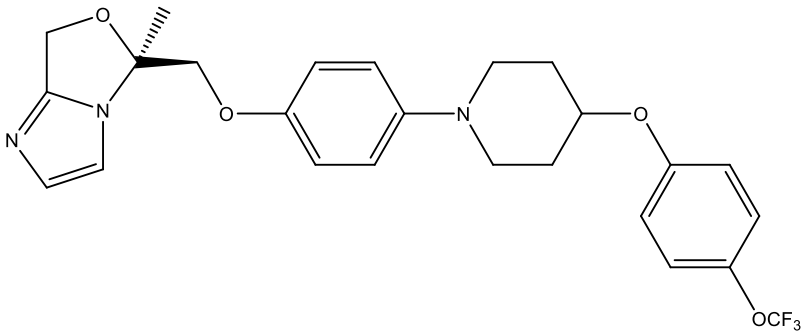
CGI 17341 (2-ethyl-5-nitro-2,3-dihydroimidazo[2,1-*b*][1,3]oxazole) is a novel lipophilic and orally active representative of the 5-nitroimidazole series of antimicrobial agents. This nitroimidazooxazole derivative is a promising novel antituberculosis compound with potent *in vitro* and *in vivo* activities (Nagargan *et*

al.,1982 and Astekar *et al.*, 1993) but is not developed because of its mutagenic properties. CGI 17341 inhibited the drug-susceptible and multi-drug-resistant strains of *Mycobacterium tuberculosis* and had no cross-resistance with isoniazid, rifampin, streptomycin, or ethambutol. While its *invitro* activity against *M. tb* was comparable to those of isoniazid and rifampin, it was superior to those of streptomycin, ciprofloxacin or norfloxacin, and oxazolidinone DuP 721.

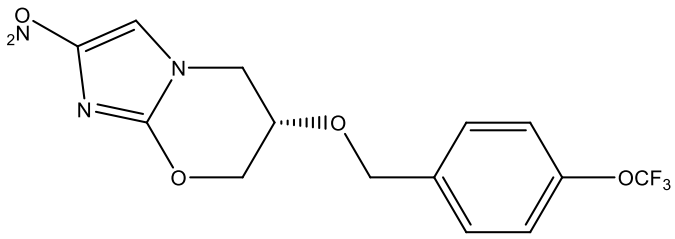


(CGI 17341)

Bicyclic nitroimidazoles such as PA-824 and OPC-67683 are currently in clinical development as a promising new class of therapeutics for tuberculosis (LiX *et a.l*, 2008).



(OPC-67683)



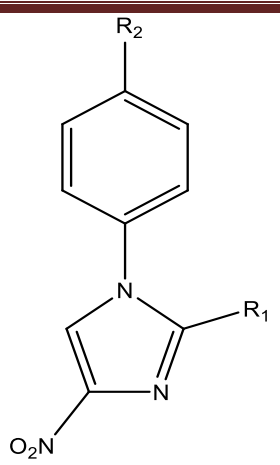
(PA-824)

CGI-17341, OPC-67683, both nitroimidazo-oxazoles and PA-824, a nitroimidazo-oxazine, have activity against aerobic and anaerobic populations of *Mycobacterium tuberculosis* (Ashtekar *et al.*,1993; Matasumoto *et al.*, 2006 and Stover *et al.*,2000). PA-824 has many attractive characteristics as a TB therapy, most notably its novel mechanism of action, its activity *in vitro* against all tested drug-resistant clinical

isolates and its activity as both a potent bactericidal and a sterilizing agent in mice. PA-824 shows no evidence of mutagenicity in genotoxicity studies, no significant cytochrome P450 interactions, and is inactive against a broad range of Gram-positive and Gram-negative bacteria. OPC-67683 had both inhibitory activity on mycolic acid biosynthesis and potent *in vitro* activity against *Mycobacterium tuberculosis*, as indicated by its low MIC range across many strains, including MDR-TB (Matasumoto *et al.,* 2006). The IC_{50} values of OPC-67683 for mycolic acid subclasses were lower than those of isoniazid (INH), and these results correlated well with the *in vitro* anti-tubercular activity of OPC- 67683 and INH. The anti-tubercular activity of nitro-imidazooxazole derivatives correlated well with their inhibitory activity against mycolic acid biosynthesis (Matasumoto *et al.,* 2006).

Thus it was concluded that the inhibitory activity of OPC-67683 against mycolic acid synthesis was a mechanism of action attributable to killing mycobacterium at least as potently as INH. PA- 824 has remarkably reduced mutagenicity compared with its parent nitroimidazole compound, CGI-17341 and it has recently been reported that PA-824 is not mutagenic in the Ames test and does not seem to be metabolized by cytochrome P450 into potentially carcinogenic substances (Barry *et al.,*2004) This suggested the possibility that its mutagenic activity will continue to be the major obstacle to its development as an anti-TB drug.

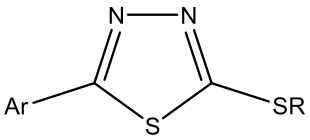
The anti-tuberculosis activity of various imidazole derivatives has been reported (Jackson *et al.,* 2000 and Mamolo *et al.,* 2003). Among 1-aryl-4-nitroimidazoles without nitro groups at 1-aryl substituent only 1-(4-chlorophenyl)-2-methyl-4-nitroimidazole **(22a)** demonstrated sufficient activity (99% of inhibition at 6.25 μg/mL), others derivatives were inactive though bromo derivatives **(22b)** were characterized by 76% and 85% inhibition, respectively (Walczak *et al.,* 2004). It was clear that electron-donating substituent on 1-nitrogen atom in *C*-nitroimidazoles diminishes, inhibition activity of the compounds. Surprisingly, it also appears that 2-methyl-4- nitroimidazoles are more active than 4-nitroimidazoles. Tuberculosis inhibition activity and cytotoxicity results of these derivatives were not better or at least similar to the control drugs INH and rifampicin (RMP).



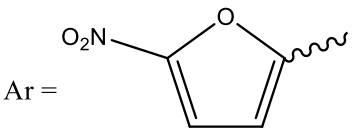
22 a, R_1 = -CH_3, R_2 = -Cl

22 b, R_1 = -H,-CH_3, R_2 =-Br

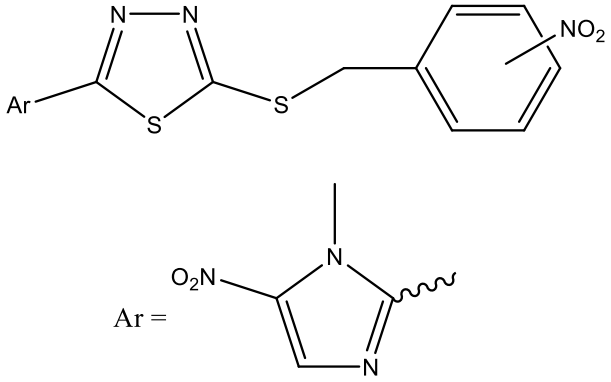
Two series of 2-(5-nitro-2-furyl)- and 2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-5-propyl, allyl and propargyl)thio-1,3,4-thiadiazoles (**23a–f)** and 2-(5-nitro-2-furyl)- and 2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-5-(nitrobenzyl)thio-1,3,4-thiadiazole derivatives (**24a–f**) were synthesized and evaluated against *Mycobacterium tuberculosis* (Foroumadi *et al.*, 2004) Among the nitrobenzylthio derivatives (**24a–f**), all the ortho, meta and para nitrobenzyl isomers in the nitrofuran series exhibited good antituberculosis activity, while the corresponding nitroimidazole analogues were completely inactive (Inhibition=0%). The compounds that bear a primary alkylthio substitution, displayed good antituberculosis activities, in the following order: *n*-propyl > ethyl > methyl (MIC=1.56-6.25 μg/mL) (Foroumadi *et al.*, 2001). Some nitroimidazole and nitrofuran derivatives have been also claimed to possess *in vitro* antibacterial, antifungal and antituberculosis activities (Gunay *et al.*, 1999).



R = *n*-propyl, aryl, or propargyl



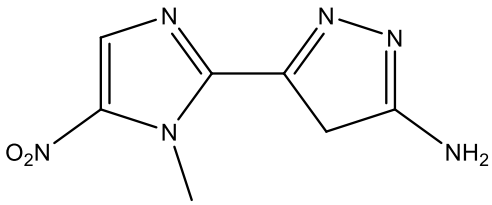
(23 a-f)



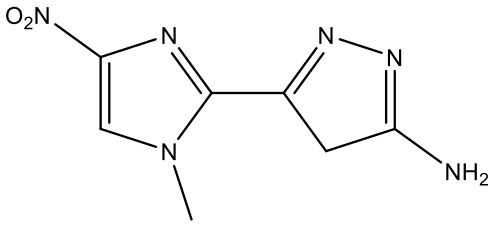
(24 a-f)

2.1.4 Trypanocidal nitroimidazoles

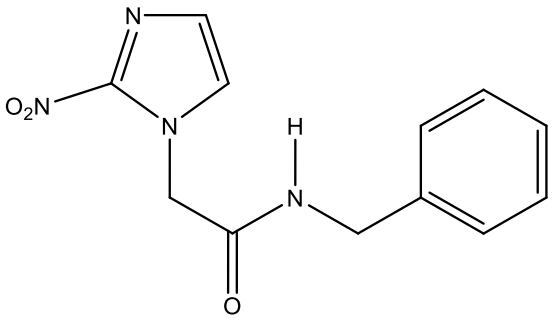
The nitroimidazole-thiadiazole derivative CL64855 (2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole, 5-nitromegazol) has a pronounced trypanocidal activity (Buschini *et al.,* 2007; Boutellie *et al.,* 1995 and Enanga *et al.,*1998) which may be due to the triggering of radical production by the compound (Viode *et al.*, 1999 and Chauviere *et al.,*2003).



(CL64855)



4-Nitromegazol

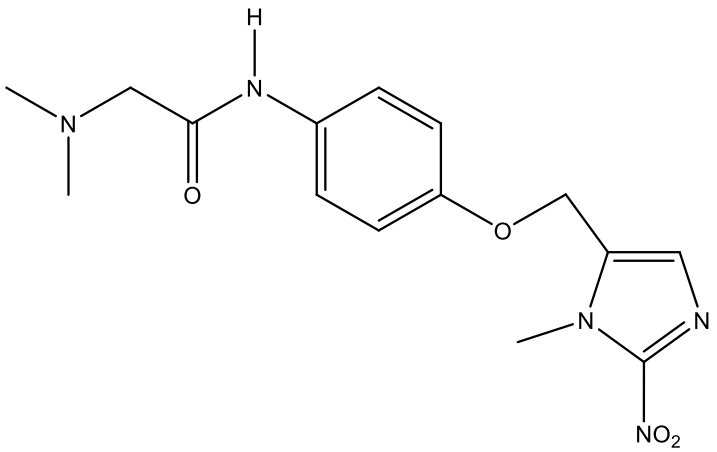


Benznidazole

Benznidazole was found mutagenic in salmonella (Voogd *et al.,* 1975 and Melo *et al.,* 1990). The location of the nitro group in position 4 makes the nitro heterocyclic compounds (such as 4-nitromegazol) totally inactive against parasite.

The treatment with 5-nitromegazol entails the production of reactive oxygen species in aerobic conditions (Boutellie *et al.,* 1999), it can induce redox cycling which explains its toxic effects, through the production of superoxide radical anion and then hydroxyl radicals (Docampo *et al.,* 1984), that are highly damaging for cellular structures. Most nitroimidazoles are mutagenic in bacteria and this mutagenicity has been attributed to nitroreductases present in these organisms. It has been argued that the lower capacity of mammalian cells to perform nitro reduction decreases the genotoxic risk of 5-nitromegazol.

However, some studies (Poli *et al.,* 2002 and Nesslany *et al*., 2004) have demonstrated its genotoxicity in mammalian cells. Because of its efficacy against several strains of *Trypanosoma cruzi* of diverse sensitivity, it has a potential role in treatment of Chagas' disease. However, 5-nitromegazol has shown mutagenicity (Ferreira *et al.,* 1986) according to the Ames assay. RO 150216, another nitroimidazole derivative, has been shown to be effective in inhibiting the culture growth of different strains of the African trypanosome (Borowy *et al.,* 1988), and *in vivo* using various animal models (Boutellie *et al.,* 1999).

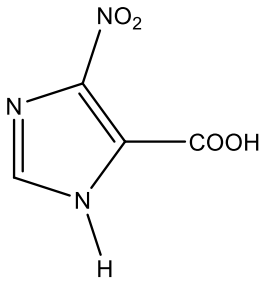


(RO 150216)

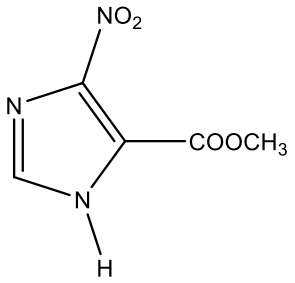
In an effort to synthesize imidazole analogs which retain biological but not toxic activities, a variety of new compounds with different substitutions on the bioisosteric imidazole and pyrazole structural frame were synthesized. Structures bear substituents able to provide some variations in the lipophilic, steric and electronic properties of the molecules. In particular, substituents with increasing hydrophobic and steric

properties, such as –COOH, –$COOCH_3$, –CH_3, –$CH_2C_6H_5$ and -$CH=CHC_6H_5$ groups were considered.

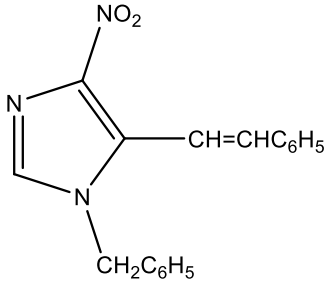
Compounds were tested on a set of tester strains of *Salmonella typhimurium*; in particular, classical nitroreductase or *O*-acetyltransferase deficient derivatives have been incorporated into the assay in order to study the metabolism and mutagenicity of nitro compounds (McCoy *et al.*, 1981 and Orr *et al.*, 1985).



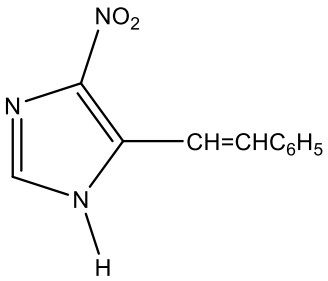
(25)



(26)



(27)



(28)

The structure-activity relationship study found that the following structural parameters are correlated with mutagenic potency: the presence of a methyl or a benzylic group on the imidazole ring, whereas the absence of a substituent in N_1 or N_3 leads to non-mutagenic compounds (derivative **25** and **26**) or to a marked decrease in mutagenicity 85%decrease with derivative (**27)** with respect to derivative (**28**).

The presence of bulky substituents, such as benzylic or styryl groups, on the imidazole ring leads to increased mutagenic activity, which implies that such substituents do not sterically hinder NO_2 metabolic activation, which would have caused inhibition of the nitroreduction or DNA binding steps critical to mutagenicity.

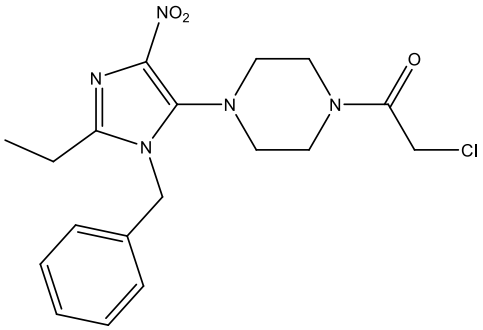
Some nitroimidazoles are reported as potent and selective histamine H-3 receptor agonists (Erik *et al.,* 1992 and Kovalainen *et al.,* 1999), mitogen-activated protein (MAP) kinases inhibitors (Farsch *et al.,* 1998), nitric-oxide synthase inhibitors (Salerno *et al.,* 1999) and antibacterial agents (Castelli *et al.,* 2000). Furthermore, 5-nitro-substituted haloimidazoles showed important biological activity as potential radiosensitizers (Adam *et al.,* 1979) and other imidazole derivatives having 5-alkylsulfonyl residues exhibited remarkable antitumour activity (Noker *et al.*, 1987).

2.1.5 Anti-HIV active nitroimidazoles

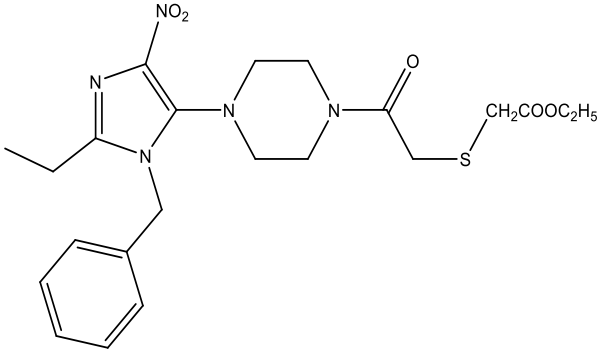
Non-nucleosides reverse transcriptase inhibitors (NNRTIs), a group of structurally diverse compounds have been reported to directly inhibit the enzyme Reverse transcriptase (RT) which plays an essential and multifunctional role in the replication of HIV-1 and thus is considered to be an attractive target for inhibition of HIV-1 replication. Synthesis of new 5-substituted piperazinyl-4-nitroimidazole derivatives and their evaluation for anti-HIV activity were reported (Al-Soud *et al.,* 2007). The target was the synthesis of new 4-nitroimidazoles, leading to inhibition of HIV by inhibition of RT and reduction of the drug-resistance strains.

Compounds **(29)** and **(30)** were found to be most active among the tested compounds inhibiting HIV replication in cell culture. The structure activity relationships of 4-nitroimidazole derivatives have suggested the importance of a piperazine group on the imidazole ring substituted by aliphatic carbonyl groups such as -$COCH_2Cl$, -$CO(CH_2)$nR or sulphonamides for potent inhibitory activity against RT.

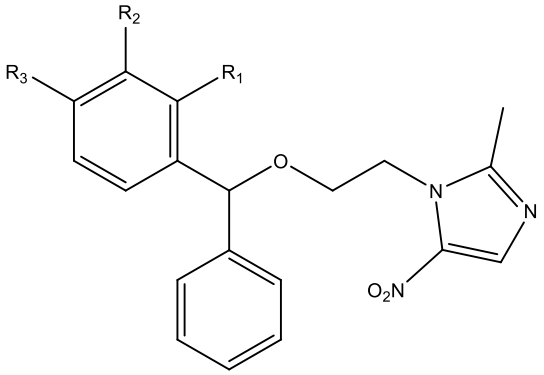
1-[2-(Diarylmethoxy) ethyl]-2-methyl-5-nitroimidazoles (DAMNIs) is a novel family of HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) active at submicromolar concentration (Silvestri *et al.,* 2000 and Martino *et al.,* 2005). Replacement of one phenyl ring of 1-[2-(diphenylmethoxy)ethyl]-2-methyl-5-nitroimidazole **(31)** with heterocyclic rings, such as 2-thienyl or 3-pyridinyl, led to novel DAMNIs with increased activity. In HIV-1 WT cell-based assay the racemic 1-{2-[α-(thiophen-2-yl)phenylmethoxy]ethyl}-2-methyl-5-nitroimidazole **(30)** (EC_{50} = 0.03 μM) proved 5 times more active than compound **(29)**.



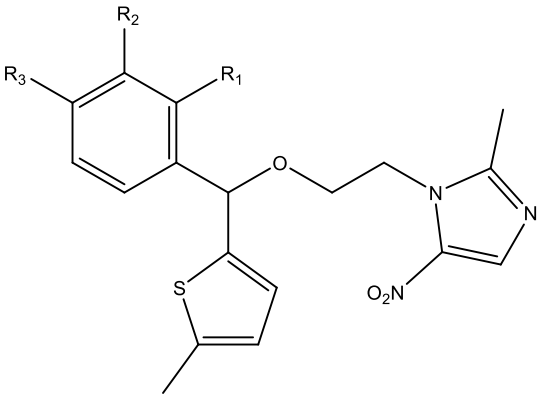
(29)



(30)



(31)



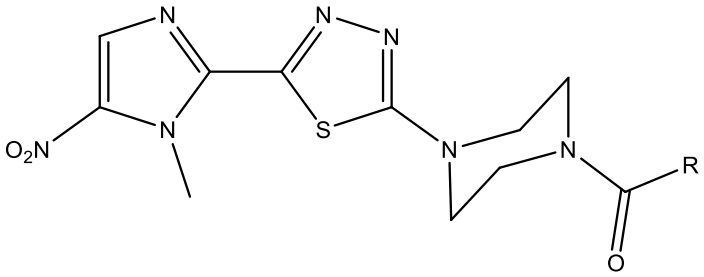
(32)

Docking experiments showed that the introduction of a chiral center would not affect the binding of both (*R*)-**30** and (*S*)-**30**. The internal scoring function of the Autodock program calculated the same inhibition constant (*K*i = 7.9 nM) for the two enantiomers. Compounds **(30)** (ID50 = 8.25 μM) were found more active than efavirenz (ID50 = 25 μM) against the viral RT carrying the K103N mutation, suggesting for these compounds, a potential use in efavirenz based anti-AIDS regimens.

2.1.6 Antileishmanial agents

A series of 1-[5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]-4-aroylpiperazines were synthesized and evaluated *in vitro* against *Leishmania major* (Poorrajab *et al.,* 2009) The most active compound was 1-[(5-chloro-2-thienyl)carbonyl]-4-[5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-1,3,4-thiadiazol-2-yl]pip

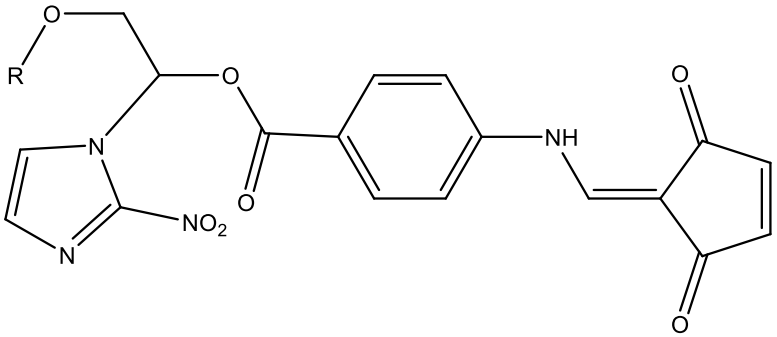
erazine **(33)** with an IC_{50} value of 9.35 ± 0.67 mM against *L. major* promastigo. In addition, this compound was effective against intracellular *L.major* and significantly decreased the infectivity index.



(33) R = 5-Cl-thiophen-2-yl

2.1.7 Nitroimidazoles as radiosensitizers

The 2-nitroimidazoles have been studied extensively for their use as radiosensitizers, hypoxic cytotoxins, and molecular markers of hypoxic regions in solid tumors (Overgarrd, 1994; Brezden *et al.*, 1994 and Koch *et al.*, 1975). Their selective hypoxic cytotoxicity and use as imaging agents for hypoxia are dependent on the bio-reduction of these compounds to reactive intermediates and the binding of these reductive species to intracellular macromolecules. Bio-reduction occurs only under extremely low oxygen tensions and, therefore, is selective for hypoxic regions (Taylor *et al.*, 1982). In contrast, the use of 2-nitroimidazoles as radiosensitizers requires the intact compound to act as an oxygen mimic and potentiate the lethal effects of ionizing radiation in hypoxic but not aerobic cells (Wardman *et al.*, 1977).



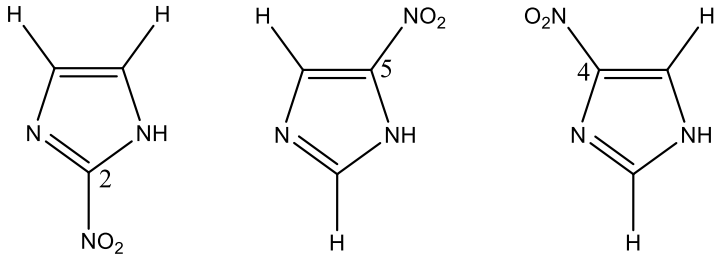
(33a)

Chiral 2-nitroimidazole derivatives containing a 2-aminomethylene-4-cyclopentene-1,3-dione moiety were designed and synthesized as antiangiogenic hypoxic cell radiosensitizers (Uto *et al.*, 2008). All of these bifunctional derivatives proved to have activity as antiangiogenic hypoxic cell radiosensitizing agents and protein tyrosine

kinase (PTK) inhibitory activities. TX-2036 was the most promising candidate for further development as an antiangiogenic hypoxic cell radiosensitizer. 2-Nitroimidazoles play a major role as bioreductive markers for tumour hypoxia and as radiosensitizers. Several 2-nitroimidazoles with immunologically-identifiable side chains have been described and conventional immuno-staining procedures can be used to locate their metabolites, bound to hypoxic cells in histological sections. Use of fluorescent immuno-reagents allows flow cytometric assessment of hypoxia and multiple color fluorescent staining allows hypoxia to be correlated with other markers on a cell by cell basis. 2-nitroimidazole markers show considerable promise for clinical use in diagnosing hypoxia and allow rational application of hypoxia-related therapies.

2.2 Chemistry of nitroimidazole

The chemistry of Nitroimidazole deals the geometry, conformation, electron affinity, and proton affinity of the parent 2-, 4-, and 5-nitroimidazoles. An understanding of various aspects of the chemistry and related biological effects is important as for example, the electron affinity decreases in the order 2-NO_2 > 3-NO_2 > 5-NO_2 which also represent the order of biological activity (wardmen *et al*., 1976).

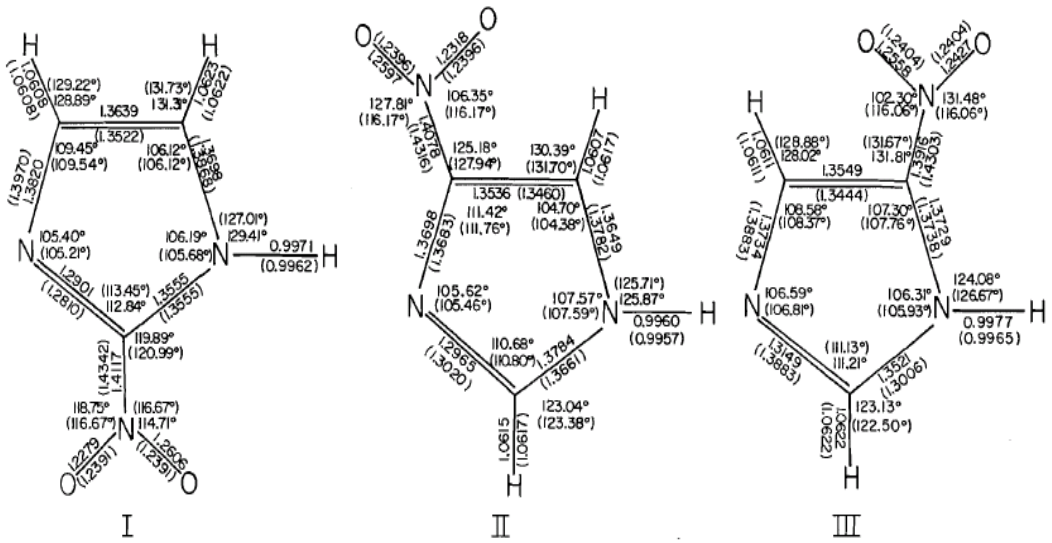


(34)

Additionally some Nitroimidazoles show enhanced radiosensitization when there is also present a group which protonated at physiological pH (Wardman *et al.*, 1976 and Anderson *et al.*, 1977). On their own, nitroimidazoles are, however, weak bases with pK, values (for the conjugate acids of 1-methyl derivatives) of -0.5, +0.6, and +2.3 for 2-nitro, 4-nitro, and 5-nitro, respectively (Galo *et al.*, 1964).

2.2.1 Geometry and conformation of nitroimidazole

The bond lengths and angles obtained with the planar ground states and the transitions state for carbon-nitro group rotation, with the rotational barriers and torsion angles in the transition state given.



(35)

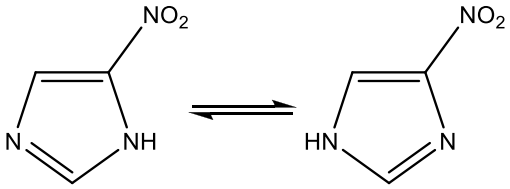
2.2.3 Dipole moments

The dipole moments for the three isomers obtained from the 3-21G calculation for both the planar and torsional forms, show an order 5-NO_2 < 2-NO_2 < 4-NO_2. This order does not match that observed for biological effects of nitroimidazoles, with the implication that with this class of agents the polarity of the parent drug is a relatively unimportant factor in determining its efficiency. The order does, however, correctly predict the experimental order of melting points. In general, as the dipole moment of a series of isomers increases, the melting point increases, and this is the case here as well. The pattern is most clearly seen with the melting points for the 1-methyl substituted nitroimidazoles.

Table 6: Melting points and calculated dipole moments (3-21G basis set) of nitroimidazoles

Parameter	value		
	2-NO_2	**4-NO_2**	**5-NO_2**
Melting point	284 ^{0}C	308 ^{0}C	--
Dipole moment	6.00	8.23	3.88

The parent 2-nitro- and 4-nitroimidazoles also show the correct order, but there is no experimental number for the 5-nitroimidazole. This is due to the fact that in condensed phases 5-nitroimidazole spontaneously tautomerizes to its 4-nitro isomer (Cavalleri, 1982)



(36)

The 4-nitro isomer is more stable because it has better resonance interactions between the nitro group and the aromatic ring. Calculations, however, indicate that there is very little difference in energy between 4-nitroimidazole and 5-nitroimidazole, the 5-nitro isomer being 0.19 kcal/mol higher in energy.

Each nitroimidazole the dipole moment is seen to decrease on proceeding from the planar ground state to the torsional transition state Table 7**.** This is expected since rotating the nitro group out of the plane of the ring prevents the conjugation of the type shown below for the 2-nitro isomer.

Table 7: Carbon-Nitrogen (NO_2) bond lengths (^0A) of planar and tortional transition state nitroimidazoles (3-21G basis set)

Form	Bond length (^0A)		
	2-NO_2	**4-NO_2**	**5-NO_2**
Planar form	1.4117	1.4079	1.3916
Transition state	1.4342	1.4316	1.4303

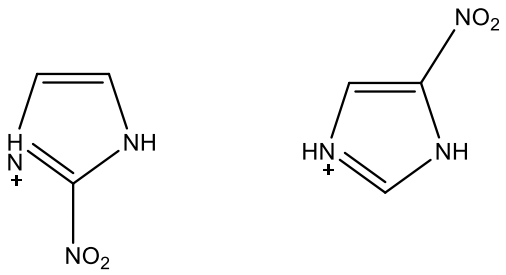
It is observed that with each nitroimidazole a small increase in the length of the carbon-nitro bond in the transition state as compared to the ground state. Interestingly, however, the changes in both the dipole moments and the bond lengths for each nitroimidazole are rather small. This would suggest that the resonance interaction has a minor effect, and that the principal contribution of the nitro group to the dipole moment is inductive effect, that is an interaction which is not affected by the loss of conjugation in the rotated form.

2.2.4 Electrostatic potentials

The relatively small degree of conjugation in nitrobenzene has also recently been established by Politzer and co-workers, through a computational analysis of the planar and rotated forms (Politzer *et al.,* 1987). The 2-nitro derivative in the molecular planes of the planar forms there are apparent large regions of negative potential which can be attributed to the lone pairs of the oxygens of the nitro group and the ring nitrogen lone pair of electrons. The most negative values (local minima) of the potential are -83.6, -86.7 and -86.5 kcal/mole for the 2-nitro, 4-nitro, and 5-nitroimidazole respectively. In the transition state when the nitro is -90^0 rotated, these large negative regions remain associated with the lone pairs, and there is little change in the absolute magnitude of the potential, the most negative values being -81.7, -83.9 and -87.6, respectively. These relatively small changes are further indicators of the unimportance of the resonance interaction.

2.2.5 Relative proton affinities

Geometry optimization of the N-3 protonated nitroimidazoles was achieved at the 3-21G basis set level. The protonated forms were in each case about 220 kcal/mol more stable than their neutral conjugate base. (Note that 4-nitroimidazole and 5-nitroimidazole have the same conjugate acid) The additional stability of the protonated forms is due to the extra N-H bond created during protonation.



(37)

Energies of the neutral and conjugate acid forms are compared in Table 8. The 4(5)-nitroimidazolium ion is calculated to be about 7 kcal/mol more stable than the 2-nitroimidazolium isomer, while the relative gas phase proton affinities of the neutral nitroimidazoles follow the order 4-NO_2 - 5-NO_2 > 2-NO_2. As illustrated in the last row of Table 8 in aqueous solution the basicity order is 5-NO_2 > 4-NO_2 > 2-NO_2. We have previously suggests that there may be salvation differences on the neutral nitroimidazoles and this could be behind the differences between gas phase and solution (Forsythan *et al.*, 1925).

Table 8: Total energies of nitroimidazoles and their N-3 protonated conjugate acids (Fock V, 1930)

parameter	value		
	2-NO_2	**4-NO_2**	**5-NO_2**
E(Im)[a,b]	-425.83174	-425.83714	-425.83725
E(3-Him^+)[b,c]	-426.18657	-426.19825	-426.19825
(E(Im)[a,b]- E(3-Him^+))[d]	-222.7	-226.6	-226.5

a Total energy in Hartrees

b Neutral parent imidazole

c N-3 protonated imidazoliun ion

2.2.5 Relative electron affinities

The radical anions of three nitroimidazoles energies are summarized in Table 9. Recognizing that without Configuration Interaction calculations, the absolute electron affinities must be regarded as being uncertain, the relative electron affinities are probably reasonably accurate as no electron pairs are formed or destroyed during the process of electron capture. These follow the order 2-NO_2 > 5-NO_2 > 4-$N0_2$, with a particularly large gap between 5-NO_2 and 4-NO_2. These numbers do correlate with the relative biological activities of differently substituted nitroimidazoles, which follow an order in both radiosensitization and hypoxic cytotoxicity of 2-NO_2 slightly more effective than 5-NO_2, with both significantly better than 4-NO_2. As discussed in the introduction, the biological effects of nitroimidazoles are believed to be closely related to the "nitro: nitro radical anion" redox cycle, and the relationship of biological activity with theoretical electron affinity is therefore not surprising. This excellent correlation does suggest, however, that theory might provide useful new information in predicting biological effects to aid in the design of new drugs. A neurotoxic side reaction is limiting the clinical effectiveness of nitroimidazoles as radiation sensitizers, and there is considerable interest in finding new molecules of similar electron affinity but lacking the nitro group.

Table 9: Total energies of nitroimidazoles and their N-3 protonated conjugate acids

parameter	value		
	2-NO_2	**4-NO_2**	**5-NO_2**
E(Im NO_2)	-425.8317	-425.8371	-425.8372
E(Im NO_2^-)	-425.8599	-425.8336	-425.8608
(E(Im NO_2^-)- E(Im NO_2))	-18.69	+2.23	-14.80

2.3 References

Adams GE, Clarke D, Flockharrt R, Jacobs S, Sehmii DS, Strattforpd J, Ardmaannd W, Watts ME. Electron-affinic sensitization II. Int J Radiat Biol 1979; 35:133-150.

Al-Soud YA, Al-Masoudi NA, Hassan HG, Clercq ED, Pannecouque C. Nitroimidazoles V. Synthesis and anti-HIV evaluation of new 5-substituted piperazinyl-4-nitroimidazole derivatives. Acta Pharm 2007;57:379-393.

Anderson RF, Patela B, Smithenin CE. Sensitivity of Nitroimidazoles. J Radiat Biol 1977;32: 471-473.

Arredondo Y, Moreno-Mafias M, Pleixats R, Palacin C, Raga MM, Castello JM, Ortiz JA. Preparation, antimicrobial evaluation and mutagenicity of differently substituted [2-hydroxyaryl]-[1- methyl-5-nitro-1H-2-idazolyl]methanols. Bioorg Med Chem Lett 1996;6:1781–1784.

Ashtekar DR, Costa-Perira R, Nagrajan K, Vishvanathan N, Bhatt AD, Rittel W. In vitro and invivo activities of the nitroimidazole CGI 17341 against Mycobacterium tuberculosis. Antimicrob Agents Chemother 1993;37:183-186.

Bagan ES, Miwa GT. Uniquely non-mutagenic substituted nitroimidazole. US 1988; 4,728,664.

Barry CE, Boshoff HI, Dowd CS. Prospects for clinical introduction of nitroimidazole antibiotics for the treatment of tuberculosis. Curr Pharm Des 2004;10:3239-3262.

Borowy NK, Nelson RT, Hirumi H, Brun R, Waithaka HK, Schwartz D, Polak A. RO150216 a nitroimidazole compound active against human and animal pathogenic African trypanosomes. Ann Trop Med Parasitol 1988;82:13-19.

Bouteille B, Chauviere G. Implication du megasol dans la chimiotherapie des trypanosomosis. Med Trop 1999;59:321-330.

Bouteille B, Marie-Daragon A, Chauviere G, DeAlbuquerque C, Enanga B, Darde ML. Effect of megazol on Trypanosoma brucei brucei acute and subacute infections in Swiss mice. Acta Trop 1995;60:73-80.

Brezden CB, McClelland RA, Rauth AM. Mechanism of the selective hypoxic cytotoxicity of 1-methyl-2-nitroimidazole. Biochem Pharmacol 1994;48:361-370.

Buschini A, Giordani F, Northfleet de Albuquerque C, Pellacani C, Pelosi G, Rossi C, Araujo TM, Zucchi D, Poli P. Trypanocidal nitroimidazole derivatives: Relationships among chemical structure and genotoxic activity. Biochem Pharmacol 2007;73:1537-1547.

Castelli M, Malagoli M, Lupo L, Roffia S, Paolucci F, Cermelli C, Zanca A, Baggio G. Cytotoxicity and probable mechanism of action of sulphimidazole. J Antimicrob Chemother 2000;46:541-550.

Cavalleri B, Ballotta R, Arioli V, Lancini G. New 5-substituted 1-alkyl-2-nitroimidazoles. J Med Chem 1973;16:557-560.

Cavalleri B, Volpe G, Arioli V. Synthesis and biological activity of some vinyl-substituted 2-nitroimidazoles. J Med Chem 1977;20:656–660.

Cavalleri B, Volpe G, Ripamonti A, Arioli V. 1-Methyl-2-nitroimidazol-5-yl derivatives, IIIrd communication. Arzneimittel forschung 1977;27:1131–1134.

Cavalleri B. Nitroimidazole Chemistry, Pharmacology And Clinical Application. Edited By Breccia A, Cavalleri B, Adams GE. Plenum Press, London. 1982 p-150.

Chauviere G, Bouteille B, Enanga B, de Albuquerque C, Croft SL, Dumas M. Synthesis and biological activity of nitro heterocycles analogous to megazol, a trypanocidal lead. J Med Chem 2003;46:427-440.

Crozet MD, Botta C, Gasquet M, Curti C, Remusat V, Hutter S, Chapelle O, Azas N, DeMeo M, Vanelle P. Lowering of 5-nitroimidazole's mutagenicity: Towards optimal antiparasitic pharmacophore. Eur J Med Chem 2009;44:653-659.

De Meo M, Vanelle P, Bernadini E, Laget M, Maldonado J, Jentzer O, Crozet MP, Dumenil G. Evaluation of the mutagenic and genotoxic activities of 48 nitroimidazoles and related imidazole derivates by the Ames test and the SOS chromotest. Environ Mol Mutagen 1992;19:167-181.

Dobias L, Cerna M, Rossner P, Sram R. Genotoxicity and carcinogenicity of metronidazole. Environ Mutagen 1994;317:177–194.

Docampo R, Moreno SN. Free radical metabolites in the mode of action of chemotherapeutic agents and phagocytic cells on *Trypanosoma cruzi*. Rev Infect Dis 1984;6:223-238.

Dubini F, Riviera L, Cocuzza C, Bellotti MG. Antibacterial, antimycotic and trichomonicidal activity of a new nitroimidazole EU 11100. J Chemother 1992;4:342-346.

Dunn LA, Boreham PFL. The in-vitro activity of drugs against *Blastocystis hominis*. J Antimicrob Chemother 1991;27:507-516.

Enanga B, Keita M, Chauviere G, Dumas M, Bouteille B. Megazol combined with suramin: a chemotherapy regimen which reversed the CNS pathology in a model of human African trypanosomiasis in mice. Trop Med Int Health 1998;3:736-741.

Erik JC, Vander, Goot, H, Sterk GJ, Timmerman H. Histamine H2-receptor agonists. Synthesis, in vitro pharmacology, and qualitative structure-activity relationships of substituted 4-and 5-(2-aminoethyl) thiazoles. J Med Chem 1992;35:3239-3246.

Farsch SC, Nick JA, Fadok, VA, Bratton, DL, Worthen, GS, Henson, PM. p38 Mitogen-activated protein kinase-dependent and -independent intracellular signal

transduction pathways leading to apoptosis in human neutrophils. J Biol Chem 1998; 273:8389-8397.

Ferreira RC, Ferreira LC. CL64,855, a potent anti-Trypanosoma cruzi drug, is also mutagenic in the Salmonella/microsome assay. Mem Inst Oswaldo Cruz 1986;81:49-52.

Fock V. The Electronic Structure of Atoms-The Hartree-Fock Method and correlation. Physik 1930;61:126.

Foroumadi A, Mirzaei M, Shafiee A. Antituberculosis agents II: Evaluation of in vitro antituberculosis activity and cytotoxicity of some 2-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole derivatives. Farmaco 2001;56:621-623.

Foroumadi A, Soltani F, Jabini R, Moshafi MH, Rasnani FM. Antituberculosis Agents X. Synthesis and evaluation of in vitro antituberculosis activity of 2-(5-nitro-2-furyl)- and 2-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazole Derivatives. Arch Pharm Res 2004;27:502-506.

Forsythan WG, Pymanj FL. Molecular structure and relative proton and electron affinities of isomeric nitroimidazoles. J Chem Soc 1925;127:57.

Fudan D. Antibacterial 5-Nitro-1-methyl-imidazolyl-3-terbutyl-2-hydroxy-aryl-carbinols. Euroresearch S.R.L 1992; EP535528.

Galloc GG, Pasqualuccc R, Radaellani P, Lancinji GC. The Ionization Constants of Some Imidazoles. J Org Chem 1964;29:862.

Gunay NS, Capan G, Ulusoy N, Ergenc N, Otuk G, Kaya D. Nitroimidazole derivatives as possible antibacterial and antifungal agents. Farmaco 1999;54:826-831.

Hadj-esfandiari N, Navidpour L, Shadnia H, Amini M, Samadi N, Faramarzi MA, Shafieea A. Synthesis, antibacterial activity, and quantitative structure–activity

relationships of new (*Z*)-2-(nitroimidazolylmethylene)-3(2*H*)-benzofuranone derivatives. Bioorg Med Chem Lett 2007;17:6354-6363.

Hrelia P, Fimognari C, Maffei F, Brighenti B, Garuti L, Burnelli S, Cantelli-Forti G. Synthesis, metabolism and structure–mutagenicity relationships of novel 4-nitro-(imidazoles and pyrazoles) in Salmonella typhimurium. Mutat Res 1998;397:293-301.

Jackson CJ, Lamb DC, Kelly DE, Kelly SL. Bactericidal and inhibitory effects of azole antifungal compounds on Mycobacterium smegmatis. FEMS Microbiol Lett 2000;192:159-162.

Khabnadideh S, Rezaei Z, Khalafi-Nezhad A, Bahrinajafi R, Mohamadi R, Farrokhroz AA. Synthesis of N-alkylated derivatives of imidazole as antibacterial agents. Bioorg Med Chem Lett 2003;13:2863-2865.

Koch CJ, Evans SM, Lord EM. Oxygen dependence of cellular uptake of F5[2-(2-nitro-1H-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl)acetamide]: Analysis of drug adducts by fluorescent antibodies vs bound radioactivity. Br J Cancer 1975;72:869-874.

Kovalainen JT, Christiaans JAM, Kotisaari S, Laitinen JT, Mannisto PT, Tuomisto L, Gynther J. Synthesis and in vitro pharmacology of a series of new chiral histamine H3-receptor ligands: 2-(RandS)-amino-3-(1*H*-imidazol-4(5)-yl)propylether derivatives. J Med Chem 1999; 42:1193-1202.

Li X, Manjunatha UH, Goodwin MB, Knox JE, Lipinski CA, Keller TH, Barry CE III, Dowd CS. Synthesis and antitubercular activity of 7-(R)-and 7-(S)-methyl-2-nitro-6-(S)-(4-(trifluoromethoxy)benzyloxy)-6,7-dihydro-5*H*-imidazo[2,1-b][1,3]oxazines, analogues of PA-824. Bioorg Med Chem Lett 2008;18:2256-2262.

Lowryan TH. Richardsomne C. Hanism A. Theory In Organic Chemistry. 2nd ed. Harper And Row, New York; 1981.p: 131.

Mamolo MG, Zampieri D, Falagiani V, Vio L, Banfi E. Synthesis and antifungal activity of (+/-)-1-(5-aryl-3-pyridin-2-yl-4,5-dihydro-pyrazol-1-yl)-2-imidazol-1-yl-ethanone. derivatives. Farmaco 2003;58:315-322.

Martino G, De Regina G, La Pasquali, A. Di, Ragno R, Bergamini A, Ciaprini C, Sinistro A, Maga G, Crespan E, Artico M, Silvestri R. Novel 1-[2-(Diarylmethoxy)ethyl]-2-methyl-5-nitroimidazoles as HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitors: A structure-activity relationship investigation. J Med Chem 2005;48:4378-4388.

Mason RP, Josephy PP. Free radical mechanisms of nitroreductase. In: D. E. Rickert (Ed.). Toxicity of Nitroaromatic Compounds, Hemisphere, Washington DC, 1985;121-140.

Matsumoto M, Hashizume H, Tomishige T, Kawasaki M, Tsubouchi H, Sasaki H, Shimokawa Y, Komatsu M. OPC-67683, a Nitro-Dihydro-midazooxazole derivative with promising action against tuberculosis invitro and in mice. PLoS Med 2006;3:2131-2144.

McCoy EC, Rosenkranz HR, Mermelstein R. Evidence for the existence of a family of bacterial nitroreductases capable of activating nitrated polycyclics to mutagens. Environ Mol Mutagen 1981;3:421-427.

Melo ME, Ferreira LC. Screening the mutagenic activities of commonly used antiparasite drugs by the Simultes, a simplified Salmonella/microsome plate incorporation assay. Rev Inst Med Trop Sao Paulo 1990;32:269-274.

Mirzaei J, Siavoshi F, Emami S, Safari F, Khoshayand MR, Shafiee A, Foroumadi A. Synthesis and in vitro anti-Helicobacter pylori activity of N-[5-(5-nitro-2-heteroaryl)-1,3,4-thiadiazol-2-yl]thiomorpholines and related compounds. Eur J Med Chem 2008;43:1575-1580.

Miwa GT, Wang Wen-Jen R, Walsh JS. Non-mutagenic 1,2-disubstituted-4-nitroimidazole compounds useful as antiprotozoal agents. 1987; US 4,675, 337.

Nagarajan K, Arya VP, George T, Sudarsanam V, Shah RK, Goud AN, Shenov SJ, Honkan V, Kulkarni YS and Rao ML. Nitroimidazoles. IV. 1-1-Methyl-5-nitroimidazolyl (2)-2-oxo-3-sulphonyl (carbamoylthiocarbamoyl) tetrahydro imidazoles. Ind J Chem 1982; 21: 928-940.

Nesslany F, Brugier S, Mouries MA, Le Curieux F, Marzin D. In vitro and in vivo chromosomal aberrations induced by megazol. Mutat Res 2004;560:147-158.

Noker P, Simpson-Herren L, Wagoner SD. Distribution of nitroimidazoles and L-phenylalanine mustard in mammary adenocarcinoma 16/C tumors. Cancer Chemother Pharm 1987;20:188-192.

Olender D, Zwawiak J, Lukianchuk V, Lesyk R, Kropacz A, Fojutowski A, Zaprutko L. Synthesis of some N-substituted nitroimidazole derivatives as potential antioxidant and antifungal agents. Eur J Med Chem 2009;44:645-652.

Orr JC, Bryant DW, McCalla DR, Quilliam MA. Dinitropyrene-resistant Salmonella typhimurium are deficient in n acetyl-CoA-acetyl-transferase. Lancet 1985;54:281-288.

Overgaard J. Clinical evaluation of nitroimidazoles as modifiers of hypoxia in solid tumors. Oncol Res 1994;6:509-518.

Poli P, de Mello MA, Buschini A, Mortara RA, de Albuquerque NC, da Silva S. Cytotoxic and genotoxic effects of megazol, an anti-Chagas' disease drug, assessed by different short-term tests. Biochem Pharmacol 2002;64:1617-1627.

Politzer P, Lane P, Jayasuriya K, Linda ND. Examination of some effects of NO2 rotation in nitrobenzene. J Am Chem Soc 1987;109:1899.

Poorrajab F, Ardestani SK, Emami S, Behrouzi-Fardmoghadam M, Shafiee A, Foroumadi, A. Nitroimidazolyl-1,3,4-thiadiazole-based anti-leishmanial agents: Synthesis and in vitro biological evaluation. Eur J Med Chem 2009; 44:1758-1762.

Purohit V, Basu AK. Mutagenicity of nitroaromatic compounds. Chem Res Toxicol 2000;8:673-692.

Salerno L, Sorrenti V, Guerrera F, Sarva MC, Siracusa MA, Di Giacomo C, Vanella A. N-substituted-imidazoles as inhibitors of nitric oxide synthase: a preliminary screening. Pharmazie 1999;54:685-690.

Scalesiani JBA, Calle YB. 1-Methyl-5-nitro-imidazoline derivatives and therapeutic compositions which contain them as active principle. 1983; EP 0103100-A (8413).

Sehgal RK, Agrawal KC. Novel nitroimidazo[2,1-b] oxazole formation from reaction of 2, 4(5)-dinitroimidazole with oxiranes. J Heterocyclic Chem 1979;16:1499-1500.

Silvestri R, Artico M, Massa S, Marceddu T, Montis F De, Colla PL. 1-[2-(diphenylmethoxy)ethyl]-2-methyl-5-nitroimidazole: a potent lead for the design of novel NNRTIs. Bioorg Med Chem Lett 2000;10:253-256.

Stover CK, Warrener P, VanDevanter DR, Sherman DR, Arain TM, Langhorne MH, Anderson SW, Towell JA, Yuan Y, McMurray DN, Kreiswirth BN, Barry CE III, Baker WR. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature 2000;405:962-966.

Sun Z and Zhang Y. Antituberculosis activity of certain antifungal and antihelmintic drugs. Tub Lung Dis 1999;79:319-320.

Taylor YC, Rauth AM. Oxygen tension, cellular respiration, and redox state as variables influencing the cytotoxicity of the radiosensitizer misonidazole. Radiat Res 1982;91:104-123.

Tessitore PT. New 2-(1-alkyl-5-nitro)-imidazolyl-1-(2-hydroxy-5-alkyl)-phenyl carbinols, method for the preparation thereof and pharmaceutical compositions containing them. 1983;EP 111,657.

Uto Y, Nagasawa H, Jin C-Zhe, Nakayama S, Tanaka A, Kiyoi S, Nakashima H, Shimamura M,Inayama S, Fujiwara T, Takeuchi Y, Uehara Y, Kirk KL, Nakata E, Hori H. Design of antiangiogenic hypoxic cell radiosensitizers: 2-Nitroimidazoles containing a 2-aminomethylene-4-cyclopentene-1,3-dione moiety. Bioorg Med Chem 2008;16:6042-6053.

Viode C, Bettache N, Cenas N, Krauth-Siegel RL, Chauviere G, Bakalara N. Enzymatic reduction studies of nitroheterocycles. Biochem Pharmacol 1999;57: 549-557.

Voogd CE, Van der Stel JJ, Jacobs JJ. The mutagenic action of nitroimidazoles II. Effects of 2-nitroimidazoles. Mutat Res 1975;31:149-152.

Walczak K, Gondela A, Suwinski J. Synthesis and anti-tuberculosis activity of N-aryl-C-nitroazoles. Eur J Med Chem 2004;39:849-853.

Wardman P, Clarke ED. Oxygen inhibition of initroreductase: Electron transfer from nitroradical anion to oxygen. Biochem Biophys Res Comm 1976;69:942.

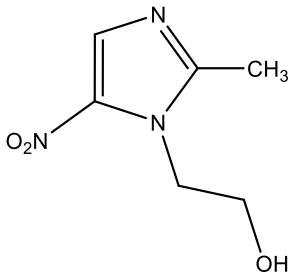
Wardman P. The Use of Nitroaromatic Compounds as Hypoxic Cell Radiosensitizers. Curr Top Radiat Res Quart 1977;11:347.

3.1 AIM OF PRESENT WORK

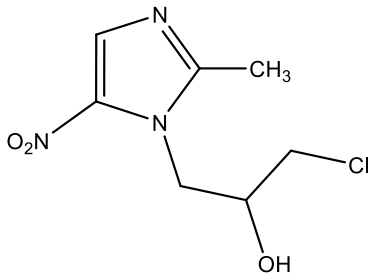
The nitro heterocyclic compounds have proved to be very effective antimicrobial agents and very valuable member of limited armamentarium of antiprotozoal agents. The mechanism of action of nitroheterocyclic agents depend upon the reduction of the nitro group to toxic radicals and binding to the protein and DNA. The two families of nitroheterocyclic, the nitroimidazole and nitrofurans are very different in their reduction characteristics.

Drug resistance to the nitroheterocyclic drugs and cross resistance leads to the development of new derivatives. A number of 5-nitoimidazole with substitution at 2nd position with various substitutes like beta lactam ring, dioxane, 2-hexahyropyrimidine, 1-formyl and 2-formyl ring, pyridinium, tetrahyropyridine derivatives and nitroimidazole with a trisubstituted ethylenic double bond are reported recently. Two of beta lactam substituted imidazole compounds exhibited 100 and 50-fold increase in activity than metronidazole against *Trichomonas* and *Entamoeba histolytica* while one of the dioxane derivative was more effective than metronidazole against *Entamoeba histolytica* and *Trichomonas vaginalis* (Vanelle *et al.,* 1991). The formyl derivative exhibited an antiparasitic spectrum with significant activity against *Entamoeba histolytica* and *Trichomonas vaginalis* (Alcade *et al.,* 1989). Such studies indicate the thrust for the future development of new nitroimidazole drugs.

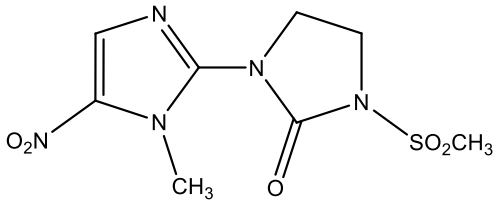
Tinidazole and Ornidazole have different side chains at the 1st position; do not differ markedly in their antimicrobial activity. Modifications at the 2nd position, however, are known to interfere with both the activity and the microbial spectrum (Nagarjan *et al.,* 1982). Compound Satranidzole, which has an imidazolidinone ring structure at the 2nd position, exerts stronger antitrichomonal activity than metronidazole (Ray *et al.,* 1982 and 1984). Satranidzole is highly active against anaerobic bacteria, even more than metronidazole but less than niridazole. Thus, the modification of the 5-nitroimidazole at the 2nd position increases not only its antitrichomonal activity but also its antibacterial activity.



Metronidazole

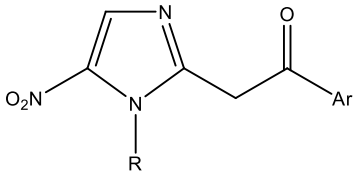


Ornidazole



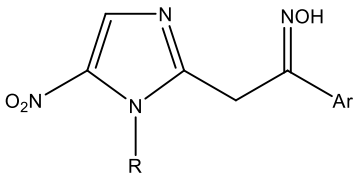
Satranidazole

In the present study the 5- nitroimidazoles like tinidazole and dimetridazole molecule is thought to be modified at 2nd position as shown in structure **39, 40, 41** and **42.**



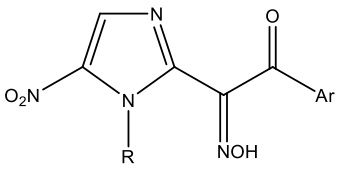
R = CH_3, $CH_2CH_2SO_2C_2H_5$
Ar = phenyl, *p*-chlorophenyl, *m*-chlorophenyl, *o*-chlorophenyl, furoyl

(39)



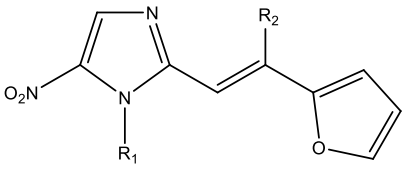
R = CH_3, $CH_2CH_2SO_2C_2H_5$
Ar = phenyl, *p*-chlorophenyl, *m*-chlorophenyl, *o*-chlorophenyl, furoyl

(40)



R = CH_3, $CH_2CH_2SO_2C_2H_5$
Ar = phenyl, *p*-chlorophenyl, *m*-chlorophenyl, *o*-chlorophenyl, furoyl

(41)



R_1 = CH_3, $CH_2CH_2SO_2C_2H_5$
R_2 = heterocyclic amine, aromatic amines, aliphatic amines

(42)

3.1 References

Alcade E, Marlos L, Dinares I, Valls N, Elguero JA, Martinez AR, Igea A, Osauna A, Ruiz-pere LM, Cifuentes J. New derivatives of 5-nitroimidazols: synthesis and antiparasitic activities. Farmaco E dizione Scientifica 1989;44:1095-1107.

Nagarajan K, Arya VP, George T, Sudarsanam V, Shah RK, Goud AN, Shenov SJ, Honkan V, Kulkarni YS and Rao ML. Nitroimidazoles IV. 1-1-Methyl-5-nitroimidazolyl (2)-2-oxo-3-sulphonyl (carbamoylthiocarbamoyl) tetrahydro imidazoles. Ind J Chem 1982;21: 928–940.

Ray DK, Chatterjee DK, Tendulkar JS. Comparative efficacy of GO 10213 and some nitroimidazoles against Trichomonas vaginalis and T. foetus in mice infected subcutaneously. Ann Trop Med Parasitol 1982;76:175–178.

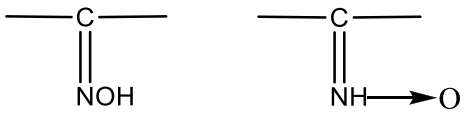
Ray DK, Tendulkar JS, Shrivastava VB, Datta AK, Nagarajan K. A metronidazole-resistant strain of Trichomonas vaginalis and its sensitivity to GO 10213. J Antimicrob Chemother 1984;14:423-426.

Vanelle P, Maldonado J, Gasquet M, Delamas F, Timon-David P, Jentzer O, Crozet MP. Studies on antiparasitic agents: effect of the lactam substitution in the 2-position on the invitro activity of 5-nitroimidazoles. J Pharm Pharmacol 1991c;43:735-736.

4.1 Introduction

4.1.1 Chemistry

The oximes are tautomeric substances in that they furnish both oximino and nitronic derivatives **(43**). In addition, when certain special structural requirements in the remainder of the molecule are satisfied, they may furnish cyclic derivatives.



(43)

An oximes is a chemical compound belonging to the imines, with the general formula $R_1R_2C=NOH$, where R_1 is an organic side chain and R_2 may be hydrogen, forming an aldoxime, or another organic group, forming a ketoxime. O-substituted oximes form a closely related family of compounds. Amidoximes are oximes of hemiaminals with general structure RC(=NOH)(NRR') (Ferdinand, 1891 and Robert *et al*., 1956).

Oximes are usually generated by the reaction of hydroxylamine and aldehydes or ketones. The term oximes date back to the 19th century, a portmanteau of the words oxygen and imide. Oximes exist as two geometric stereoisomers: a syn isomer and an anti isomer. Aldoximes, except for aromatic aldoximes, exist only as a syn isomer, while ketoximes can be separated almost completely and obtained as a syn isomer and an anti isomer.Oximes have three characteristic bands in the infrared spectrum, at wave numbers 3600 (O-H), 1665 (C=N) and 945 (N-O) (Reusch *et al*., 1945).

Oximes can be synthesized by condensation of an aldehyde or a ketone with hydroxylamine. The condensation of aldehydes with hydroxylamine gives aldoxime, and ketoxime is produced from ketones and hydroxylamine. Generally, oximes exist as colorless crystals and are poorly soluble in water. Oximes can also be obtained from reaction of nitrites such as isoamyl nitrite with compounds containing an acidic hydrogen atom. Examples are the reaction of ethyl acetoacetate and sodium nitrite in acetic acid (Fischer, 1943 and 1955) the reaction of methyl ethyl ketone with ethyl nitrite in hydrochloric acid (Semon *et al*., 1943) and a similar reaction with propiophenone (Walter *et al*., 1943), the reaction of phenacyl chloride (Nathan *et al*., 1955), the reaction of malononitrile with sodium nitrite in acetic acid (Ferris *et al*., 1943).

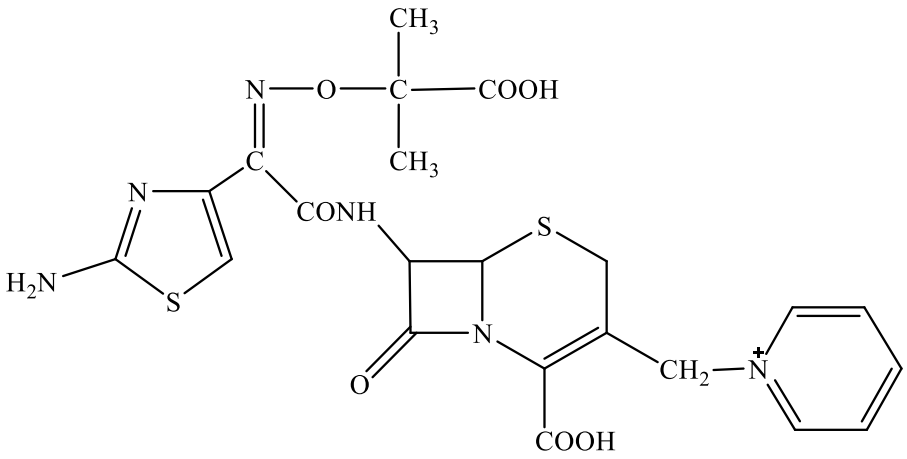
The hydrolysis of oximes proceeds easily by heating in the presence of various inorganic acids, and the oximes decompose into the corresponding ketones or aldehydes, and hydroxylamines. The reduction of oximes by sodium amalgam or hydrogenation produces amines. The reduction of aldoximes gives both primary amines and secondary amines.

Generally oximes can be changed to the corresponding amide derivatives by treatment with various acids. This reaction is called Beckmann rearrangement. In this reaction, a hydroxyl group is exchanged with the group that is in the anti position of the hydroxyl group. The amide derivatives that are obtained by Beckmann rearrangement can be transformed into a carboxylic acid by means of hydrolysis (base or acid catalyzed). And an amine by Hoffman degradation of the amide in the presence of alkali hypoclorites at 80 ^{0}C, the degradation is itself prone to side reactions namely, the formation of biurets or, cyanate polymers, to avoid this side reaction strict temperature control is necessary, the reaction must be conducted at sufficient temperature to isomerise the cyanate to the isocyante. Beckmann rearrangement is used for the industrial synthesis of caprolactam.

The Ponzio reaction (Giacomo, 1906) concerning the conversion of *m*-nitrobenzaldoxime to *m*-nitrophenyldinitromethane with dinitrogen tetroxide, was the result of research into TNT-like high explosives (Louis *et al*., 1946). Dimethylglyoxime is a reagent for the analysis of nickel and a popular ligand in its own right. Typically a metal reacts with two equivalents of $dmgH_2$ concomitant with ionization of one proton.

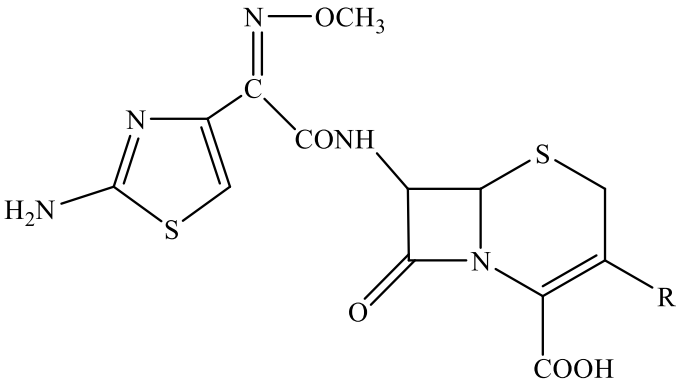
4.1.2 Biological importance

Oximes are used as antidotes for nerve agents. A nerve agent inactivates acetylcholinesterase molecules by phosphorylation of the molecule. Oximes compounds can reactivate acetylcholinesterase by attaching to the phosphorus atom and forming an oxime-phosphonate which then splits away from the acetylcholinesterase molecule. The most effective oxime nerve-agent antidotes are pralidoxime (also known as 2-PAM), obidoxime, methoxime (Aaron, 2007). Effectiveness of the oxime treatment depends on the particular nerve agent used (Kassa, 2002). Perillartine, the oxime of perillaldehyde is used as an artificial sweetener in Japan, as it is 2000 times sweeter than sucrose. Salicylaldoxime is a chelator. Glyoxime, produced via the condensation of glyoxal with hydroxylamine (Michelman *et al*., 1965), forms highly energetic copper, lead and silver salts like copper, lead and silver glyoximate respectively (Urben. 1999). However these compounds are too unstable to be of any commercial value. Diaminoglyoxime, a glyoxime derivative, is a key synthetic precursor, used to prepare various compounds, containing the highly reactive furazan ring. Some of the clinically important cephalosporin of the oxime class is shown in structure **(44)** and **(45)**.

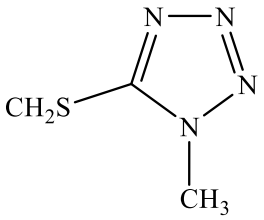
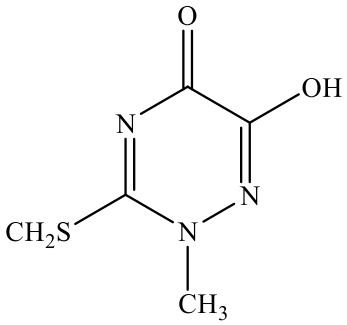


(44)

Ceftazmide



(45)

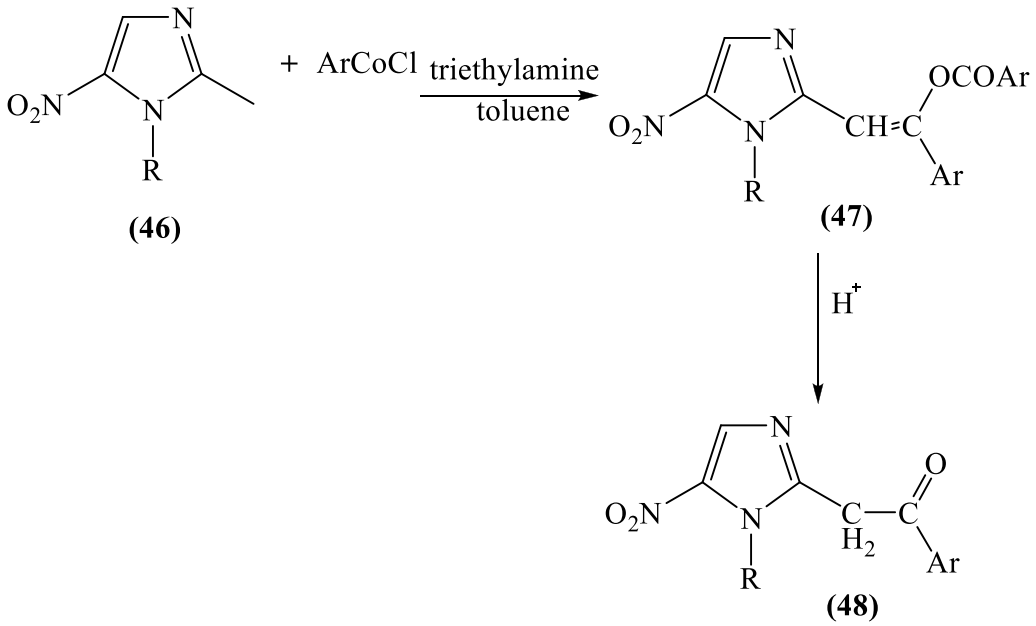
Name	**R**
Ceftizoxime	H
Cefotaxime	CH_2OCOCH_3
Cefmenoxime	
Ceftrioxzone	

4.2 Results and Discussion

4. 2.1 Synthetic approach

4.2.1.1 Synthesis of 2-(1-(substituted)-5-nitro-1*H*-imidazol-2-yl)-1-(substituted) ethanone

2-(1-(substituted)-5-nitro-1*H*-imidazol-2-yl)-1-(substituted)ethanone **(48)** was synthesized by activation of methyl group at 2nd of **(46)** by sterically hindered base and subsequent electrophilic substitution with acid chlorides afforded **(47)** as intermediate which upon acidic hydrolysis yields the desired product (Albright *et al*., 1973).

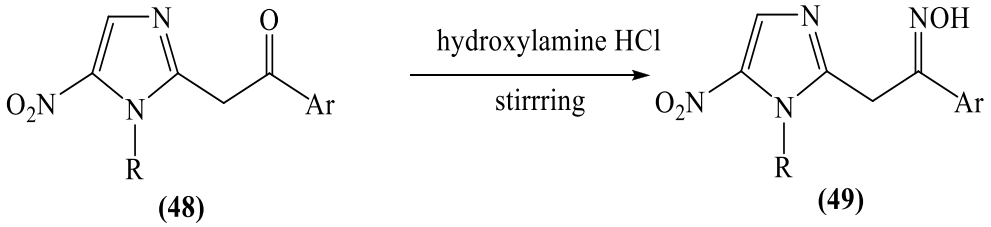


(46) **(47)** **(48)**

R = CH_3, $CH_2CH_2SO_2C_2H_5$
Ar = phenyl, *p*-chlorophenyl, *m*-chlorophenyl, *o*-chloro phenyl, furoyl

4.2.1.2 Synthesis of 2-(1-(substituted)-5-nitro-1*H*-imidazol-2-yl)-1-substituted ethanoneoxime (Albright *et al*., 1973)

The active carbonyl group of 2-(1-(substituted)-5-nitro-1*H*-imidazol-2-yl)-1-(substituted)ethanone **(48)** were reacted with hydroxylamine HCl yielding, 2-(1-(substituted)-5-nitro-1*H*-imidazol-2-yl)-1-substitutedethanoneoxime **(49)**

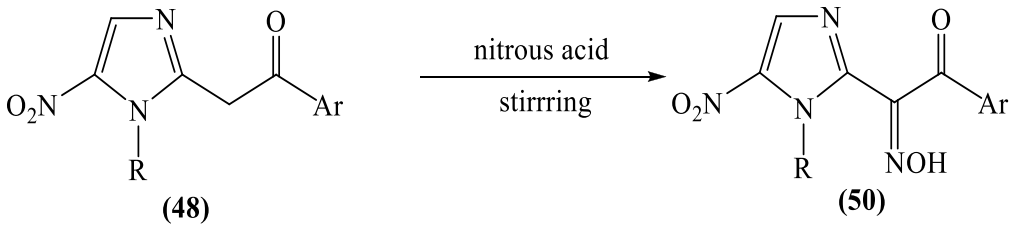


(48) **(49)**

R = CH_3, $CH_2CH_2SO_2C_2H_5$
Ar = phenyl, *p*-chlorophenyl,
m-chlorophenyl, *o*-chlorophenyl,
furoyl

4.2.1.3 Synthesis of 1-(substituted-2-yl)-2-(hydroxyimino)-2-(1-(substituted)-5-nitro-1*H*-imidazol-2-yl)ethanone (Albright *et al*., 1973)

The active methyl group of 2-(1-(substituted)-5-nitro-1H-imidazol-2-yl)-1-(substituted)ethanone **(48)** were reacted with nitrous acid affording 1-(substituted-2-yl)-2-(hydroxyimino)-2-(1-(substituted)-5-nitro-1*H*-imidazol-2-yl)ethanone **(50)**



(48) **(50)**

R = CH_3, $CH_2CH_2SO_2C_2H_5$
Ar = phenyl, *p*-chlorophenyl,
m-chlorophenyl, *o*-chlorophenyl,
furoyl

4.2.2 Physical and spectral characteristics

The oximes derivatives of 5-nitroimidazole compounds were pale yellow to orange color and soluble in dichloromethane, chloroform and partially soluble in methanol and ethanol. All the compounds were recrystallized from ethanol having melting point ranging from 130-235 ^{0}C.

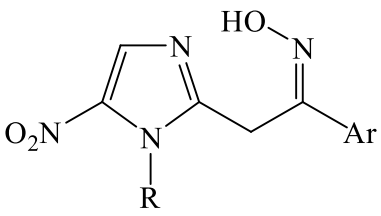
4.2.2.1 IR spectra (KBr, Cm^{-1})

Broad peak around 3600-3000cm^{-1} was observed; signify the presence of oxime (NOH) group. The other peaks were observed in IR spectra as, C-H (2995-2800 cm^{-1}), C=O (1720-1680 cm^{-1}), C=N (1665 cm^{-1}), NO_2 (1570-1540 & 1366-1358 cm^{-1}) and C-N of nitro group (945-800 cm^{-1}).

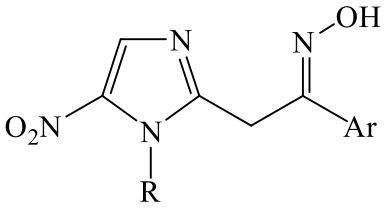
4.2.2.2 ^{1}H NMR spectra (DMSO-d6, δ ppm)

The ^{1}HNMR (DMSO-d_6) spectra of 2-(1-(substituted)-5-nitro-1*H*-imidazol-2-yl)-1-substitutedethanoneoxime, displays the triplet of methyl group (-$SO_2CH_2\underline{CH_3}$) resonates at δ 1.28-1.40 ppm, quartet of methylene group (-$SO_2\underline{CH_2}CH_3$) resonates at δ 3.15 ppm, triplet for (-$CH_2\underline{CH_2}SO_2CH_2CH_3$) resonates at δ 3.66-3.89 ppm, singlet for (-N=C-$\underline{CH_3}$) resonates at δ 4.26-4.30, triplet for (imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$) resonates at δ 4.96 ppm, all the aromatic proton resonates between δ 6.54-7.94 , the singlet for (1$\underline{H}$ nitroimidazole ring) observed between δ 7.87-8.1 ppm due to the electron withdrawing nitro group.

The ^{1}HNMR spectra 2-(1-(substituted)-5-nitro-1*H*-imidazol-2-yl)-1-substitutedethanoneoxime may show the presence of double peaks which may represents geometric isomers *(E)* and *(Z)*of the oxime.

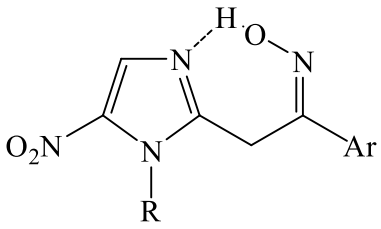


(51)



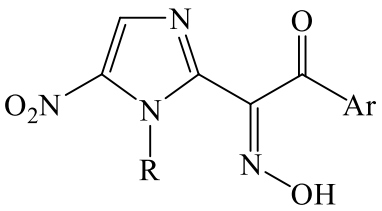
(52)

But due to hydrogen bonding between oxime group and nitrogen of nitroimidazole as shown in **(53),** single peak were observed as singlet at δ 13.0 ppm.

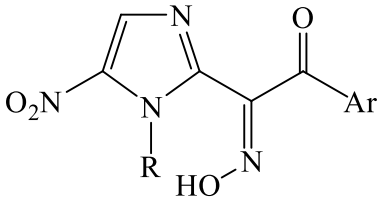


(53)

The 1HNMR spectra 1-(substituted-2-yl)-2-(hydroxyimino)-2-(1-(substituted)-5-nitro-1*H*-imidazol-2-yl)ethanone may also show the presence of double peaks which represent geometric isomers *(E)* and *(Z)* of the oxime, but due to hydrogen bonding

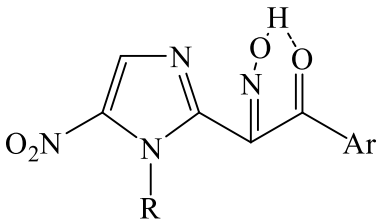


(54)



(55)

between carbonyl and oxime of nitroimidazole, singlet were observed between δ 12.9-13.0 ppm at downfield as shown in (**56)**

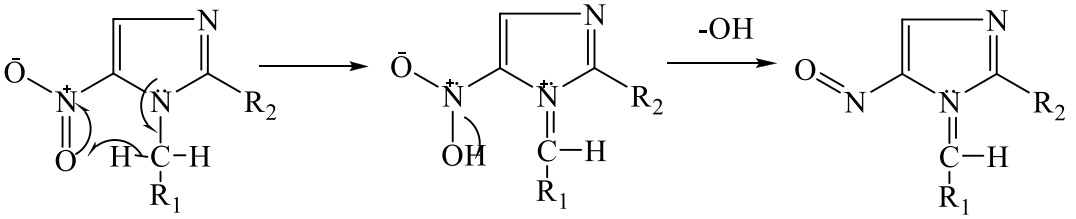


(56)

4.2.2.3 Mass spectra (m/z)

The molecular ions, M+1, M+2 were observed. The fragmentation routes primarily involved losses of OH (M-17), NO (M-30), NO_2 (M-46) and HNO_2 (M-47) from the molecular ion, which are characteristic of compounds.

The possible fragmentation route for nitroimidazole is shown below,



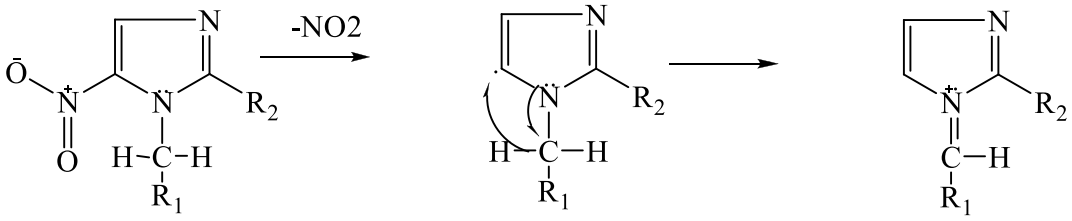
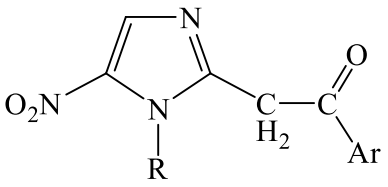

-NO2

Table 10 Physical characteristics of 2-(1-(substituted)-5-nitro-*1H*-imidazol-2-yl)-1-(substituted)ethanone



(57)

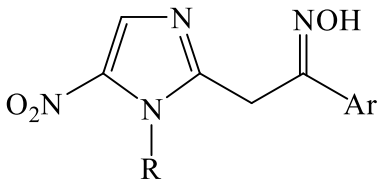
Code	R	Ar	m.p. (^{0}C)	Yield (%)*	Mol. formula	Mol. weight
57-a	$-CH_2CH_2SO_2C_2H_5$	phenyl	146-148 (147-149)	80	$C_{15}H_{17}N_3O_5S$	351.38
57-b	$-CH_2CH_2SO_2C_2H_5$	*p-cl* phenyl	152-154	72	$C_{15}H_{16}ClN_3O_5S$	385.82
57-c	$-CH_2CH_2SO_2C_2H_5$	*m-cl* phenyl	156-158	65	$C_{15}H_{16}ClN_3O_5S$	385.82
57-d	$-CH_2CH_2SO_2C_2H_5$	*o- cl* phenyl	180-182	45	$C_{15}H_{16}ClN_3O_5S$	385.82
57-e	$-CH_2CH_2SO_2C_2H_5$	furoyl	165-166	75	$C_{13}H_{15}N_3O_6S$	341.34
57-f	$-CH_3$	furoyl	130-132	78	$C_{10}H_9N_3O_4$	235.20

*Solvent of recrystallization; ethanol

TABLE 11 Spectral characteristics of 2-(1-(substituted)-5-nitro-*1H*-imidazol-2-yl)-1-(substituted)ethanone

Code	IR (KBr, Cm^{-1})	^{1}HNMR (DMSO-d6, δ ppm)	MASS (m/z)
57-a	2987-2800 (CH_2), 1720 (C=O), 1541,1345 (NO_2)	δ 1.28(t,3H,$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.89 (t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 2H, N=C-$\underline{CH_2}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.56-7.94 (m, 5H, Ar-$\underline{H}$), δ 7.81 (s,1H, nitroimidazole ring)	352 .10 (M+1)
57-b	2977-2850 (CH_2), 1705(C=O), 1565,1365 (NO_2)	δ1.28(t,3H,$SO_2CH_2\underline{CH_3}$), δ 3.20 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.72 (t, 2H,-$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.30 (s, 3H, N=C-$\underline{CH_3}$), δ 4.90 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.60-7.84 (m, 4H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring)	387.24 (M+2)
57-c	2977-2850 (CH_2), 1700(C=O), 1565,1365 (NO_2)	δ 1.30(t,3H,$SO_2CH_2\underline{CH_3}$), δ 3.18 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.28 (s, 3H, N=C-$\underline{CH_3}$), δ 4.90 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.50-7.86 (m, 4H, Ar-$\underline{H}$), δ 7.87 singlet for (1H, imidazole ring).	387.05 (M+2)
57-d	2977-2850 (CH_2), 1700(C=O), 1565,1365 (NO_2)	δ1.38(t,3H,$SO_2CH_2\underline{CH_3}$), δ 3.18 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.70(t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.30 (s, 3H, N=C-$\underline{CH_3}$), δ 4.92(t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.44-7.88 (m, 4H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring)	387 .12 (M+2)
57-e	3050-2850 (CH_2), 1690(C=O), 1550,1360 (NO_2)	δ 1.40 (t,3H,-$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.75 (d, 1H, 4-furoyl), δ 7.59(s, 1H, 5-furoyl), δ 8.1 (s,1H, nitroimidazole ring)	341.34 ($M^{+)}$
57-f	2980-2850 (CH_2), 1710(C=O), 1555,1362 (NO_2)	...	235.20 (M^+)

Table 12 Physical characteristics of 2-(1-(substituted)-5-nitro-1*H*-imidazol-2-yl)-1-substitutedethanoneoxime



(58)

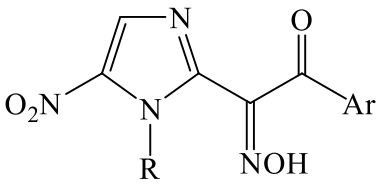
Code	R	Ar	m.p. (^{0}C)	Yield (%)*	Mol. formula	Mol. weight
58-a	$-CH_2CH_2SO_2C_2H_5$	phenyl	202-205	63	$C_{15}H_{18}N_4O_5S$	366.39
58-b	$-CH_2CH_2SO_2C_2H_5$	*p-cl* phenyl	210-212	57	$C_{15}H_{17}ClN_4O_5S$	400.84
58-c	$-CH_2CH_2SO_2C_2H_5$	*m-cl* phenyl	216-219	53	$C_{15}H_{17}ClN_4O_5S$	400.84
58-d	$-CH_2CH_2SO_2C_2H_5$	*o- cl* phenyl	240-245	48	$C_{15}H_{17}ClN_4O_5S$	400.84
58-e	$-CH_2CH_2SO_2C_2H_5$	furoyl	170-176	65	$C_{13}H_{16}N_4O_6S$	356.35
58-f	$-CH_3$	furoyl	145-147	65	$C_{10}H_{10}N_4O_4$	250.21

*Solvent of recrystallization; ethanol

TABLE 13 Spectral characteristics of 2-(1-(substituted)-5-nitro-1*H*-imidazol-2-yl)-1-substitutedethanoneoxime

Code	IR (KBr, Cm^{-1})	^{1}HNMR (DMSO-d6, δ ppm)	MASS (m/z)
58-a	3600-3000 (N-OH), 2977-2800 (CH_2), 1555,1362 (NO_2)	δ 1.45 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.19 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.64 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 2H, N=C-$\underline{CH_2}$), δ 4.66 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.56-7.94 (m, 5H, Ar-$\underline{H}$), δ 7.87 (s,1H, imidazole ring), δ 12.6 (s,1H, NO$\underline{H}$)	366.00 (M^+)
58-b	3600-3100 (N-OH), 2977-2800 (CH_2), 1555,1362 (NO_2)	δ 1.45 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.19 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.64 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.66 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.56-7.94 (m, 4H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring), δ 12.5 (s,1H, NO$\underline{H}$)	401.64 (M+2)
58-c	3670-3150 (N-OH), 2977-2800 (CH_2), 1555,1362 (NO_2)	δ 1.45 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.19 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.64 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.66 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.56-7.94 (m, 4H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring), δ 12.5 (s,1H, NO$\underline{H}$)	…
58-d	3610-3150 (N-OH), 2977-2800 (CH_2), 1555,1362 (NO_2)	δ 1.45 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.19 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.64 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.66 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.56-7.94 (m, 4H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring), δ 12.5 (s,1H, NO$\underline{H}$)	…
58-e	3605-3000 (N-OH), 2977-2800 (CH_2), 1555,1362 (NO_2)	δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.76 (d, 1H, 4-furoyl), δ 7.59 (s, 1H, 5-furoyl), δ 7.87 (s,1H, nitroimidazole ring) δ 13.0 (s,1H, NO$\underline{H}$)	356.00 (M^+)
58-f	3600-3000 (N-OH), 2977-2800 (CH_2), 1555,1362 (NO_2)	δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (s, 3H, imidazole-$\underline{CH_3}$), δ 6.54 (d, 1H, 3-furoyl), δ 6.76 (d, 1H, 4-furoyl), δ 7.59(s, 1H, 5-furoyl), δ 7.87 (s,1H, nitroimidazole ring), δ 7.87 (s,1H, nitroimidazole ring), δ 12.9 (s,1H, NO$\underline{H}$)	250.50 (M^+)

Table 14: Physical characteristics of 1-(substituted-2-yl)-2-(hydroxyimino)-2-(1-(substituted)-5-nitro-1*H*-imidazol-2-yl)ethanone



(59)

Code	R	Ar	m.p. (^{0}C)	Yield (%)*	Mol. formula	Mol. weight
59-a	$-CH_2CH_2SO_2C_2H_5$	phenyl	192-194	52	$C_{15}H_{16}N_4O_6S$	380.38
59-b	$-CH_2CH_2SO_2C_2H_5$	*p-cl* phenyl	204-206	48	$C_{15}H_{15}ClN_4O_6S$	414.82
59-c	$-CH_2CH_2SO_2C_2H_5$	*m-cl* phenyl	210-213	35	$C_{15}H_{15}ClN_4O_6S$	414.82
59-d	$-CH_2CH_2SO_2C_2H_5$	*o- cl* phenyl	232-235	32	$C_{15}H_{15}ClN_4O_6S$	414.82
59-e	$-CH_2CH_2SO_2C_2H_5$	furoyl	198-202	45	$C_{13}H_{14}N_4O_7S$	370.34
59-f	$-CH_3$	furoyl	150-151	50	$C_{10}H_8N_4O_5$	264.19

*Solvent of recrystallization; ethanol

TABLE 15: Spectral characteristics of 1-(substituted-2-yl)-2-(hydroxyimino)-2-(1-(substituted)-5-nitro-1*H*-imidazol-2-yl)ethanone

Code	IR (KBr, Cm^{-1})	^{1}HNMR (DMSO-d6, δ ppm)	MASS (m/z)
59-a	3600 -3000(N-OH), 2977-2800 (CH_2), 1720 (C=O), 1541,1345 (NO_2)	δ 1.39 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.25 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.73 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.20 (s, 3H, N=C-$\underline{CH_3}$), δ 5.18 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.60-7.84 (m, 5H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring), δ 12.8 (s,1H, NO$\underline{H}$ hydrogen bonded with oxygen)	380.00 (M^+)
59-b	3610-3050 (N-OH), 2977-2800 (CH_2), 1720 (C=O), 1545,1365 (NO_2)	δ 1.48 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.25 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.73 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.20 (s, 3H, N=C-$\underline{CH_3}$), δ 5.18 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.60-7.84 (m, 4H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring) δ 12.8 (s,1H, NO$\underline{H}$ hydrogen bonded with oxygen)	---
59-c	3615-3000 (N-OH), 2977-2800 (CH_2), 1710 (C=O), 1548,1355 (NO_2)	δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.60-7.84 (m, 4H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring) δ 12.8 (s,1H, NO$\underline{H}$ hydrogen bonded with oxygen)	----
59-d	3605-3000 (N-OH), 2977-2800 (CH_2), 1710 (C=O), 1541,1345 (NO_2)	δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.60-7.84 (m, 4H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring) δ 12.8 (s,1H, NO$\underline{H}$ hydrogen bonded with oxygen)	416.12 (M+2)
59-e	3600 -3000(N-OH), 2960-2800 (CH_2), 1720 (C=O), 1561,1350 (NO_2)	δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.75 (d, 1H, 4-furoyl), δ 7.59 (s, 1H, 5-furoyl), δ 7.87 (s,1H, nitroimidazole ring) δ 13.0 (s,1H, NO$\underline{H}$)	370.34 (M^+)
59-f	3600-3180 (N-OH), 2977-2800 (CH_2), 1710 (C=O), 1540,1340 (NO_2)	δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (s, 3H, imidazole-$\underline{CH_3}$), δ 6.54 (d, 1H, 3-furoyl), δ 6.78 (d, 1H, 4-furoyl), δ 7.59(s, 1H, 5-furoyl), δ 7.87 (s,1H, nitroimidazole ring) δ 12.9 (s,1H, NO$\underline{H}$ hydrogen bonded with oxygen)	264.65 (M^+)

4.3 Experimental

Metronidazole, dimetridazole, tinidazole, triethylamine, toluene, acetonitrile, methanol and acid chlorides of LR grade were obtained from Lincon laboratory Pvt. Ltd., Ahmedabad and SD Fine chemicals Pvt. Ltd Ahmedabad were used after purification. All the melting points were determined in open capillaries and are uncorrected. Thin layer chromatography was performed on microscopic slides (2x7.5cm) coated with silica gel G and spots were visualized by normal TLC and exposure to iodine vapor. IR spectra were recorded in KBr on SHIMADZU Fourier Transform Infrared 8400S spectrophotometer. Mass spectra were recorded on Micromass Q-T, TOF MS $ES^{+}4.73e^{3.}$ Nuclear Magnetic Resonance spectra (^{1}H NMR) were recorded in DMSO-d_6 on BRUKER AVANCE II at 400 MHz and the chemical shift are given in parts per million, downfield from Tetramethyl silane (TMS) was used as internal standard.

Synthesis of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-phenylethanone (57-a) (Molvi et al, 2006)

To a mixture of 4.94 g (0.020 mole) of 1-(2-(ethylsulfonyl)ethyl)-2-methyl-5-nitro-1*H*-imidazole **(46)**, 17.5 mL of toluene and 8.09g (0.080mole) of triethylamine was added with occasional cooling 7.7 g (0.55 mole) of benzoyl chloride. The mixture was stirred for 22 hr, diluted with 10 mL of ether and chilled. The mixture was filtered and the solid washed with three 10 mL portions ether and four 10 mL portion water to give intermediate. The whole crude intermediate was taken in to 10 mL of water, 15 mL of ethanol and 10 mL of concentrated hydrochloric acid and refluxed for 2-4 hr. The solution was chilled and poured on to the ice. The solid was filtered, washed with water and recrystallized from ethanol to gave 5.63g (80% yield), yellow crystals of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-phenylethanone **(57-a)**, m.p. 146-148 ^{0}C.

Analysis:

TLC	:Toluene: acetonitrile (4:1); Rf value: 0.67
IR (KBr, cm^{-1})	:2987-2800 (CH_2), 1720 (C=O), 1541,1345 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.28(t,3H,$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H,-$SO_2\underline{CH_2}CH_3$), δ 3.89 (t, 2H,-$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$),δ4.96(t,2H,imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.56-7.94 (m, 4H, Ar-H), δ 7.81 (s,1H, nitroimidazole ring)
Mass (m/z)	:352.10 (M+1)

Synthesis of 1-(4-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanone (57-b)

To a mixture of 4.94 g (0.020 mole) of 1-(2-(ethylsulfonyl)ethyl)-2-methyl-5-nitro-1*H*-imidazole **(46)**, 17.5 mL of toluene and 8.09g (0.080mole) of triethylamine was added with occasional cooling 9.62 g (0.055 mole) of *p*-Chlorobenzoyl chloride. The mixture was stirred for 24 hr, diluted with 10 mL of ether and chilled. The mixture was filtered and the solid washed with three 10 mL portions ether and four 10 mL portion water to give intermediate. The whole crude intermediate was taken in to 10 mL of water, 15 mL of ethanol and 10 mL of concentrated hydrochloric acid and

refluxed for 2-4 hr. The solution was chilled and poured on to the ice. The solid was filtered, washed with water and recrystallized from ethanol to gave 5.56g (72% yield), pale yellow crystals of 1-(4-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanone **(57-b),** m.p. 152-154 ^{0}C.

Analysis:

TLC	:Toluene: acetonitrile (4:1); Rf value: 0.69
IR (KBr, cm^{-1})	:2977-2850 (CH_2), 1705 (C=O), 1565, 1365 (NO_2)
NMR (δ , ppm)(DMSO-d_6)	:δ 1.28 (t, 3H, $SO_2CH_2\underline{CH_3}$), δ 3.20 (q, 2H,-$SO_2\underline{CH_2}CH_3$), δ 3.72 (t, 2H,-$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.30 (s, 3H, N=C-$\underline{CH_3}$),δ4.90 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.60-7.84 (m, 4H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring)
Mass (m/z)	:387.24 (M+2)

Synthesis of 1-(3-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanone (57-c)

To a mixture of 4.94 g (0.020 mole) of 1-(2-(ethylsulfonyl)ethyl)-2-methyl-5-nitro-1*H*-imidazole **(46)**, 17.5 mL of toluene and 8.09g (0.080mole) of triethylamine was added with occasional cooling 9.62 g (0.055 mole) of *m*-Chlorobenzoyl chloride. The mixture was stirred for 24 hr, diluted with 10 mL of ether and chilled. The mixture was filtered and the solid washed with three 10 mL portions ether and four 10 mL portion water to give an intermediate. The whole crude intermediate product was taken in to 10 mL of water, 15 mL of ethanol and 10 mL of concentrated hydrochloric acid and refluxed for 2 hr. The solution was chilled and poured on to the ice. The solid was filtered, washed with water and recrystallized from ethanol to gave 5.06 g (65% yield), yellow crystals of 1-(3-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanone **(57-c)**, m.p. 156-158 ^{0}C.

Analysis:

TLC	:Toluene: acetonitrile (4:1); Rf value: 0.62
IR (KBr, cm^{-1})	:2977-2850 (CH_2), 1700(C=O),1565,1365 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ1.30 (t,3H,$SO_2CH_2\underline{CH_3}$), δ3.18 (q, 2H,-$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.28 (s, 3H,

N=C-$\underline{CH_3}$), δ 4.90 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.50-7.86 (m, 4H, Ar-$\underline{H}$), δ 7.87 singlet for (1H, imidazole ring)

Mass (m/z) :387.05 (M+2)

Synthesis of 1-(2-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanone (57-d)

To a mixture of 4.94 g (0.020 mole) of 1-(2-(ethylsulfonyl)ethyl)-2-methyl-5-nitro-*1H*-imidazole **(46)**, 17.5 mL of toluene and 8.09g (0.080mole) of triethylamine was added with occasional cooling 9.62 g (0.055 mole) of *o*-Chlorobenzoyl chloride. The mixture was stirred for 36 hr, diluted with 10 mL of ether and chilled. The mixture was filtered and the solid washed with three 10 mL portions ether and four 10 mL portion water to give an intermediate. The whole crude intermediate product was taken in to 10 mL of water, 15 mL of ethanol and 10 mL of concentrated hydrochloric acid and refluxed for 2-4 hr. The solution was chilled and poured on to the ice. The solid was filtered, washed with water and recrystallized from ethanol gave 3.50g (45% yield), yellow crystals of 1-(2-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanone **(57-d)**, m.p. 180-182 ^{0}C.

Analysis:

TLC :Toluene: acetonitrile (4:1); Rf value: 0.60

IR (KBr, cm^{-1}) :2977-2850 (CH_2), 1700(C=O),1565,1365 (NO_2)

NMR (DMSO-d_6, δ ppm) :δ 1.38(t,3H,$SO_2CH_2\underline{CH_3}$), δ 3.18 (q, 2H, -$SO_2\underline{CH_2}CH_3$), 3.70(t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.30 (s, 3H, N=C-$\underline{CH_3}$), δ 4.92(t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.44-7.88 (m, 4H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring)

Mass (m/z) :387.12 (M+2)

Synthesis of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone (57-e)

To a mixture of 4.94 g (0.020 mole) of 1-(2-(ethylsulfonyl)ethyl)-2-methyl-5-nitro-1*H*-imidazole **(46)**, 17.5 mL of toluene and 8.09g (0.080mole) of triethylamine was added with occasional cooling 7.17 g (0.055 mole) of furoyl chloride. The mixture

was stirred for 18 hr, diluted with 10 mL of ether and chilled. The mixture was filtered and the solid washed with three 10 mL portions ether and four 10 mL portion water to give an intermediate. The whole crude intermediate product was taken in to 10 mL of water, 15 mL of ethanol and 10 mL of concentrated hydrochloric acid and refluxed for 2 hr. The solution was chilled and poured on to the ice. The solid was filtered, washed with water and recrystallized from ethanol to gave 5.12g (75% yield) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(57-e)**, m.p. 165-166 ^{0}C.

Analysis:

TLC	: Toluene: acetonitrile (4:1); Rf value: 0.56
IR (KBr, cm^{-1})	: 3050-2850 (CH_2), 1690(C=O),1550,1360 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.75 (d, 1H, 4-furoyl), δ 7.59(s, 1H, 5-furoyl), δ 8.10 (s,1H, nitroimidazole ring)
Mass (m/z)	: 341.34 (M^+)

Synthesis of 1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)ethanone (57-f)

To a mixture of 2.82 g (0.020 mole) of 1,2-dimethyl-5-nitro-1*H*-imidazole **(46)**, 17.5 mL of toluene and 8.09g (0.080mole) of triethylamine was added with occasional cooling 9.62 g (0.055 mole) of furoyl chloride. The mixture was stirred for 18hr, diluted with 10 mL of ether and chilled. The mixture was filtered and the solid washed with three 10 mL portions ether and four 10 mL portion water to give an intermediate. The whole crude intermediate product was taken in to 10 mL of water, 15 mL of ethanol and 10 mL of concentrated hydrochloric acid and refluxed for 2 hr. The solution was chilled and poured on to the ice. The solid was filtered, washed with water and recrystallized from ethanol to gave 3.67g (78% yield) of 1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)ethanone **(57-f)**, m.p. 130-132 ^{0}C.

Analysis:

TLC : Toluene: acetonitrile (4:1); Rf value: 0.50

IR (KBr, cm^{-1}) : 2980-2850 (CH_2), 1710 (C=O),1555,1362 (NO_2)

Mass (m/z) : 235.20 (M^+)

Synthesis of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1H-imidazol-2-yl)-1-phenylethanoneoxime (58-a)

To a mixture of 1.72 g (0.0050 mole) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-phenylethanone **(57-a)**, 0.41 g (0.0060 mole) of hydroxylamine hydrochloric acid in 25 mL ethanol and 5 mL of water, was heated on steam bath for 10 minutes and allowed to stand at room temperature. The solvent was removed by vacuum and the residue was triturated with ammonium hydroxide to bring the pH at 7.0, the solid was filtered and recrystallized form ethanol gave 1.12 g, (63% yield) pale yellow crystal of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-phenylethanoneoxime **(58-a)**, m.p. 202-205 ^{0}C.

Analysis:

TLC : Toluene: methanol (4:1); Rf value: 0.44

IR(KBr,cm^{-1}) :3600-3000 (N-OH),2977-2800 (CH_2),1555,1362 (NO_2)

NMR (DMSO-d_6, δ ppm) :δ 1.45 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.19 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.64 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.66 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.56-7.94 (m, 5H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring), δ 12.6 (s,1H, NO$\underline{H}$)

Mass (m/z) : 366.39 (M^+)

Synthesis of 1-(4-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanoneoxime (58-b)

To a mixture of 1.92g (0.0050mole) of 1-(4-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanone **(57-b)**, 0.41 g (0.0060 mole) of hydroxylamine hydrochloric acid in 25 mL ethanol and 5 mL of water was heated on steam bath for 10 minutes and allowed to stand at room temperature. The solvent was removed by vacuum and the residue was triturated with ammonium hydroxide to bring the pH at 7.0, the solid was filtered and recrystallized form ethanol gave 1.13 g

(53% yield) pale yellow crystal of 1-(4-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanoneoxime **(57-b),** m.p. 210-212 ^{0}C.

Analysis:

TLC	: Toluene: methanol (4:1); Rf value: 0.49
IR (KBr, cm^{-1})	:3600-3100(N-OH),2977-2800(CH_2),1555,1362(NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.45 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.19 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.64 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.66 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.56-7.94 (m, 4H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring), δ 12.5 (s,1H, NO$\underline{H}$)
Mass (m/z)	: 401.64 (M+2)

Synthesis of 1-(3-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanoneoxime (58-c)

To a mixture of 1.92g (0.0050mole) of 1-(3-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanone **(57-c)**, 0.41 g (0.0060 mole) of hydroxylamine hydrochloric acid in 25 mL ethanol and 5 mL of water was heated on steam bath for 10 minutes and allowed to stand at room temperature. The solvent was removed by vacuum and the residue was triturated with ammonium hydroxide to bring the pH at 7.0, the solid was filtered and recrystallized form ethanol gave 1.05 g (53 % yield) pale yellow crystal of 1-(3-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanoneoxime **(58-c),** m.p. 216-219 ^{0}C.

Analysis:

TLC	: Toluene: methanol (4:1); Rf value: 0.52
IR (KBr, cm^{-1})	: 3670-3150(N-OH),2977-2800(CH_2), 1555,1362 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ1.45 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.19 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.64 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.66 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.56-7.94 (m, 4H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring), δ 12.5 (s,1H, NO$\underline{H}$)

Synthesis of 1-(2-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanoneoxime (58-d)

To a mixture of 1.92 g (0.0050 mole) of 1-(2-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanone **(57-d)**, 0.41 g (0.0060 mole) of hydroxylamine hydrochloric acid in 25 mL ethanol and 5 mL of water was heated on steam bath for 10 minutes and allowed to stand at room temperature. The solvent was removed by vacuum and the residue was triturated with ammonium hydroxide to bring the pH at 7.0, the solid was filtered and recrystallized form ethanol gave 0.95 g (48% yield) pale yellow crystal of 1-(2-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanoneoxime **(58-d),** m.p. 240-245 ^{0}C.

Analysis:

TLC	: Toluene: methanol (4:1); Rf value: 0.50
IR (KBr, cm^{-1})	:3610-3150(N-OH), 2977-2800 (CH_2),1555,1362 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.45 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.19 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.64 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.66 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.56-7.94 (m, 4H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring), δ 12.5 (s,1H, NO$\underline{H}$)

Synthesis of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanoneoxime (58-e)

To a mixture of 1.70 g (0.0050mole) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(57-e)**, 0.41 g (0.0060 mole) of hydroxylamine hydrochloric acid in 25 mL ethanol and 5 mL of water was heated on steam bath for 10 minutes and allowed to stand at room temperature. The solvent was removed by vacuum and the residue was triturated with ammonium hydroxide to bring the pH at 7.0, the solid was filtered and recrystallized form ethanol gave 1.15 g (65% yield) pale yellow crystal of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanoneoxime **(58-e),** m.p. 170-176 ^{0}C.

Analysis:

TLC	: Toluene: methanol (4:1); Rf value: 0.56
IR (KBr,cm^{-1})	:3605-3000(N-OH), 2977-2800 (CH_2),1555,1362 (NO_2)

NMR (DMSO-d_6, δ ppm)	:δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.76 (d, 1H, 4-furoyl), δ 7.59(s, 1H, 5-furoyl), δ 7.87 (s,1H, nitroimidazole ring) δ 13.0 (s,1H, NO<u>H</u>)
Mass (m/z)	: 356.00 (M^+)

Synthesis of 1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)ethanoneoxime (58-f)

To a mixture of 1.12 g (0.0050mole) of 1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)ethanone **(57-f)**, 0.41 g (0.0060 mole) of hydroxylamine hydrochloric acid in 25 mL ethanol and 5 mL of water was heated on steam bath for 10 minutes and allowed to stand at room temperature. The solvent was removed by vacuum and the residue was triturated with ammonium hydroxide to bring the pH at 7.0, the solid was filtered and recrystallized form ethanol gave 0.77 g (65% yield) pale yellow crystal of 1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)ethanoneoxime **(58-f),** m.p. 145-147 ^{0}C.

Analysis:

TLC	: Toluene: methanol (4:1); Rf value: 0.40
IR (KBr, cm^{-1})	:3600-3000 (N-OH), 2977-2800 (CH_2),1555,1362 (NO_2)
NMR (DMSO-d_6, δ ppm)	: δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (s, 3H, imidazole-$\underline{CH_3}$), δ 6.54 (d, 1H, 3-furoyl), δ 6.76 (d, 1H, 4-furoyl), δ 7.59(s, 1H, 5-furoyl), δ 7.87 (s,1H, nitroimidazole ring), δ 7.87 (s,1H, nitroimidazole ring), δ 12.9 (s,1H, NO<u>H</u>)
Mass (m/z)	:250.50 (M^+)

Synthesis of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-2-(hydroxyimino)-1-phenylethanone (59-a)

To 10.5g (0.030 mole) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-phenylethanone **(57-a),** was added 30 mL of concentrated sulfuric acid. To the solution was added 5.1g (0.060 mole) of potassium nitrite, in three portions with occasional cooling. The mixture was stirred at room temperature for 45 minutes and poured onto ice. The solid was filtered, washed with water and recrystallized from ethanol to gave 5.91g (52% yield) orange crystals of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-2-(hydroxyimino)-1-phenylethanone **(59-a),** m.p. 192-194 ^{0}C.

Analysis:

TLC	: Toluene: methanol (4:1); Rf value: 0.64
IR (KBr, cm^{-1})	:3600 -3000(N-OH), 2977-2800 (CH_2),1720 (C=O), 1541, 1345 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.39 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.25 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.73 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.20 (s, 3H, N=C-$\underline{CH_3}$), δ 5.18 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.60-7.84 (m, 5H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring),δ 12.8 (s,1H, NO$\underline{H}$ hydrogen bonded with oxygen)
Mass (m/z)	:380.00 (M^+)

Synthesis of 1-(4-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-2- hydroxyimino)ethanone (59-b)

To 11.57g (0.030 mole) of 1-(4-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanone **(57-b),** was added 30 mL of concentrated sulfuric acid. To the solution was added 5.1g (0.060 mole) of potassium nitrite, in three portions with occasional cooling. The mixture was stirred at room temperature for 45 minutes and poured onto ice. The solid was filtered, washed with water and recrystallized from ethanol to gave 5.97g (48% yield) orange crystals of 1-(4-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-2-hydroxyimino)ethanone **(59-b),** m.p. 204-206 ^{0}C.

Analysis:

TLC : Toluene: methanol (4:1); Rf value: 0.64

IR (KBr, cm^{-1}) : 3610-3050 (N-OH), 2977-2800 (CH_2), 1720 (C=O),1545,1365 (NO_2)

NMR (DMSO-d_6, δ ppm) :δ 1.48 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.25 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.73 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.20 (s, 3H, N=C-$\underline{CH_3}$), δ 5.18 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.60-7.84 (m, 5H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring),δ 12.8 (s,1H, NO$\underline{H}$ hydrogen bonded with oxygen)

Synthesis of 1-(3-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-2- hydroxyimino)ethanone (59-c)

To 11.57g (0.030 mole) of 1-(3-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanone **(57-c),** was added 30 mL of concentrated sulfuric acid. To the solution was added 5.1g (0.060 mole) of potassium nitrite, in three portions with occasional cooling. The mixture was stirred at room temperature for 45 minutes and poured onto ice. The solid was filtered, washed with water and recrystallized from ethanol to give 4.35g (35% yield) orange crystals of 1-(4-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-2-hydroxyimino)ethanone **(59-c),** m.p. 210-213 ^{0}C.

Analysis:

TLC :Toluene: acetonitrile (4:1); Rf value: 0.64

IR (KBr, cm^{-1}) :3615-3000 (N-OH), 2977-2800 (CH_2), 1710 (C=O),1548,1355 (NO_2)

NMR (DMSO-d_6, δ ppm) :δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.60-7.84 (m, 4H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring) δ 12.8 (s,1H, NO$\underline{H}$ hydrogen bonded with oxygen)

Synthesis of 1-(2-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-2- hydroxyimino)ethanone (59-d)

To 11.57g (0.030 mole) of 1-(2-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanone **(57-d),** was added 30 mL of concentrated sulfuric acid. To the solution was added 5.1g (0.060 mole) of potassium nitrite, in three portions with occasional cooling. The mixture was stirred at room temperature for 45 minutes and poured onto ice. The solid was filtered, washed with water and recrystallized from ethanol to give 3.98g (32% yield) orange crystals of 1-(2-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-2-hydroxyimino)ethanone **(59-d),** m.p. 232-235 ^{0}C.

Analysis:

TLC	: Toluene: acetonitrile (4:1); Rf value: 0.64
IR (KBr, cm^{-1})	: 3605-3000 (N-OH), 2977-2800 (CH_2), 1710 (C=O), 1541, 1345 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 7.60-7.84 (m, 4H, Ar-$\underline{H}$), δ 7.87 (s,1H, nitroimidazole ring) δ 12.8 (s,1H, NO$\underline{H}$ hydrogen bonded with oxygen)
Mass (m/z)	: 416.12 (M+2)

Synthesis of 1-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-2-(furan-2-yl)ethane-1,2-dioneoxime (59-e)

To 10.24g (0.030 mole) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(57-e),** was added 30 mL of concentrated sulfuric acid. To the solution was added 5.1g (0.060 mole) of potassium nitrite, in three portions with occasional cooling. The mixture was stirred at room temperature for 45 minutes and poured onto ice. The solid was filtered, washed with water and recrystallized from ethanol to give 4.99g (45% yield) orange crystals of 1-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1H-imidazol-2-yl)-2-(furan-2-yl)ethane-1,2-dioneoxime **(59-e),** m.p.198-202 ^{0}C.

Analysis:

TLC	: Toluene: acetonitrile (4:1); Rf value: 0.64
IR (KBr, cm^{-1})	: 3600 -3000(N-OH), 2960-2800 (CH_2), 1720 (C=O), 1561, 1350 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.75 (d, 1H, 4-furoyl), δ 7.59(s, 1H, 5-furoyl), δ 7.87 (s,1H, nitroimidazole ring) δ 13.0 (s,1H, NO<u>H</u> hydrogen bonded with oxygen)
Mass (m/z)	: 370.34 (M^+)

Synthesis of 1-(furan-2-yl)-2-(hydroxyimino)-2-(1-methyl-5-nitro-1H-imidazol-2-yl)ethanone (59-f)

To 7.05g (0.030 mole) of 1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)ethanone **(57-f),** was added 30 mL of concentrated sulfuric acid. To the solution was added 5.1g (0.060 mole) of potassium nitrite, in three portions with occasional cooling. The mixture was stirred at room temperature for 45 minutes and poured onto ice. The solid was filtered, washed with water and recrystallized from ethanol to gave 5.45 (50% yield) orange crystals of 1-(furan-2-yl)-2-(hydroxyimino)-2-(1-methyl-5-nitro-1H-imidazol-2-yl)ethanone **(59-f),** m.p. 150-151 ^{0}C.

Analysis:

TLC	: Toluene: acetonitrile (4:1); Rf value: 0.64
IR (KBr, cm^{-1})	: 3600-3180 (N-OH), 2977-2800 (CH_2), 1710 (C=O), 1540, 1340 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (s, 3H, imidazole-$\underline{CH_3}$), δ 6.54 (d, 1H, 3-furoyl), δ 6.78 (d, 1H, 4-furoyl), δ 7.59(s, 1H, 5-furoyl), δ 7.87 (s,1H, nitroimidazole ring) δ 12.9 (s,1H, NO<u>H</u> hydrogen bonded with oxygen)
Mass (m/z)	: 264.65 (M^+)

4.4 References

Aaron R. New Nerve Gas Antidotes. Wired magazine 2007;11:27-28.

Albright DJ, Shepherd RG. Reaction of 1,2-Dimethyl-5-nitroimidazole, Novel methods of conversion of the 2-methyl group to a nitrile. J Het Chem 1973;10:899-907.

Ferdinand T. Ueber die Einwirkung von Benzolsulfonsäurechlorid auf Amidoxime. Chemische Berichte 1891;24:4162-4167.

Ferris JP, Sanchez RA, Mancuso RW. p-toluenesulfonate. Org Synth 1943;5:32-33.

Fischer H. 2,4-Dimethyl-3,5-dicarbethoxypyrrole. Org Synth 1943;2: 202.

Fischer H. Kryptopyrrole. Org Synth 1955;3: 513.

Giacomo P. Einwirkung von Stickstofftetroxyd auf Benzaldoxim. J Prakt Chem 1906;73:494-496.

Kassa J. Review of oximes in the antidotal treatment of poisoning by organophosphorus nerve agents. Journal of Toxicology-Clinical Toxicology 2002;40: 803-805.

Louis F. Fieser, Williamvon E, Doering. Aromatic-Aliphatic Nitro Compounds. III. The Ponzio Reaction; 2,4,6-Trinitrobenzyl Nitrate. J Am Chem Soc 1946;68:2252-2254.

Michelman J, Michelman JS. Furazan. J of Org Chem 1965;30:1854-1859.

Molvi KI, Sudarsanam V and Haque N: Synthesis and Antibacterial Activity of Some 2-Substituted Tinidazole Analogues. Ethiop Pharm J 2007;25:35-42.

Nathan L, Walter H, Hartung. ω-chloroisonitrosoacetophenone. Org Synth 1955;3:191-193.

Reusch W. Infrared Spectroscopy. Virtual Textbook of Organic Chemistry. Michigan StateUniversity.http://www.cem.msu.edu/~reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.html.

Robert P, Omer O. Notes-The Reaction of Phosphorus-Containing Enzyme Inhibitors with Some Hydroxylamine Derivatives. J Org Chem 1956; 21:1186-1189.

Semon WL, Damerell VR. Dimethoxyglyoxime. Org Synth 1943;2:204-205.

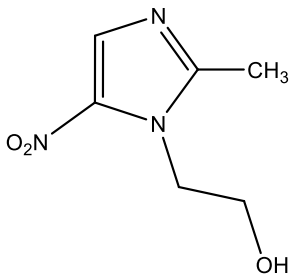
Urben P. Bretherick's Handobook of Reactive Chemical Hazards, 5ed. Butterworth-Heinemann; 1999. P: 799-781.

Walter H, Hartung, Frank C. Isonitrosopropiophenone. Org Synth 1943;2: 363.

5.1 Introduction

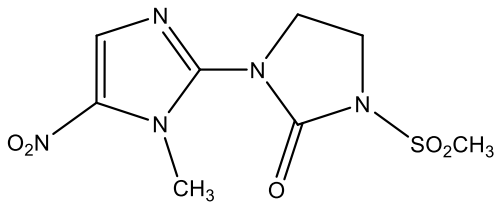
A significant advance in the chemotherapy of protozoan infections was the discovery of metronidazole **(60)**. This drug not only proved very effective in the treatment of trichomoniasis in humans (Cosar *et al.,* 1959 and 1966) but was also found to be a highly potent amoebicide, when given in higher dose (Powell *et al.,* 1966). Niridazole (Wilhelm *et al.,* 1966) has been proven to be powerful chemotherapeutics agent for the treatment of schistosomiasis (Lambert *et al.,* 1969) and amoebiasis (Jarumilinta, 1966). The development of Satranidzole **(61)** is also proven to be the potent antiprotozoal agent.

All the above compounds have been concerned with the modification or functionalization at the N atom at 2nd position of 5-nitroimidazole.



Metronidazole

(60)



Satranidazole

(61)

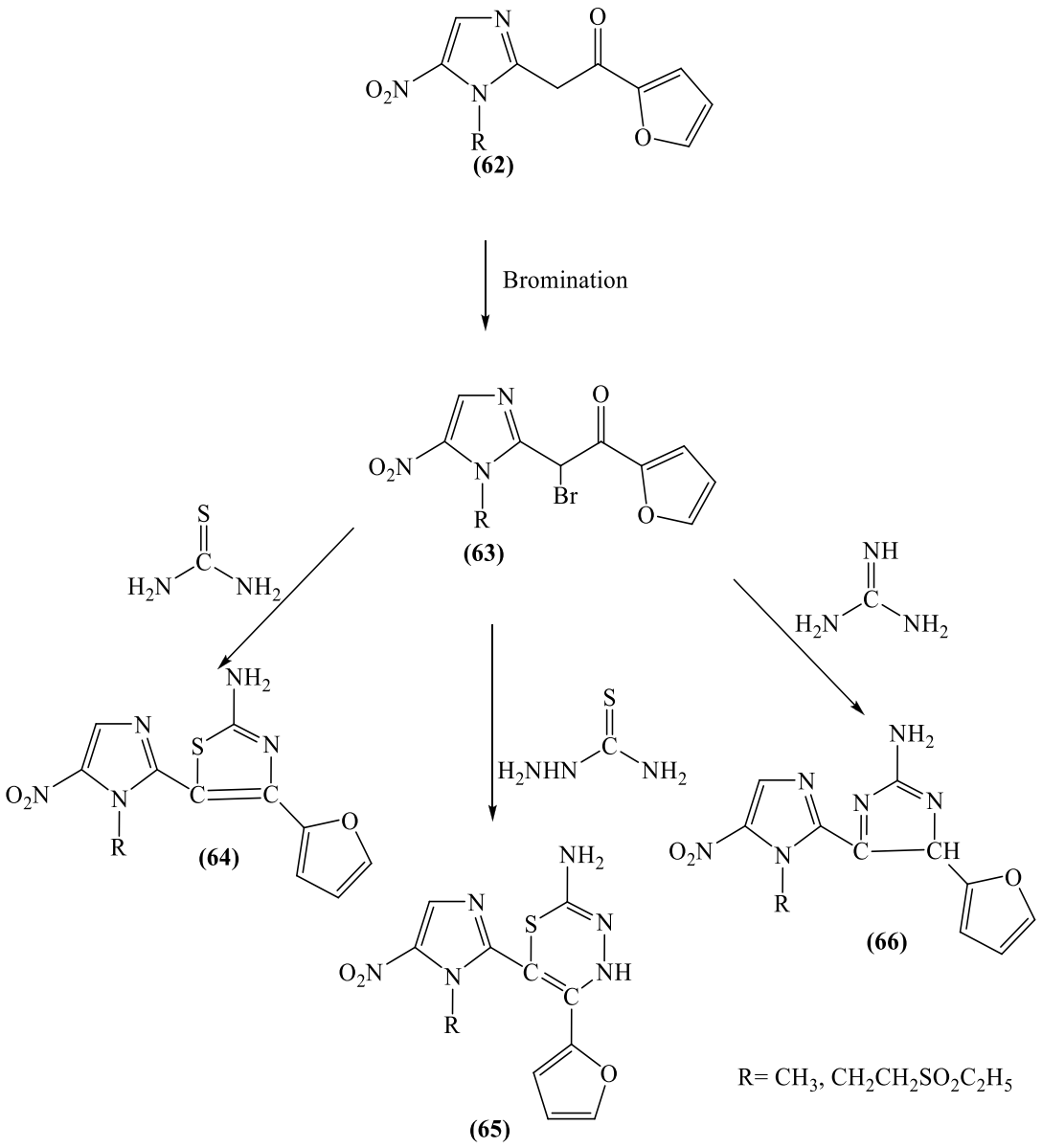
Compound Satranidzole, which has an imidazolidinone ring structure at the 2nd position, exerts stronger antitrichomonal activity than metronidazole. Satranidzole is highly active against anaerobic bacteria, even more than metronidazole but less than niridazole. Thus, the modification of the 5-nitroimidazole at the 2nd position especially cyclization increases not only its antitrichomonal activity but also its antibacterial activity.

It was thought of modify the synthesized 5-nitroimidazole drugs at 2nd position with various heterocyclic rings such as aminothizole, aminothidizine and aminoimidazole.

5.2 Results and Discussion

5.2.1 Synthetic approach

The active methyl group at 2nd position of 1-(furan-2-yl)-2-(1-(substituted)-5-nitro-1*H*-imidazol-2-yl)ethanone **(62)** were brominated yielding 2-bromo-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(63)** which upon condensation reaction with semicarbazide, thiosemicarbazide and guanidine hydrochloride afforded the respective compound **(64, 65, 66)** as shown in scheme V.



Scheme V

5.2.2 Physical and spectral characteristics

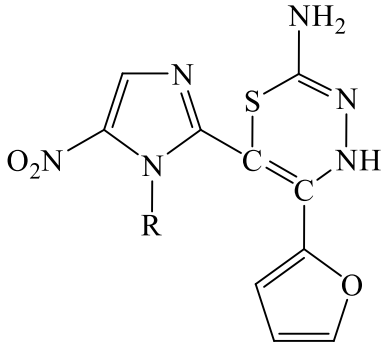
The compounds were pale yellow to orange color and soluble in methanol and partially soluble in ethanol. All the compounds were recrystallized from ethanol-ethylacetate mixture (2:1) and shows meting point in the range of 165-247 ^{0}C.

5.2.2.1 IR spectra (KBr, cm^{-1})

Intense absorption band around 3300-3400 cm^{-1} indicates the presence of the primary amine group. The stretching of methyl group was observed at 3000-2900 cm^{-1}. Other common peaks observed in IR spectra were 1720-1680 cm^{-1}(C=O), 1665 cm^{-1} (C=N), 1570-1540 and 1366-1358 cm^{-1} (NO_2) and C-N of nitro group at 945-800 cm^{-1}.

5.2.2.2 ^{1}H NMR spectra (DMSO-d$_6$, δ ppm)

The ^{1}H NMR (DMSO-d$_6$) spectra of 5-nitroimidazole substituted with various heterocyclic rings , displays the triplet of methyl group (-$SO_2CH_2\underline{CH_3}$) resonates at δ 1.28-1.40 ppm, quartet of methylene group (-$SO_2\underline{CH_2}CH_3$) resonates at δ 3.15 ppm, triplet for (-$CH_2\underline{CH_2}SO_2CH_2CH_3$) resonates at δ 3.66-3.89 ppm, singlet for (-N=C-$\underline{CH_3}$) resonates at δ 4.26-4.30, triplet for (imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$) resonates at δ 4.96 ppm, all the aromatic proton resonates between δ 6.54-7.94 , the singlet for (1H nitroimidazole ring) observed between δ 7.87-8.1 ppm due to the electron withdrawing nitro group. All the cyclized heterocycles shows singlet 7.5 ppm observed due to primary amine group as shown in **(65)**.



(65)

5.2.2.3 Mass spectra (m/z)

The molecular ions (M^+), M+1, M+2 were observed. The fragmentation routes primarily involved losses of OH (M-17), NO (M-30), NO_2 (M-46) and HNO_2 (M-47) from the molecular ion, which are characteristic of compounds.

The possible fragmentation route for nitroimidazole is shown below.

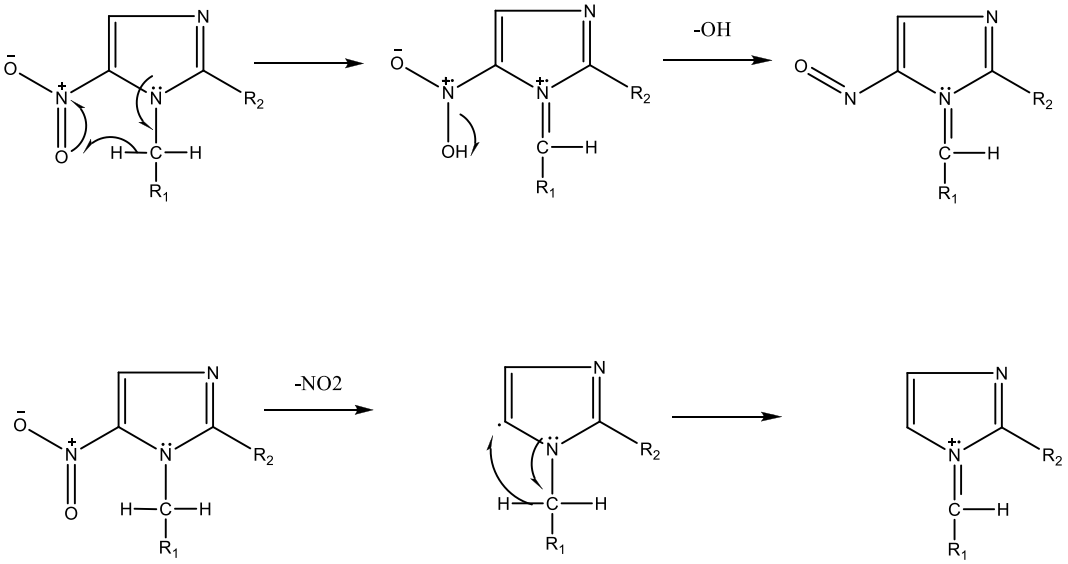
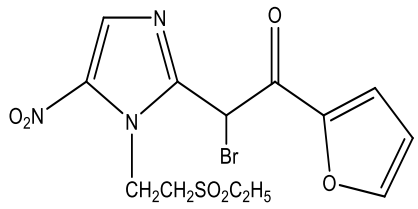
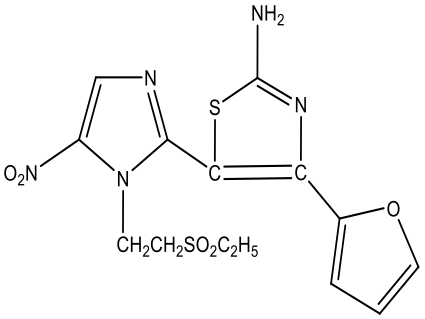
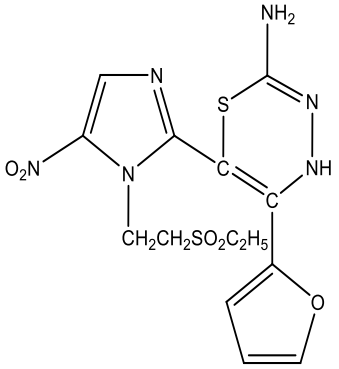
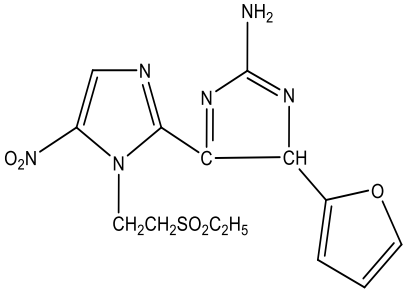
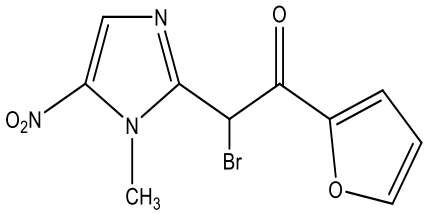
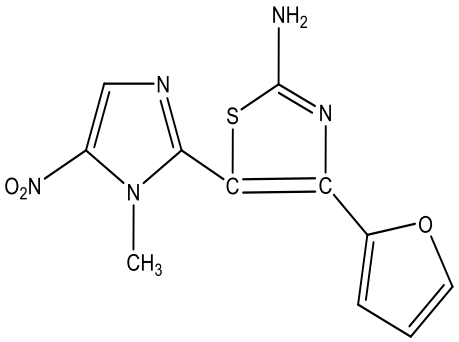
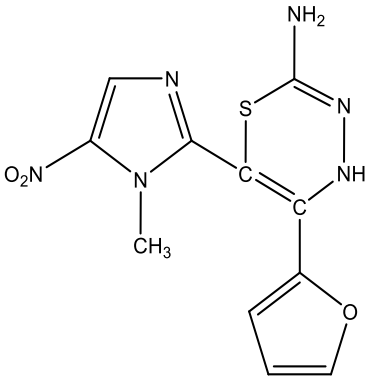
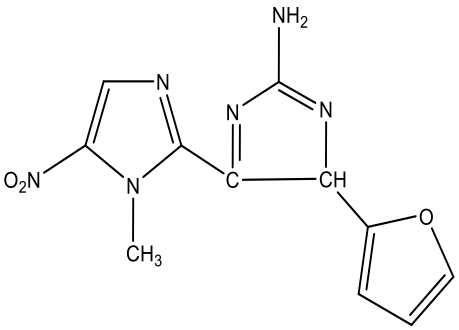

-OH
-NO2

Table 16: Physical characteristics of the 5-nitroimidazole substituted with various heterocyclic rings

Code	Structure	m.p. (^{0}C)	Yield (%)*	Mol. Formula	Mol. weight
67 a		165-167	75	$C_{13}H_{14}BrN_3O_6S$	418.98
67 b		210-212	52	$C_{14}H_{15}N_5O_5S_2$	397.05
67 c		245-247	45	$C_{14}H_{16}N_6O_5S_2$	412.44
67 d		202-205	32	$C_{14}H_{16}N_6O_5S$	380.38

* Solvent of recrystallization; ethanol-ethylacetate (2:1)

Table 17: Physical characteristics of the 5-nitroimidazole substituted with various heterocyclic rings

Code	Structure	m.p. (^{0}C)	Yield (%)*	Mol. formula	Mol. weight
68 a		150-153	72	$C_{10}H_8BrN_3O_4$	312.97
68 b		167-169	55	$C_{11}H_9N_5O_3S$	291.04
68 c		195-197	40	$C_{11}H_{10}N_6O_3S$	306.30
68 d		180-183	32	$C_{11}H_{10}N_6O_3$	274.24

* Solvent of recrystallization; ethanol-ethylacetate (2:1)

Table 18: Spectral characteristics of 5-nitroimidazole substituted with various heterocyclic rings

Code	IR (KBr, Cm^{-1})	^{1}HNMR (DMSO-d_6, δ ppm)	MASS (m/z)
67-a	2854-2800 (CH_2), 1718 (C=O), 1554,1350 (NO_2)	δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.74 (d, 1H, 4-furoyl), δ 7.87 (s, 1H, 5-furoyl), δ 7.90 (s,1H, nitroimidazole ring)	418.28 ($M^{+\cdot}$)
67-b	3395-3320 (NH_2), 2900-2854, (CH_2) 1554,1350 (NO_2)	δ 1.24 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 2.93 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.24 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.6 (s, 3H, N=C-$\underline{CH_3}$), δ 4.66 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.40 (d, 1H, 3-furoyl), δ 6.70 (d, 1H, 4-furoyl), δ 7.28 (s, 1H, 5-furoyl), δ 7.50 (s, 1H, $N\underline{H_2}$, aminothiazole), δ 7.90 (s,1H, nitroimidazole ring)	398.50 ($M^{+\cdot}$)
67-c	3370-3315 (NH_2), 2895 -2854 (CH_2) 1554,1350 (NO_2)	δ 1.26 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 2.95 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.28 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.65 (s, 3H, N=C-$\underline{CH_3}$), δ 4.86 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.42 (d, 1H, 3-furoyl), δ 6.75 (d, 1H, 4-furoyl), δ 7.30 (s, 1H, 5-furoyl), δ 7.48 (s, 1H, $N\underline{H_2}$, aminothiadiazine), δ 7.90 (s,1H, nitroimidazole ring)	412.64 ($M^{+\cdot}$)
67-d	3390-3325 (NH_2), 2980-2854 (CH_2) 1554,1350 (NO_2)	δ 1.28 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 2.90 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.26 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.50 (s, 3H, N=C-$\underline{CH_3}$), δ 4.80 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.40 (d, 1H, 3-furoyl), δ 6.72 (d, 1H, 4-furoyl), δ 7.30 (s, 1H, 5-furoyl), δ 7.50 (s, 1H, $N\underline{H_2}$, aminoimidazole), δ 7.98 (s,1H, nitroimidazole ring)	380.58 ($M^{+\cdot}$)

Table 19: Spectral characteristics of 5-nitroimidazole substituted with various heterocyclic rings

Code	IR (KBr, Cm^{-1})	^{1}HNMR (DMSO-d_6, δ ppm)	MASS (m/z)
68-a	2977-2800 (CH_2), 1718 (C=O), 1541,1345 (NO_2)	δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.86 (s, 3H, imidazole-$\underline{CH_3}$), δ 6.54 (d, 1H, 3-furoyl), δ 6.74 (d, 1H, 4-furoyl), δ 7.59(s, 1H, 5-furoyl), δ 7.97 (s,1H, nitroimidazole ring)	312.97 (M^+)
68-b	3398 -3310(NH_2), 2854 (CH_2) 1554,1350 (NO_2)	δ 4.50 (s, 3H, N=C-$\underline{CH_3}$), δ 4.80 (t, 2H, imidazole-$\underline{CH_2}$CH $_2SO_2CH_2CH_3$), δ 6.40 (d, 1H, 3-furoyl), δ 6.72 (d, 1H, 4-furoyl), δ 7.30 (s, 1H, 5-furoyl), δ 7.50 (s, 1H, $N\underline{H_2}$, aminoimidazole), δ 7.98 (s,1H, nitroimidazole ring)	292.40 (M+1)
68-c	3395 -3323(NH_2), 2854 (CH_2) 1554,1350 (NO_2)	δ 4.63 (s, 3H, N=C-$\underline{CH_3}$), δ 4.66 (t, 2H, imidazole-$\underline{CH_2}$CH $_2SO_2CH_2CH_3$), δ 6.40 (d, 1H, 3-furoyl), δ 6.72 (d, 1H, 4-furoyl), δ 7.32 (s, 1H, 5-furoyl), δ 7.48 (s, 1H, $N\underline{H_2}$, aminothiadiazine), δ 8.10 (s,1H, nitroimidazole ring)	306.30 (M^+)
68-d	3365-3324 (NH_2), 2854 (CH_2) 1554,1350 (NO_2)	δ 4.50 (s, 3H, N=C-$\underline{CH_3}$), δ 4.80 (t, 2H, imidazole-$\underline{CH_2}$CH $_2SO_2CH_2CH_3$), δ 6.40 (d, 1H, 3-furoyl), δ 6.72 (d, 1H, 4-furoyl), δ 7.30 (s, 1H, 5-furoyl), δ7.50 (s, 1H, $N\underline{H_2}$, aminoimidazole), δ 7.98 (s,1H, nitroimidazole ring)	---

5.3 Experimental

Dimetridazole, tinidazole, triethylamine, toluene, acetonitrile, methanol, acid chlorides, semicarbazide, thiosemicarbazide and guanidine hydrochloride of LR grade were procured from Lincon lab Pvt. Ltd. Ahmedabad, SD Fine chemicals and Thomas Baker. It was used after purification. All the melting points were determined in open capillaries and are uncorrected. Thin layer chromatography was performed on microscopic slides (2x7.5cm) coated with silica gel G and spots were visualized by normal TLC and exposure to iodine vapor. IR spectra were recorded in KBr on SHIMADZU Fourier Transform Infrared 8400S spectrophotometer. Mass spectra were recorded on Micromass Q-T, TOF MS $ES^{+}4.73e^{3}$. Nuclear Magnetic Resonance spectra (^{1}H NMR) were recorded in DMSO-d_6 on BRUKER AVANCE II at 400 MHz and the chemical shift are given in parts per million, downfield from Tetra methyl silane (TMS) was used as internal standard.

Synthesis of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone (57-e)

To a mixture of 4.94 g (0.020 mole) of 1-(2-(ethylsulfonyl)ethyl)-2-methyl-5-nitro-1*H*-imidazole **(46)**, 17.5 mL of toluene and 8.09g (0.080mole) of triethylamine was added with occasional cooling 7.17 g (0.055 mole) of furoyl chloride. The mixture was stirred for 18 hr, diluted with 10 mL of ether and chilled. The mixture was filtered and the solid washed with three 10 mL portions ether and four 10 mL portion water to give an intermediate. The whole crude intermediate product was taken in to 10 mL of water, 15 mL of ethanol and 10 mL of concentrated hydrochloric acid and refluxed for 2 hr. The solution was chilled and poured on to the ice. The solid was filtered, washed with water and recrystallized from ethanol to gave 5.12g (75% yield) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(57-e)**, m.p. 165-166 ^{0}C.

Analysis:

TLC	: Toluene: acetonitrile (4:1); Rf value: 0.56
IR (KBr, cm^{-1})	: 3050-2850 (CH_2), 1690(C=O),1550,1360 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.75 (d, 1H, 4-furoyl), δ 7.59(s, 1H, 5-furoyl), δ 8.10 (s,1H, nitroimidazole ring)
Mass (m/z)	: 341.34 (M^+)

Synthesis of 2-bromo-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone (67a)

To a solution of 3.42 g (0.01mole) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(57e)**, in 30 mL dry chloroform was added under stirring a solution of bromine in chloroform over 30 minutes. The reaction mixture was stirred for one hr. The solvent was removed by vacuum and the residual, were filtered and recrystallized from ethanol-ethyl acetate mixture (2:1) gave 3.14 g (75% yield), orange crystal of 2-bromo-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(67a),** m.p. 165-167 ^{0}C.

Analysis:

TLC	: Toluene: methanol (4:1); Rf value: 0.44
IR (KBr, cm^{-1})	: 2854-2800 (CH_2), 1718 (C=O), 1554,1350 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.74 (d, 1H, 4-furoyl), δ 7.87 (s, 1H, 5-furoyl), δ 7.90 (s,1H, nitroimidazole ring)
Mass (m/z)	: 418.28 (M^+)

Synthesis of 5-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-4-(furan-2-yl)thiazol-2-amine (67 b)

A mixture of 4.18 g (0.01mole) of 2-bromo-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-*1H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(67a)** , 0.76 g (0.01 mole) of semicarbazide in 25 mL ethanol was refluxed for one hr and allowed to stand at room temperature for 24 hr. The solid was filtered and recrystallized from ethanol-ethylacetate mixture (2:1) gave 2.05 g (52% yield), pale yellow crystal of 5-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-4-(furan-2-yl)thiazol-2-amine **(67 b),** m.p.210-212 ^{0}C.

Analysis:

TLC	: Toluene: methanol (4:1); Rf value: 0.35
IR (KBr, cm^{-1})	: 3395-3320 (NH_2), 2900-2854, (CH_2) 1554,1350 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.24 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 2.93 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.24 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.6 (s, 3H, N=C-$\underline{CH_3}$), δ 4.66 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.40 (d, 1H, 3-furoyl), δ 6.70 (d, 1H, 4-furoyl), δ 7.28 (s, 1H, 5-furoyl), δ 7.50 (s, 1H, $N\underline{H_2}$, aminothiazole), δ 7.90 (s,1H, nitroimidazole ring)
Mass (m/z)	: 398.50 (M+)

Synthesis of 6-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-5-(furan-2-yl)-4*H*-1,3,4-thiadiazin-2-amine (67 c)

A mixture of 4.18 g (0.01mole) of 2-bromo-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(67a)** , 0.91 g (0.01 mole) of thiosemicarbazide in 25 mL ethanol was refluxed for one hr and allowed to stand at room temperature for 24 hr. The solid was filtered and recrystallized from ethanol-ethylacetate mixture (2:1) 1.86 g (45% yield) pale yellow crystal of 6-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1H-imidazol-2-yl)-5-(furan-2-yl)-4*H*-1,3,4-thiadiazin-2-amine **(67c),** m.p.245-247 ^{0}C.

Analysis:

TLC	: Toluene: methanol (4:1); Rf value: 0.32
IR (KBr, cm^{-1})	: 3370-3315 (NH_2), 2895-2854 (CH_2) 1554,1350 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.26 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 2.95 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.28 (t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.65 (s, 3H, N=C-$\underline{CH_3}$), δ 4.86 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.42 (d, 1H, 3-furoyl), δ 6.75 (d, 1H, 4-furoyl), δ 7.30 (s, 1H, 5-furoyl), δ 7.48 (s, 1H, $N\underline{H_2}$, aminothiadiazine), δ 7.90 (s,1H, nitroimidazole ring)
Mass (m/z)	:412.64 (M^+)

Synthesis of 5-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-4-(furan-2-yl)-4*H*-imidazol-2-amine (67d)

A mixture of 4.18 g (0.01mole) of 2-bromo-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(67a)**, 0.95g (0.01 mole) of guanidine hydrochloride in 25 mL ethanol was refluxed for 1 hr and allowed to stand at room temperature for 24 hr. The solid was filtered and recrystallized from ethanol-ethylacetate mixture (2:1) gave 1.20 g (32% yield) pale yellow crystal of 5-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-4-(furan-2-yl)-4*H*-imidazol-2-amine **(67d),** m.p.202-205 ^{0}C.

Analysis:

TLC	: Toluene: methanol (4:1); Rf value: 0.40

IR (KBr, cm^{-1})	: 3390-3325 (NH_2), 2980-2854 (CH_2) 1554,1350 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.28 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 2.90 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.26 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.50 (s, 3H, N=C-$\underline{CH_3}$), δ 4.80 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.40 (d, 1H, 3-furoyl), δ 6.72 (d, 1H, 4-furoyl), δ 7.30 (s, 1H, 5-furoyl), δ 7.50 (s, 1H, $N\underline{H_2}$, aminoimidazole), δ 7.98 (s,1H, nitroimidazole ring)
Mass (m/z)	:380.58 (M^+)

Synthesis of 1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)ethanone (57 f)

To a mixture of 2.82 g (0.020 mole) of 1,2-dimethyl-5-nitro-1*H*-imidazole **(57 f)**, 17.5 mL, of toluene, and 8.09g (0.080mole) of triethylamine was added with occasional cooling, 9.62 g (0.055 mole) of furoyl chloride. The mixture was stirred for 18 hr, diluted with 10 mL of ether and chilled. The mixture was filtered and the solid washed with three 10 mL portions ether and four 100 mL portion water to give an intermediate. The whole crude intermediate product was taken in to 10 mL of water, 15 mL of ethanol and 10 mL of concentrated hydrochloric acid and refluxed for 2-4 hours. The solution was chilled and poured on to the ice. The solid was filtered, washed with water and recrystallized from ethanol to gave 3.67g (78% yield) of 1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)ethanone **(57-f)**,m.p. 148-149 ^{0}C.

Analysis:

TLC	: Toluene: acetonitrile (4:1); Rf value: 0.50
IR (KBr, cm^{-1})	: 2980-2850 (CH_2), 1710(C=O),1555,1362 (NO_2)
Mass (m/z)	: 235.20 (M^+)

Synthesis of 2-bromo-1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)ethanone (68 a)

To a solution of 2.35g (0.01mole) of 1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)ethanone **(57 f)**, in 30 mL dry chloroform was added under stirring a solution of bromine in chloroform over 30 minutes. The reaction mixture was stirred for 1 hr. The solvent was removed by vacuum and the residual were filtered and recrystallized from

ethanol-ethyl acetate mixture gave 2.24g (72% yield) orange crystal of 2-bromo-1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)ethanone **(68 a),** m.p.150-153 ^{0}C.

Analysis:

TLC	: Toluene: methanol (4:1); Rf value: 0.49
IR (KBr, cm^{-1})	: 2977-2800 (CH_2), 1718 (C=O),1541,1345 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.86 (s, 3H, imidazole-$\underline{CH_3}$), δ 6.54 (d, 1H, 3-furoyl), δ 6.74 (d, 1H, 4-furoyl), δ 7.59(s, 1H, 5-furoyl), δ 7.97 (s,1H, nitroimidazole ring)
Mass (m/z)	:312.97 (M^+)

Synthesis of 4-(furan-2-yl)-5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)thiazol-2-amine (68 b)

A mixture of 3.13g (0.01mole) of 2-bromo-1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)ethanone **(68 a)**, 0.76 g (0.01 mole) of semicarbazide in 25 mL ethanol was refluxed for one hr and allowed to stand at room temperature for 24 hr. The solid was filtered and recrystallized from ethanol gave 1.60 g (55% yield) of pale yellow crystal of 4-(furan-2-yl)-5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)thiazol-2-amine **(68 b),** m.p.167-169 ^{0}C.

Analysis:

TLC	: Toluene: methanol (4:1); Rf value: 0.45
IR (KBr, cm^{-1})	: 3398 -3310(NH_2), 2854 (CH_2) 1554,1350 (NO_2)
NMR (DMSO-d_6, δ ppm)	: δ 4.50 (s, 3H, N=C-$\underline{CH_3}$), δ 4.80 (t, 2H, imidazole-$\underline{CH_2}$CH $_2$$SO_2CH_2CH_3$), δ 6.40 (d, 1H, 3-furoyl), δ 6.72 (d, 1H, 4-furoyl), δ 7.30 (s, 1H, 5-furoyl), δ 7.50 (s, 1H, $N\underline{H_2}$, aminothiazole), δ 7.98 (s,1H, nitroimidazole ring)
Mass (m/z)	:292.40 (M+1)

Synthesis of 5-(furan-2-yl)-6-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-4*H*-1,3,4-thiadiazin-2-amine (68 c)

A mixture of 3.13 g (0.01mole) of 2-bromo-1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)ethanone **(68 a)**, 0.91 g (0.01 mole) of thiosemicarbazide in 25 mL

ethanol was refluxed for 1 hr and allowed to stand at room temperature for 24 hr. The solid was filtered and recrystallized from ethanol gave 1.23g (40% yield) pale yellow crystal of 5-(furan-2-yl)-6-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-4*H*-1,3,4-thiadiazin-2-amine **(68 c),** m.p.195-197 ^{0}C.

Analysis:

TLC	: Toluene: methanol (4:1); Rf value: 0.40
IR (KBr, cm^{-1})	: 3395-3323 (NH_2), 2854 (CH_2) 1554,1350 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 4.63 (s, 3H, N=C-$\underline{CH_3}$), δ 4.66 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.40 (d, 1H, 3-furoyl), δ 6.72 (d, 1H, 4-furoyl), δ 7.32 (s, 1H, 5-furoyl), δ 7.48 (s, 1H, $N\underline{H_2}$, aminothiadiazine), δ 8.10 (s,1H, nitroimidazole ring)
Mass (m/z)	:306.30 (M^+)

Synthesis of 4-(furan-2-yl)-5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-4*H*-imidazol-2-amine (68 d)

A mixture of 2.35 g (0.01mole) of 2-bromo-1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)ethanone **(68 a)**, 0.95 g (0.01 mole) of guanidine hydrochloride in 25 mL ethanol was refluxed for 1 hr and allowed to stand at room temperature for 24 hr. The compounds was filtered and recrystallized from ethanol gave 0.88 g (32% yield) pale yellow crystal of 4-(furan-2-yl)-5-(1-methyl-5-nitro-1*H*-imidazol-2-yl)-4*H*-imidazol-2-amine **(68 d),** m.p.180-183 ^{0}C.

Analysis:

TLC	: Toluene: methanol (4:1); Rf value: 0.42
IR (KBr, cm^{-1})	: 3365-3324 (NH_2), 2854 (CH_2) 1554,1350 (NO_2)
NMR (DMSO-d_6, δ ppm)	: δ 4.50 (s, 3H, N=C-$\underline{CH_3}$), δ 4.80 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.40 (d, 1H, 3-furoyl), δ 6.72 (d, 1H, 4-furoyl), δ 7.30 (s, 1H, 5-furoyl), δ 7.50 (s, 1H, $N\underline{H_2}$, aminoimidazole), δ 7.98 (s,1H, nitroimidazole ring)

5.4 References

Cosar C, Crisan C, Horclois R, Jacob R M, Robert J, Tchelitcheffs S, Vaupre R. Nitroimidazoles. Arzneium-Forsch 1966;16:23-25.

Cosar C, Juolu J, Benazer M. Therapeutic efficacy of new nitroimidazoles. Ann Inst Pastuer Paris 1959; 96:238-241.

Jarumilinta R, Activity of Ciba 32644-Ba in amoebic liver abscess in man. Acta Trop Suppl. 1966;9:102-109 .

Lambert CR, Wilhelm M, Striebel H, Kradolfer F, Schmidt P. Eine neue gegen Bilharziose und Amoebiase wirksame Verbindung . Experientia 1964;20 :1964, 452-456.

Powell SJ, MacLeod I, Wilmot AJ, Eldsdon-Dew R. Metronidazole in amoebic dysentery and liver abscess. Lancet 1966; 2:1329-1332.

Wilhelm M, Marquardt FH, Meier Kd, Schmidt P, Helv. The synthesis of the first examples of Class H mesoionic xanthine acyclonucleosides is described. Chim Acta 1966;49:2443-2445.

6.1 Introduction

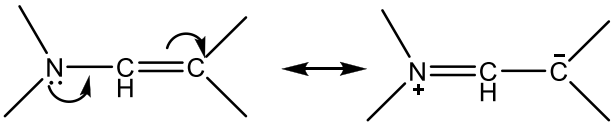
The class of compounds designated as enamines, although known for many years, has only recently gained recognition by organic chemists as an extremely important group of intermediates in organic syntheses. During the last decade, an increasing number of investigators have reported new ways by which enamines may be formed and in which they react. In the past few years, especially, a prodigious number of articles have appeared describing enamines and their advantageous use as intermediates and blocking groups in organic syntheses.

6.1.1 Chemistry of enamine

6.1.1.1 The nature of enamine

The name enamine usually refers to a, α β-unsaturated tertiary amines, >C=C-N<. Although this is the customary usage, the name enamine really implies any C=C-N linkage. In this sense the name could be applied to the pyrrole family, the pyridine family and even aniline and its derivatives, all of which are certainly "ene-amines." Except for some indoles and pyrroles, however, such compounds do not in general display the typical properties of "enamines," probably as a result of the increased resonance in these fully unsaturated systems. It is concerned with α β-unsaturated tertiary amines, the "enamines" that have evoked so much interest in the past few years.

It should be at once apparent that enamines may be represented by two important resonance forms, the second of which is derived from the first by the conjugation of the unshared pair of electrons of the nitrogen atom with the double bond.

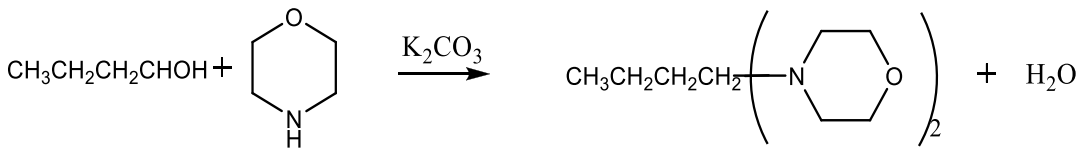


(69)

6.1.1.2 Preparations of enamine

The classical preparation of simple enamines (Mannich *et al*., 1936) involves the mixing of an aldehyde and a secondary amine, in the presence of some dehydrating material which removes the water eliminated in the condensation of the amine and the aldehyde, to give a diamine with both amine residues on the same carbon atom. When the aldehyde has at least one hydrogen on the carbon atom adjacent to the carbonyl

group, these diamines cannot, in general, be distilled without decomposition: they split off one mole of amine and yield an unsaturated amine. The formation of 1-morpholino-1-butene from butyraldehyde and morpholine is an example.



(70)

In this reaction, secondary cyclic amines give better results than dialkyl amines which are better in turn than aryl alkyl amines. Also, best yields are obtained when an excess of amine is avoided. Most simple aldehydes give enamines in 50-90% yield or unsaturated aldehydes also react with secondary amines to give ene-diamines (Mannich *et al*., 1936) which decompose upon heating or exposing to air.

At present, the most common method of preparing enamines (Herr *et al*., 1952) involves heating the ketone or aldehyde with a small excess of amine in benzene at reflux. A moisture trap is used to collect the water of reaction which forms an azeotrope with the benzene. Under these conditions, there is no evidence for the formation of the diamino compound and the reaction appears to a direct condensation of one mole of the carbonyl compound and one mole of amine with elimination of water.

Some amines show a preference for a particular type of carbonyl function for example, piperidine forms enamines preferentially with steroidal aldehydes in the presence of steroidal ketone groups, whereas pyrrolidine is selective to carbonyl in steroids in the presence of C_{I7} carbonyl groups (Spero *et al*., 1956).

Thus formation of the pyrrolidine enamine could be used to protect a C3 keto group in steroidal syntheses and the formation of a piperidine enamine of a steroidal aldehyde group could be used to protect it, provided the original carbonyl functions could be regenerated from the respective enamines. The stability of acyclic enamines is not great; and upon treatment with aqueous acid and heat they readily decompose into the original carbonyl compound and amine (Mannichc *et al*., 1936 and Herr *et al*., 1953).

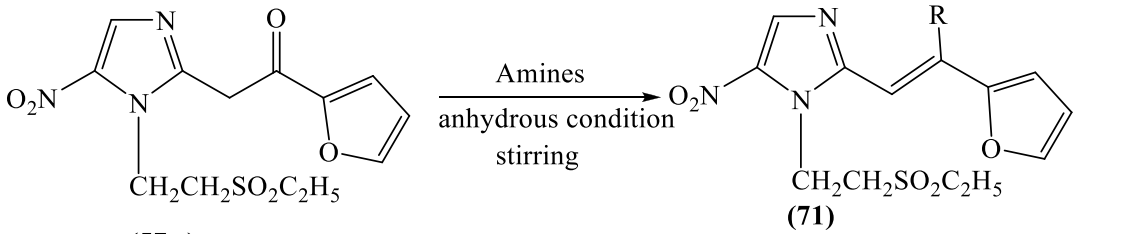
Treatment of enamines with hydrogen sulfide instead of aqueous acid gives gem-dimercaptans which can be reduced to mercaptans with lithium aluminum hydride. This sequence has been used as the preferred method of synthesis of 4-*t*-butylcyclohexylmercaptan (mixture of stereoisomer) from 4-tbutylcyclohexanone.

6.2 Results and discussion

6.2.1 Synthetic approach

Synthesis of 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinylsubstitutedamine

The active carbonyl group at 2nd-postion of 1-(furan-2-yl)-2-(1-(substituted)-5-nitro-1*H*-imidazol-2-yl)ethanone **(57e)** were reacted with various amines in anhydrous condition gave 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinylsubstitutedamine **(71)**



(57 e)

(71)

R= morpholine, pyrrolidine, piperizine, piperidine,methylpiperizine, dimethyl amine, diethyl amine, aniline, isonidzide, norfloxacin

6.2.2 Physical and spectral characteristics

The 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinylsub stitutedamine were having orange color and are soluble in dichloromethane, chloroform and partially soluble in methanol and ethanol. All the compounds were recrystallized from chloroform-methanol mixture (3:1) and melting point was found in the range of 120-250 ^{0}C.

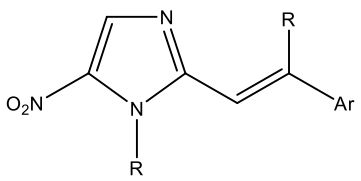
6.2.2.1 IR spectra (KBr, cm^{-1})

The stretching of methyl group was observed at 3000-2900 cm^{-1}. Other common peaks observed in IR spectra were 1665 cm^{-1} (C=N), 1570-1540 and 1366-1358 cm^{-1} (NO_2) and C-N of nitro group at 945-800 cm^{-1}.

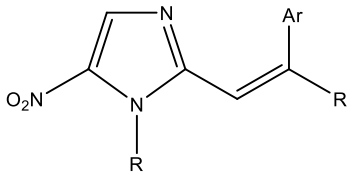
6.2.2.2 ^{1}HNMR spectra (DMSO-d6, δ ppm)

The ^{1}H NMR (DMSO-d_6) spectra of1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinylsubstitutedamine, displays the triplet of methyl group (-$SO_2CH_2\underline{CH_3}$) resonates at δ 1.28-1.40 ppm, quartet of methylene group (-$SO_2\underline{CH_2}CH_3$) resonates at δ 3.15 ppm, triplet for (-$CH_2\underline{CH_2}SO_2CH_2CH_3$) resonates at δ 3.66-3.89 ppm, singlet for (-N=C-$\underline{CH_3}$) resonates at δ 4.26-4.30, triplet for (imidazole-$\underline{CH_2}CH\ _2SO_2CH_2CH_3$) resonates at δ 4.96 ppm, all the aromatic proton resonates between δ 6.54-7.94, the singlet for (1H nitroimidazole ring) observed between δ 7.87-8.1 ppm due to the electron withdrawing nitro group. The carboxylic peak is observed downfield at δ 9.0-10 ppm.

The ^{1}HNMR spectra 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl(substituted amine) should show of double peaks geometric isomers *(E)* and *(Z)*of the enamine.



(72)



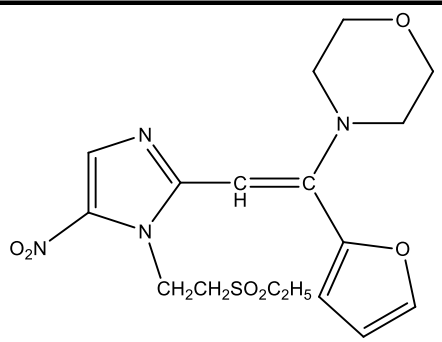
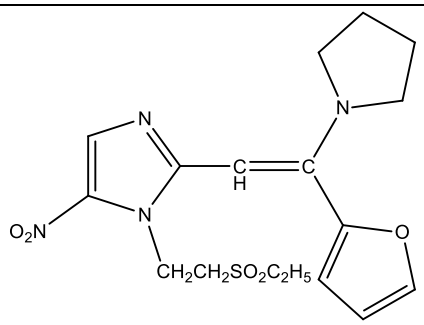
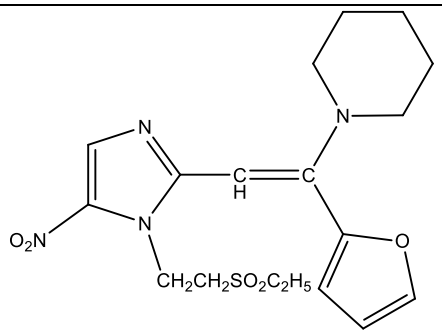
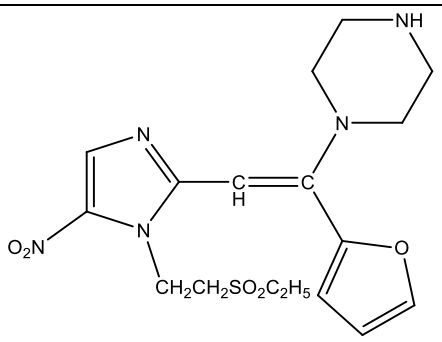
(73)

The peak observed in actual spectra was having single molecules suggesting that out of two isomer one isomer exist during the synthesis.

6.2.2.3 Mass spectra (m/z)

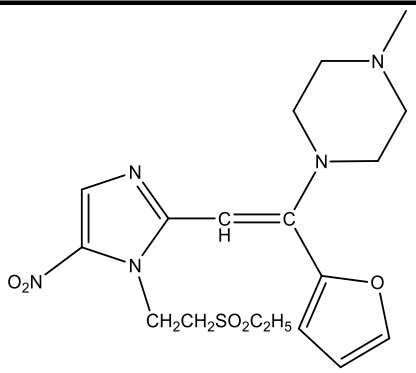
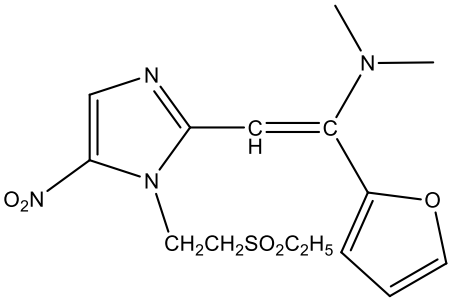
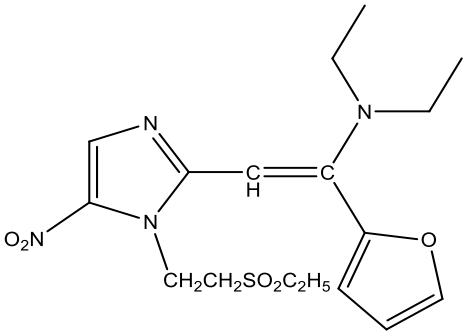
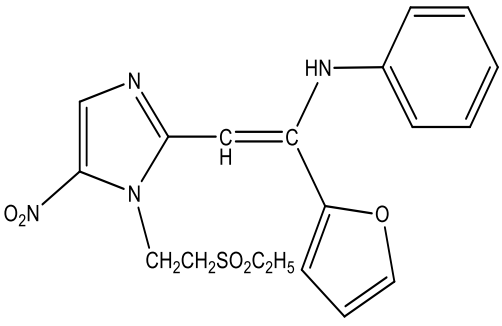
The molecular ions, M+1, M+2 were observed. The fragmentation routes primarily involved losses of OH (M-17), NO (M-30), NO_2 (M-46) and HNO_2 (M-47) from the molecular ion, which are characteristic of compounds.

Table 20: Physical characteristics of 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinylsubstitutedamine

Code	Structure	m.p. (^{0}C)	% Yield	Mol. formula	Mol. weight
74 a		120-122	75	$C_{15}H_{22}N_3O_6S$	410.13
74 b		136-138	72	$C_{17}H_{22}N_4O_5S$	394.13
74 c		130-134	70	$C_{18}H_{24}N_4O_5S$	408.15
74 d		125-127	78	$C_{17}H_{23}N_5O_5S$	409.14

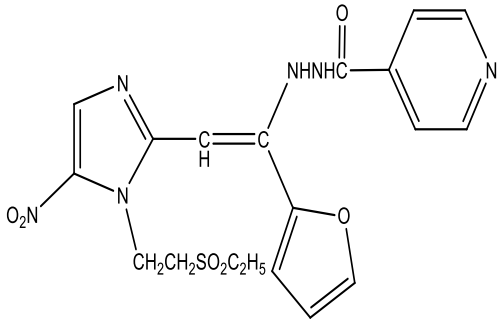
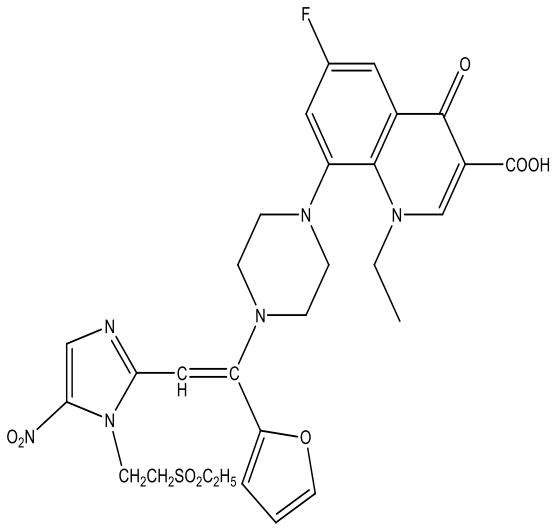
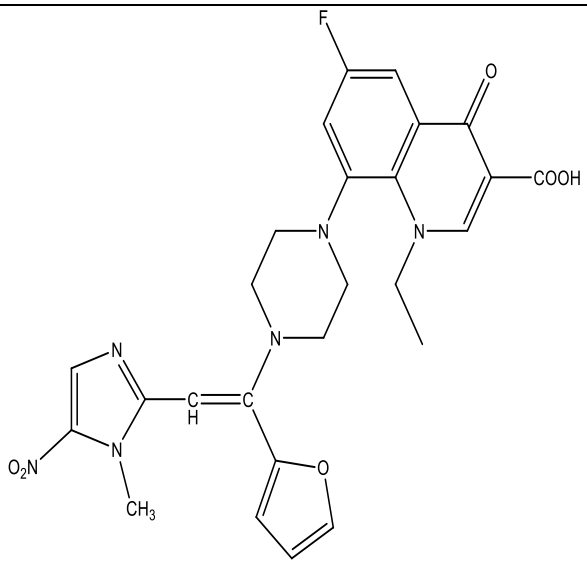
* Solvent of recrystallization; chloroform: methanol (3:1)

Table 21: Physical characteristics of 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinylsubstitutedamine

Code	Structure	m.p. (^{0}C)	% Yield	Mol. formula	Mol. weight
74 e		126-129	78	$C_{18}H_{25}N_5O_5S$	423.16
74 f		140-142	72	$C_{15}H_{20}N_4O_5S$	368.12
74 g		144-146	65	$C_{17}H_{24}N_4O_5S$	396.15
74 h		129-131	67	$C_{19}H_{20}N_4O_5S$	416.12

* Solvent of recrystallization; chloroform: methanol (3:1)

Table 22: Physical characteristics of 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinylsubstitutedamine

Code	Structure	m.p. (^{0}C)	% Yield	Mol. formula	Mol. weight
74 i		134-136	62	$C_{19}H_{20}N_6O_6S$	460.12
74j		250-252	52	$C_{29}H_{31}FN_6O_8S$	642.19
74k		240-242	50	$C_{26}H_{25}FN_6O_6$	536.18

* Solvent of recrystallization; chloroform: methanol (3:1)

Table 23:Spectral characteristics of 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinylsubstitutedamine

Code	IR (KBr,Cm^{-1})	^{1} NMR (DMSO-d_6, δ ppm)	MASS (m/z)
74-a	2958-2825(CH_2) 1540,1364 (NO_2)	---	410.13 (M^+)
74-b	2977-2800(CH_2) 1560,1368(NO_2)	δ 1.43 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 1.48 (t, 2H, -pyrrolidine) δ 3.13 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.49 (t, 2H, pyrrolidine) δ 3.58 (t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.6 (s, 3H, N=C-$\underline{CH_3}$), δ 4.85 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.64 (m, 1H, 4-furoyl), δ 6.97(s, 1H, 5-furoyl), δ 8.07 (s,1H, nitroimidazole ring)	394.13 (M^+)
74-c	2977-2800(CH_2) 1540,1364 (NO_2)	δ 1.43 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 1.48 (t, 2H, piperidine) δ 3.13 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.49 (t, 2H, piperidine) δ 3.58 (t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.6 (s, 3H, N=C- $\underline{CH_3}$), δ 4.85 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.64 (m, 1H, 4-furoyl), δ 6.97(s, 1H, 5-furoyl), δ 8.07 (s,1H, nitroimidazole ring)	…
74-d	2977-2800(CH_2) 1550,1364 (NO_2)	δ 1.48 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.13 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.51 (t, 2H, piperazine) δ 3.58 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.60 (s, 3H, N=C-$\underline{CH_3}$), δ 4.85 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.55 (d, 1H, 3-furoyl), δ 6.64 (d, 1H, 4-furoyl), δ 6.97 (s, 1H, 5-furoyl), δ 8.09 (s,1H, nitroimidazole ring)	…
74-e	2947-2810(CH_2) 1550,1364 (NO_2)	---	423.16 (M^+)
74-f	2977-2800(CH_2) 1550,1364 (NO_2)	δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.20 (s, 3H, -N-dimethyl), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.754 (d, 1H, 4-furoyl), δ 7.59(s, 1H, 5-furoyl), δ 7.87 (s,1H, nitroimidazole ring)	368.12 (M^+)

Table 24:Spectral characteristics of 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinylsubstitutedamine

Code	IR (KBr,Cm^{-1})	^{1} NMR (DMSO-d_6, δ ppm)	MASS (m/z)
74-g	2977-2800 (CH_2), 1550,1364 (NO_2)	…	396.15 (M^+)
74-h	3200 (–NH), 2977-2800 (CH_2), 1550,1364 (NO_2)	δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.04 (s, 1H, -anilino),δ 6.54 (d, 1H, 3-furoyl), δ 6.64 (d, 1H, 4-furoyl), δ 6.97(s, 1H, 5-furoyl), δ 7.37-7.69 (multiplet of aromatic ring) δ 8.05(s,1H, nitroimidazole ring)	…
74-i	3300-3100 (NH_2 and -NH), 2900-2800 (CH_3), 1700 (C=O), 1550, 1360 (NO_2)	δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.754 (d, 1H, 4-furoyl), δ 7.59(s, 1H, 5-furoyl), δ 7.87 (s,1H, nitroimidazole ring)	460.12 (M^+)
74-j	3600 -3200 (broad peak of COOH), 1720 (C=O), 1550,1362 (NO_2)	δ 1.48 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 2.48 (q, 2H, piperazine) δ 3.30 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, - $CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 3.90 (s, 3H, N=C-$\underline{CH_3}$), δ 4.40 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.51 (m, 1H, 3-furoyl), δ 6.98 (d, 1H, 4-furoyl), δ 7.07 (d, 1H, 5-furoyl), δ 7.58 (s, 1H, quinolone), δ 7.93 (s,1H, F-quinolone), δ 7.96 (s,1H, nitroimidazole ring), δ 11.25 (s,1H, COO$\underline{H}$ quinolone)	642.19 (M^+)
74 k	3600-3100 (broad peak of COOH), 1720 (C=O), 1550,1362 (NO_2)	δ 2.48 (q, 2H, piperazine), δ 3.90 (s, 3H, N=C-$\underline{CH_3}$), δ 4.40 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.51 (m, 1H, 3-furoyl), δ 6.98 (d, 1H, 4-furoyl), δ 7.07 (d, 1H, 5-furoyl), δ 7.58 (s, 1H, quinolone), δ 7.93 (s,1H, F-quinolone), δ 7.96 (s,1H, nitroimidazole ring), δ 11.05 (s,1H, COO$\underline{H}$ quinolone)	…

6.3 Experimental

Tinidazole, triethylamine, toluene, acetonitrile, methanol, various amines, Isoniazide, norfloxacin and acid chlorides of were obtained from Lincon laboratory Pvt. Ltd. Ahmedabad, SD Fine chemical and Thomas Baker. It was used after purification. All the melting points were determined in open capillaries and are uncorrected. Thin layer chromatography was performed on microscopic slides (2x7.5 cm) coated with silica gel G and spots were visualized by normal TLC and exposure to iodine vapor. IR spectra were recorded in KBr on SHIMADZU Fourier Transform Infrared 8400S spectrophotometer. Mass spectra were recorded on Micromass Q-T, TOF MS $ES^{+}4.73e^{3}$. Nuclear Magnetic Resonance spectra (^{1}H NMR) were recorded in DMSO-d_6 on BRUKER AVANCE II at 400 MHz and the chemical shift are given in parts per million, downfield from Tetra methyl silane (TMS) was used as internal standard.

Synthesis of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone (57-e)

To a mixture of 4.94 g (0.020 mole) of 1-(2-(ethylsulfonyl)ethyl)-2-methyl-5-nitro-1*H*-imidazole **(46)**, 17.5 mL of toluene and 8.09g (0.080mole) of triethylamine was added with occasional cooling 7.17 g (0.055 mole) of furoyl chloride. The mixture was stirred for 18 hr, diluted with 10 mL of ether and chilled. The mixture was filtered and the solid washed with three 10 mL portions ether and four 10 mL portion water to give an intermediate. The whole crude intermediate product was taken in to 10 mL of water, 15 mL of ethanol and 10 mL of concentrated hydrochloric acid and refluxed for 2 hr. The solution was chilled and poured on to the ice. The solid was filtered, washed with water and recrystallized from ethanol gave 5.12g (75% yield) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(57-e)**, m.p. 165-166 ^{0}C.

Analysis:

TLC	: Toluene: acetonitrile (4:1); Rf value: 0.56
IR (KBr, cm^{-1})	: 3050-2850 (CH_2), 1690 (C=O),1550,1360 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.75 (d, 1H, 4-furoyl), δ 7.59(s, 1H, 5-furoyl), δ 8.10 (s,1H, nitroimidazole ring)
Mass (m/z)	: 341.34 (M^+)

Synthesis of 4-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)morpholine (74 a)

To a mixture of 3.42 g (0.01 mole) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(57 e)**, 12.0 mL, of chloroform and 2.00g of dry sodium sulfate was added drop wise 0.87 g (0.01 mole) of morpholine under stirring at room temperature. The mixture was stirred for 1 hr; the solvent was removed under vacuum. The residue was recrystallized form chloroform-methanol mixture to gave 3.08g (75% yield), orange crystal of 4-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)morpholine **(74 a)**, m.p. 120-122 ^{0}C.

Analysis:

TLC : Toluene: acetonitrile (4:1); Rf value: 0.64

IR (KBr, cm^{-1}) : 2958-2825(CH_2), 1540, 1364 (NO_2)

Mass(m/z) : 410.13 (M^+)

Synthesis of 1-(2-(ethylsulfonyl)ethyl)-2-(2-(furan-2-yl)-2-(pyrrolidin-1-yl)vinyl)-5-nitro-1*H*-imidazole (74 b)

To a mixture of 3.42 g (0.01 mole) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(57 e)**, 12.0 mL, of chloroform and 2.00g of dry sodium sulfate was added drop wise 0.71 g (0.01 mole) of pyrrolidine under stirring at room temperature. The mixture was stirred for 1 hr; the solvent was removed under vacuum. The residue was recrystallized from chloroform-methanol mixture (3:1) gave 2.83g (72% yield), orange crystal of 1-(2-(ethylsulfonyl)ethyl)-2-(2-(furan-2-yl)-2-(pyrrolidin-1-yl)vinyl)-5-nitro-1*H*-imidazole **(74 b)**, m.p. 136-138 ^{0}C.

Analysis:

TLC : Toluene: acetonitrile (4:1); Rf value: 0.60

IR (KBr, cm^{-1}) : 2977-2800(CH_2), 1560,1368 (NO_2)

NMR (DMSO-d_6, δ ppm) :δ 1.43 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 1.48 (t, 2H, -pyrrolidine) δ 3.13 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.49 (t, 2H, pyrrolidine) δ 3.58 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.6 (s, 3H, N=C-$\underline{CH_3}$), δ 4.85 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.64 (m, 1H, 4-furoyl), δ 6.97(s, 1H, 5-furoyl), δ 8.07 (s,1H, nitroimidazole ring)

Mass (m/z) : 394.13 (M^+)

Synthesis of 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)piperidine (74 c)

To a mixture of 3.42 g (0.01 mole) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(57 e)**, 12.0 mL, of chloroform and 2.00g of dry sodium sulfate was added drop wise 0.85 g (0.01 mole) of pepridine under stirring at room temperature. The mixture was stirred for 1.5 hr; the solvent was

removed under vacuum. The residue was recrystallized form chloroform-methanol mixture (3:1) gave 2.86g (70% yield), orange crystal of 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)piperidine **(74c)**, m.p. 130-134 ^{0}C.

Analysis:

TLC	: Toluene: acetonitrile (4:1); Rf value: 0.64
IR (KBr, cm^{-1})	: 2977-2800 (CH_2), 1540,1364 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.43 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 1.48 (t, 2H, piperidine) δ 3.13 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.49 (t, 2H, piperidine) δ 3.58 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.6 (s, 3H, N=C-$\underline{CH_3}$), δ 4.85 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.64 (m, 1H, 4-furoyl), δ 6.97 (s, 1H, 5-furoyl), δ 8.07 (s,1H, nitroimidazole ring)

Synthesis of 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)piperazine (74 d)

To a mixture of 3.42 g (0.01 mole) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(57 e)**, 12.0 mL, of chloroform and 2.00g of dry sodium sulfate was added 0.86 g (0.01 mole) of piperazine under stirring at room temperature. The mixture was stirred for 1 hr, the solvent was removed under vacuum. The residue was recrystallized form chloroform-methanol mixture (3:1) gave 3.19g (78% yield), orange crystal of 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)piperazine **(74 d)**, m.p. 125-127 ^{0}C.

Analysis:

TLC	: Toluene: acetonitrile (4:1); Rf value: 0.64
IR (KBr, cm^{-1})	:2977-2800 (CH_2), 1550,1364 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.48 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.13 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.51 (t, 2H, piperazine) δ 3.58 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.60 (s, 3H, N=C-$\underline{CH_3}$), δ 4.85 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.55 (d, 1H, 3-furoyl), δ 6.64 (d, 1H, 4-furoyl), δ 6.97 (s, 1H, 5-furoyl), δ 8.09 (s,1H, nitroimidazole ring)

Synthesis of 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)-4-methylpiperazine (74 e)

To a mixture of 3.42 g (0.01 mole) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(57 e)**, 12.0 mL, of chloroform and 2.00g of dry sodium sulfate was added 0.99 g (0.01 mole) of *N*-methylpiperazine under stirring at room temperature. The mixture was stirred for 1 hr; the solvent was removed under vacuum. The residue was recrystallized form chloroform-methanol mixture (3:1) gave 3.30g (78% yield), orange crystal of 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)-4-methylpiperazine **(74 e)**, m.p. 126-129 ^{0}C.

Analysis:

TLC	: Toluene: acetonitrile (4:1); Rf value: 0.64
IR (KBr, cm^{-1})	:2977-2800 (CH_2), 1550,1364 (NO_2)
Mass (m/z)	:423.16 (M^+)

Synthesis of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)-*N*,*N*-dimethylethenamine (74 f)

To a mixture of 3.42 g (0.01 mole) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(57 e)**, 12.0 mL, of chloroform and 2.0g of dry sodium sulfate was added drop wise 0.45 g (0.01 mole) of dimethylamine under stirring at room temperature. The mixture was stirred for 1 hr; the solvent was removed under vacuum. The residue was recrystallized form chloroform-methanol mixture (3:1) gave 2.65g (72% yield), orange crystal of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)-*N*,*N*-dimethylethenamine **(74 f)**, m.p. 140-142 ^{0}C.

Analysis:

TLC	: Toluene: acetonitrile (4:1); Rf value: 0.64
IR (KBr, cm^{-1})	:2977-2800 (CH_2), 1550,1364 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.20 (s, 3H, -N-dimethyl), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.754 (d, 1H,

4-furoyl), δ 7.59 (s, 1H, 5-furoyl), δ 7.87 (s,1H, nitroimidazole ring)

Mass(m/z) :368.12 (M^+)

Synthesis of *N*,*N*-diethyl-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethenamine (74 g)

To a mixture of 3.42 g (0.01 mole) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(57 e)**, 12.0 mL, of chloroform and 2.00g of dry sodium sulfate was added drop wise 0.75 g (0.01 mole) of diethylamine under stirring at room temperature. The mixture was stirred for 1 hr; the solvent was removed under vacuum. The residue was recrystallized form chloroform-methanol mixture gave 2.57g (65% yield), orange crystal of *N*,*N*-diethyl-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethenamine **(74 g)**, m.p. 144-146 ^{0}C.

Analysis:

TLC : Toluene: acetonitrile (4:1); Rf value: 0.64

IR (KBr, cm^{-1}) : 2977-2800(CH_2), 1550,1364 (NO_2)

Mass (m/z) :396.15 (M^+)

Synthesis of *N*-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)aniline (74 h)

To a mixture of 3.42 g (0.01 mole) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(57 e)**, 12.0 mL, of chloroform and 2.00g of dry sodium sulfate was added drop wise 0.93 g (0.01 mole) of aniline under stirring at room temperature. The mixture was stirred for 1 hr; the solvent was removed under vacuum. The residue was recrystallized form chloroform-methanol mixture (3:1) gave 2.79g (67% yield), orange crystal of *N*-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)aniline **(74 h)**, m.p. 129-131 ^{0}C.

Analysis:

TLC : Toluene: acetonitrile (4:1); Rf value: 0.64

IR (KBr, cm^{-1}) :3200 (sec.NH), 2977-2800 (CH_2), 1550,1364 (NO_2)

NMR (DMSO-d_6, δ ppm) :δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH\ _2SO_2CH_2CH_3$), δ 6.04 (s, 1H, -anilino),δ 6.54 (d, 1H, 3-furoyl), δ 6.64 (d, 1H, 4-furoyl), δ 6.97(s, 1H, 5-furoyl), δ 7.37-7.69 (multiplet of aromatic ring) δ 8.05 (s,1H, nitroimidazole ring)

Synthesis of *N*-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)isonicotinohydrazide (74 i)

To a mixture of 3.42 g (0.01 mole) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(57 e)**, 12.0 mL, of chloroform and 2.00 g of dry sodium sulfate was added drop wise 1.37 g (0.01 mole) of isonicotinohydrazide under stirring at room temperature. The mixture was stirred for 2 hr; the solvent was removed under vacuum. The residue was recrystallized form chloroform-methanol mixture gave 2.85g (62% yield), orange crystal of *N*-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)isonicotinohydrazide **(74 i)**, m.p. 134-136 ^{0}C.

Analysis:

TLC : Toluene: acetonitrile (4:1); Rf value: 0.64

IR (KBr, cm^{-1}) :3300-3100 (NH_2 & -NH), 2900-2800 (CH_3), 1700 (C=O), 1550,1360 (NO_2),

NMR (DMSO-d_6, δ ppm) :δ 1.40 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 3.15 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 4.26 (s, 3H, N=C-$\underline{CH_3}$), δ 4.96 (t, 2H, imidazole-$\underline{CH_2}CH\ _2SO_2CH_2CH_3$), δ 6.54 (d, 1H, 3-furoyl), δ 6.754 (d, 1H, 4-furoyl), δ 7.59 (s, 1H, 5-furoyl), δ 7.87 (s,1H, nitroimidazole ring)

Mass (m/z) :460.12 (M^+)

Synthesis of 1-ethyl-8-(4-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (74 j)

To a mixture of 3.42 g (0.01 mole) of 2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)ethanone **(57 e)**, 12.0 mL of chloroform and 2.00 g of dry sodium sulfate was added drop wise 3.33 g (0.01 mole) of 1-ethyl-6-fluoro-8-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylicacid under stirring at room temperature. The mixture was stirred for 1 hr; the solvent was removed under vacuum. The residue was recrystallized form chloroform-methanol mixture (3:1) gave 3.34g (52% yield), white crystal of 1-ethyl-8-(4-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylicacid **(74 j)**, m.p. 250-252 ^{0}C.

Analysis:

TLC	: Toluene: acetonitrile (4:1); Rf value: 0.64
IR (KBr, cm^{-1})	:3600-3200 (broad peak of COOH), 1720 (C=O), 1550, 1362 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 1.48 (t, 3H, -$SO_2CH_2\underline{CH_3}$), δ 2.48 (q, 2H, piperazine) δ 3.30 (q, 2H, -$SO_2\underline{CH_2}CH_3$), δ 3.66 (t, 2H, -$CH_2\underline{CH_2}SO_2CH_2CH_3$), δ 3.90 (s, 3H, N=C-$\underline{CH_3}$), δ 4.40 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.51 (m, 1H, 3-furoyl), δ 6.98 (d, 1H, 4-furoyl), δ 7.07 (d, 1H, 5-furoyl), δ 7.58 (s, 1H, quinolone), δ 7.93 (s,1H, F-quinolone), δ 7.96 (s,1H, nitroimidazole ring), δ 11.25 (s,1H, COO<u>H</u> quinolone)
Mass (m/z)	:642.19 (M^+)

Synthesis of 1-ethyl-6-fluoro-8-(4-(1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)vinyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylicacid (74 k)

To a mixture of 2.35 g (0.01 mole) of 1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)ethanone **(57 f)**, 12.0 mL, of chloroform and 2.00 g of dry sodium sulfate was added drop wise 3.33 g (0.01 mole) of 1-ethyl-6-fluoro-8-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid under stirring at

room temperature. The mixture was stirred for 1 hr; the solvent was removed under vacuum. The residue was recrystallized form chloroform-methanol mixture (3:1) gave 2.68 g (50% yield), white crystal of 1-ethyl-6-fluoro-8-(4-(1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)vinyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carbox ylicacid **(74 k)**, m.p. 240-242 ^{0}C.

Analysis:

TLC	: Toluene: acetonitrile (4:1); Rf value: 0.64
IR (KBr, cm^{-1})	:3600-3100 (broad peak of COOH), 1720 (C=O), 1550, 1362 (NO_2)
NMR (DMSO-d_6, δ ppm)	:δ 2.48 (q, 2H, piperazine), δ 3.90 (s, 3H, N=C-$\underline{CH_3}$), δ 4.40 (t, 2H, imidazole-$\underline{CH_2}CH_2SO_2CH_2CH_3$), δ 6.51 (m, 1H, 3-furoyl), δ 6.98 (d, 1H, 4-furoyl), δ 7.07 (d, 1H, 5-furoyl), δ 7.58 (s, 1H, quinolone), δ 7.93 (s,1H, F-quinolone), δ 7.96 (s,1H, nitroimidazole ring), δ 11.05 (s,1H, COO$\underline{H}$ quinolone)

6.4 References

Herr ME, Heyl FW. Enamine Derivatives of Steroidal Carbonyl Compounds I, J Am Chem Soc 1952;74: 3627-3630.

Herr ME, Heyl FW. Enamine Derivatives of Steroidal Carbonyl Compounds II. J Am Chem Soc 1953;75:1918-1920.

Mannich C, Davidsen H. Simple enamines with nitrogen in tertiary combination. Chem Ber 1936;69B:2106-2112.

Mannich C, Handke K, Roth, K. Reaction of dimethylamine with acrolein in the presence of a dehydrating agent. Chem Be. 1936;69:2113-2115.

Spero G B, Thompson JL, Magerlein B J, Hanze A R, Murray HC, Sebek OK, Hogg J A. Adrenal hormones and related compounds IV 6-methyl steroids. J Am Chem Soc 1956;78:6213-6214.

7.1 Introduction

7.1.1 Susceptibility testing of anaerobes

Antimicrobial therapy of anaerobic infections is usually determined empirically because cultivation and isolation of clinically important anaerobic bacteria are relatively slow and results of susceptibility tests are usually not available for 2 to 4 days. For the most part, this is a satisfactory approach because with many anaerobes there is reasonable level of predictability of antimicrobial susceptibility that serves as the basis for selection of appropriate initial therapy.

Routine susceptibility testing of all anaerobic isolates is not recommended, but there are a number of circumstances when determination of the susceptibility of individual isolates is of great importance. Susceptibility tests are usually needed for patients with serious infections such as endocarditis or brain abscess or infections requiring prolonged therapy such as osteomyelitis, or infections also needed to monitor the susceptibility of commonly isolated species so that changing patterns of resistance can be detected and the empirical basis of therapy can reflect any changes in antibiogram.

Methods similar to those used for anaerobic and facultative bacteria are useful for estimating the susceptibility of anaerobes to various antimicrobial agents, Broth dilution tests, both macro and micro, and agar dilution tests are currently being used. Agar diffusion tests are not recommended because of the complexities and variation introduced by the slow and varying growth rates of anaerobic bacteria.

7.1.2 Broth dilution methods

Macrodilution test

Conventional broth dilution tests are convenient if small numbers of strains are to be tested or if the bacteria, such as *Clostridium* spp., spread on the surface of agar plates. They are also useful if one wishes to determine bactericidal as well as bacteriostatic activity of drugs. Dilution of the drug are prepared in a suitable medium is not kept under reduced conditions; it should be boiled out or reduced in an anaerobic atmosphere prior to use. An inoculums is added so that the final concentration of bacteria is between 10^5 and 10^6 CFU/mL.

Procedure:

Serial two-fold dilutions of the antimicrobial agent were prepared in Brucella broth containing fildes enrichment (5%) or hemin (5µg/mL) and vitamin K_1 (0.1 µg/mL). A limited number of concentrations of drugs that are clinically relevant may be selected to simplify the procedure. Table 25, indicates the solvents for stock solutions of antibiotics.

Table 25: Solvents for stock solutions of antimicrobial agents

Antimicrobial Agent	Solvent
Ampicillin	Phosphate buffer 0.1 M, pH 8
Carbenicillin	Water
Cefamandole	Water
Cefoperazone	Water
Cefoxitin	Water
Cephalothin	Phosphate buffer 0.1 M, pH 8
Chloramphenicol	Phosphate buffer 0.1 M, pH 6
Clindamycin	Water
Erythromycin	Ethanol[a]
Impenem	Phosphate buffer 0.01 M, pH 7.2
Metronidazole	Water
Moxalactam	Water
Penicillin G	Water
Tetracycline	Water
Vancomycin	0.05 N HCl[b]

[a] Erythromin is dissolved in 1:10 of final volume of 95% ethanol, then brought to the proper volume with water.

[b] Stock solution is made with 0.05 N HCl, further dilutions are made in water.

With rapidly growing strains, the inoculum was prepared as follows:

1. Three or four colonies or a 3 mm loopful of the strain to be tested are picked from an overnight culture on a blood agar plate and inoculated in to a tube of 5 to 6 mL supplemented thioglycollate medium without indicator (BBL 135 C). This medium was enriched with hemin (5µg/mL) and vitamin K_1 (0.1 µg/mL) prior to sterilization plus $NaHCO_3$ (1mg/mL) added just prior to use. This medium will henceforth be referred to as THCK.

2. After 4 to 9 hr incubation at 37 ^{0}C, the culture is diluted in Brucella broth (supplemented as above) to the turbidity of the 0.5 McFarland standard (10^8 CFU/mL), and was then further diluted 1:200.

With slower growing strains, colonies are picked from 2 to 3 days old blood agar plate culture, inoculated into THCK and incubated overnight prior to dilution for the test. A volume of inoculum equal to the amount of broth containing the drug is added, and tests were incubated at 37 ^{0}C in Gas Pak jars for approximately 48 hr. All inoculated broth containing no antimicrobial agent was included as a growth control for each strain tested and a tube of uninoculated broth was also included with each day's tests. Tests on control strains of known susceptibility should also be included. The minimum inhibitory concentration (MIC) was read as the lowest concentration of drug showing no visible growth. Bactericidal endpoints were determined by streaking 0.1 mL of material from each tube to a blood agar plate. Plates were incubated anaerobically for 48 hr and the minimum bactericidal concentration (MBC) was read as the lowest concentration of drug resulting in fewer than 25 colonies (99.9% killing rate).

Microdilution test

Microdilution tests are miniaturized variations of the broth dilution procedure described previously. A variety of manual, semi automated, and automated devices are available which allow the preparation of large numbers of two-fold dilution series of antimicrobial agents economically and rapidly.

Plastic trays containing eight or more rows of small, flat-bottomed U-or V- shaped wells are used. Dilutions may be prepared by adding 50 µL of broth to each well with

one of several varieties of dispensing pipettes or semi automated dispensers. Special calibrated loops are then used to transfer 50 µL of stock solutions of antimicrobial agents to the first well of each row, and then the contents of these wells are mixed by twirling the loops. Next 50 µL are transferred to the wells in the second row, mixed, transferred to the next row, of wells and continued untill the dilution series is completed. Alternatively, a multichannel dispenser may be used to dispense 50 or 100 µL of dilutions of antimicrobial agents to the wells of the trays. After trays are filled, they may be sealed and frozen at 20 ^{0}C or colder. Self-defrosting freezer units should not be used since the fluctuation of temperature during the defrost cycle contributes to rapid deterioration of the antimicrobial agents. Trays stored at -20 ^{0}C are stable for up to 4 months; trays stored at -60 ^{0}C are stable for up to a year. Quality control must be performed periodically to monitor shelf life.

The inoculum is grown in the same manner as for the broth dilution test, adjusted to the turbidity of the 0.5 McFarland standards, and is then further diluted so that the final density of bacteria in each well is approximately 10^5 to 10^6 CFU/mL. The exact dilution used will vary depending upon the volume of broth in the wells and the volume of inoculum delivered. Alternatively, a suspension may be prepared from growth on a plate not more than 72 hr old, adjusting the density as previously noted.

Trays are removed from the freezer, allowed to thaw, and then inoculated. Thawing and pre-conditioning the plates in an anaerobic atmosphere for 4 hr or more will enhance the growth of some of the more fastidious anaerobes but is usually not necessary for strains of *B.fragilis* group. When metronidazole is part of the test system, tray must be preconditioned to ensure optimum activities of metronidazole.Tests are incubated in an anaerobic jar for 48 hr. The MIC is read as the lowest concentration of drug showing no visible growth. Interpretation of the endpoint with some of the cephalosporin against some strains of the *B.fragilis* group can be difficult since there sometimes is a small button of growth in several wells beyond the larger button exhibited in the growth control well. Our practice has been to read the endpoint as the concentration in the well where a drastic change is the size of the button occurs. With metronidazole a tiny button of precipitate occurs in all wells. Again, the endpoint is read as the concentration in the well where a drastic change in the size of the button occurs.

Commercially prepared frozen trays are available from Micro-Media systems. These have been shown to be satisfactory and are convenient for the laboratory which does small numbers of test. Other systems, utilizing lyophilized antimicrobial agents, are being developed, but are not yet approved for the use with anaerobes. When commercially available test trays are used the manufacturer's directions for test performance and quality control should be followed for reliable, reproducible results (Fine gold, 1994).

7.1.3 Broth-disk test

Modifications of broth tests that simplify procedures for microbiologists in clinical laboratories have been gaining widespread acceptance. Selected concentrations of antimicrobial agents relevant to the clinical situation are incorporated into liquid media by means of elution from paper disks. A practical procedure described by Kurzynski and co-workers places antimicrobial agent disks into thioglycollate medium (BBL 135 C) prepared in screw-capped tubes and incubates the tests under aerobic conditions. For strains of pigmented *Bacteroides*, a 0.5 mL supplement of nine parts of rabbit serum and one part of hemin-menadione stock solution (500 µg of hemin, 50 µg of menadione) is added. Disks are added to the thioglycollate medium as indicated in Table 26.

Tubes are inoculated with 0.1 mL of an actively growing culture of the organism (24 to 48 hr) to be tested. Alternatively, several colonies picked from a plate not more than 72 hr old may be suspended in a broth medium to achieve a 0.5 McFarland standard or greater. One tube of medium without antibiotic should be inoculated with each organism tested to serve as a growth control. After inoculation, tubes are gently inverted 2 or 3 times to ensure adequate mixing of antibiotics and inoculum. Tubes are incubated for 18 to 24 hr (occasionally 48 hr may be required). Susceptibility to the drugs is indicated by the absence of growth or less than 50% of the growth as compared to the control tube (Fine gold, 1994).

Table 26: Preparation of broth-disk tubes

Drugs	Disk Content [b]	No. of disks 5 mL medium	Test µg/mL [a, b]
Ampicillin	10	2	4
Carbenicillin	100	3	60
Cefoperazone	75	2	30
Other cephalosporins[c]	30	3	18
Chloramphenicol	30	3	18
Clindamycin[d]	2	8	3.2
Metronidazole	80	1	16
Mezlocillin	75	4	60
Penicillin G	10	1	2
Pipericillin	100	3	60
Tetracycline	5	3	3
Ticracillin	75	4	60

[a] concentrations listed are not always the same as those recommended by Kurzynskin and co-workers. Other concentrations can be obtained by altering the number of disks or the volume of medium. If more than 4 disks are used, they should be reduced prior to addition to the medium.

[b] content or concentration in µg/mL.

[c] Includes cefamandole, cefotaxime, cefoxitin, moxalactam.

[d] A 10µg disk may become available and would be preferable. Two 10 µg disks added to 5 mL of medium will give a concentration 4 µg/mL once 10µg disk added to 5 mL of medium will give a concentration of 2µg/mL. If oral therapy is to be used, the lower concentration should be tested.

7.1.4 Agar dilution test

Wadsworth Method

Agar dilution tests are convenient when large numbers of strains are to be tested. Test results are often easier to read, and there is the advantage of visual detection of contaminants. Dilutions of drugs are prepared and incorporated into Brucella agar supplemented with vitamin K_1 and either 5% defribnated sheep blood or 5% defribnated sheep blood that has been laked (lysed) by freezing and thawing. The concentrations of antimicrobial agents may be made in a series of two-fold dilutions or may be a set of concentrations relevant to the clinical situation.

The inoculum is grown in the same manner as for the broth dilution test, adjusted to the turbidity of the 0.5 McFarland standards and applied to the plates without further dilution. The inoculum is applied by means of a steer's replicator. Two plates of the medium being used in the test agent-containing medium as controls. One of these is incubated aerobically to detect aerobic contamination. Strains of known susceptibility should be included as controls. The test and growth control plates are incubated anaerobically at 37 ^{0}C in Gas Pak jars for approximately 48 hr. The MIC is read as the lowest concentration of drug yielding no growth, a barely visible haze, or one discrete colony (Fine gold, 1994).

7.2 Results and Discussion

The sensitivity of about 20 compounds selected on the basis of their one electron reduction potential from all the three series of synthesized nitroimidazole were checked against Gram positive and Gram negative anaerobes by macro broth dilution method.

Table 27: Inhibition cut off value against Gram positive and Gram negative anaerobe

Sr. No.	Compound Code	Organism tested (Inhibition cut off value)	
		Clostridium sporogenus **(Gram positive anaerobe)**	***Bacteriodes fragililis*** **(Gram negative anaerobe)**
1	**57- e**	>20 µg/mL	10 µg/mL
2	**58-e**	>20 µg/mL	10 µg/mL
3	**59-e**	>20 µg/mL	10 µg/mL
4	**58-b**	>20 µg/mL	10 µg/mL
5	**59-b**	>20 µg/mL	10 µg/mL
6	**58-d**	>20 µg/mL	10 µg/mL
7	**59-d**	**10 µg/mL**	10 µg/mL
8	**57-f**	>20 µg/mL	10 µg/mL
9	**67-a**	>20 µg/mL	10 µg/mL
10	**67-b**	>20 µg/mL	10 µg/mL
11	**67-c**	**10 µg/mL**	10 µg/mL
12	**74-k**	**10 µg/mL**	10 µg/mL
13	**74-b**	>20 µg/mL	10 µg/mL
14	**74-d**	**10 µg/mL**	10 µg/mL
15	**74-e**	>20 µg/mL	10 µg/mL
16	**74-c**	>20 µg/mL	10 µg/mL
17	**74-i**	**10 µg/mL**	10 µg/mL
18	**74-h**	**10 µg/mL**	10 µg/mL
19	**74-f**	>20 µg/mL	10 µg/mL
20	**74-j**	**10 µg/mL**	10 µg/mL
21	**Metronidazole (Control)**	20 µg/mL	10 µg/mL

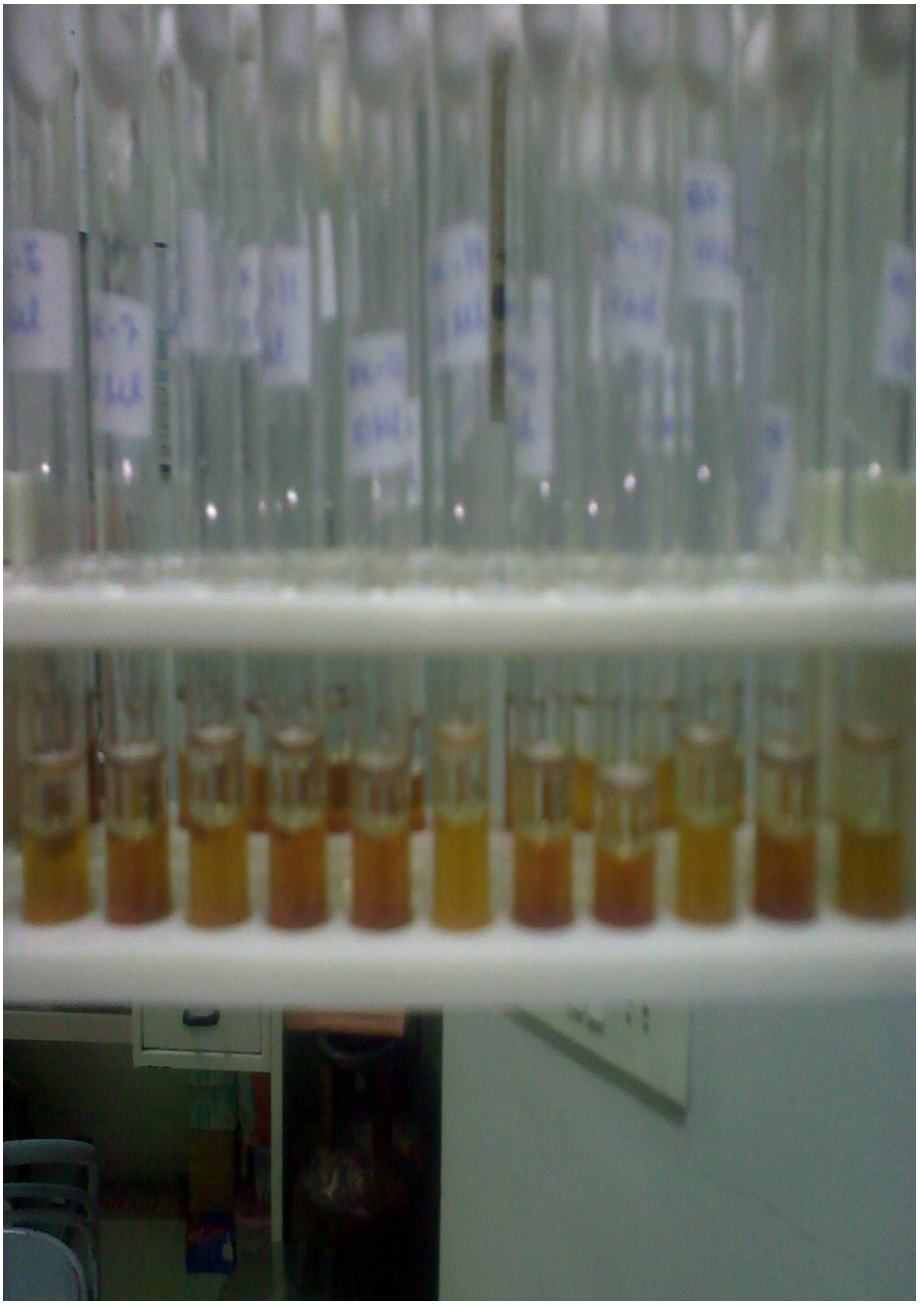


Figure 4: Antianaerobic sensitivity against *C. sporogenus* at 10 and 20 µg/mL

Figure 5: Antianaerobic sensitivity against *B. fragilis* at 10 and 20 µg/mL

The inhibition cut off value (sensitivity) of about 20 compounds selected from all the three series of synthesized nitroimidazoles were carried out against *Clostridium sporogenus* and *Bacteriodes fragilis* using metronidazole as control drug. All the compounds sensitivity was found same as the metronidazole (control standard) at 10 µg/mL concentration against the Gram negative bacteria, *Bacteriodes fragilis* which was shown in Fig 4 and 5, the transparent broth tubes indicating absence of growth of bacteria while the turbidity indicates its growth.

Some of the synthesized compounds were found more potent then metronidazole at 20 µg/mL concentrations against Gram positive bacteria i.e., *C. sporogenus*, the compound number were (59-d, 67-c, 74-k, 74-d, 74-I, 74-h, 74-j). Compound 59-d belongs to the nitroimidazole-oxime derivative; 1-(2-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanone while compound number 67-c is 6-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-5-(furan-2-yl)-4*H*-1,3,4-thiadiazin-2-amine. Compounds 74-k, 74-d, 74-I, 74-h and 74-j were belonging to 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinylsubstitute damine series.

Thus most potent series against Gram positive anaerobe; *C. sporogenus* was found to be nitroimidazole-enamine series i.e., 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinylsubstitutedamine which were more active as compared to the metronidazole.

The compound 74-k i.e., 1-ethyl-6-fluoro-8-(4-(1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)vinyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylicacid) were dimetridazole-norfloxacin enamine derivatives and 74-j i.e., 1-ethyl-8-(4-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylicacid, belongs to tinidazole-norfloxacin enamine derivatives, thus it indicates, increase in the potency of activity against gram positive anaerobe by fusion of quinolone moiety with nitroimidazole drugs, which acts as hybrid antibacterial to show dual activity against both anaerobic and aerobic bacteria due to nitroimidazole and quinolone moiety in a single molecule.

Surprisingly it was found that Isoniazide-tinidazole enamine derivatives compound 74-i that was *N*-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)isonicotinohydrazide were inactive against Gram positive organism while active against Gram negative organism.

7.3 Reference

Finegold SM: Wadsworth Anaerobic Bacteriology Manual, Star Publishing Company, Fourth edition 1986:79-90.

A) ONE ELECTRON REDUCTION POTENTIAL

8.1 Introduction to one electron reduction potential of 5-nitroimidazole

Nitroimidazole comprises a large group with useful clinical activity as antibacterial, antiprotozoal and anticancer agents. With all nitro compounds their activity is solely depend upon reduction of nitro group, the metabolic products of which are responsible for the DNA damage. The key to understanding the basis of selective toxicity of these drugs lies in the knowledge of the range of reduction potentials exhibited by such compounds. Fig 6 shows the redox potential of electron affinity exhibited by nitroaromatic compounds (Wardman *et al.,* 1983).

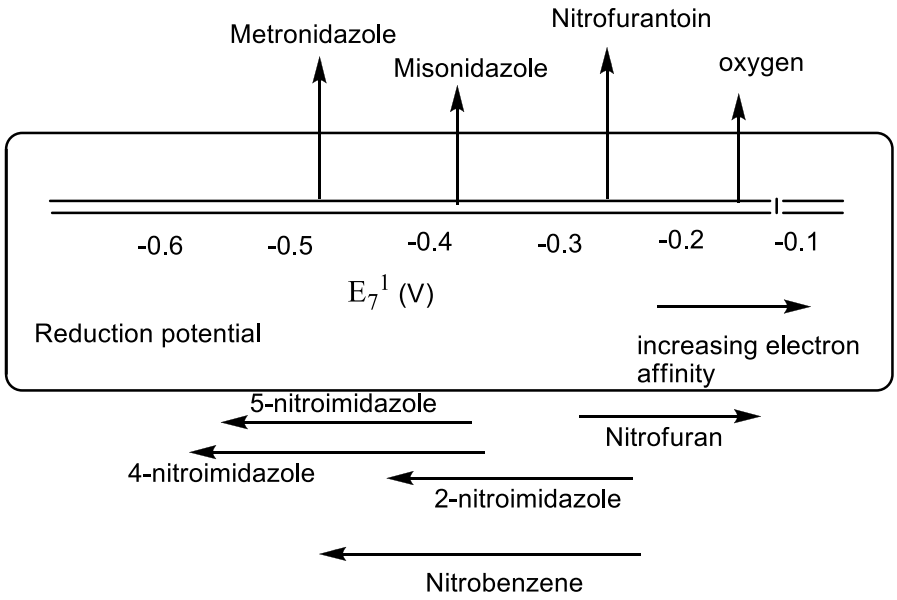


Fig 6: The redox spectrum showing the elctron affinity (E_7^1) of nitroaromatic drugs. Oxygen is the most electron affinic and drugs of lower potential are less easily reduced

The boundary falls at -0.35 V to the right of this division, redox reactions are easily performed under the aerobic conditions; in fact the most negative of redox reactions carried out by the aerobic cell is that involving NAD(NADP)-NADH(NADPH) couples at -0.35 V. Thus redox reactions more negative (lower) than -0.35 V is not possible under aerobic conditions but are possible under anaerobic conditions.

Consequently anaerobes have evolved special electron-transfer proteins to deal with low-potential redox reactions, characterized by the possession of iron-sulfur proteins such as ferredoxins and rubredoxins. Since the voltage span for the acceptance of a single electron is 60 mV, one may expect some weak electron transfer reactions down to the potential of -0.4 V in aerobes but below we enter the domain of anaerobic

organism. Even between the values of -0.35 and -0.4 V the aerobic cell would be considerably acid and hypoxic, so it is not surprising that the 2-nitroimidazoles being selectively reduced under hypoxia but having redox potential too positive to be usefully considered as possible antianerobic drugs.

The redox potential of bioreducible drugs is therefore crucial beginning in understanding how these compounds may be applied to combat diseases which flourish under redox environment (Zahoor *et al*., 1986). Wardman and colleagues have performed the quantitative structure-activity relationship on the 2-nitroimidazole radio sensitization activity shows a good relation between electron affinities (measured as one-electron reduction potential, E_7^1 of half wave reduction potential $E_{1/2}$) and the concentration required to produce fixed, relevant biological response.

Hypoxic cells are more resistant to ionizing radiation than well oxygenated ones, and their presence in human tumors may lead to the failure of radiotherapy. In addition to their ability as radiosensitizers, nitroimidazoles are selectively cytotoxic to hypoxic cells (Adam *et al.,* 1978). The basis of structure-activity relationships in the development of electron-affinic nitroheterocyclic hypoxic cell radiosensitizers is a linear correlation of the type:

$$-\log C = b_0 + b_1E + b_2 \log P + b_3 (\log P)^2 \ldots\ldots\ldots\ldots\ldots \quad (1)$$

C = the drug concentration required to cause specific relevant biological effect.

E= the electron affinity usually expressed as the one-electron reduction potential (E_7^1)

P= the octanol/water partition coefficient of the drugs

b_0, b_1, b_2 and b_3 = constants

It has shown that there is negligible effect of lipophilicity (P) on radiosentitizing and cytotoxicity. Thus the coefficient b_2 and b_3 may be omitted.

$$-\log C = bo + b_lE \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

It is well-established that the E_7^1 value correlates positively with radiosensitization efficiency (Adams *et al.,* 1976 and 1979a) aerobic cytotoxicity, mutagenicity and hypoxic cytotoxicity (Adam *et al.,* 1975). The more electron-affinic the drug (the more positive the E_7^1 value) the greater the radiosensitization and cytotoxicity, which varies in general by an order of magnitude for each 100 mV change in E_7^1. These correlations suggest that redox processes are involved both in radiosensitization and

cytotoxicity, but do not indicate a common mechanism, since radiosensitization is a fast process occurring in a few milliseconds (Adam *et al.,* 1975) and is temperature-independent, whereas cytotoxicity is relatively slow and is temperature-dependent (Strantford *et al.,* 1977).

These criteria of nitroimidazole cytotoxicity to mammalian cells also apply to their effect on anaerobic microorganisms. However, the correlation of cytotoxicity and electron affinity is, in this case, a negative one: that is, the less electron-affinic the drug the greater its cytotoxicity, which generally doubles for each 100 mV decrease in E_7^1 (Reynolds *et al.,* 1981). The evidence that toxicities to hypoxic mammalian cells and to anaerobic microorganisms depend upon the reduction of the nitro group (Edward *et al.,* 1980). In anaerobic environments, metronidazole and other nitroheterocyclic drugs are activated by reduction of the nitro group to a radical anion which has been shown to cause damage to DNA. Metronidazole has a very low redox potential so activation only occurs in environments with a suitably low redox potential. Within strictly anaerobic bacteria and protozoa, electrons produced by the pyruvate: ferredoxins oxidoreductase complex derived from the decarboxylation of pyruvate are passed on to the low redox electron carrier protein ferredoxin, which in turn reduces another component, usually H^+, which acts as the terminal electron acceptor. Metronidazole is activated by accepting electrons from reduced ferredoxin (Edwards *et al.,* 1993).

8.2 Experimental

8.2.1 Methods

A) Electrochemical method

Reported $E_{1/2}$ as the Polarographic half-wave potential (HWPs) in mV measured against Ag/AgCl reference electrode at pH 7.0 and E_7^1 as one-electron reduction potential (OERPs) in mV measured against the normal hydrogen electrode (Knox *et al*., 1981). Table 28 shows the $E_{1/2}$ and E_7^1 of nine standard nitroimidazole which were considered in the study along with their structure and DNA damage.

B) Computational method

The molecular geometries of each of the nine compounds in Table 28 were built by using standard bond lengths and angles with Chem. Office Ultra 11.00 (Chem Draw ultra; 2008). These structures were initially optimized using MM_2 force field method until the root mean square gradient value becomes smaller than 0.001 Kcal/mol. The resulting optimized structure was processed through the Extended Huckel partial atomic charges on oxygen of nitro group in nitroimidazole (Mladenovic *et al*.., 2010), as the electronic descriptors, which were correlated against the observed HWPs, OERPs and DNA damage by using multidimensional linear regression analysis.

The partial charges on the oxygen of nitro group were calculated as observed in the figure 7.

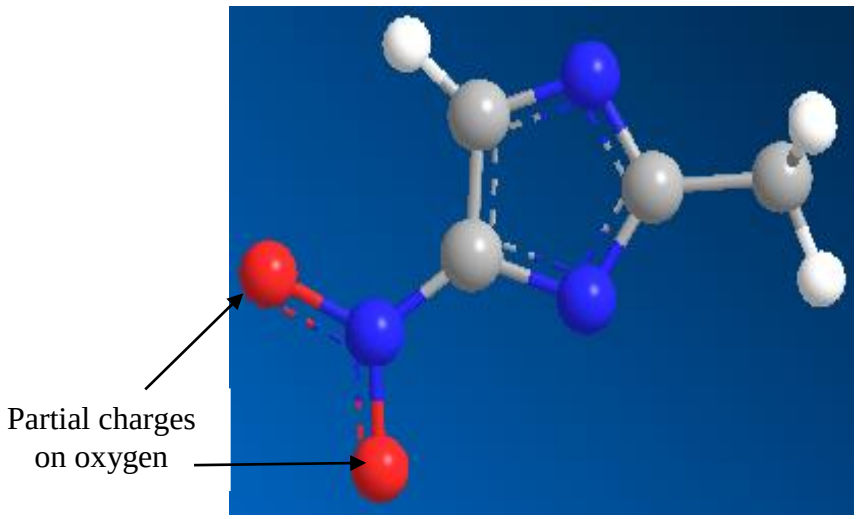


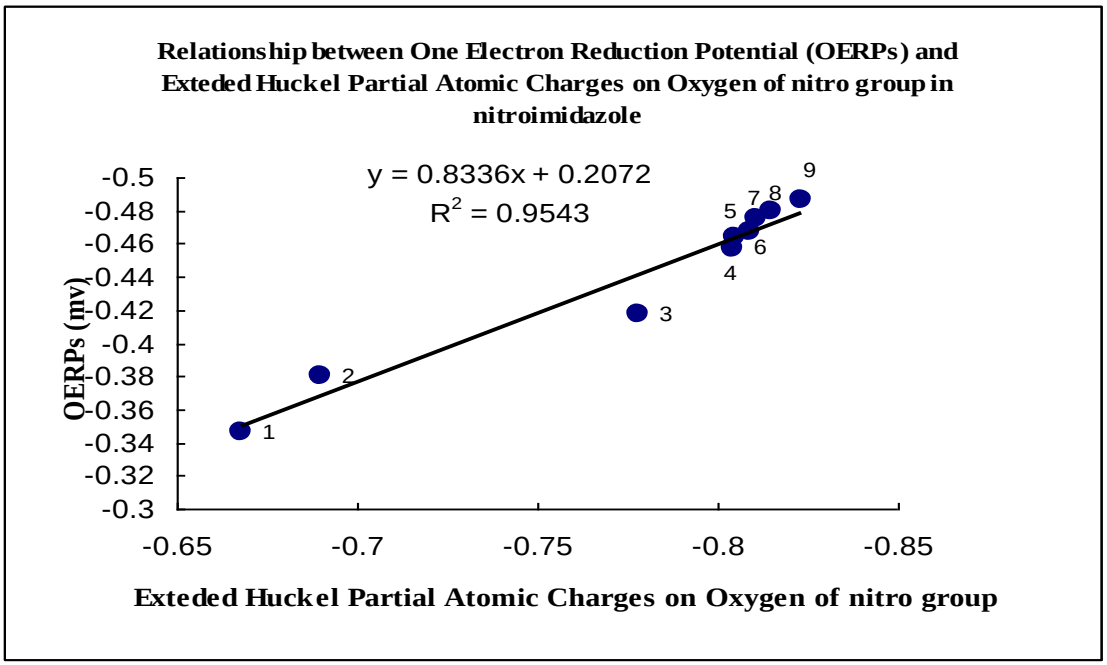
Fig 7: Extended Huckel partial atomic charges on oxygen of nitro group in 5-nitroimidazole

Table 28: Electronic property of nitroimidazole

Half wave potentials (HWPs), One-electron reduction potentials (OERPs), DNA damage and Extended Huckel partial atomic charges on oxygen of nitro group in nitroimidazole.

No	Drug	$E_{1/2}$ (HWPs) (Obsd) (mV)	E_7^1 (OERPs) (Obsd) (mV)	Log DNA damage (Obsd)	Extended Huckel Partial Atomic Charges on oxygen of nitro group
1	Pimonidazole	-180	-346	-0.660	-0.668
2	Benznidazole	-200	-380	-0.569	-0.690
3	Azomycin	-374	-418	-0.222	-0.778
4	Nimorazole	-345	-457	-0.393	-0.804
5	Tinidazole	-340	-464	0.104	-0.805
6	Ornidazole	-345	-467	-0.131	-0.809
7	Dimetridazole	-388	-475	0.017	-0.811
8	Secnidazole	-390	-480	0.013	-0.815
9	Metronidazole	-382	-486	-0.022	-0.823

The correlation between one electron reduction potential and Exteded Huckel partial atomic charges on oxygen of nitro group of nitroimidazole is established as shown in graph 1.

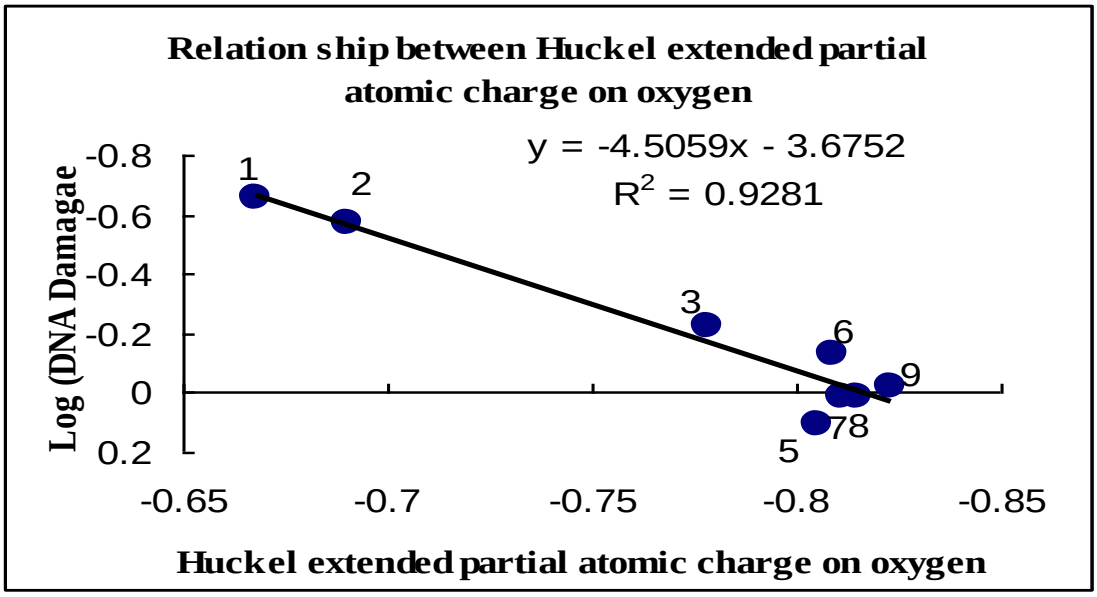


Graph: 1 One electron reduction potential of standard nitroimidazole and partial charge on oxygen

Table 29: Validation of the model by regression analysis for one electron reduction potential of standard nitroimidazole and partial charge on oxygen

Description	r	r^2	r^2 (adj)	F value	Least squared error
OREPS= 0.8336 * (Partial charge) + 0.2072	0.9768	0.9542	0.9477	146.0945	0.0112

The DNA damage activity of standard nitroimidazole were calculated showing a good correlation having negative slope as shown in the graph 2



Graph: 2 Partial charges on oxygen of standard nitroimidazole and DNA damage

Table 30: Validation of the regression analysis for Partial charges on oxygen of standard nitroimidazole and DNA damage

Description	r	r^2	r^2 (adj)	F value	Least squared error
Log DNA damage = -4.5059 * (Partial charge) - 3.6752	0.9633	0.9281	0.9160	77.41	0.08248

8.3 Results and Discussion

The reported E_7^1 (One electron reduction potential) of nitroimidazoles was found in the range of -257 to -500 mV which indicate its active range against anaerobic bacteria and various other biological activity. Good correlation between one electron reduction potential and calculated Extended Huckel partial atomic charge on oxygen of nitro group was obtained having, r = 0.9768, r^2=0.9542, $r^2_{(adjusted)}$ = 0.9477, F value = 146.0945, standard error = 0.0112.The correlation between Extended Huckel partial atomic charge on oxygen and Log DNA damage activity was also found out having r=0.9633, r^2=0.9281, $r^2_{(adjusted)}$ = 0.9160, F value = 77.41, standard error = 0.08248.

It was well-established that the E_7^1 value correlates significantly with radiosensitization efficiency (Adams *et al.*, 1976, 1979a), aerobic cytotoxicity, mutagenicity and hypoxic cytotoxicity (Adam *et al.*, 1975) of various standard nitroimidazole drugs. The more electron-affinic the drug (the more positive the E_7^1 value) the greater the radiosensitization and cytotoxicity, which varies in general by an order of magnitude for each 100 mV change in E_7^1.

Thus Computed Extended Huckel partial atomic charge on oxygen can be used to correlates significantly radio sensitization efficiency, aerobic cytotoxicity, mutagenicity, hypoxic cytotoxicity, DNA damage and anaerobic activity of nitroimidazole class drugs prior to its synthesis.

Thus it was clear that electronic parameters were navigating the biological activity of various nitroimidazole and also influencing the availability of partial negative charges on oxygen of the nitro group thus it can acts as the predictor of electron affinity and various biological acitity of the nitroimidazole drugs.

Thus, Extended Huckel partial atomic charge on oxygen was founded as the novel electronic parameter which can be regressed in QSAR with other physicochemical parameter like steric and lipophilic in the further study.

B) 8.4 QSAR

8.4.1 Introduction to QSAR

Most molecular discoveries today are the results of an iterative, three-phase cycle of design, synthesis and test. Analysis of the results from one iteration provides information and knowledge that enables the next cycle of discovery to be initiated and further improvement to be achieved. A common feature of this analysis stage is the construction of some form of model which enables the observed activity or properties to be related to the molecular structure. Such models are often referred to as Quantitative Structure Activity Relationships (Selassie *et al.,* 2003).

Quantitative structure-activity relationship (QSAR) studies unquestionably are of great importance in modern chemistry and biochemistry. The concept is to transform searches for compounds with desired properties using chemical intuition and experience into a mathematically quantified and computerized form. QSAR methods are characterized by two assumptions with respect to the relationship between chemical structure and the biological potency of compounds. The first is that one can derive a quantitative measure from the structural properties significant to the biological activity of a compound. The properties are assumed to be physicochemical such as partition coefficient or sub structural such as presence or absence of certain chemical features. The other assumption is that one can mathematically describe the relationship between biological property (one can wishes to optimize and the molecular property calculated from the structure) (Martin *et al.,* 1981). QSAR's general mathematical form is represented by the following equation.

Biological Activity = f (Physicochemical Property) …… (3)

8.4.2 Objectives of QSAR

QSAR attempts to correlate structural, chemical and physical properties with biological activity by various statistical approaches.(Eriksson *et al.,* 2003) QSAR models are scientific credible tool for predicting and classifying biological activities of untested chemicals. QSAR is an essential tool for lead development (optimization). The growing trend is to use QSAR early in drug discovery process as a screening and enrichment tool to eliminate development of those chemicals lacking "drug like" properties or those predicted to elicit a toxic response (Perkins *et al.,* 2003).

8.4.3 Historical development

QSAR modeling born in toxicology field. In 1863, A.F.A Cross observed that the toxicity of alcohols in mammals increased as the solubility of alcohols in water decreased (Borman *et al.*, 1990). Crum-Brown and Fraser expressed the idea that the physiological action of substance was function of its chemical composition and constitution and published equation (4) in 1868 which is considered to be the first formulation of a quantitative structure-activity relationship: the "physiological activity" Φ was expressed as a function of the chemical structure C (Crum-Brown *et al.*, 1868).

$$\Phi = f(C) \ldots\ldots (4)$$

A few decades later, in 1893, Richet showed that the cytotoxicities of a diverse set of simple organic molecules were inversely related to their water solubilities (Richet *et al.,* 1893). In 1900's, Meyer and Overton independently suggested that narcotic (depressant) action of a group of organic compounds paralleled their oil/water partition coefficient (Meyer *et al.,* 1899 and Oveton *et al.,* 1901). Hammet in mid 1930s correlated the electronic properties of organic acids and bases with their equilibrium properties. He found that a relationship resulted when different substitutions were made to aromatic compounds. The technique was introduced by Hansch et al. in the early 1960s (Hansch *et al.*, 1963 and Fujita *et al.*, 1964). The approach stemmed from linear free-energy relationship in general and the Hammett equation in particular (Hammet, 1935). It is based on the assumption that differences in physicochemical properties accounts for the differences in biological activities of compounds. According to this approach, the changes in physicochemical properties that affect the biological activities of a set of congeners are of major three types viz. electronic, steric, and hydrophobic (Hansch, 1955).

Hansch Analysis

Corwin Hansch may be the best known as the father of concept of quantitative structure activity relationship, described the quantitative correlation of the physicochemical properties of molecules with their biological activities. It is also known as linear free energy relationship (LFER) or extra thermodynamic approach. Its basic assumption is that the effect of substituents on the strength of interactions between drug and its receptor and other biomolecules is an additive combination of

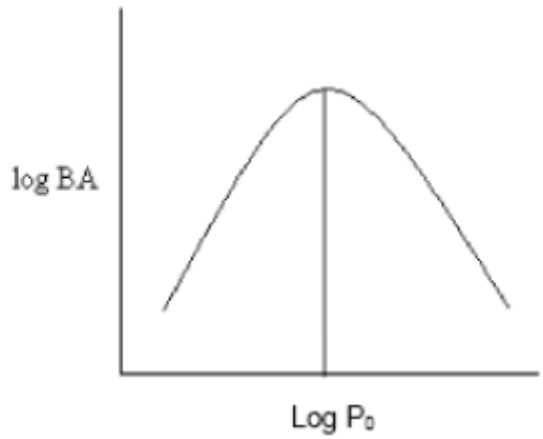
the effect of the substituents on various types of simpler model intermolecular interactions. From physical chemistry, the interactions were assumed to be electrostatic, steric and hydrophobic in nature (Hansch 1993). He suggested the linear and non-linear dependence of biological activity on different parameters.

$$\log BA = a(\log P) + b\sigma + cEs + d \text{ .....linear (4)}$$

$$\log BA = a(\log P)^2 + b(\log P) + c\sigma + dEs + e \text{ .....non-linear (5)}$$

Where logBA is the logarithmic form of biological activity, a-e are constants determined for particular biological activity by multiple linear regression analysis. Log P, σ, Es represents lipophilic, electronic and steric properties, respectively. Equation (4) was developed from the two assumptions as proposed by the Hansch.

1. The transport of a drug from the site of application to its site of action depends on the lipophilicity of the drugs (in a non-linear manner).



2. The binding affinity (interaction) of drug to its biological counter part, such as an enzyme or receptor depends on the lipophilicity, the electronic properties and other linear free energy related properties.

Free Wilson analysis

The Free-Wilson approach is truly a structure-activity based methodology because it incorporates the contribution made by various structural fragments to the overall biological activity (Wilson, 1964; Kubyini, 1979 and Rekker, 1984). Indicator variables are used to denote the presence or absence of a particular structural feature. It is represented by equation (6).

$$BA = \Sigma\, aiXi + \mu \text{ …….. (6)}$$

Where BA is the biological activity, μ is the overall activity; ai is the contribution of each structural feature, xi denotes the presence xi = 1 or absence xi = 0 of a particular structural fragment. This mathematical model incorporated symmetry equation to minimize linear dependence between variables. This approach was easy to apply; it had its drawbacks, mostly centered on the large number of parameters and subsequent loss of the statistical degree of freedom. In 1971, in an attempt to deal with limitations of these approaches, Fujita and Ban proposed a simplified approach that solely focused on the additivity of group contribution.

$$\log A/A_0 = \Sigma\, GiXi \quad \ldots\ldots\ldots\ldots\ldots\ldots(7)$$

where A and A_0 represents the biological activity of the substituted and unsubstituted compounds respectively, while Gi was the activity of the ith sustituent and Xi had the value of 1 or 0 that corresponded to the presence or absence of that substituents (Fujita *et al.*, 1971).

8.4.4 Molecular descriptors used in QSAR

Molecular descriptors can be defined as a numerical representation of chemical information encoded within a molecular structure via mathematical procedure (David, 2002). This mathematical representation has to be invariant to the molecule's size and number of atoms to allow model building with statistical methods.

The information content of structure descriptors depends on two major factors:

(1) The molecular representation of compounds.

(2) The algorithm which is used for the calculation of the descriptor (Gasteiger, 2003)

The three major types of parameters initially suggested were,

(1) Hydrophobic

(2) Electronic

(3) Steric

Table 31: Molecular descriptors used in QSAR

Type	Descriptors
Hydrophobic Parameters	Partition coefficient (log P)
	Hansch's substitution constant (π)
	Hydrophobic fragmental constant (f,f')
	Distribution coefficient (log D)
	Apparent log P
	Capacity factor in HPLC (log k', log k'W)
	Solubility parameter (log S)
	Retention factor (RM)
Electronic Parameters	Hammett constant (σ, σ +,σ -)
	Taft's inductive (polar) constant (σ*)
	Swain and Lupton field parameter
	Ionization constant (pKa , ΔpKa)
	Chemical shifts: IR, NMR
	Dipole moment
Steric Parameters	Taft's steric parameter (Es)
	Molar volume (MV)
	Vander waals radius
	Vander waals volume
	Molar refractivity (MR)
	Molecular weight (MW), Parachor, Sterimol
Quantum chemical descriptors	Atomic net charge (Qσ, Qπ)
	Superdelocalizability
	Energy of highest occupied molecular orbital (HOMO)
	Energy of lowest unoccupied molecular orbital (LUMO)
Topological descriptors	Connectivity indices chi (χ), kappa (K) shape indices
	Winner index, Zagreb index
Spatial Descriptor	Jurs descriptors, Shadow indices, Radius of Gyration,
	Principle moment of inertia

Table 32: Classification of descriptor based on the dimensionality of their molecular representation

Molecular representation	Descriptor	Example
0D	Atom count, bond counts, molecular weight, sum of atomic properties	Molecular weight, average molecular weight number of - atoms, hydrogen atoms, carbon atoms, hetero-atoms, non-hydrogen atoms, double bonds, triple bonds, aromatic bonds, rotatable bonds, rings, 3-membered ring, 4-membered ring, 5-membered ring, 6-membered ring
1D	Fragments counts	Number of: primary C, secondary C, tertiary C, quaternary C, secondary carbon in ring, tertiary carbon in ring, quaternary carbon in ring, unsubstituted aromatic carbon, substituted carbon, number of H-bond donor atoms, number of H-bond acceptor atoms, unsaturation index, hydrophilic factor, molecular refractivity
2D	Topological descriptors	Zagreb index, Wiener index, Balaban index, connectivity indices chi (χ), kappa (K) shape indices
3D	Geometrical descriptors	Radius of gyration, E-state topological Parameters, 3D Wiener index, 3D Balaban index

8.4.5 Methods of QSAR

Many different approaches to QSAR have been developed since Hansch's seminal works. QSAR methods can be analyzed from two view points:

(1) The types of structural parameters that are used to characterize molecular identities starting from different representation of molecules, from simple chemical formulas to 3D conformations,

(2) The mathematical procedure that is employed to obtain the quantitative relationship between these structural parameters and biological activity.

8.4.5.1 2D QSAR

Methods of 2D QSAR have been divided principally into the following:

a. Hansch linear free energy relationship (LFER) model

b. Mathematical models, viz Free-Wilson and Fujita-ban methods.

8.4.5.2 3D QSAR (Affinities correlated with 3-dimensional structure)

Molecular shape analysis (MSA)

Molecular shape analysis utilizes matrices which include common overlap steric volume and potential energy fields between pairs of superimposed molecules which were successfully correlated to the activity of series of compounds. The MSA, using common volumes, also provides some insights regarding the receptor-binding site, shape and size (Sugiyama *et al.,* 1996).

Molecular topological difference (MTD)

Simons and his coworkers developed a quantitative 3D-approach, the minimal steric (topologic) difference approach. Minimal topological difference uses a 'hypermolecule' concept for molecular alignment which correlated vertices (atoms) in the hypermolecule (a superposed set of molecules having common vertices) to activity differences in the series (Montsenigos *et al.,* 1989; Simon *et al.,* 1980 and Simon *et al.,* 1984).

Comparative molecular moment analysis (COMMA)

COMMA is a unique alignment independent approach. The 3D QSAR analysis utilizes a succinct set of descriptors that would simply characterize the three

dimensional information contained in the movement descriptors of molecular mass and charge up to and inclusive of second order (Silverman *et al.,* 1996).

Hypothetical Active Site Lattice (HASL)

Inverse grid based methodology developed in 1986-88, that allows mathematical construction of a hypothetical active site lattice which can model enzyme-inhibitor interaction and provides predictive structure-activity relationship for a set of competitive inhibitors. Computer-assisted molecule to molecule match which makes the use of multidimensional representation of inhibitor molecules. The result of such matching are used to construct a hypothetical active site by means of a lattice of points which is capable of modelling enzyme-inhibitor interactions (Doweyko, 1988).

Self Organizing Molecular Field Analysis (SOMFA)

SOMFA utilizes a self-centered activity, i.e., dividing the molecule set into actives (+) and inactives (-), and a grid probe process that penetrates the overlaid molecules, the resulting steric and electrostatic potentials are mapped onto the grid points and are correlated with activity using linear regression (Robinson et al ., 1999).

Comparative Molecular Field Analysis (COMFA)

The comparative molecular field analysis a grid based technique, most widely used tools for three dimensional structure-activity relationship studies, was introduced in 1988, is based on the assumption that since, in most cases, the drug-receptor interactions are non covalent, the changes in biological activities or binding affinities of sample compound correlate with changes in the steric and electrostatic fields of these molecules. These field values are correlated with biological activities by partial least square (PLS) analysis (Hoskuldsson, 1966).

Comparative Molecular Similarity Indices (COMSIA)

COMSIA is an extension of COMFA methodology where molecular similarity indices can serve as a set of field descriptors in a novel application of 3D QSAR (Leach *et al.*, 2007).

8.4.5.3 Statistical Methods

Statistical methods are the mathematical foundation for the development of QSAR models. The application of multivariate analysis, data description, classification, and regression modelling, are combined with the ultimate goal of interpretation and prediction of non-evaluated or non-synthesized compounds.

Discriminant Analysis

The aim of discriminant analysis is to try and separate the molecules into their constituent classes. Discriminant analysis finds a linear combination of factor that best discriminate between different classes. Linear discriminant analysis was used for the analysis rather than multiple linear regressions since the biological activity data were not on a continuous scale of activity but rather were classified into two groups: active and inactive. It is used to obtain a qualitative association between molecular descriptor and biological property (Contrera *et al.,* 2005).

Cluster Analysis

Cluster analysis is the process of dividing a collection of objects (molecules) into groups (or cluster) such that the objects within a cluster are highly similar whereas objects in different clusters are dissimilar. When applied to a compound dataset, the resulting clusters provide an overview of the range of structural types within the dataset and a diverse subset of compounds can be selected by choosing one or more compounds from each cluster. Clustering methods can be used to select diverse subset of compounds from larger dataset. The clustering methods most widely applied to compound selection include k-means clustering, non-hierarchiral clustering and hierarchial clustering (Grigoras, 1990).

Principle Component Analysis

The dimensionality of a data set is the number of variables that are used to describe each object. Principle Components Analysis (PCA) is a commonly used method for reducing the dimensionality of data set when there are significant correlations between some or all of the descriptors (Gasteiger *et al.,* 2003). PCA provides a new set of variables (the principle component) which represent most of the information contained in the independent variables (Lauria *et al.,* 2009).

Quantum Mechanical Methods

Quantum mechanical techniques are used to obtain accurate molecular properties such as electrostatic potential or polarizabilities that are only available with much lower resolution from classical mechanical techniques or those (ionization potential or electron affinities etc.) that can be obtained only quantum mechanically. The methods used commonly are divided into three categories: semi-empirical molecular orbital theory, density functional theory (DFT) and *ab-initio* molecular orbital theory (Pople, 1970). Quantum chemical methods can be applied to quantitative structure-activity relationship by direct derivation of electronic descriptors from molecular wave function (Todeschini *et al.,* 2000). There is no single method that works best for all problems. Besides above mentioned methods, statistical modelling techniques aims to develop correlation models between independent variables (molecular descriptors) and dependent variable (biological property) which include simple linear regression, multiple linear regression, principle component regression, partial least squares (PLS) regression, genetic function approximation (GFA) and genetic partial least squares (GPLS).

8.4.6 Recent advances in structure-activity relationships

8.4.6.1 3D Pharmacophore modeling

Pharmacophore modeling is powerful method to identify new potential drugs. Pharmacophore models are hypothesized on the 3D arrangement of structural properties such as hydrogen bond donor and acceptor properties, hydrophobic groups and aromatic rings of compounds that bind to the biological target (Langer *et al.,* 2004). The pharmacophore concept assumes that structurally diverse molecules bind to their receptor site in a similar way, with their pharmacophoric elements interacting with the same functional groups of the receptor (Sotriferr *et al.,* 2003). Pharmacophore generation utilizes Hiphop and Hypogen hypothesis (Discovery Studio 2.1, Accelrys Inc. USA). Hiphop is common feature-based pharmacophore modeling and Hypogen is activity-based pharmacophore modeling, it is used in virtual screening of databases in lead identification/lead optimization phase.

8.4.6.2 4D QSAR

4D QSAR can be interpreted as a feasible extension of 3D QSAR to address uncertainities during the alignment process. 4D QSAR concept approaches the alignment issue by incorporating molecular and spatial variety by representing each molecule in different conformation, orientations, tautomers, stereoisomers or protonation states. Two different class of 4D concept have been developed: one class of QSAR makes use of large ensemble of structurally similar conformations. In the second, QSAR approach a small set of diverse ligand configurations represents independent alternatives for the QSAR modeling (Lill *et al.,* 2007).

8.4.6.3 5D QSAR (Induced fit modeling)

Flexible-protein docking involves allowance for the flexibility of the binding pocket, while docking a small molecule-ligand is now-a-days considered as state of the art. The adaptation of this philosophy to the area of QSAR is still in its infancy. To simulate induced fit in an explicit manner, simulation of a topological adaptation of the model of the binding site surface to the individual ligand molecule has been devised where the surface of the binding site model can slightly shrink or expand depending on the size and topology of the ligand binding to it. As the identification of the correct magnitude and mechanism of induced fit is not possible in absence of the structure of the true target protein, different induced fit protocols (e.g. magnitude

dependent on steric, electrostatic, hydrogen bond or lipophilic potential) are presented as alternative scenarios (5D QSAR) to the QSAR (Lill *et al.*, 2007).

8.4.6.4 6D QSAR

6D QSAR is an extension of 5D QSAR. It allows for the evaluation of multiple representation of different solvation models.

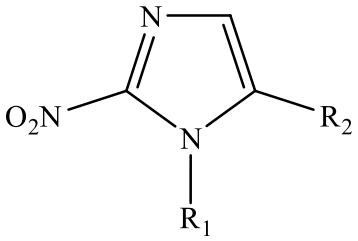
8.4.7 QSAR Study of a series of potent nitroimidazole as anti-anaerobic activity

8.4.7.1 Selection of training and test sets

In the present study, a series of substituted Nitroimidazole derivatives, reported by Reynolds et al. as antianerobic activity, was selected (Reynolds *et al.*, 1981) twenty five compounds were randomly divided into training and test sets, the former set consisting of 15 compounds and the remaining 6 compounds were taken as test set. Structures of all the compounds used for QSAR analysis and their activity against *Bacteriod fragilis*, in milimolar concentrations, mmolar/L was given in Table 33. For every compound of the series, the experimental values of microbiological activity are used in the negative logarithmic scale (pMIC) to achieve normal distribution. The structures of all compounds used in this study were sketched by using Visualizer module of Discovery studio 2.1 software (Accelrys Inc., USA). An energy minimization of all the compounds was done using Smart Minimizer method until the root mean square gradient value becomes smaller than 0.001 kcal/mol followed by geometry optimization by semi empirical MOPAC-AM1 method (Astin Method-1).

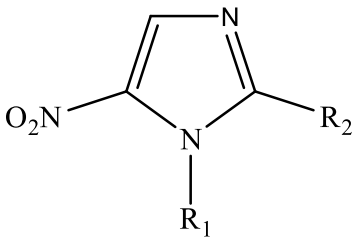
The molecular geometries of each of the twenty five nitroimidazole compounds were built by using standard bond lengths and angles with Chem. Office Ultra 11.00 (Chem Draw ultra, 2008). These structures were initially optimized using MM_2 force field method until the root mean square gradient value becomes smaller than 0.001 Kcal/mol. The resulting optimized structure was processed through the Extended Huckel Partial Atomic Charges on oxygen of nitro group in nitroimidazole (Mladenovic *et al.*, 2010). Further, optimized structures for all compounds were aligned with compound 1 and these structures were used for calculation of various descriptors.

Table 33: Nitroimidazole and their pMIC values

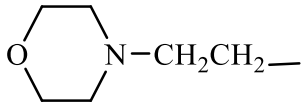


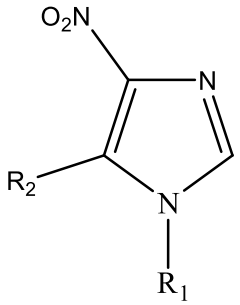
2-Nitroimidazoles

No.	R_1	R_2	MIC mmol/L	pMIC
1	$-CH_3$	-CHO	0.0035	2.455932
2	$-CH_3$	$-CH=N(O)CH_3$	0.005	2.30103
3	$-CH_3$	$-COOCH_3$	0.0035	2.455932
4	$-CH_2CH_2SO_2CH_3$	-H	0.005	2.30103
5	$-CH_2CONHCH_2C_6H_5$	-H	0.0035	2.455932
6	$-CH_2CH(OH)CH_2F$	-H	0.0035	2.455932
7	$-CH_2CH(OH)CH_2C1$	-H	0.0023	2.638272
8	$-CH_2CH(OH)CH_2OCH_3$	-H	0.0023	2.638272
9	$-CH_2CH(OH)CH_2OCH(CH3)_2$	-H	0.0023	2.638272
10	$-CH_2CH(OH)CH_2OCH_2CH=CH_2$	-H	0.0023	2.638272
11	$-CH_2CH(OH)CH_2OC2H_5$	-H	0.0023	2.638272
12	$-CH_3$	$-CH=CH_2$	0.0000023	5.638272
13	$-CH_3$	$-CH_2OH$	0.0015	2.823909
14	-H	-H	0.005	2.30103



5-Nitroimidazoles

No.	R_1	R_2	MIC mmol/L	pMIC
15		-H	0.0023	2.638272
16	$-CH_2CH_2SO_2CH_2CH_3$	$-CH_3$	0.00015	3.823909
17	$-CH_2CH_2OH$	$4\text{-}FC_6H_4$	0.00035	3.455932
18	$-CH_2CH(OH)CH_2C1$	$-CH_3$	0.0005	3.30103
19	$-CH_3$	CH_2OCONH_2	0.0001	4
20	$-CH_3$	CH_3	0.0015	3.5
21	$-CH_2CH_2OH$	CH_3	0.0001	4
22	-H	CH_3	0.230	0.638272



4-Nitroimidazoles

No.	R_1	R_2	MIC mmol/L	pMIC
23	$-CH_3$	-Cl	0.230	0.638272
24	-H	-H	0.100	1
25	$-CH_2CH_2$ $(OH)CH_2OCH_3$	-Cl	0.100	1

8.4.7.2 Descriptors selection

A common practice in QSAR study is to build QSAR models with descriptors which are not inter correlated and observe lesser degree of multi-collinearity. This is achieved by determining correlation between variable (descriptor) of interest and other variables in the regression equation, with value 1 implying high multi-collinearity. To further check inter-correlation of descriptor variance inflation factor (VIF) analysis was performed. VIF value higher than 10 indicates high multi-collinearity. Various physicochemical descriptors like lipophilic, steric, electronic and quantum mechanical descriptors were calculated using Calculate molecular properties protocol of the Discovery Studio 2.1. Theoretical ClogP calculations were carried out using WINDOWS based ClogP software program (version 4.0, Bio Byte Corp, Claremont, CA). A correlation matrix of the molecular descriptors was prepared and highly correlated descriptors with a correlation value of 0.8 or above were removed from the study. Remaining descriptors were used to develop QSAR models. Descriptors included developed QSAR models are listed and described in Table 34 and Table 35.

Table 34: Descriptor used in QSAR models

No.	Descriptor	Definition
1	CLogP	Log of the octanol-water partition coefficient
2	Molar volume	Steric parameter
3	HOMO	Energy of highest occupied molecular orbital (HOMO)
4	LUMO	Energy of lowest unoccupied molecular orbital (LUMO)
5	Partial charges	Electronic parameter, Huckel Extended Atomic partial charge on oxygen of nitro group

The four compounds were not used in the QSAR model generation that is all 4-nitroimidazole and compound No 12 was considered as outlier.

Table 35: Physicochemical descriptors used in training (1-10,15-19) and test (11-14 , 20-22) compounds

No.	Huckel Extended Atomic partial charges	HOMO	LUMO	CLogP	Molecular Volume
1	-0.742	-5.504	0.69	-0.733	91.92
2	-0.737	-4.932	1.945	-0.809	145.77
3	-0.762	-4.747	1.827	-1.077	139.6
4	-0.746	-4.727	1.245	0.059	98.78
5	-0.768	-4.751	4.52	-1.47	99.46
6	-0.758	-4.654	0.508	-0.99	67.91
7	-0.759	-4.951	4.535	-1.232	145.08
8	-0.776	-6.366	0.821	-0.255	156.4
9	-0.747	-5.248	1.237	0.39	150.23
10	-0.767	-4.642	1.808	-0.786	137.54
11	-0.777	-5.045	1.317	-1.443	114.56
12	-0.737	-5.161	0.801	0.412	112.84
13	-0.78	-4.276	2.656	-0.941	93.63
14	-0.773	-4.759	1.594	-1.48	110.44
15	-0.836	-4.304	2.93	-1.146	79.57
16	-0.852	-4.477	2.284	-0.328	97.41
17	-0.84	4.475	9.027	-2.679	69.62
18	-0.823	-4.71	2.146	-1.428	125.19
19	-0.84	-4.663	0.41	-0.92	107.01
20	-0.835	-4.846	0.17	-1.805	136.85
21	-0.844	-5.929	0.643	0.321	103.92
22	-0.848	-5.704	0.951	0.575	150.57
23	-0.823	-5.774	0.117	-0.637	109.75
24	-0.814	-4.941	1.161	-1.128	134.11
25	-0.837	-5.044	0.362	-1.095	131.36

8.4.7.3 Generation of QSAR models

Fifteen compounds from 5-nitroimidazole series and 2-nitroimidazole series were randomly selected to generate QSAR model. QSAR model were built using multiple linear regression protocol of the Discovery Studio 2.1. Statistical qualities of the generated models were judged by parameters such as regression coefficient (r^2), adjusted r^2 (r^2 adj), cross-validated r^2 (r^2cv),

Table 36: Generation of QSAR models along with their statistics

Eq. No.	Description	r	r^2	r^2 (adj)	r^2 (pred)	Least-squared error
1	pMIC = - 7.747+ 0.0082* (CLogP) + 0.000175 * (M V) - 13.34 * (PC)	0.927	0.860	0.822	0.756	0.0040
2	pMIC = -7.248+ 0.00788*(CLogP) + 0.001106 * (LUMO) - 12.98 * (PC)	0.924	0.854	0.814	0.692	0.0042
3	pMIC = -7.238+ 0.104*(CLogP) - 12.96 * (PC)	0.924	0.853	0.828	0.768	0.0042
4	pMIC = -6.743 - 12.21* (PC)	0.915	0.837	0.825	0.762	0.046

PC=Partial charge on oxygen, MV = Molar volume

8.4.7.4 Validation of QSAR models

Model validation is one of the most important aspects of QSAR analysis. Validation is necessary in QSAR methodology to prove that generated models are acceptable for their intended purpose. Once the regression equation is obtained, it is important to evaluate robustness and predictive capacity or validity of the model before using the model for interpretation and prediction of the biological activity. Validation of a QSAR model is the process by which the predictive ability of a QSAR and the mechanistic basis are assessed for practical purpose. There are two techniques to determine reliability of the generated models, internal and external validation.

To check the inter-correlation of descriptors, variance inflation factor (VIF) analysis was performed. VIF value is calculated from $1/1\text{-}r^2$, where r^2 is the multiple correlation coefficient of one descriptor's effect regressed on the remaining molecular descriptors. VIF value larger than 10 signals towards chance-correlation and hide the information of descriptors by inter-correlation of descriptors (Jaiswal *et al.*, 2004).

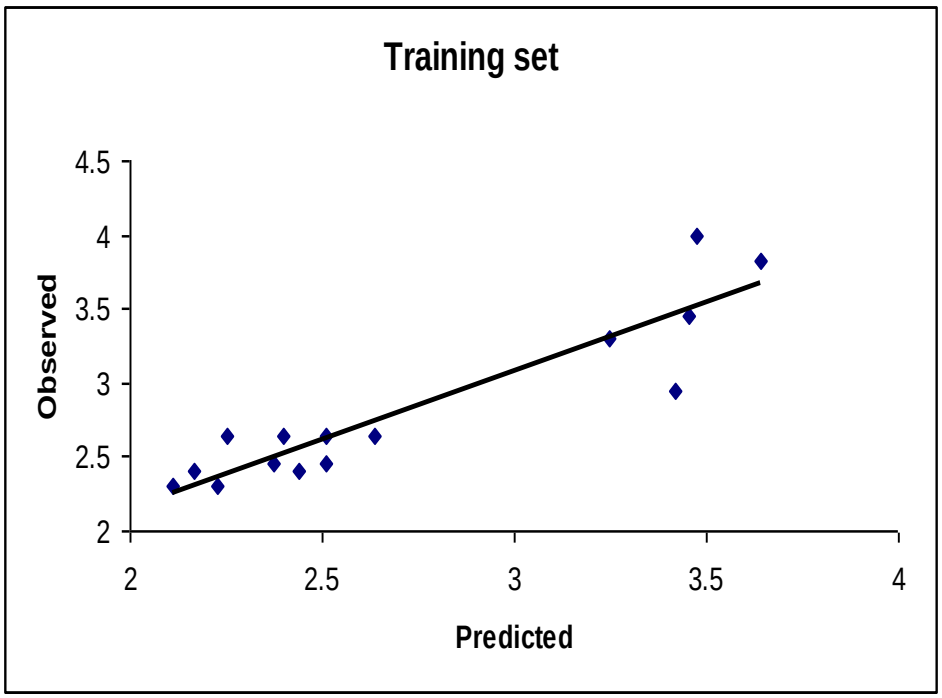
8.4.7.4.1 Internal Cross-validation

Internal validation uses the dataset from which the model is derived and it is required to check internal consistency and stability. To determine quality of the model internally A Cross-Validation (CV) technique is extensively employed. Cross-Validation methods employed as internal validation method are Leave-one-out, Leave-Some-Out or Leave- Many-Out. The quality of the model is analyzed by the value of correlation coefficient of the cross-validation procedure, that is, r^2cv. The commonly accepted value for a satisfactory QSAR model is $r^2cv > 0.5358$.

The most common approach of validation is to examine the residuals which are calculated from difference between observed and predicted biological activity (Table 37, Graph 3).

Table 37: Internal validation of training compound as per equation no 1

Comp No.	pMIC (observed)	pMIC (predicted)	Residual
1	2.4	2.168355	0.231645
2	2.3	2.110456	0.189544
3	2.4	2.440679	-0.04068
4	2.3	2.22941	0.07059
5	2.46	2.510472	-0.05047
6	2.46	2.375486	0.084514
7	2.64	2.400347	0.239653
8	2.64	2.637119	0.002881
9	2.64	2.254468	0.385532
10	2.64	2.509404	0.130596
15	2.94	3.416768	-0.47677
16	3.82	3.640037	0.179963
17	3.46	3.455816	0.004184
18	3.3	3.249019	0.050981
19	4.0	3.476783	0.523217



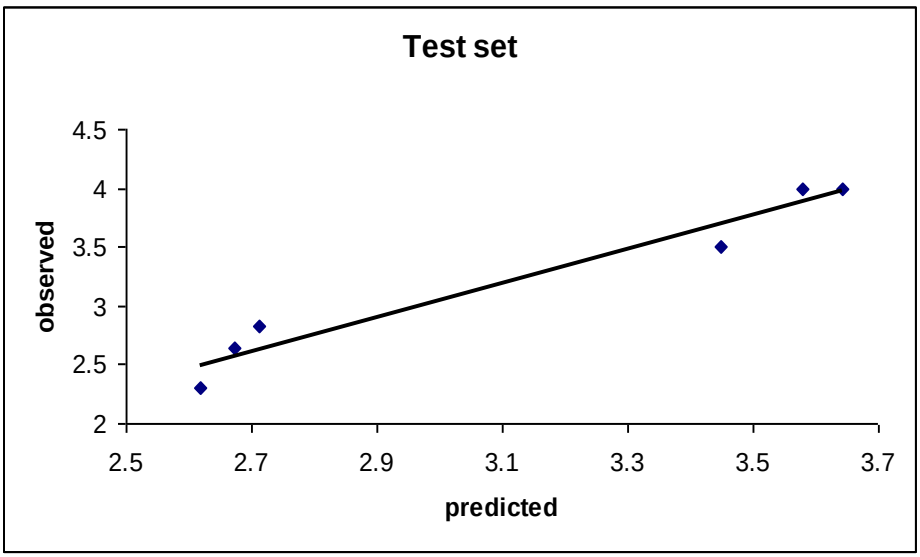
Graph 3: Plot of observed Vs predicted pMIC values for training set compounds (as per Equation 1)

8.4.7.4.2 External Cross-validation

Any model with excellent statistical characteristics (like r^2, r^2cv) and satisfactory predictions may lack true relationship between molecular descriptors and target property. To avoid chance correlation, a reliable validation procedure must be carried out. The ultimate validation of the model is examined by means of external validation. The quality of QSAR model is mostly determined by its ability to perform predictions of objects not included in the training sets. The real validation of QSAR model was carried out by examining residuals and predictive r^2 (r^2pred) value using test set compounds. The actual activity, predicted activity and residuals for test set compounds are shown in Table 38 and Graph no 4

Table 38: External cross-validation of test compounds as per Equation 1

Comp no.	pMIC (observed)	pMIC (predicted)	Residual
11	2.64	2.672823	-0.03282
13	2.82	2.713401	0.106599
14	2.3	2.618459	-0.31846
20	3.5	3.447364	0.052637
21	4	3.579259	0.420741
22	4	3.642632	0.357368



Graph 4: Plot of Observed Vs Predicted pMIC values for test set compounds (as per Equation 1)

The predicted activity of test set compounds was found to be very close to their actual activity as shown in Table 38, which indicates robustness of model. The residual observation is not only optimum criteria to validate the model. Further, the external predictability of the model was evaluated by calculating r^2pred value for a given model r^2pred is the predicted correlation coefficient calculated from the predicted activity of all the test set compounds and it is calculated by equation

$$r^2_{pred} = 1 - \frac{\Sigma (Y_{Pred(test)} - Y_{Obs(test)})^2}{\Sigma (Y_{Obs(test)} - \overline{Y}_{training})^2}$$

Where, Ypred (test) and Yobs (test) are the predicted and observed activity values, respectively, of the test set compounds and Ytraining is the mean activity value of the training set. A value of r^2 pred greater than 0.5 may be taken as an indicator of good external predictability (Roy *et al.*, 2007).

8.4.7.5 Discussion

In the present study, we have screened 5 preselected descriptors for 15 nitroimidazole derivatives multiple linear regression analysis. The inter-correlation of the descriptors used in the selected models was very low. The correlation matrix for the descriptors used is shown in Table 39.

Table 39: Inter correlation matrix of descriptors used in QSAR models

	partial charge	HOMO	LUMO	Clogp	molar volume	PMIC
partial charge	1					
HOMO	0.021	1				
LUMO	-0.022	0.832742	1			
Clogp	-0.188	-0.58237	-0.54162	1		
molar volume	-0.002	-0.43778	-0.30464	0.247901	1	
PMIC	-0.308	0.239394	0.207906	0.007626	-0.2698	1

The intercorrelation matrix was prepared the LUMO and HOMO is intercorrelated so one of the parameter HOMO was omitted from the model generation. To further check the inter-correlation of descriptors, variance inflation factor (VIF) analysis was performed. The VIF values of these descriptors were found to be 0.0001 (ClogP), 0.0001 (Molar volume), 0.0059 (partial charges). All the VIF values were found to be less than 10. Thus, from the VIF analysis, it is clear that the descriptors used in the final models have low inter-correlation.

The models were also evaluated for their capacity to predict the activity of training set and test set compounds, i.e., internal and external cross-validation, respectively. The results for the Equation 1 are summarized in Table 35 and 36. Graph 4 and 5 depicts the plots of observed vs. predicted activity for training and test set compounds, respectively. The models displayed satisfactory r^2pred. For all the models, r^2pred was found to be in the acceptable range. As expected, electronic parameter (Huckel extended partial atomic charges on oxygen) of compounds, with linear relationship, emerged as an indispensable descriptor for Nitroimidazole along with other structural, spatial and lipophilic descriptors.

8.5 References

Adams GE, Clarke ED, Flockhart IR. Structure-activity relationships in the development of hypoxic cell radiosensitizers I. Sensitization efficiency. Int J Radiat Biol 1979a;35:133-138.

Adams GE, Clarke ED, Gray P. Structure-activity relationships in the development of hypoxic cell radiosensitizers II. Cytotoxicity and therapeutic ratio. Int J Radiat Biol 1979b;35:151-154.

Adams GE, Flockhart IR, Smithen CE, Stratford IJ, Wardman P, Watts ME. Electron-affinic sensitization VII. A correlation between structures, one-electron reduction potentials, and efficiencies of nitroimidazoles as hypoxic cell radiosensitizers. Radiat Res 1976;67:9-12.

Adams GE, Fowler JF, Wardan P. Hypoxic cell sensitizers in radiobiology and radiotherapy. Br J Cancer 1978;37:(Suppl. III).

Adams GE, Michael BD, Asquith JC, Shenoy MA, Watts ME, Whillans DW. Rapid mixing studies on the timescale of radiation damage in cells. In Radiation Research: Biomedical, Chemical and Physical Perspectives. Ed. Nygaard et al. London: Academic Press; 1975 p:478.

Borman S. New QSAR techniques eyed for environmental assessments. Chem Eng News 1990;68:20-23.

Chem 3D Ultra 11.0, Chem Office Ultra, 2008. Cambridge scientific software: Cambridge soft corporate headquarters, 100 Cambridge Park Drive Cambridge, MA 02140, USA, 2008.

Contrera JF, Maclaughlin P, Hall LH, Kier LB. QSAR modeling of carcinogenic risk using discriminant analysis and topological molecular descriptors. Curr Drug Discov Technol 2005;2:55-67.

Crum-Brown A, Fraser TR. On the connection between chemical constitution and physiological action. Part 1. On the physiological action of the ammonium bases, derived from Strychia Brucia, Thebaia, Codeia, Morphia and Nicotia. Trans R Soc Edinburgh 1868;25:151-157.

David A, Winkler. The role of quantitative structure-activity relationships (QSAR) in biomolecular discovery. Brief Bioinform 2002;3:73-76.

Doweyko AM. The Hypothetical active lattice. An Approach to modeling active sites from data on inhibitor molecules. J Med Chem 1988;31:1396-1406.

Edwards DI. Nitroimidazole drugs-action and resistance mechanisms. I. Mechanisms of action. J Antimicrob Chemother 1993;31:9–20.

Edwards DI, Knox RJ, Rowley DA, Skolimowski IM, Knight RC. The biochemistry of nitroimidazole drug action. In The Host Invader Interplay. Ed. Van den Bossche. Amsterdam: Elsevier/North Holland; 1980b.p:673.

Eriksson L. Methods for reliability and uncertainty assessment and for applicability evaluations of classification and regression-based QSAR Environ Health Perspectives 2003;111:1361-1367.

Free SM, Wilson JW. A mathematical contribution to structure-activity studies. J Med Chem 1964;7:395-399.

Fujita T, Ban T. Structure activity studies of phenylethylamines as substrate of biosynthetic enzymes of sympathetic transmitters. J Med Chem 1971;14:148-152.

Fujita, T, Iwasa J, Hansch C. A new substituent constant, derived from partition coefficients. J Am Chem Soc 1964;86:5175-5183.

Gasteiger J, Engel T. Representation of chemical compounds. In Chemoinformatics: A Texbook. Eds.; John Wiley Publishing Co.: New York; 2003. p:403.

Grigoras S. A structural approach to calculate physical properties of pure organic substances: the critical temperature, critical volume and related properties. J Comp Chem 1990;11:493-510.

Hammett LP. Some relations between reaction rates and equilibrium constants. Chem Rev 1935;17:125-137.

Hansch C. A Quantitative Approach to Biochemical Structure–Activity Relationships. Acc Chem Res 1969;2:232-239.

Hansch, C. Quantitative structure-activity relationships and the unnamed science. Acc Chem Res 1993;26:147-153.

Hansch C, Fujita T, Geiger E, Streich M. The correlation of biological activity of plant growth regulators and chlormycetin derivatives with Hammett constant and partition coefficient. J Am Chem Soc 1963;85:2817-2819.

Hansch. C, Leo A. Exploring QSAR. In Fundamentals and Application in Chemistry and Biology; Hellen, S., Ed.; Am Chem Soc: Washington, DC, 1;1995. p:580.

Hoskuldsson A. In Prediction Methods in Science and Technology. Thor Publishing Co: Copenhagen. 1996.

Jaiswal M, Khadikar PV, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors: the first QSAR study on inhibition of tumor-associated isoenzyme IX with aromatic and heterocyclic sulfonamides. Bioorg Med Chem Lett 2004;14:3283-3290.

Knox RJ, Knight RC, Edwards DI. Interaction of Nitroimidazole Drugs Wit h DNA In Vitro:Structure-Activit y Relat ionships. Br J Cancer 1981;44:741-754.

Kowar TR. Genetic Function Approximation Experimental Design: A new method for experimental design. J Chem Inf Comput Sci 1998;38:858-866.

Kubinyi H. Lipophilicity and biological activity; drug transport and distribution in model system and biological system. Arzeneim-Forsch 1979;29:1067-1069.

Langer T, Wolber G. Pharmacophore definition and 3D searches. Drug Discovery Today: Technologies 2004;3:203-209.

Lauria A. Ippolito M, Almerico AM. Combined use of PCA and QSAR/QSPR to predict the drugs mechanism of action. An application to the NCI ACAM Database. QSAR. Comb Sci 2009;28:387-395.

Leach AR, Gillet VJ. Similarity methods. In An Introduction toChemoinformatics, Eds.; Springer Publishing Co.: The Netherlands; 2007. p:126.

Lill MA. Multi dimension QSAR in drug discovery. Drug Discovery Today 2007;12:1013-1017.

Martin YC. A practitioner's perspective of the role of quantitative structure activity analysis in medicinal chemistry. J Med Chem 1981;24:229-237.

Meyer H. Weiche eigenschdft der anaesthetica bedingt iher narkotische wirkung. Naunyn schmiedebergs Arch Exp Pathol Pharmacol 1899;42:109-119.

Mladenovic M, Vukovic N, Sukdolak S and Solujic S: Design of Novel 4-hydroxy-chromene-2-one derivatives as antimicrobial agents. Molecules 2010;15:4294-4308.

Montsenigos A, Ciubotariu D, Chiriac A, Simon Z. Rev Roumaine Chimie 1989;34:2101-2106.

Oveton E. Studien Uber die Narkose, Zugleich in Beitrag Zur allgemeinen Parmakologie Verlag von Gustav Fischer, Jena, Germany, 1901.

Perkins R, Fang H, Tong W, Welsh WJ. Quantitative structure-activity relationship methods perspectives on drug discovery and toxicology. Environ Toxico Chem 2003;22:3-5.

Pople JA. In Approximate Molecular Orbital Theory. Eds.; McGraw Hill Publishing Co: New York;1970.

Rekker RF. In Theoretical Drug Design Methods, Elsevier Publishing Co.:New York; 1984. p:256.

Reynolds AV. The activity of nitrocompounds against Bacteroides fragilis is related to their electron affinity. J Antimicrobial Chemother 1981;8:91-97.

Richet C, Seancs CR. QSAR and Molecular structure. Soc Biol Ses Fil 1893;9:775-758.

Robinson DD, Winn PJ, Lyne PD, Richards WG. Self-organizing molecular field analysis: a tool for structure-activity studies. J Med Chem 1999; 42:573-583.

Rogers D, Hopfinger AJ. Application of genetic function approximation to quantitative structure-activity relationships and quantitative structure-property relationships. J Chem Inf Comput Sci 1994;34:854-886.

Roy PP, Roy K. On some aspects of variable selection for partial least squares regression models. QSAR Comb Sci 2007;27:302-313.

Selassie CD. History of structure activity relationships. In Burger's Medicinal chemistry and drug discovery 6th ed. Vol 1; Abraham, D. J., Eds.; John Wiley & Sons, Inc: Virginia, 2003; pp 1-48.

Silverman BD, Platt DE. Comparative molecular moment analysis (CoMMA):3D-QSAR without molecular superposition. J Med Chem 1996;39(11):2129-2140.

Simon Z. MTD and Hyperstructure Approaches. In 3D QSAR and Drug Design Eds.; Escom Publishing Co: The Netherlands;1993. p: 307-319.

Simon Z, Chiriac A, Holban S, Ciubotariu D, Mihalas GI. Minimum Steric Difference. In The MTD Methods for QSAR Studies Eds.; John Wiely Publishing Co.: New York;1984. p: 7.

Simon Z, Dragomir N, Plauchitiu MG, Holban S, Glatt H, Kerek F. Receptor site mapping for cardiotoxic aglicones by the minimal steric difference method. Eur J Med Chem 1980;15:521-527.

Sotriffer C, Klebe G. Docking and scoring functions/virtual screening. In Burger's Medicinal Chemistry and Drug Discovery, 6th ed.; Abraham, D. J.,Eds.; John Wiley Publishing Co: New York; 2003.p: 281-.332.

Stratford IJ, Adams GE. The effect of hyperthermia on differential cytotoxicity of a hypoxic cell radiosensitizer Ro-07-0582 on mammalian cells in vitro. Br J Cancer 1977;35: 307-315.

Sugiyama Y, Ohtani II, Isobe M, Takai A, Ubukata M, Isono K. Molecular shape analysis and activity of tautomycin, a protein phosphatase inhibitor. Bioorg Med Chem Lett 1996;6:3-8.

Todeschini R, Consonni V. In Handbook of Molecular Descriptors. Eds.;Wiley-VCH: Weinheim, 2000.

Wardman P. In chemotherapeutic strategy ed. Edwards DI and Hiscocck DR, Macmillan press, London; 1983.p:173.

Zahoor A, Knight RC, Whitty PW, Edwards DI. Satranidazole: mechanism of action on DNA and structure-activity correlations. J Antimicro chemother 1986;18:17-25.

CONCLUSION

The inhibition cut off value (sensitivity) of about 20 compounds from all the three series of synthesized nitroimidazole were selected on the basis of their one electron reduction potential, were carried out against *Clostridium sporogenus* & *Bacteriodes fragilis* using metronidazole as control drug.

All the compounds sensitivity was found same as the metronidazole (control standard) at 10 µg/mL concentration against the Gram negative bacteria; *B. fragilis*. Some of the synthesized compounds were found more potent then metronidazole at 20 µg/mL concentrations against Gram positive bacteria i.e., *C. sporogenus*, the compound number were (59-d, 67-c, 74-k, 74-d, 74-I, 74-h, 74-j). Compound 59-d belongs to the nitroimidazole-oxime derivative; 1-(2-chlorophenyl)-2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)ethanone while compound number 67-c is 6-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-5-(furan-2-yl)-4*H*-1,3,4-thiadiazin-2-amine. Compounds 74-k, 74-d, 74-I, 74-h and 74-j were belonging to 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinylsubstitutedamine series.

Thus it was concluded that most potent series against Gram positive anaerobe; *C. sporogenus* was nitroimidazole-enamine series i.e., 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinylsubstitutedamine which were more active as compared to the metronidazole.

The compound 74-k i.e., 1-ethyl-6-fluoro-8-(4-(1-(furan-2-yl)-2-(1-methyl-5-nitro-1*H*-imidazol-2-yl)vinyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylicacid) were dimetridazole-norfloxacin enamine derivatives and 74-j i.e., 1-ethyl-8-(4-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylicacid, belongs to tinidazole-norfloxacin enamine derivatives, thus it indicates the increase in the potency of activity against gram positive anaerobe by fusion of quinolone moiety with nitroimidazole drugs, which acts as hybrid antibacterial to show dual activity against both anaerobic and aerobic bacteria due to nitroimidazole and quinolone moiety in a single molecule. Surprisingly it was found that Isoniazide-tinidazole enamine derivatives compound 74-i that was *N*-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-

imidazol-2-yl)-1-(furan-2-yl)vinyl)isonicotinohydrazide were inactive against Gram positive organism while active against Gram negative organism.

QSAR study of literature nitroimidazole class drug, establish significant correlation between antibacterial activity and Extended huckel partial atomic charges on oxygen of nitro group present in nitroimidazole against *Bacteriodes fragilis* for the reported nitroimidazole compounds. The correlation is reverse to those obtained for cytotoxicity, mutagenicity and DNA damage acitivity because the more electron affinity of the drugs were found in between -0.80 to -0.84 (Extended huckel partial atomic charges on oxygen of nitro group present in nitroimidazole) have greatest potency against the test organism and also its is range of reduction for nitroimidazole in anaerobic condition. The correlation was exactly reverse for the toxicity of aerobic bacteria, it concludes that most electron affinic drug are reduced in the aerobic condition while compounds having less electron affinity and having low reduction potential were showing activity against anaerobes. The addition of partition coefficient that was lipophilic parameter values produced positive correlation, imparting better regressed line and increasing the correlation. However the molar volume and other steric factor were not influencing the activity of nitroimidazoles. The other electronic parameters such as LUMO, HOMO was found to be producing no significant change in the R value.

It was concluded that nitroimidazole falling under -0.80 to -0.84 (Extended huckel partial atomic charges on oxygen of nitro group present in nitroimidazole) are considered to be safest drug for anaerobic infections. If Extended huckel partial atomic charges on oxygen of nitro group present in nitroimidazole less, than -0.84 is not reduced under anaerobic condition and greater than that -0.80 probably cytotoxicity in nature and produce DNA damage. All the 2-nitroimidazole taken in study shows value of Extended Huckel partial atomic charges on oxygen of nitro group present in nitroimidazole greater than -0.80, imparting cytotoxicity. That is shown in Fig 6, below.

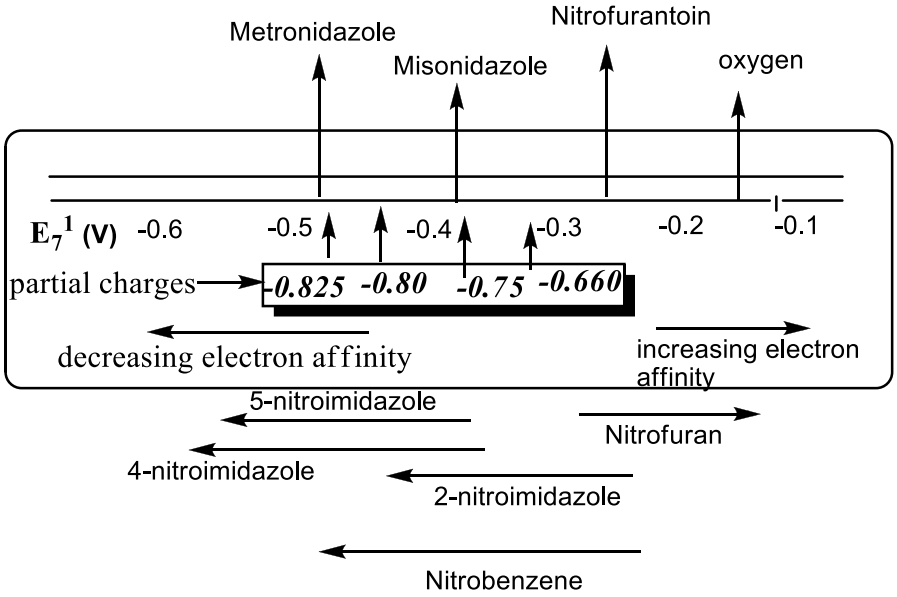


Fig 6 (modified): The redox spectrum showing the elctron affinity (E_7^1) of nitroaromatic drugs and calculated Extended Huckel partial atomic charges on oxygen showing an active range as (-0.80 to -0.825) for various biological activities of nitroimidazole drugs.

Thus it was concluded that as the one electron reduction potential can governs the DNA damage capacity, cytoxicity, mutagenicity, good anaerobic activity and aerobic toxicity. Computed Extended huckel partial atomic charges on oxygen of nitro group present in nitroimidazole can be used to correlates all above biological activity significantly, of nitroimidazole class drugs before synthesizing the compounds and with out taking any polarographic half wave reduction potential or one electron reduction potential practically.

SUMMARY

Chapter one, describes the brief overview of five member heterocycles-nitroimidazole its classification, mechanism of action, toxicity and resistance. It also emphasis on the anaerobic infection, its type and latest strategy for treatment.

Chapter two, highlights the review of nitroimidazole class drugs, were it describes biological review and chemistry. Biological review was regarding nitroimidazole-activity against bacteria, mycobacterium, HIV, protozoa and radio sensitivity. The chemistry reveals the geometry and conformation of nitroimidazole, its dipole moment, electrostatic potentials, relative proton affinities and relative electron affinities influencing the activity and toxicity of the drugs.

Chapter three describes the aim of present work. Its discusses the main aim of investigations. The investigation was aimed to; 1) synthesis of 2-substituted nitroimidazole and to carry out anti-anaerobic activity 2) to develop QSAR model for the nitroimidazole class drugs.

Chapter four indicates synthesis of some oxime derivatives of nitroimidazole and their characterization by IR, MASS and ^{1}HNMR spectral analysis.

Chapter five indicates synthesis of nitroimidazole substituted with various heterocyclic rings and their characterization by IR, MASS and ^{1}HNMR spectral analysis.

Chapter six indicates synthesis of various enamine derivatives of nitroimidazole and their characterization by IR, MASS and ^{1}HNMR spectral analysis.

Chapter seven, describes the anti-anaerobic activity of twenty compounds from all three synthesized nitroimidazole series, selected on the basis of one electron reduction potential, against Gram positive anaerobic bacteria (*C. sporogenus*) and Gram negative anaerobic bacteria *(B. fragilis),* all compounds potency were found same as metronidazole against Gram negative bacteria, while some of the compounds were active against Gram positive bacteria and were found more potent as compared to metronidazole.

Chapter eight reveals, establishment of electronic physiochemical parameter; Extend huckel partial atomic charges on oxygen of nitroimidazole and QSAR study of literature nitroimidazole derivatives. The QSAR study shows that Extend huckel partial charge on oxygen of nitroimidazoles act as powerful electronic parmeter to predict the biological activity, DNA damage, cytotoxicity, mutagenicity of nitroimidazole class drugs.

Chapter nine describes the conclusion of the research project. It was concluded as,

1) The most potent series against Gram positive anaerobe; *C. sporogenus* was nitroimidazole-enamine series i.e., 1-(2-(1-(2-(ethylsulfonyl)ethyl)-5-nitro-1*H*-imidazol-2-yl)-1-(furan-2-yl)vinylsubstitutedamine which were more active as compared to the metronidazole.

2) QSAR study concludes that as the one electron reduction potential can governs the DNA damage capacity, cytoxicity, mutagenicity, good anaerobic activity and aerobic toxicity. Computed Extended huckel partial atomic charges on oxygen of nitro group present in nitroimidazole can be used to correlates all above biological activity significantly, for nitroimidazole class drugs, before synthesizing the compounds and with out taking any polarographic half wave reduction potential or one electron reduction potential practically.

PHARMA SCIENCE MONITOR
AN INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES

EXTENDED HUCKEL PARTIAL ATOMIC CHARGES OF NITROIMIDAZOLE AND PREDICTION OF DNA DAMAGE

B.R.Prajapati *[1], A.K.Seth[2], K.I.Molvi[3], I.S.Rathod[4], B.N.Suhagia[5], M.M.Mansuri,[6] D.G.Desai[7]

[1,4 & 5] L.M.College of Pharmacy, Navrangpura, Ahmedabad-09, Gujarat-India.
[2] Department of Pharmacy, Sumandeep Vidyapeeth, Pipariya,Gujarat-India.
[3] Ali-Allana College of Pharmacy, Department of Pharmaceutical Medicinal Chemistry, Akkalkuawa, Nandurbar, 425415 – India.
[6,7] P&M Kansara Pharmacy College, Pipariya, Gujarat –India

ABSTRACT

Nine nitroimidazoles drugs representing different class were found in the literature to have their respective half wave potential and one electron reduction potential measure under near-identical conditions. The structures of these nine nitroimidazoles were optimized, and resulting physicochemical parameters were regressed against one electron reduction potential (OERPs). A very significant linear correlation (QSAR) was found between OERPs and Extended Huckel Partial Atomic Charges. These findings suggest that it may be possible to estimate OERPs of nitroimidazole in advance to its synthesis. Insofar as the OERPs correlating to DNA damage, radiosensitization efficiency, aerobic cytotoxicity, mutagenicity and hypoxic cytotoxicity; the Extended Huckel Partial Atomic Charges can predict well all these biological activity prior to synthesis of novel nitroimidazoles.

Keywords: One electron reduction potential, nitroimidazole, partial charge, half wave potential.

INTRODUCTION

Nitroimidazole comprises a large group with useful clinical activity as antibacterial, antiprotozoal and anticancer agents. With all nitro compounds their activity is solely depend upon reduction of nitro group, the metabolic products of which are responsible for the DNA damage. The key to understanding the basis of selective toxicity and biological activity of these drugs lies in the knowledge of the range of polarographic determined one electron reduction potentials exhibited by such compounds. Fig: 1 shows the one electron reduction potential of electron affinity exhibited by nitroaromatic compounds [1].

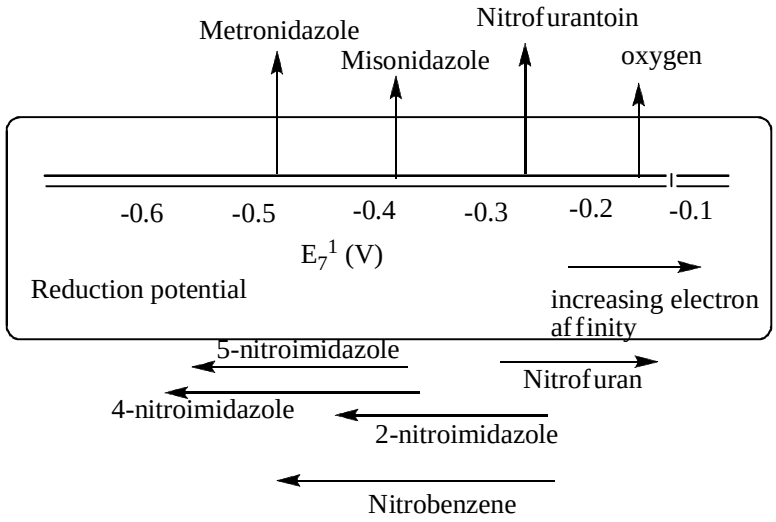


Figure:1 The redox spectrum showing the elctron affinity (E_7^1) of nitroaromatic drugs. Oxygen is the most electron affinic and drugs of lower potential are less easily reduced

The boundary falls at -0.350 V to the right of this division, redox reactions are easily performed under the aerobic conditions; in fact the most negative of redox reactions carried out by the aerobic cell is that involving NAD(NADP)-NADH(NADPH) couples at -0.35V. Thus redox reactions more negative (lower) the -0.350 V is not possible under aerobic conditions, but are possible under anaerobic conditions. The redox potential of bioreducicble drugs is therefore crucial beginning in understanding how these compounds may be applied to combat diseases which flourish under redox environment [2].

The basis of structure-activity relationships in the development of electron-affinic nitroheterocyclic hypoxic cell, radiosensitizers, DNA damage and anaerobic activity is a linear correlation of the type:

$$-\log C = bo + bi\ E + b_2 \log P + b_3 (\log P)^2 \ldots\ldots\ldots\ldots\ldots \quad (1)$$

C = the drug concentration required to cause specific relevant biological effect.

E= the electron affinity usually expressed as the one-electron redox potential (E_7^1)

P= the octanol/water partition coefficient of the drugs

Adams et al. has shown that there is negligible effect of lipophilicity (P) on radiosentitizing and cytotoxicity. Thus the coefficient b2 and b3 may be omitted.

$$-\log C = bo + b_1E \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

Thus it is well-established that the E_7^1 value correlates positively with radiosensitization efficiency[3], aerobic cytotoxicity [4] , mutagenicity[5] and hypoxic cytotoxicity[6]. The more electron-affinic the drug (the more positive the E_7^1 value) the greater the radiosensitization and cytotoxicity, which varies in general by an order of magnitude for each 100mV change in E_7^1.

The objective of the research work is to find out some physicochemical parameter which can correlates with experimental determined one electron reduction potential.

MATERIAL AND METHODS

A) Electrochemical method

Knox et al [7] reported the $E_{1/2}$ as the Polarographic half-wave potential (HWPs) in volts measured against Ag/AgCl reference electrode at pH 7.0 and E_7^1 as one-electron reduction potential (OERPs) in volts measured against the normal hydrogen electrode. Table I, shows the $E_{1/2}$ and E_7^1 of nine standard nitroimidazole compounds considered in the study along with their structure and DNA damage.

B) Computational method

The molecular geometries of each of the nine compounds in Table I were built by using standard bond lengths and angles with Chem. Office Ultra 11.00 (Chem Draw ultra 2008)[8]. These structures were initially optimized using MM_2 force field method until the root mean square gradient value becomes smaller than 0.001 Kcal/mol. The resulting optimized structure was processed through the Extended Huckel Partial Atomic Charges on oxygen of nitro group in nitroimidazole [9], LUMO and HOMO as electronic parameters. The CLogp and Molar volume was considered as lipophilic and steric parmeter in the study. The various physicochemical parmeters were regressed against one electron reduction potential and Log DNA damage using multiple linear regression analysis.

RESULTS AND DISCUSSION

The calculated Extended Huckel Partial Atomic Charges on oxygen of nitro group in nitroimidazole as depicted in the figure:2, shows a good correlation with one electron

reduction potential while other parmeters like Clog P, LUMO, HOMO and molar volume does not contributes significantly.

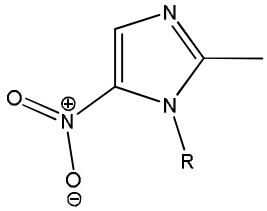


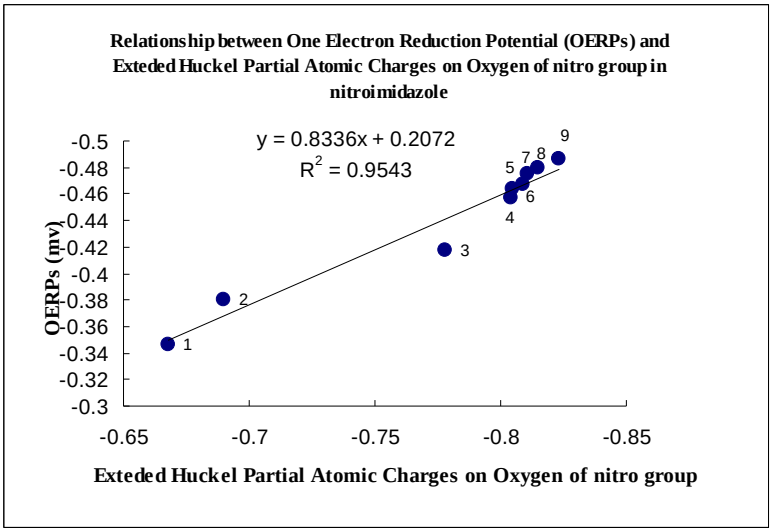
Figure: 2
Extended Huckel Partial Atomic Charges on oxygen of Nitroimidazole.

Thus Extended Huckel Partial Atomic Charges on oxygen of nitro group is considered as important parmeter in generation of model while omitting the lipophilic and steric physicochemical parameters.

TABLE 1: ELECTRONIC PARAMETRES OF NITROIMIDAZOLE

No	Drug	$E_{1/2}$(V) (HWPs) (Obsd)	E_7^1 (V) (OERPs) (Obsd)	Log DNA damage (Obsd)	Extended Huckel Partial Atomic Charges on oxygen of nitro group
1	Pimonidazole	-0.180	-0.346	-0.660	-0.668
2	Benznidazole	-0.200	-0.380	-0.569	-0.690
3	Azomycin	-0.374	-0.418	-0.222	-0.778
4	Nimorazole	-0.345	-0.457	-0.393	-0.804
5	Tinidazole	-0.340	-0.464	0.104	-0.805
6	Ornidazole	-0.345	-0.467	-0.131	-0.809
7	Dimetridazole	-0.388	-0.475	0.017	-0.811
8	Secnidazole	-0.390	-0.480	0.013	-0.815
9	Metronidazole	-0.382	-0.486	-0.022	-0.823

The correlation between one electron reduction potential and Extended Huckel partial atomic charges on oxygen of nitro group of nitroimidazole is established as shown in graph 1.

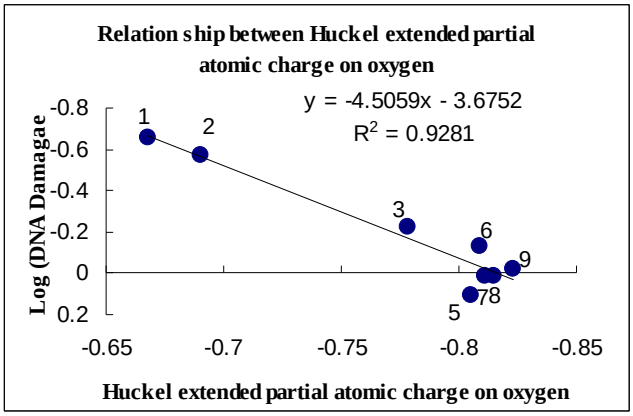


Graph: 1

TABLE 2: VALIDATION OF ONE ELECTRON REDUCTION POTENTIAL OF STANDARD NITROIMIDAZOLE AND EXTENDED HUCKEL PARTIAL ATOMIC CHARGE ON OXYGEN.

Description	**r**	**r^2**	**r^2 (adj)**	**F value**	**Least squared error**
OERPS=0.8336*(Partialcharge) +0.2072	0.9768	0.9542	0.9477	146.0945	0.0112

The DNA damage activity of standard nitroimidazole, showing a good correlation with Extended Huckel Partial atomic charge as shown in the graph 2.



Graph: 2

TABLE 3: VALIDATION OF EXTENDED HUCKEL PARTIAL ATOMIC CHARGES ON OXYGEN OF STANDARD NITROIMIDAZOLE AND DNA DAMAGE

Description	r	r^2	r^2 (adj)	F value	Least squared error
Log DNA damage = -4.5059* (Partial charge) -3.6752	0.9633	0.9281	0.9160	77.41	0.08248

The reported E_7^1 (One electron reduction potential) of compound was found in the range of -0.257 V to -0.500 V which indicate the range of active nitroimidazole. A good correlation between one electron reduction potential and calculated Extended Huckel partial atomic charge on oxygen of nitro group was obtained having, r = 0.9768 , r^2=0.9542, $r^2_{(adjusted)}$ = 0.9477, F value = 146.0945, standard error = 0.0112 as shown in graph 1. Further the direct correlation between Calculated Huckel Extended partial negative charge on oxygen and Log DNA damage activity is also established. r = 0.9633, r^2=0.9281, $r^2_{(adjusted)}$ = 0.9160, F value = 77.41, standard error = 0.08248 as per graph 2. The active range of various nitroimidazole class drugs as per Extended Huckel partial atomic charge on oxygen of can be established as -0.778 to -0.820.

CONCLUSION

Computed Extended Huckel partial atomic charge on oxygen can be used to predict radiosensitization efficiency, aerobic cytotoxicity, mutagenicity and hypoxic cytotoxicity, DNA damage and anaerobic activity of various novel nitroimidazole class drugs prior to synthesis. The advantage of this model is that it does not require any experimentally calculated one electron reduction potential.

AKNOWLEDGEMENT

The authors are thankful to, Department of Medicinal Chemistry, L.M.College of Pharmacy Ahmedabad, Gujarat, India, for providing necessary facilities.

REFERENCES

1. Wardman P. In chemotherapeutic strategy ed. Edwards DI and Hiscocck DR, Macmillan press, London 1983;173.

2. Zahoor A, Knight RC, Whitty PW and Edwards DI: Satranidazole: mechanism of action on DNA and structure-activity correlations. J Antimicro chemother 1986;18(1):17-25.
3. Adams GE, Flockhart IR, Smithen CE, Stratford IJ, Wardman P and Watts ME: Electron-affinic sensitization VII. A correlation between structures, one-electron reduction potentials, and efficiencies of nitroimidazoles as hypoxic cell radiosensitizers. Radiat Res 1976;67:9.
4. Adams GE, Clarke ED and Flockhart IR: Structure-activity relationships in the development of hypoxic cell radiosensitizers. I. Sensitization efficiency. Int J Radiat Biol 1979a;35:133.
5. Adams GE, Clarke ED, Gray P & 4 others: Structure-activity relationships in the development of hypoxic cell radiosensitizers II. Cytotoxicity and therapeutic ratio. Int J Radiat Biol 1979b;35:151.
6. Adams GE, Michael BD, Asquith JC, Shenoy MA, Watts ME and Williams DW: Rapid mixing studies on the timescale of radiation damage in cells. In Radiation Research: Biomedical, Chemical and Physical Perspectives. Ed. Nygaard et al. London: Academic Press 1975; 478.
7. Knox RJ, Knight RC and Edwards DI: Interaction of Nitroimidazole Drugs With DNA In Vitro:Structure-Activity Relationships. Br J Cancer 1981;44:741.
8. *Chem 3D Ultra 11.0, Chem Office Ultra, 2008*. Cambridge scientific software: Cambridge soft corporate headquarters, 100 Cambridge Park Drive Cambridge, MA 02140, USA, 2008.
9. Mladenovic M, Vukovic N, Sukdolak S and Solujic S: Design of Novel 4-hydroxy-chromene-2-one derivatives as antimicrobial agents. Molecules 2010;15:4294-4308.

For Correspondence:
Beenkumar R. Prajapati
E-mail: beenprajapati26@yahoo.co.in