"Synthesis And Pharmacological Evaluation Of Some Substituted Coumarin Derivatives"

Thesis submitted for the degree of

Doctor of Philosophy (Ph.D.) in

Pharmaceutical Sciences

BY

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JULY 2011

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Certificate

I certify that the thesis titled **"SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME SUBSTITUTED COUMARIN DERIVATIVES"** submitted for the Degree of Doctor of Philosophy by **Mr. MUSTAKIM M. MANSURI** 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.

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At last I am thankful to almighty God, the creator of world, without whom nothing is possible.

July 2011 (Mustakim M. Mansuri)

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ABBREVIATIONS

cAMP	3',5'-cyclic monophosphate
ADME	Absorption, distribution, metabolism and excretion
AChE	Acetylcholinesterase
AA	Arachidonic acid
FAS-II	Bacterial fatty acid synthase
COX	Cyclooxygenase
CDK	Cyclin dependent kinase
°C	Degree celcius
DMARDs	Disease modifying anti-rheumatoid drugs
ECM	Extracellular matrix
Gm	Gram
HTS	High throughput screening
hr	Hour
HPETEs	Hydro peroxy eicosa tetra enoic acid
Ig-E	Immunoglobulin E
IBD	Inflammatory Bowl Disorder
IL	Interleukin
LT	Leukotrienes
LO	Lipoxygenase
FAS-I	Mammalian fatty acid synthase
MIF	Migration inhibitor factor
MIC	Minimum inhibitory concentration
M.W.	Molecular weight
MAO	Mono amino oxidase
NME	New medicinal entity

NSAIDS	Non steroidal anti-inflammatory drugs
PPM	Parts per million
%	Percentage
PDE	Phosphodiestrase
PAF	Platelet activating factor
PPP	Platelet poor plasma
PRP	Platelet rich plasma
PC	Prostacycline
PG	Prostaglandins
QSAR	Quantitative structure activity relationship
ROS	Reactive oxygen species
Rf	Retention factor
r.t.	Room temperature
TMS	Tetramethyl silane
TLC	Thin layer chromatography
TX	Thromboxanes
TOX	Toxicology
TNF	Tumor necrosis factor
Vc	Volume of oedema in control compounds
Vt	Volume of oedema in test compounds
Add-MMa	1-(α-carbomethoxy-β-amino-thiocrotonoyl)-aniline
Add-MMe	1-(α-carbomethoxy-β-amino-thiocrotonoyl)-benzoyl amine
Add-MMg	1-(α-carbomethoxy-β-amino-thiocrotonoyl)-ethyl amine
Add-MMf	1-(α-carbomethoxy-β-amino-thiocrotonoyl)-furoyl amine
Add-MMd	1-(α-carbomethoxy-β-amino-thiocrotonoyl)-p-bromo aniline

Add-MMc	1-(α-carbomethoxy-β-amino-thiocrotonoyl)-p-chloro aniline
Add-MMb	1-(α-carbomethoxy-β-amino-thiocrotonoyl)-p-methyl-aniline
Add-M2	2-chloro-N-(morpholine carbothioly)-benzamide
Add-Pz2	2-chloro-N-(piperazine-1-thioly)-benzamaide
Add-P2	2-chloro-N-(piperidin-1-thioly)-benzamaide
MM3M1	3-(2-morpholin-4-yl-4-phenyl-thiazole-5-carbonyl)-chromen-2-one.
MM3M2	3-(4-(2-chloro-phenyl)-(2-morpholin—4-phenyl-thiazole-5-carbonyl)-chromen-2-one.
MM3P2	3-(4-(2-chloro-phenyl)-(2-piperidin-1-yl-thiazole-5-carbonyl)-chromen-2-one.
MM3M3	3-(4-(3-chloro-phenyl)-(2-morpholin—4-phenyl-thiazole-5-carbonyl)-chromen-2-one.
MM3P3	3-(4-(3-chloro-phenyl)-(2-piperidin-1-yl-thiazole-5-carbonyl)-chromen-2-one.
MM3M4	3-(4-(4-Chloro-phenyl)-(2-Morpholin—4-phenyl-thiazole-5-carbonyl)-chromen-2-one.
MM3P4	3-(4-(4-Chloro-phenyl)-(2-Piperidin-1-yl-thiazole-5-carbonyl)-chromen-2-one.
MM3Pz1	3-(4-Phenyl-2-piperazin-1-yl-thiazole-5-carbonyl)-chromen-2-one.
MM3P1	3-(4-Phenyl-2-piperidin--1-yl-thiazole-5-carbonyl)-chromen-2-one.
MMmPz1	3-[2-(4-Methyl-piperazin-1-yl)-4-phenyl-thiazole-5-carbonyl]-chromen-2-one.
MM3Pz2	3-[4-(2-Chloro-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one.
MM3Pz3	3-[4-(3-Chloro-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one.
MM3Pz4	3-[4-(4-Chloro-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one.
Add-M3	3-chloro-N-(morpholine carbothioly)-benzamaide
Add-Pz3	3-chloro-N-(piperazine-1-thioly)-benzamaide

Add-P3	3-chloro-N-(piperidin-1-thioly)-benzamaide
Add-M4	4-chloro-N-(morpholine carbothioly)-benzamaide
Add-Pz4	4-chloro-N-(piperazine-1-thioly)-benzamaide
Add-P4	4-chloro-N-(piperidin-1-thioly)-benzamaide
MM1f	Methyl-2-(2-furoylamino)-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate.
MM1d	Methyl-2-(4-bromoanilino)-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate.
MM1c	Methyl-2-(4-chloroanilino)-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate.
MM1b	Methyl-2-(4-methylanilino)-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate.
MM1a	Methyl-2-anilino-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate.
MM1e	Methyl-2-benzoylamino-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate.
MM1g	Methyl-2-ethylamino-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate.
ENA-1	methyl-β-amino crotonate
Add-mPz1	N-(4-methylpiperazine-1-thioyl)benzamide
Add-M1	N-(morpholine-4-thioyl) benzamide
Add-Pz1	N-(piperazine-1-thioyl) benzamide
Add-P1	N-(piperidin-1-thioyl) benzamide

ABSTRACT

Inflammation occurs as a defensive response, which induces physiological adaptations to limit tissue damage and remove the pathogenic infections. Diseases caused by inflammation are an important factor of morbidity and mortality in humans. It is a fundamental pathologic process consisting of a dynamic complex of cytological and chemical reactions that occur in the affected blood vessels and adjacent tissues (connective tissues), in response to an injury or abnormal stimulation caused by a physical chemical or biological agents resulting in;

(i) The local reactions and resulting morphologic changes.

(ii) The destruction or removal of the injurious material.

(iii) The responses that lead to repair and healing.

The so called “cardinal signs” of rubor (redness) calor (heat), tumor (swelling) dolor (pain).

Platelets provide the initial haemostatic plug at sites of vascular injury. They also participate in reaction that leads to atherosclerosis and pathological thrombosis in numerous animal studies. Antagonists of platelet function have thus been used in attempts to prevent thrombosis and to alter the natural history of atherosclerotic vascular disease. As one of the cyclooxygenase product is thromboxane $A_{2.}$ Ryn reported aspirin as dual inhibitor as it block production of thromboxane A_2 by covalently acetylating a serine residue near the active site of cyclooxygenase, it is used as antiplatelet agent.

Thamotharan indicated that the coumarin compounds have good anti-inflammatory activity. Coumarin compounds having significant bleeding tendency. Coumarin compound cannot use as alone therapy due to its slow onset of action. An immediately acting anti-coagulant such as heparin is always co-administered with coumarin derivative for treatment of active thrombosis.

Corinne and coworker reported that substitution at 3^{rd} position of coumarin nucleus modulated AChE as well as MAO-B inhibitory activity. Substitution with methyl

groups at 3rd and 4th position led to more active compounds toward both AChE and MOA-B enzymes.

Pillai A and Molvi KI have reported novel tetrasubstiuted thiophene as dual inhibitor enzymes in inflammation pathway (COX and LOX). Similarly novel substituted thiazole derivatives also reported by Franclin. Substituted coumarin derivatives inhibiting more than one pathway was found to be effective as an anti-inflammatory chemical entity is also reported by above workers.

The number of antibacterial compounds available in market are belongs to penicillin derivatives and sulpha drugs, which belongs to sulphur containing heterocyclic ring (thiazole and thiazolidine).

Multi-drug treatment of inflammatory conditions associated with microbial infections poses a unique problem especially for patients with impaired liver or kidney functions. Husain A and coworker have reported anti-inflammatory and analgesic compounds with antimicrobial activity. Therefore, from the pharmacoeconomic and patient compliance points of view, the mono therapy with a drug having anti-inflammatory and antimicrobial activities is highly desirable.

In view of above reports the investigation was aimed to synthesize new alternative and more effective 3-substituted coumarin liked at 5th position to thiophene and thiazole respectively. In anticipation that 3-substituted coumarin moiety will be acting as anti-platelet and five membered sulphur containing heterocyclic compounds (thaizole and thiophene) acting as anti-inflammatory and antibacterial. (Figure A).

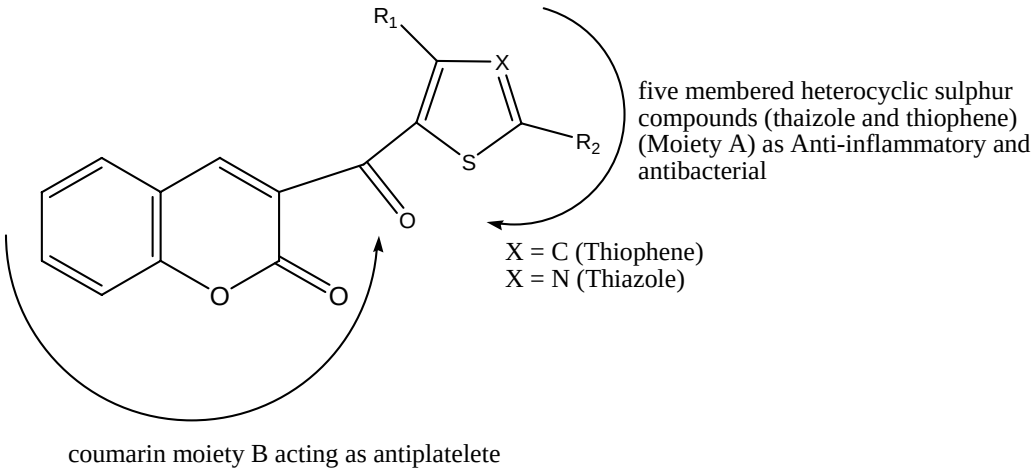

Figure A: Basic structure of targeted compounds.

All the synthesized tetra-substituted thiophene-coumarin compounds (MM1a- MM1g) were evaluated for their invivo anti-inflammatory and analgesic activity. On the basis of structure-activity relationship studies of synthesized thiophene compounds it can be concluded that presence of -Cl group in anilino moiety (MM1c), benzoyl (MM1e) and 2-furoyl moiety (MM1f) attached to -NH at the second position of the thiophene contributes to anti-inflammatory and analgesic activity profile of the candidates.

Among all the synthesized coumarin-thiazole derivatives, basic structural difference is at 2^{nd} position namely morpholine, piperidine, and piparazine and N-methyl piperazine. Antibacterial activity in all piperidino compounds against gram negative microorganism i.e. *Escherichia coli* was found comparable and consistent. Whereas, almost similar results were observed in all other compounds against gram positive microorganism i.e. *Staphylococcus aureus* and *Bacillus subtilis*. The anti-platelet activity was found consistent and comparably greater in all the phenyl substituted piperidino compounds. Whereas it was moderate to poor in morpholino, piperazino,N-methyl-piperazino compounds. Based on this results it is concluded that an increase in activity in piperidino compounds against gram negative bacteria and anti-platelet activity may be because of lipophilic nature of piperidine ring.

CHAPTER I
INTRODUCTION TO INFLAMMATION

1.1 Introduction:

Discovery and development of drug is a laborious process. Drug discovery covers everything from basic disease mechanisms, the validation of targets, the chemistry and screening that accompany the discovery and optimization of lead compounds through down stream ADME/TOX (absorption, distribution, metabolism, excretion and toxicology), clinical studies to marketing of new medicinal entity (NME) in an appropriate and convenient dosage form. It takes 10-15 years and $500-800 million to bring a drug to market (Editorial 2003)**.** The human and other genome projects and the rapidly expanding proteome studies provide enormous opportunities to obtain a greater understanding of the chemical basis and the molecular pathways and entities responsible or have a causal in health and disease. Molecular biology, automated bioassays and rapid developments in combinatorial chemistry accelerate drug discovery process and greater understanding of the physiological mechanism has made it possible for a more mechanistic approach to research and start from a rationally argued manner.

The aim of modern drug research is to design excellent drugs in terms of spectrum, profile of activity, absorption characteristics, potency, and duration of action and with minimum adverse effects. To achieve these goals, emerging new drug discovery technologies such as genomics, high throughput screening (HTS), miniaturization, structure based drug design, rational drug design, and sometimes serendipity has give rise to a plethora of biological targets and chemical leads to explore. The time chart of drug discovery and development is outlined in Figure1.1 (David 1995).

From the standpoint of market potential and unmet medical need, the therapeutic areas of,

(1) Inflammation/pain/immunology modulation
(2) Cardiovascular
(3) Central nervous system and
(4) Anti-invectives are considered to be important cornerstones or pillars of pharmaceutical research.

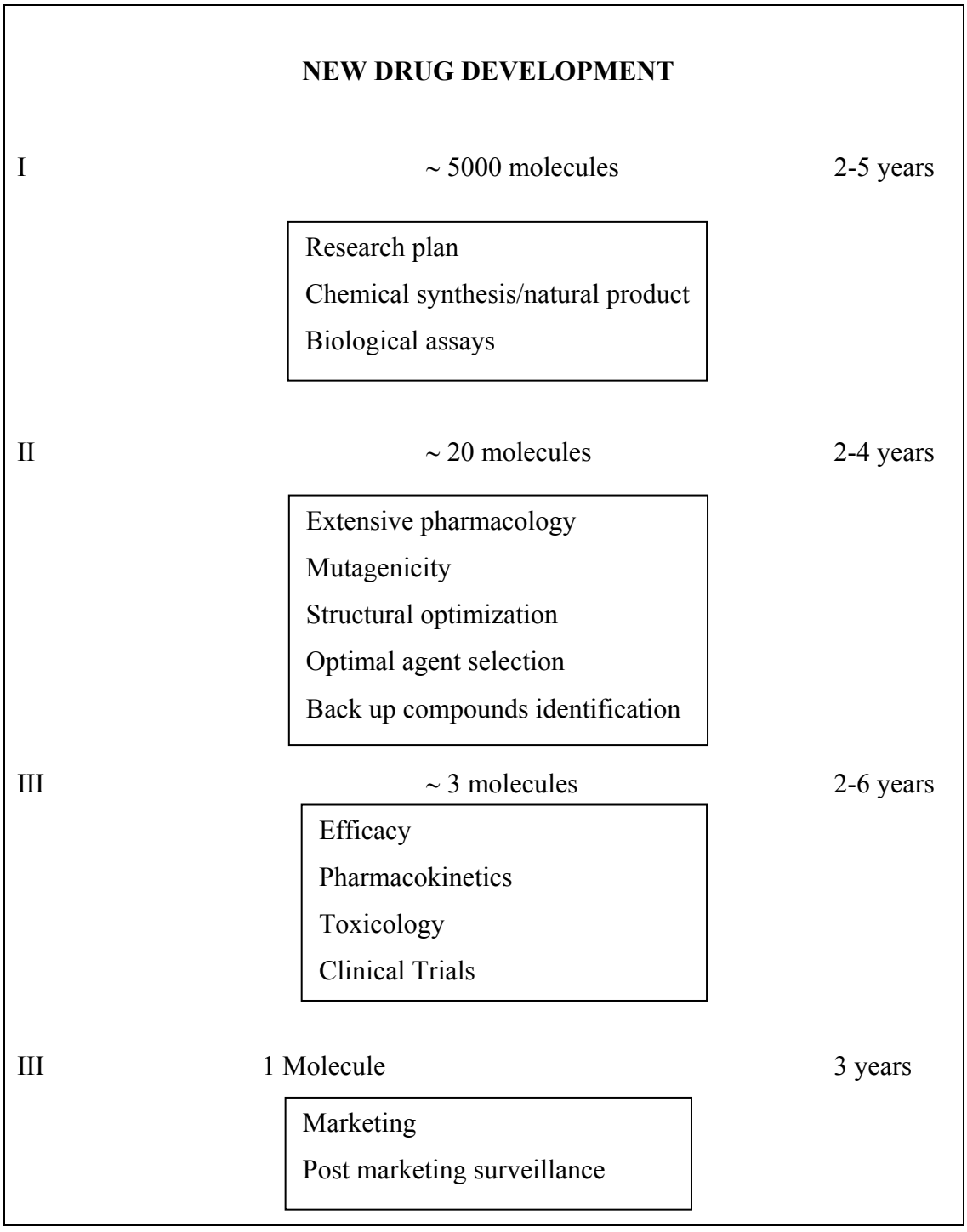

Figure 1.1: Time chart of drug discovery and development.

Inflammation/pain/immunology therapeutic areas are major ones from the view point of patients, primarily because the existing drugs for these areas often tend to treat the symptoms and do not address the root cause of the disease process.

1.2 Inflammation :

Inflammation occurs as a defensive response, which induces physiological adaptations to limit tissue damage and remove the pathogenic infections. Diseases caused by inflammation are an important factor of morbidity and mortality in humans. It is a fundamental pathologic process consisting of a dynamic complex of cytologic and chemical reactions that occur in the affected blood vessels and adjacent tissues (connective tissues), in response to an injury or abnormal stimulation caused by a physical chemical or biological agents resulting in;

(i) The local reactions and resulting morphologic changes.

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The so called "cardinal signs" of rubor (redness) calor (heat), tumor (swelling) dolor (pain) (Paul & Diana 1988).

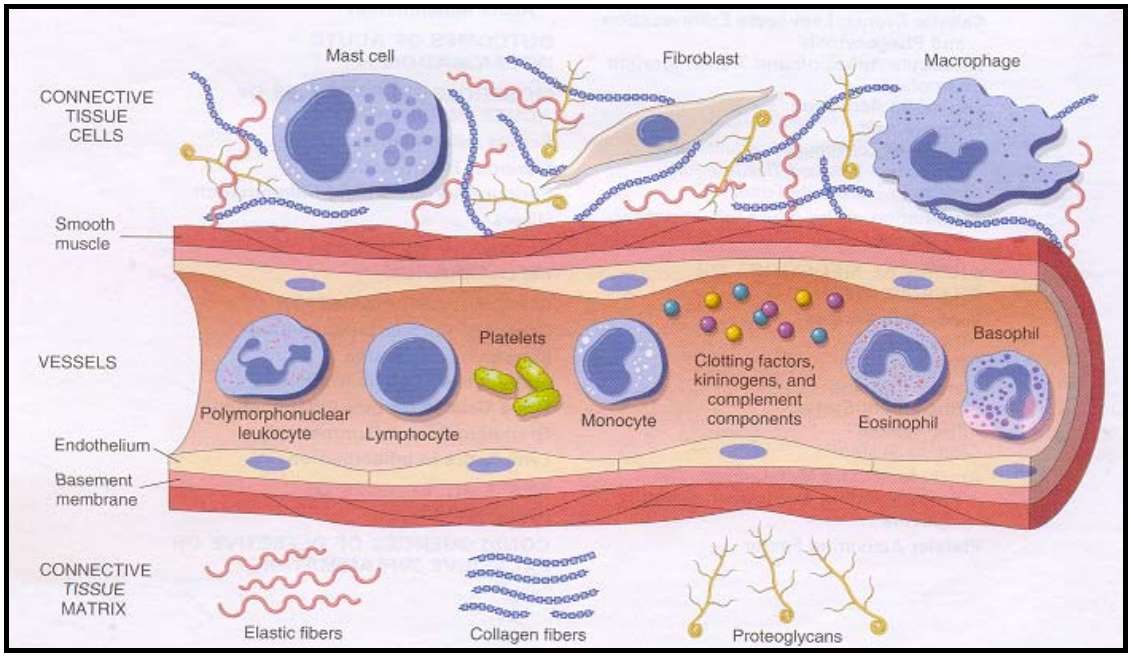

Figure 1.2: The component of acute and chronic inflammatory responses-circulating cells and proteins, cells of blood vessels and proteins of the extracellular matrix.

The inflammatory response (Paul & Diana 1988) has many players, these include circulating cells and plasma proteins, vascular cells and extracellular matrix of the surrounding connective tissue (figure 1.2). The circulating cells include bone marrow-

derived polymorphonuclear leukocytes (neutrophils), eosinophils and basophils, lymphocytes and monocytes, and platelets, the circulating proteins include clotting factors, kininogens, and complement components, largely synthesized by the liver. The vascular wall cells include the endothelial cells in direct contact with the blood. The connective tissue cells include sentinels to invasion such as mast cells, macrophages and lymphocytes. The extra cellular matrix (ECM) consists of fibrous structural proteins (e.g., collagen and elastin).

Inflammatory disorders include rheumatoid arthritis, osteoarthritis, inflammatory bowl diseases, retinitis, multiple sclerosis, psoriasis and atherosclerosis (Delves & Riott 2000). Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of acute and chronic inflammation, pain and fever.

Inflammation is divided into two basic patterns, acute inflammation and chronic inflammation.

1.2.1 Acute inflammation:

This is characterized by fluid and plasma proteins exudation and a predominantly neutrophilic leukocyte accumulation.

This process has two major components.

(1) Vascular changes:

Alterations in vessel caliber resulting in increased blood flow (vasodilatation) and structural changes that permits plasma proteins to leave the circulation (increased vascular permeability).

(2) Cellular events:

Emigration of the leukocytes from the microcirculation and accumulation in the focus of injury (cellular recruitment and activation); these events includes **(a)** Migration and rolling of leukocytes **(b)** Adhesion and transmigration between endothelial cells and **(c)** Migration in interstitial tissues toward a chemotactic stimulus.

1.2.2 Chronic inflammation:

These type of inflammation of prolonged duration (weeks to months to years) and is typified by influx of lymphocytes and macrophages with associated vascular proliferation and scarring. Chronic inflammation is characterized by the following (Fig.1.3).

(a) Infiltration with mononuclear (chronic inflammatory) cells including macrophages, lymphocytes, and plasma cells.

(b) Tissue destruction largely directed by the inflammatory cells.

(c) Repair involving new vessel proliferation (angiogenesis) and fibrosis.

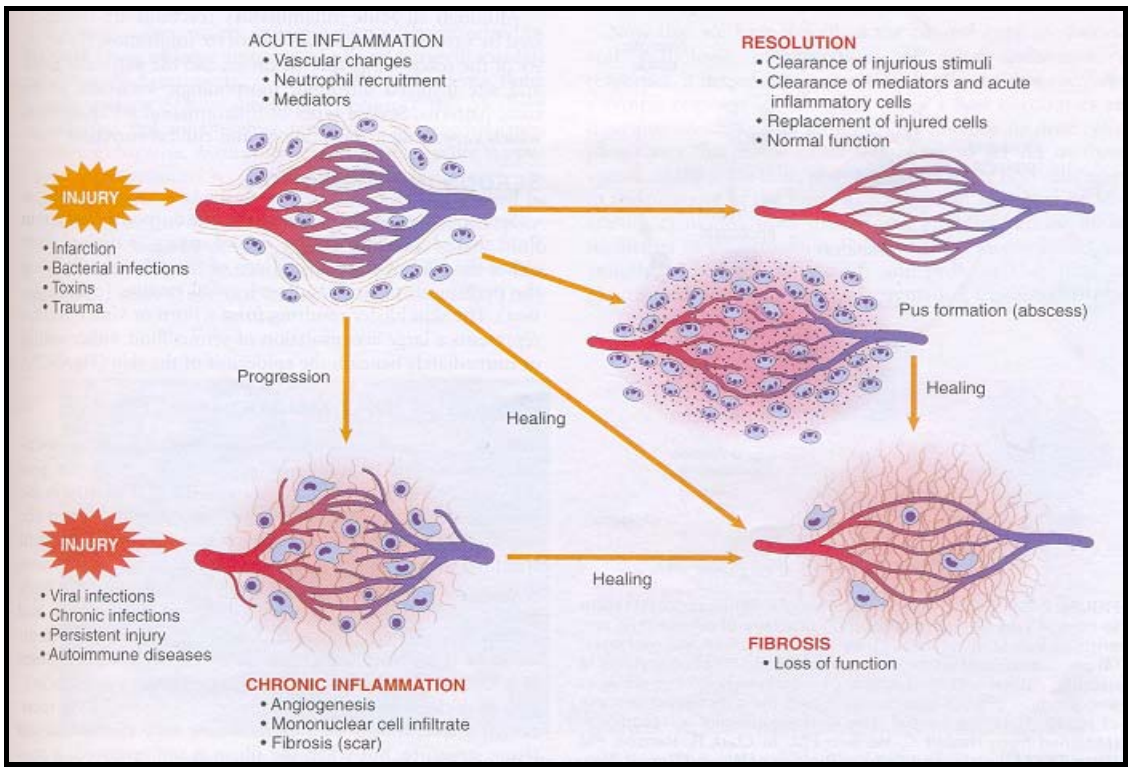

Figure 1.3: Outcomes of acute inflammation-resolution, healing by fibrosis, or chronic inflammation

1.2.2.1 Chronic inflammatory cells and mediators:

(a) Macrophages:

Macrophages constituting the critical mainstay and heart of chronic inflammation, and are tissue cells that derive from circulating blood monocytes after their emigration from the bloodstream. Their product include acid and neutral proteases, complement components and coagulation factors, reactive oxygen species and nitric oxide (NO), eicosanoids (Arachidonic Acid metabolites) and cytokines (IL-1, TNF).

(b) Lymphocytes, Plasma cells, Eosinophils and Mast cells:

Both *T* and *B* lymphocytes migrate into inflammatory sites; Lymphocytes are mobilized in the setting of any specific immune stimulus as well as in non-immune mediated inflammation. Plasma cells are the terminally differentiated end product of B-cell activation. Eosinophils are characteristically found in inflammatory sites around parasitic infection or as part of immune reactions mediated by immunoglobulin E (*Ig*E), typically associated with allergies. Mast cells are sentinel cells widely distributed in connective tissues throughout the body and can participate in both acute and chronic inflammatory responses.

1.2.2.2 Clinically chronic inflammatory diseases may fall into four categories:

a) Rheumatoid (arthritis)
b) Respiratory (asthma)
c) Cutaneous (psoriasis) and
d) Gastrointestinal inflammation.

a) Rheumatoid arthritis:

A chronic auto-immune disorder that causes the joint to become painful, swollen, stiff and deformed. It occurs at the age of 40 to 60 years more commonly. Rheumatoid arthritis is estimated to affect 1% of the world's population of a total disease burden of more than $ 65 billion (Kumar & Robbins 2009). In India, anti-inflammatory, analgesic, anti-rheumatic therapeutic areas have a market share of 7.8 billion rupees. In rheumatoid arthritis, inflammation accompanies thickening of the synovial membrane or joint lining, causing the whole joint to look swollen. The inflamed joint lining enters and damages bone and cartilage, and inflammatory cells release an enzyme called protease, which gradually digests bones and cartilages. The space between the joint diminishes and the joint loses shape and alignment.

b) Respiratory:

Respiratory inflammatory disorder (asthma) affects approximately 5% of the population in most industrialized countries (Paris 1995). Asthma is caused by an airway inflammation activated by allergen. Mast cell releases various mediators of inflammation like cytokines including Tumor necrosis factor (TNF), prostaglandins (PG), leukotrienes (LT), platelet activating factor (PAF) etc. Asthmatic inflammation

is characterized by bronchial hyperactivity. The chronic results are airway edema, smooth muscle hypertrophy, epithelial shedding etc. Asthmatic inflammation is strongly inhibited by corticosteroids e.g., beclomethasone, budenoside, flunisolide, triamcinolone hexacetonide and prednisone are commonly used as anti asthmatic (Goodman and Gilman 1996). The inhibitors of leukotrienes like ziluton and safirlukast are also used as anti asthmatic agents (Lipworth 1999).

c) Cutaneous:

Epidemiologically, psoriasis is one of the most common cutaneous diseases; affecting 1 to 2 % of general population (Asadullah 1999). Mostly glucocorticosteroids, NSAIDs such as aspirin and indomethacin, DMARDs, such as hydroxychloroquine, methotrexate and azathioprine are used as anti-psoriatic drugs (Harrison, 12th edition).

d) Gastrointestinal:

Inflammatory bowel disease is a general term for a group of chronic inflammatory disorders of unknown etiology involving the gastrointestinal tract. Numbers of mediators such as prostaglandins, cytokines, and reactive oxygen species are proposed to be in the pathogenesis of this disease. 5-amino salicylic acid and its derivatives, glucocorticoids such as budenoside and some immunomodulators like azathioprine, 6-mercaptopurine, methotrexate and cyclosporine are most commonly used drugs for inflammatory bowel diseases (IBD) (Egan & Sandborn 1998).

1.3 Chemical mediators of inflammation:

Mediators may be circulating in the plasma (typically synthesized by the liver), or they may be produced locally by cells at the site of inflammation (Fig 1.4). Plasma derived mediators (complement, kinins, coagulation factors) circulate as inactive precursors that must undergo proteolytic cleavage to acquire their biologic properties. Cell-derived mediators are normally sequestered in intracellular granules that are secreted upon activation (e.g., histamine in mast cells) or are synthesized de novo in response to a stimulus (e.g., prostaglandins).

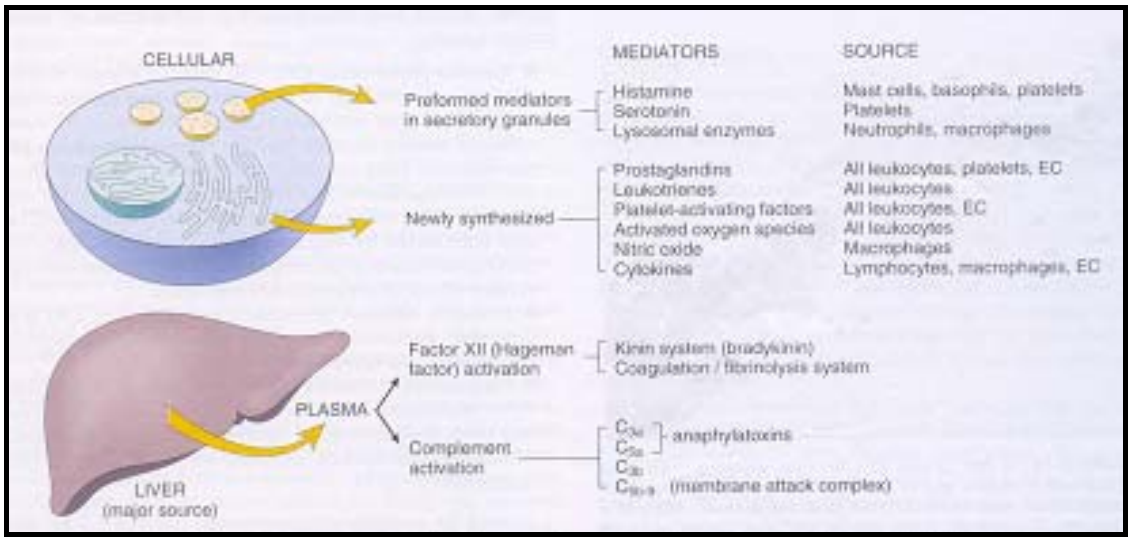

Figure 1.4: Chemical mediators of inflammation.

1.3.1 Vasoactive Amines:

1.3.1.1 Histamine

Histamine is released in response to a variety of stimuli,

- Physical injury such as trauma or heat;
- Immune reactions involving binding of IgE antibodies to Fc receptors on mast cells;
- C3a and C5a fragments of complement, the so called anaphylatoxins;
- leukocyte derived histamine-releasing proteins;
- Neuropeptides (e.g., substance P) and
- Certain cytokines (e.g., IL-1 and IL-8).

1.3.1.2 Serotonin (5-hydroxytryptamine):

Serotonin is vasoactive mediator with effects similar to those of histamine. It is found primarily within platelet dense body granules (along with histamine, adenosine diphosphate and is released during platelet aggregation.

1.3.2 Neuropeptides:

Like the vasoactive amines, neuropeptides can initiate inflammatory responses; these are small proteins, such as substance *P*, that transmit pain signals, regulate vessel tone, and modulate vascular permeability. Nerve fibers that secrete neuropeptides are especially prominent in the lung and gastrointestinal tract.

1.3.3 Plasma proteases:

Many of the effects of inflammation are mediated by three interrelated plasma-derived factors like the kinins, the clotting system and complement (Figure 1.5).

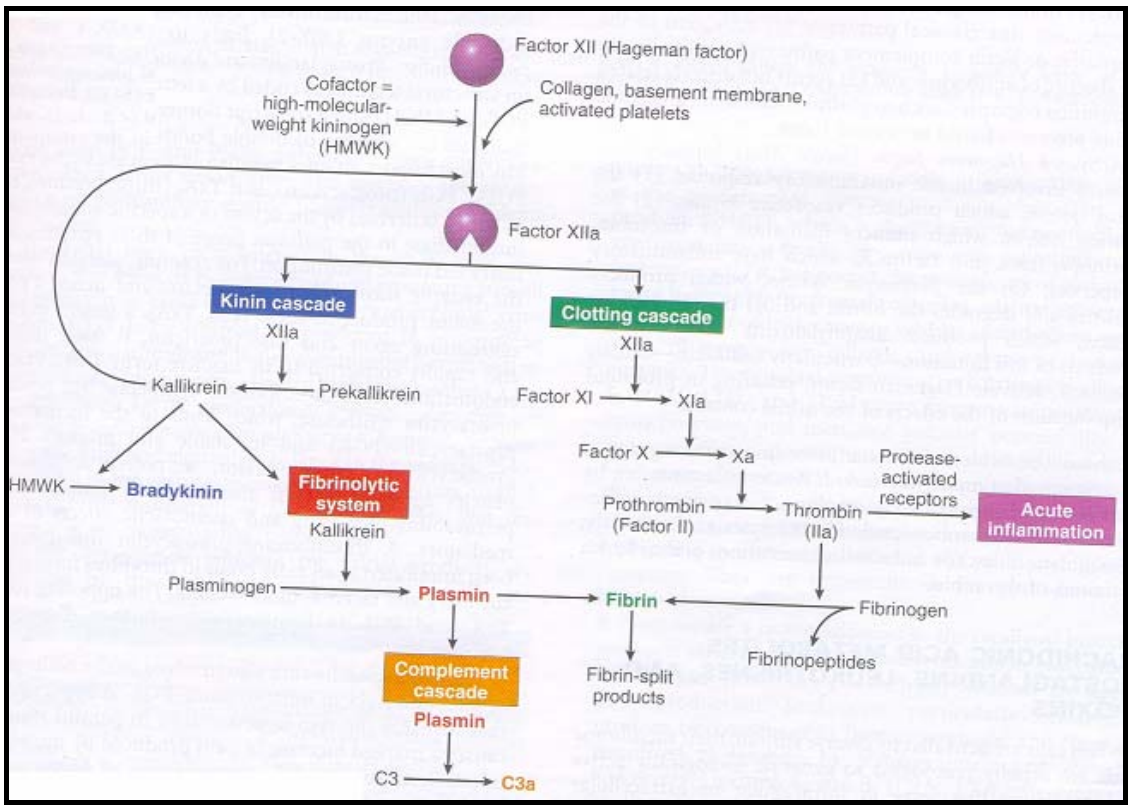

Figure 1.5: Interrelationships between the four plasma mediator system triggered by activation of factor XII (Hagman factor).

Note that thrombin induces inflammation by binding to protease-activated receptors (Principally PAR-1) on platelets, endothelium, smooth muscle cells and other cells.

1.4 Targets for anti-inflammatory activity:

As has been described earlier, there are many chemical mediators of the inflammatory and allergic response. Different mediators may be particular importance in the different inflammatory and allergic conditions. NSAIDs inhibit cyclooxygenase (COX) and thus the synthesis of prostaglandins (PG) and thromboxanes (TX) will affect mainly, and possibly only, those aspects of inflammation in which these mediators play a major role. As a result of decreasing the generation of PGE_2 and PGI_2 they will reduce in particular the vasodilatation and erythema.

1.4.1 Cyclooxygenase (COX) inhibitors:

Bio-synthesis of prostaglandins, prostacyclins and thromboxanes is accomplished in a stepwise manner from arachidonic acid through a ubiquitous complex of microsomal enzymes (Figure 1.6) (Goodman and Gilman 1996). The first enzyme in this synthetic pathway is prostaglandin synthase, also called cycloxygenase (COX), which catalyses the oxygenation of arachidonic acid to prostaglandin. The current clinically useful non-steroidal anti-inflammatory drugs (NSAIDs) are considered to act by inhibiting the enzyme cyclooxygenase (COX). (Vane 1971, Smith and Wills 1971). It was also observed that cyclooxygenase activity dramatically increased in inflammatory states and that cellular cyclooxygenase activity can be induced by inflammatory cytokines such as interleukin-1, TNF-alpha or bacterial endotoxin (Raz *et al.,* 1988). The increase in COX protein synthesis was blocked by glucocorticoids, where as the level of free arachidonic acid did not appreciably change (Masferrer *et al.,* 1994, 1990).

This observation led to the hypothesis that a second form of COX enzyme (COX-2) existed besides COX-1(Xie *et al.,* 1991, Kujubu *et al.,* 1991). These two distinct isoenzymes show about 60% amino acid sequence identical in mammalian cells. The two isoenzymes are very similar with respect to their catalytic properties, affinities for arachidonic acid and structure of the active site, with minor difference in their requirement for activation, hydroperoxide and preferences for fatty acid substrates (O'Banion *et al.,* 1991). Both enzymes are sensitive to inhibition by conventional NSAIDs. As a class, the currently available commercial NSAIDs e.g. aspirin, ibuprofen**,** indomethacin, naproxen**,** diclofenac sodium**,** piroxicam, etc. (Figure 1.8)**,** have been found to inhibit, non-selectively, both forms of the enzyme (Smith *et al.,* 1996, Herchman 1996, Mitchell *et al.,* 1993, Gierse 1995).

The observation that COX-2 is associated with inflammatory condition and that COX-1 is mainly expressed as constitutive enzyme has provided the rationale for the development of selective COX-2 inhibitors in order to reduce the risk of gastric irritation and ulceration associated with the chronic use of NSAIDs (Griwold and Adams 1996, Richardson and Emery 1996).

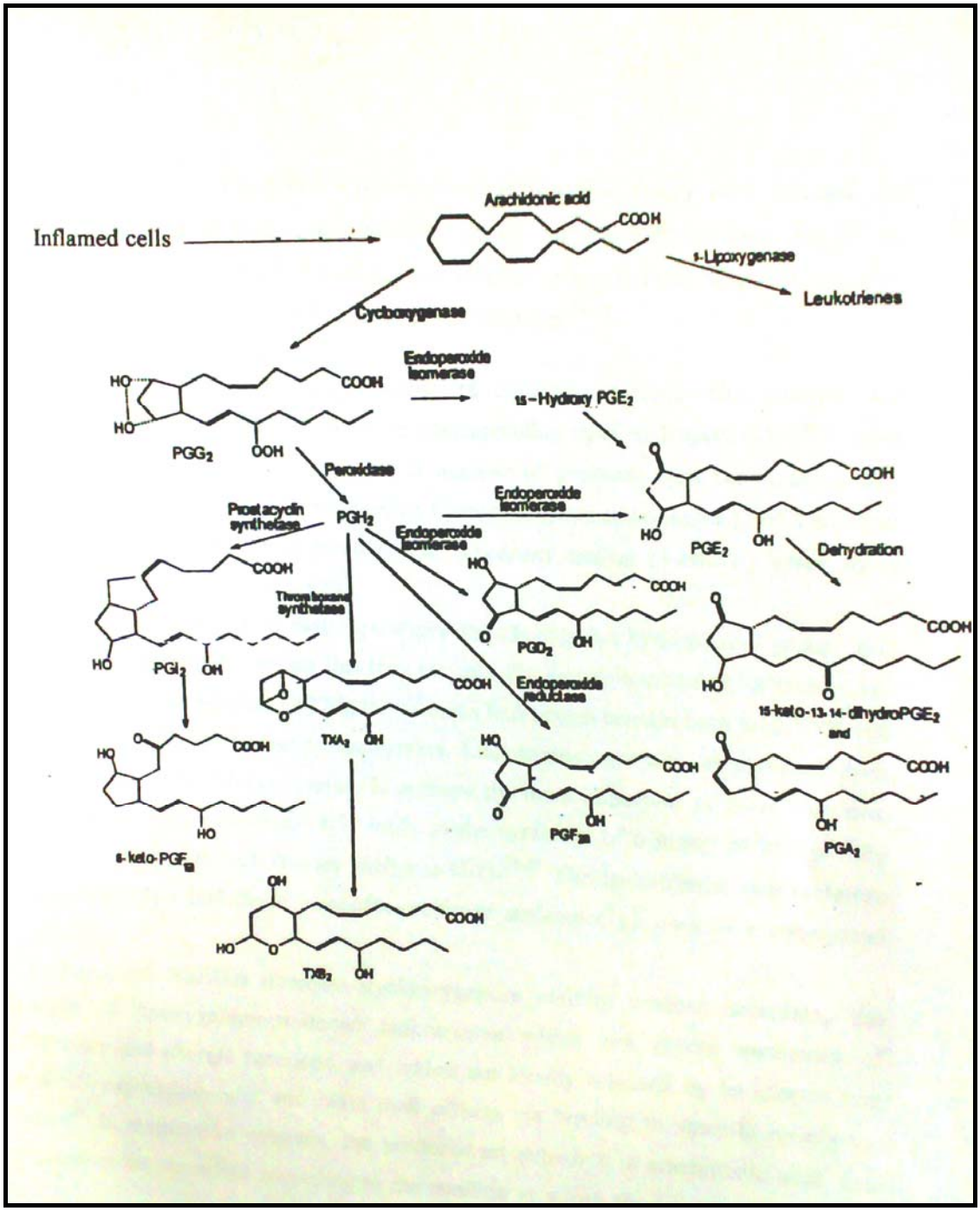

Figure 1.6: Biosynthesis of prostaglandins, prostacyclins and thromboxanes from arachidonic acid.

1.4.2 Lipoxygenase inhibitors:

Lipoxygenase are a family of cytosolic enzymes that catalyze the oxygenation of polyenic fatty acids to the corresponding lipid hydroperoxides (Samuelsson 1983, Needlem *et al.,* 1986, Sigal 1991). This enzyme metabolizes arachidonic acid to a number of products with the hydroperoxy group in different positions. There are called hydroperoxyeicosatetraenoic (HPETEs) acid and a part of which simply

reduced to the 5-hydroxy analog (5-HETE) either by a peroxides or non-enzymatic reaction.

Lipoxygenase differ in their specificity for placing the hydroperoxy group, and tissues differ in the Lipoxygenase that they contain. For example, platelets have only 12-Lipoxygenase and synthesize 12-HPETE, whereas leukocytes contain both 5-HPETE and 12-HPETE. In addition to the prostaglandins, Leukotrienes are also synthesized from arachidonic acid. The 5-Lipoxygenase is perhaps the most important of these enzymes, since metabolism of arachidonic acid leads to the synthesis of a group of biologically active lipids known as leukotrienes (Figure 1.7) (Samuelsson 1983, Needlem *et al.,* 1986, Sigal 1991)**.** The leukotrienes nomenclature arises from the fact that these leucocyte-produced molecules all contain a conjugated triene region.

NSAIDs decrease cyclooxygenase activity without decreasing the generation of Lipoxygenase-produced leukotrienes which are potent mediators of inflammatory and allergic reactions, and which are locally released by leukocytes and other 5-LO expressing cells and exert their effects via binding to specific membrane receptors (Steinhilber 1999). In mammalian systems, the predominant substrate is arachidonic acid, and lipoxygenases are classified according to the position at which they oxidize arachidonic acid.

The mammalian 5-lipoxygenase pathway (Figure 1.7) (McMillan and Walker 1992) produces the potent biological mediators leukotriene B_4 (LTB_4) and the peptidoleukotrienes (LTC_4, LTD_4 or LTE_4). Peptidoleukotrienes are powerful bronchoconstrictor agents while leukotriene B_4 is a potent chemotactic agent for a variety of leucocytes and can induce inflammatory reaction in humans (Barnes *et al.,* 1984). Leukotrienes are involved in the initiation and maintenance of a variety of inflammatory diseases (Zakrewski *et al.,* 1985, Bisgaard *et al.,* 1984, Rae *et al.,* 1982). In view of these properties, leukotrienes have been proposed as important mediators in allergic and inflammatory disorders. Inhibitors of 5-Lipoxygenase, by blocking leukotriene synthesis, have therapeutic potential in a range of allergic and inflammatory conditions such as asthma, allergic rhinitis, rheumatoid arthritis, ulcerative colitis and psoriasis.

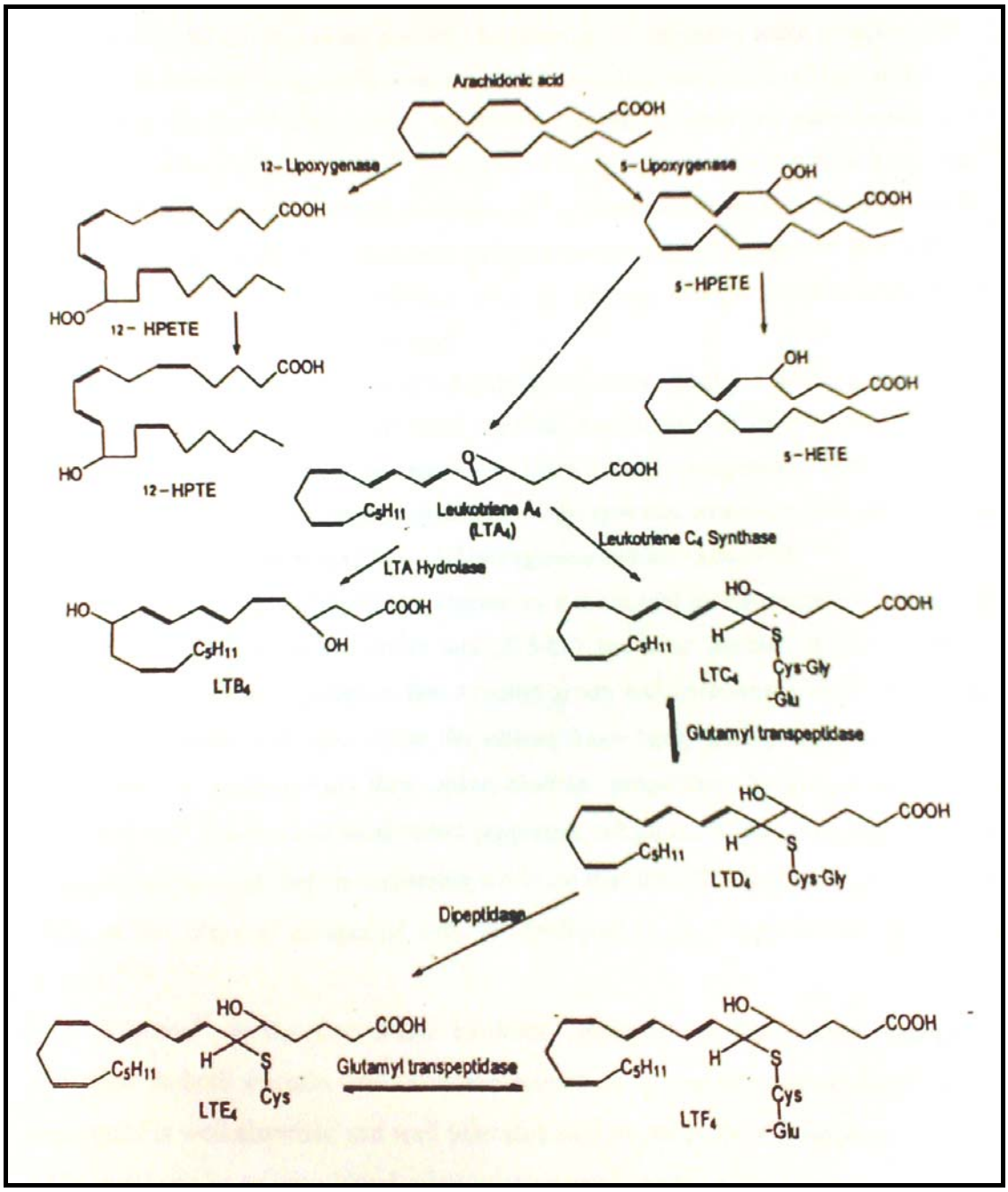

Figure 1.7: The mammalian 5-Lipoxygenase pathway.

Inhibitors of 5-LO can be classified under three classes (Redox inhibitors, Iron-ligand inhibitors. Non-redox inhibitors) based on their mechanisms of enzyme inhibition. 5-lipoxygenase is particularly susceptible to inhibition by compounds with low redox potentials. Rational design of inhibitors containing a functional group capable of interacting with the iron present at the active site of 5-LO has been fruitful. One of the most- powerful metal ligand groups is the carbonyl group and hydroxamic acid

moiety, thus hydroxamic acids and their close derivatives have been widely described as 5-LO inhibitors. In addition to their metal-binding properties, hydroxamates and N-hydroxyureas also possess weak redox properties, which are likely to be enhanced in the presence of iron, and there is increasing evidence that the 5-LO inhibitory activity of this class of this class of compound may be mediated in the whole or in part by these effects (Riendeau *et al.,* 199, Rouzer *et al.,* 1991).

Zileuton (McMillan and Walker 1992) is the first 5-LO inhibitor, presently in the market, brings about reduction in both oedema and bronchoconstriction in the airways of asthmatics. The compound is well absorbed and well tolerated and produced dose dependent inhibition of LTB_4 synthesis in ex vivo blood when administered orally to volunteers. A double-blind study in rheumatoid arthritis demonstrated that zileuton, a urea derivative, at 800mg twice daily produced significant relief of symptoms.
In addition to inhibition of leukotriene synthesis of some compounds, some other compounds act at receptor level, and block leukotriene effect. There are 2 types of leukotriene receptors those for leukotriene B_4 and those for cysteinyl leukotrienes. In human airways, leukotrienes C_4, D_4 and E_4 all activate cysteinyl leukotrienes ($cysLT_1$) receptors (Smith 1996). Montelukast (selective LTD_4 receptor antagonist,) (David 1999) and Zafirlukast (cysteinyl leukotriene antagonist) are used in treatment of asthma and allergic rhinitis.

1.4.3 Tumor necrosis factor (TNF-α):

Over production of TNF-α can lead to autoimmunity, malignancy or inflammatory and immunopathological disease (Barbara *et al.,* 1996, Tracey 1995, Tracey and Cerami 1994). Thalidomide (alpha-N-pthalimidoglutarimide) is an immuno modulator and anti-inflammatory drug. It is clinically useful in a number of conditions through its ability to inhibit selectively TNF-α synthesis. It is the drug of choice in the treatment of erythema nodosum leprosum (Sampaio 1993) an acute inflammatory complication often seen in patients with lepromatous leprosy, rheumatoid arthritis and HIV (Schuler and Ehninger 1995, Ehinger 1993).

1.4.4 Phosphodiestrase inhibitors:

It is well established that level of intracellular adenosine 3',5'-cyclic monophosphate (cAMP) is an important factor in the inflammatory response to a variety of stimuli. Its role in the production of proinflammatory cytokines, such as interleukin-10 has been proved. More recent investigations have demonstrated that PDE_4 inhibition can be a potential novel therapy in asthma. Compounds which inhibit cAMP phosphodiestrase-4, the enzyme responsible for the breakdown of cAMP, have been suggested as anti-TNF-α agents (Lowe III *et al.,* 1992, Torphy *et al.,* 1993, Palfreyman 1984).

1.4.5 Platelet activating factor (PAF):

The synthesis of PAF may be stimulated during antigen-antibody reaction or by a variety of agents, including chemotactic agents, thrombin, collagen and other autocoids and it also stimulates its own production. Platelets, neutrophils, monocytes, mast cells, eosinophils, renal mesangial cells, renal medullary cells and vascular endothelial cells synthesize PAF. PAF is a potent bronchoconstrictor, it favors accumulation of eosinophils in the lung, it causes tracheal and bronchial edema, and it stimulates the secretion of mucus. PAF antagonists inhibit the binding of PAF to its receptor and block its actions selectively. Among these antagonists are analogs of PAF with modification in the 3- position of the glycerol backbone, a number of natural plant products, and surprisingly, triazolobenzodiazepines such as alprazolam and trizolam. The development of PAF antagonists is still in its early stages. However, the involvement of PAF in inflammation, asthma and reproduction suggests that such antagonist may have therapeutic application.

1.4.6 Free radicals and scavengers:

Oxygen sustains life, but oxygen is not absolutely friendly (Chatterjee *et al.,* 1995). The function of oxygen is to act as a terminal electron acceptor. Usually oxygen receives four electrons on a concerted fashion to produce water, but often it is reduced by single electron to produce oxygen free radical ($O_2^{\cdot -}$) and reactive oxygen species (ROS), such as H_2O_2, OH^- and ferryl. ROS is highly toxic and extremely damaging to biological systems. If not properly scavenged, it results in oxidative damage such as lipid peroxidation, protein oxidation and DNA damage. Oxidative damage has been implicated in several degenerative diseases including inflammation, arthritis, cataract, atherosclerosis, cardiovascular diseases, cancer as well as ageing (Ames *et al.,* 1993,

1983). A number of studies have implicated oxygen free radicals in the induction and prolongation of the inflammatory process (Halliwell *et al.*, 1989, Roberfroid *et al.,* 1987). Many non-steroidal anti-inflammatory agents are also known to act either by inhibiting the production of free radicals or by scavenging them (Halliwell *et al.,* 1989, Roberfroid *et al.,* 1987, Freeman *et al.,* 1982).

As is apparent from these information's inflammatory diseases are polygenic multifactorial disorders with the inflammatory cascade consisting of several steps in series and in parallel and having a number of rate-limiting steps.

1.5 Classification of NSAIDs:

NSAIDs are classified as under (Foye 1989, Rang and Dale 1991);

a) Salicylates and their derivatives e.g. aspirin.
b) p-Amino phenol derivatives e.g., phenacetin, paracetamol.
c) Pyrazolone derivatives phenylbutazone.
d) Indole acetic acid derivatives e.g., indomethacin, sulinac.
e) Phenylacetic acid derivatives e.g., diclofenac, felbinac, bromfenac.
f) Fenamates e.g., flufenamic acid, mefenamic acid, meclofenamic acid.
g) Propionic acid derivatives e.g., ibuprofen, fenoprofen, ketoprofen.
h) Oxicams e.g.,piroxicam,meloxicam.
i) Sulfanilide derivatives,e.g., nimesulide.
j) Alkanones e.g., nabumetone.

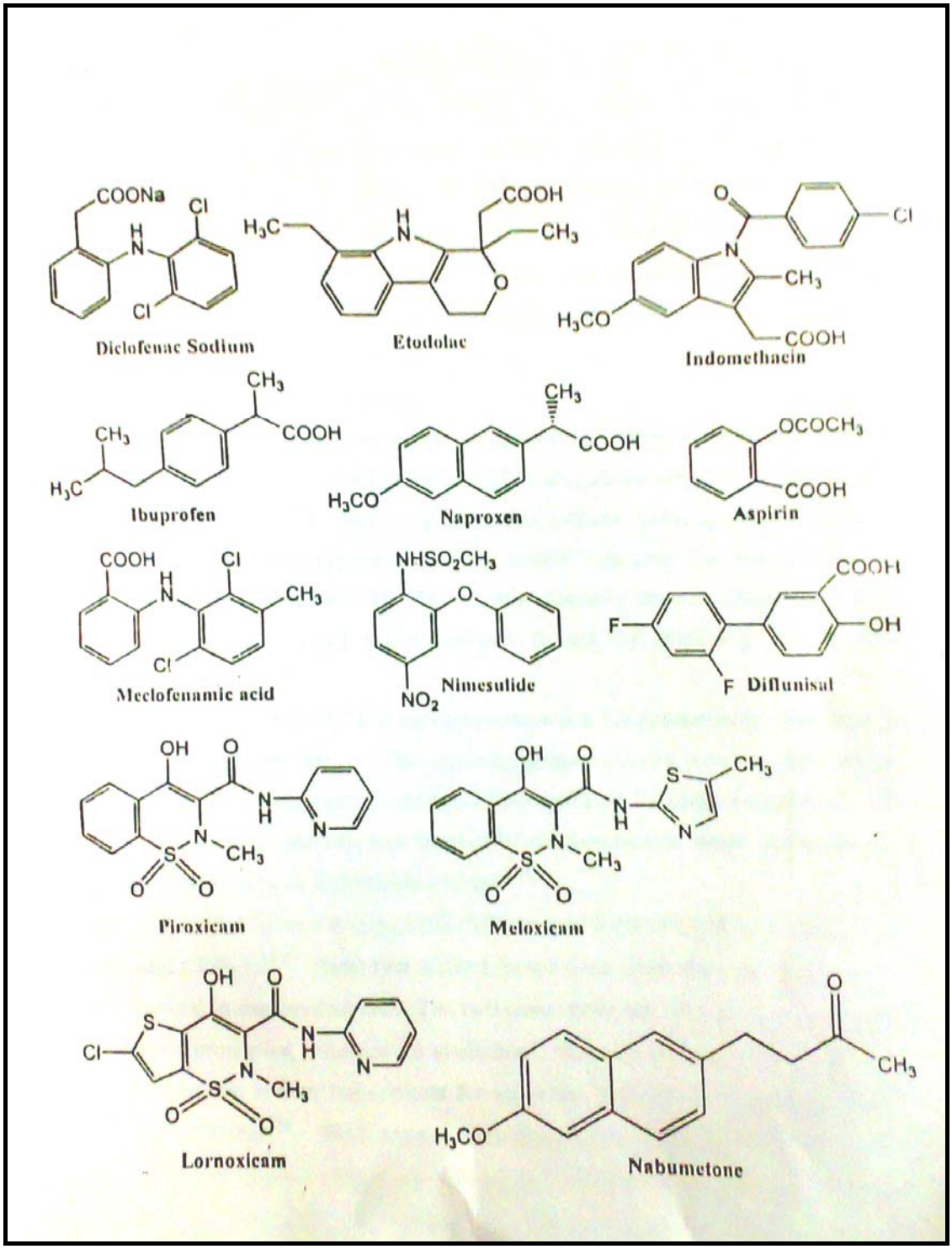

Figure 1.8: Structure of some commercially available NSAIDs.

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CHAPTER II

INTRODUCTION TO DRUG DESIGN AND AIM OF PRESENT WORK

2.1 Drug Designing:

Research is all about transforming the options to useful results and conclusive discoveries. The entire gamuts of options available for new drug discovery are listed below;

- Chemical leads are used as models or templates by the designed chemical synthesis approach.
- Macromolecular structure-based computer assisted drug design.
- Natural product lead based.
- Mimetics of endogenous small molecules.
- New uses of old drugs.
- Astute clinical observations leading to break through (e.g. discovery of sulphonylurea oral antidiabetic drugs).
- New ideas, untested hypothesis, raw data analysis for unusual observations.
- Refinement of old ideas (e.g. subtypes of receptor modulation).
- Serendipity.
- Biotechnology derived products.

Here we have used chemical-lead based drug design approach for designing molecules. The literature suggests that one of the thrust areas in research is the synthesis and biological characterization of small molecule heterocycles. In designing new candidates the two factors to be carefully assessed are;

a) Appropriate pharmacophore [minimum structural feature essential for activity].

b) Juxta positioning and tethering into appropriate molecular scaffolding.

These together provide the appropriate binding site to the macromolecular target. The appropriate features in the candidate are;

a) [H] bond donor,

b) [H] bond acceptor,

c) acidic centre,

d) basic centre,

e) hydrophobic features,

f) aromatic regions.

2.1.1 Rational basis of drug design:

The careful and judicious utilization of various scientific disciplines is very important for an efficient drug development program. Drug designing demands thorough understanding of the biological processes involved in the disease process. The complexity involved in drug designing is reflected, when we realize that only 1 in 5000 compounds that are tested makes it all the way to the drug shelves. If the ligand, which we design, binds to the target (bio-macromolecule) then a biological action, favorable or detrimental to the body, can be expected. Any drug has to surpass minimum number of conceptual steps before it can interact with the bio-macromolecule which we are talking about (Figure 2.1).

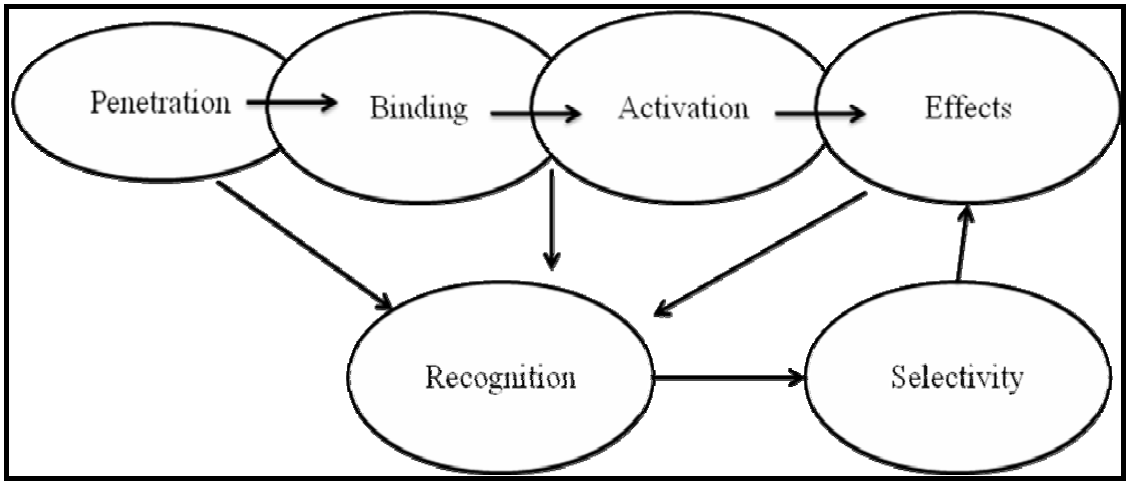


Figure 2.1: Conceptual steps drug to surpass before it interact with bio-macromolecule.

2.1.2 Hit to lead Journey:

Early phase of drug discovery (William 1996) goes through the process of identifying actives developing them to hits and then to chemical leads and ultimately lead optimization to delineate developable drug like candidates (Figure 2.2).

An active is any substance, which shows reproducible activity in any screen. A hit is an active substance, which shows reproducible activity in a relevant bioassay, has a confirmed structure, and is of high purity and for which specificity data has been generated. A lead is a hit with genuine structure activity relationship, which suggests that more potent and selective compounds can be found (Figure 2.3).

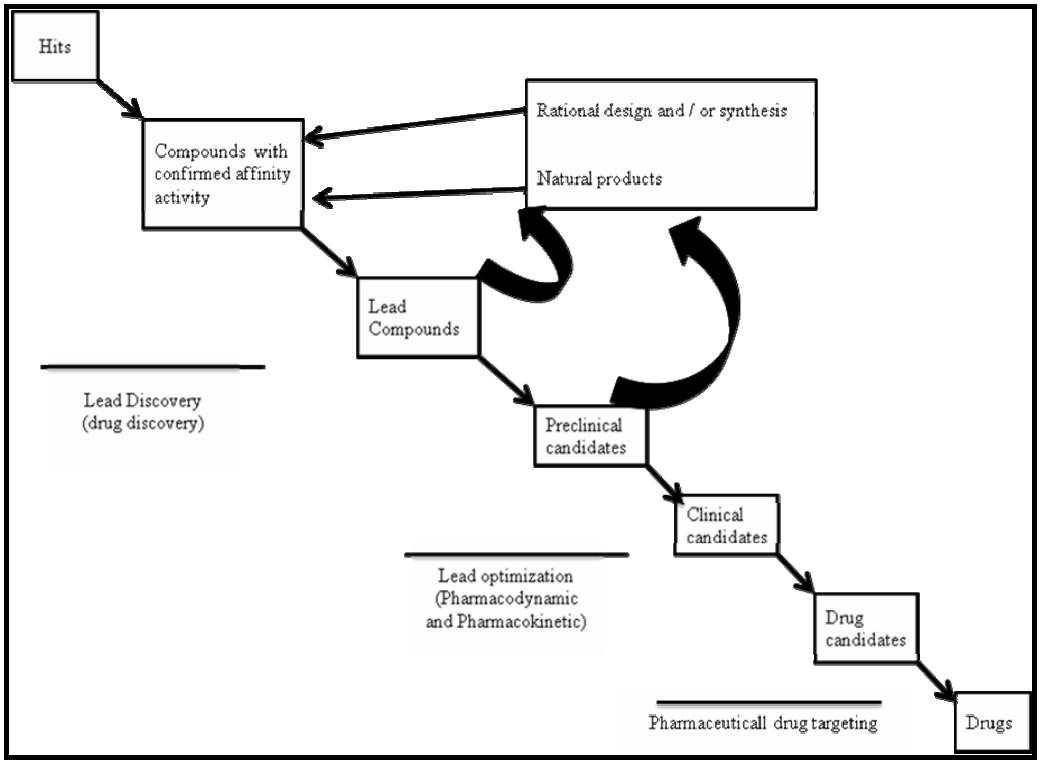


Figure 2.2: Healthy schematic representation of the long road from hits and active compounds to actual (i.e. therapeutically used) drugs.

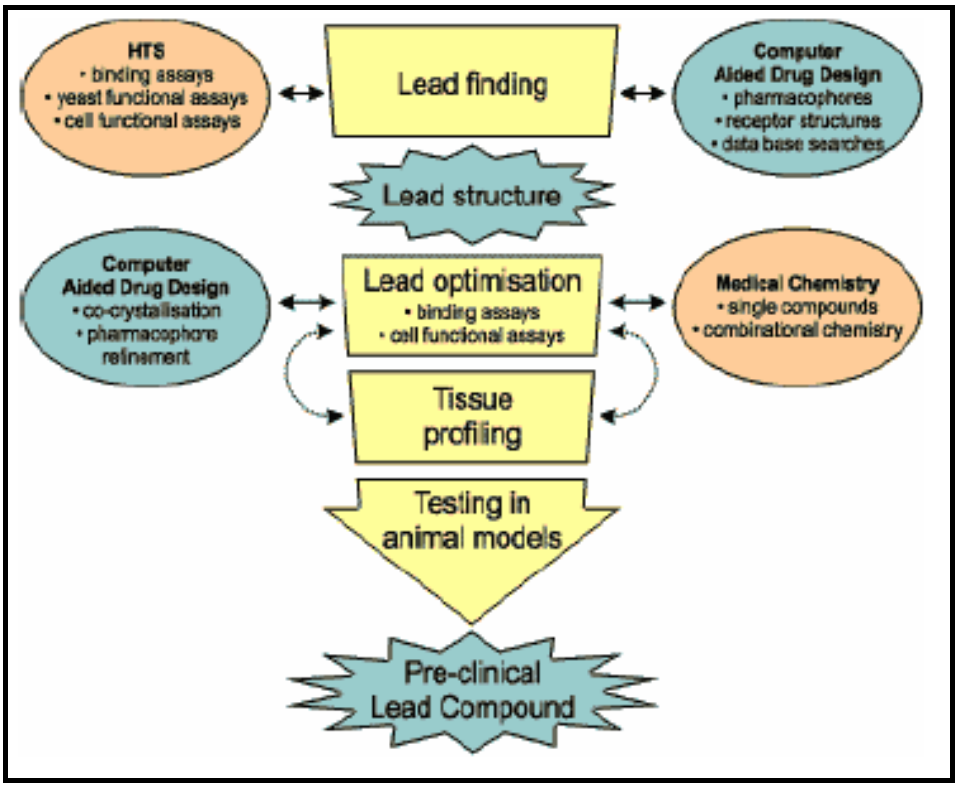


Figure 2.3: Diagram of lead finding, optimization to pre-clinical Lead compound.

It is important to note that vast majority of screening actives fails to become leads or even hits, and the sooner that compounds can be eliminated, the more efficiently solid leads can be discovered (William 1996). These chemical lead like candidates are utilized,

1. To find the pharmacophore,
2. To enhance the selectivity,
3. To separate structure based to property based mechanism of actions and
4. To enhance the potency.

Thus the main objective in hit to lead chemistry, which is a key element in new and faster lead generation, is ultimately to end up with a preclinical compound.

Drug design can be broadly classified into two categories;

2.1.3 Direct drug design:

If the structure of the target which we aim at is known, designing a ligand complimentary to the knowledge obtained from the structure of the target, is called target based drug design or structure based drug design, which is a direct type of drug design. The three dimensional structure of the therapeutic target is used to speed the discovery and refinement of lead compounds. It is cost competitive with conventional methods and can be coupled with high throughput screening and combinatorial chemistry. Thus direct drug design is a useful tool for both lead development and optimization. Further, docking efforts can be used to speed up lead screening process. The development of HIV protease inhibitors is a classical outcome of structure based drug design.

2.1.4 Indirect drug design:

Many receptors are not amenable to receptor based drug design, as in cases where we don't know much about the receptor structure. In such cases, a lead compound or active ligand must be found, and the structure of the ligand will guide the drug discovery process. This type of drug design, where a lead compound or an active ligand guide the drug discovery process to reach the receptor sites is called lead based drug design or ligand based drug design. Literature has many instances, where the chemical modification of known drugs and experimental candidates has led to improved drugs. ACE inhibitors were successfully developed without any knowledge of the enzyme structure, and were designed on an assumed mechanistic homology of

carboxy-peptidase A (Simon 2003). The development of Timoprazole to Omeprazole is the other example for indirect drug design (olbe 2003)**.** The development of the oxicam series and the propionic acid series of anti-inflammatory drugs are another examples of chemical modification of known drugs in the same class, which resulted in better and safer drugs. Especially in the field of inflammation where we don't know much about the binding sites of several enzymes involved in the cascade, indirect drug design is an appropriate approach. It will be worth modifying a known ligand, taking into consideration of the pharmacophoric requirements needed for eliciting the required anti-inflammatory action. In the process of developing a lead compound, an antagonist to a known agonist, or an anti-metabolite from a known substrate, a large number of avenues for systematic molecular modifications are available in literature. In this context the phenomenon called bioisosterism is widely used as a powerful tool in drug designing approach for setting a lead.

2.2 Bioisosterism:

Bioisosterism represents one approach used by the medicinal chemists for rational modification of lead compounds into safer and more clinically effective agents. The concept of bioisosterism is often considered to be qualitative and intuitive. Bioisosteres are groups or molecules, which have chemical and physical similarities producing broadly similar biological activity. The bioisosteric replacements often provide the foundation for the development of QSAR studies (Lipinski 1986, Hansch 1981). Medicinal chemistry is rich in examples where the concept of bioisosterism has given fruitful drug candidates. β-Adrenoceptor agonists in which a 3- hydroxyl group has been replaced with bioisosteric groups include albuterol (3-CH2OH), soterenol (3-NHSO2CH3), and carbuterol. (Figure 2.4)

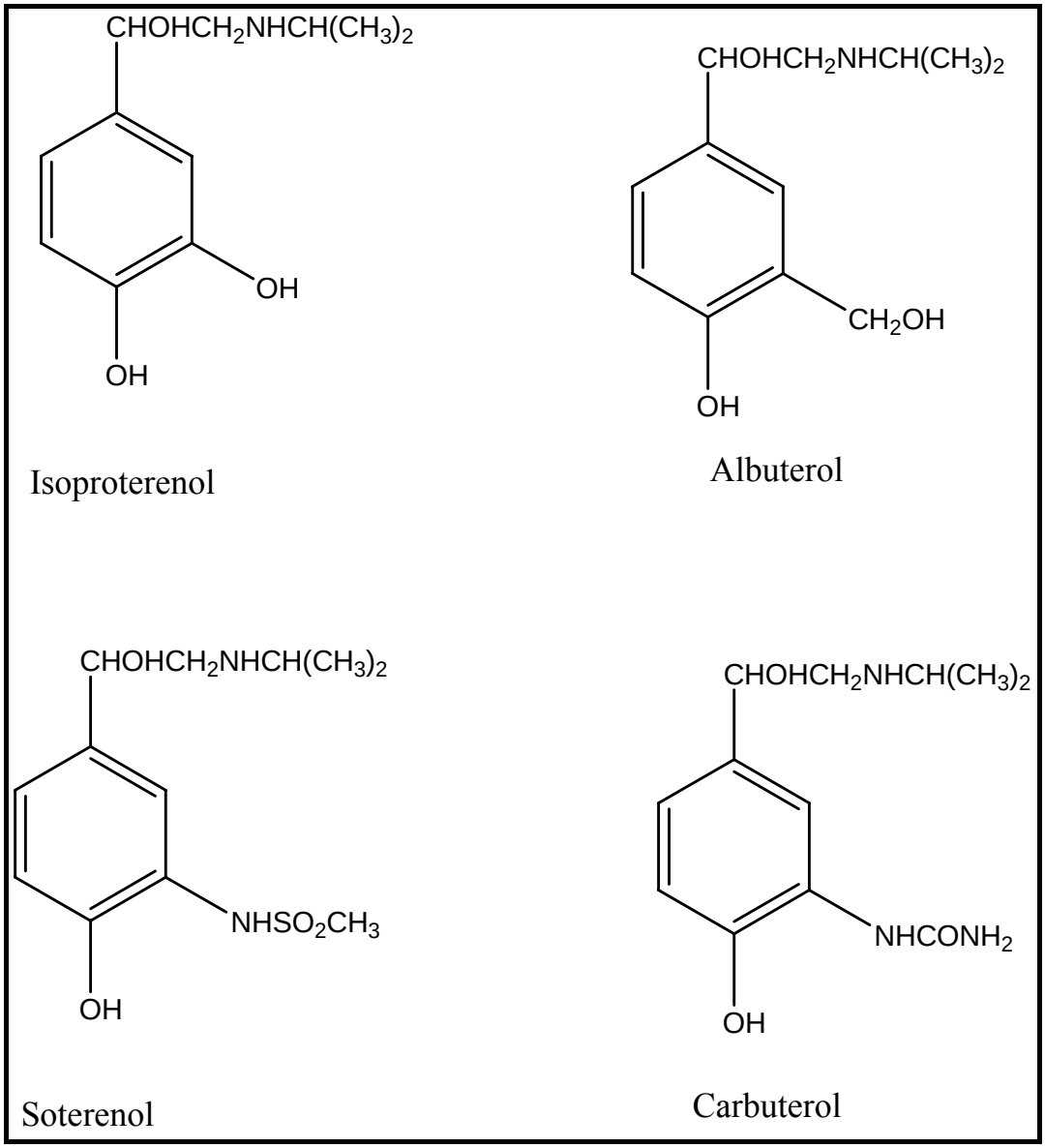


Figure 2.4: Bioisosterism concept examples of some β-adrenoreceptor agonist.

A well-known example of classical isosteric substitution of an amino for a hydroxyl group is illustrated by aminopterin wherein the hydroxyl substituent of folic acid has been substituted by an amino group (Williams 1983)**.** Replacement of the thiourea moiety in buriamide by bioisosteric features had given rise to block buster H2-receptor antagonist antiulcer drugs cimetidine and ranitidine (The Merck Index, 12th edition) (Figure 2.5).

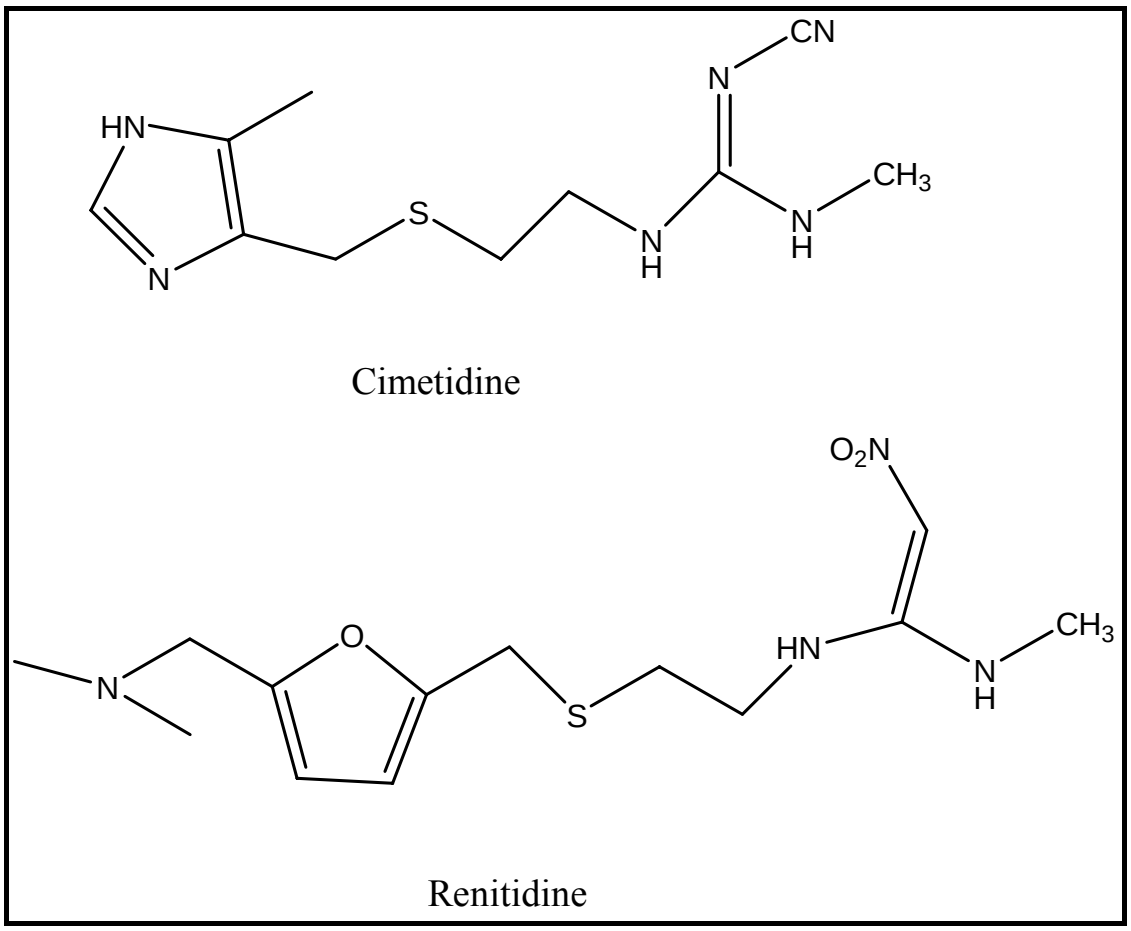


Figure 2.5: Bioisosterism concept examples of some antiulcer drugs.

A number of NSAID hydroxamic acid analogs like ibuproxamibuprofen, Oxamethacin and indomethacin (Figure 2.6) possess anti-inflammatory activity (Orzalesietal.1980).

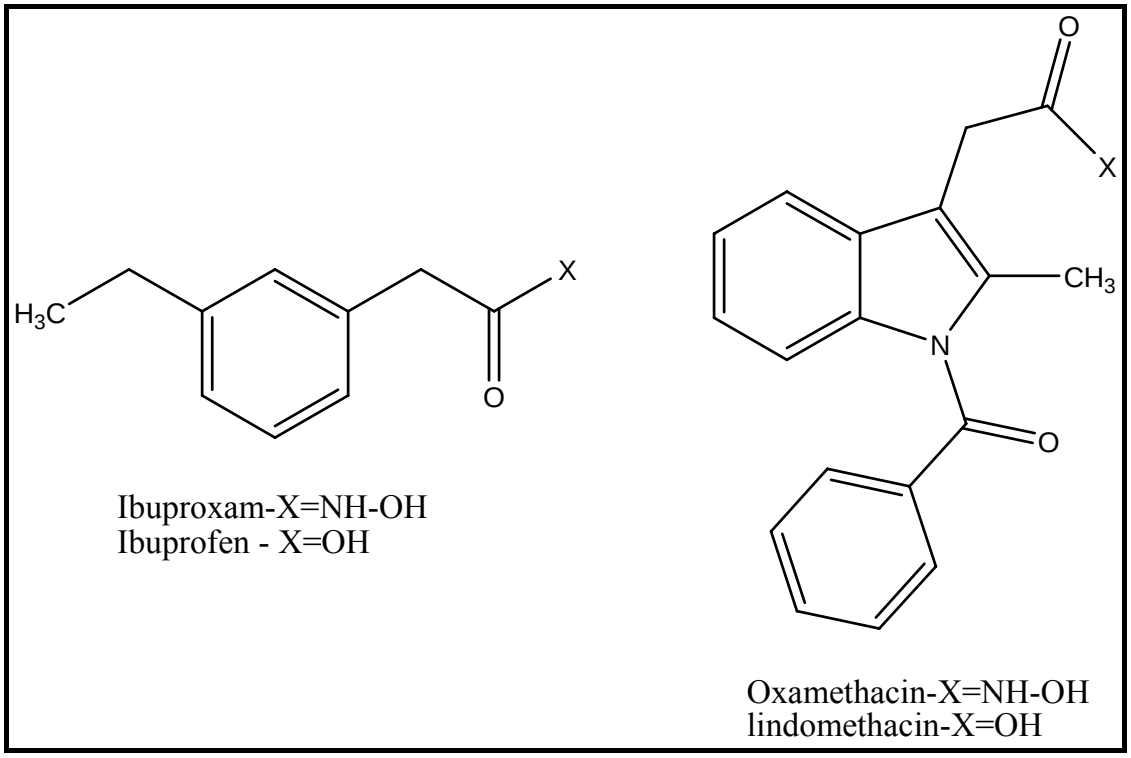


Figure 2.6: Bioisosterism concept examples of some anti-inflammatory drugs.

The SO2NHCO moiety was proposed as a carbonyl isoster based on similar hypoglycemic activities of gliburide and carboxylic acid (Brown and Foubister 1984) (Figure 2.7)**.**

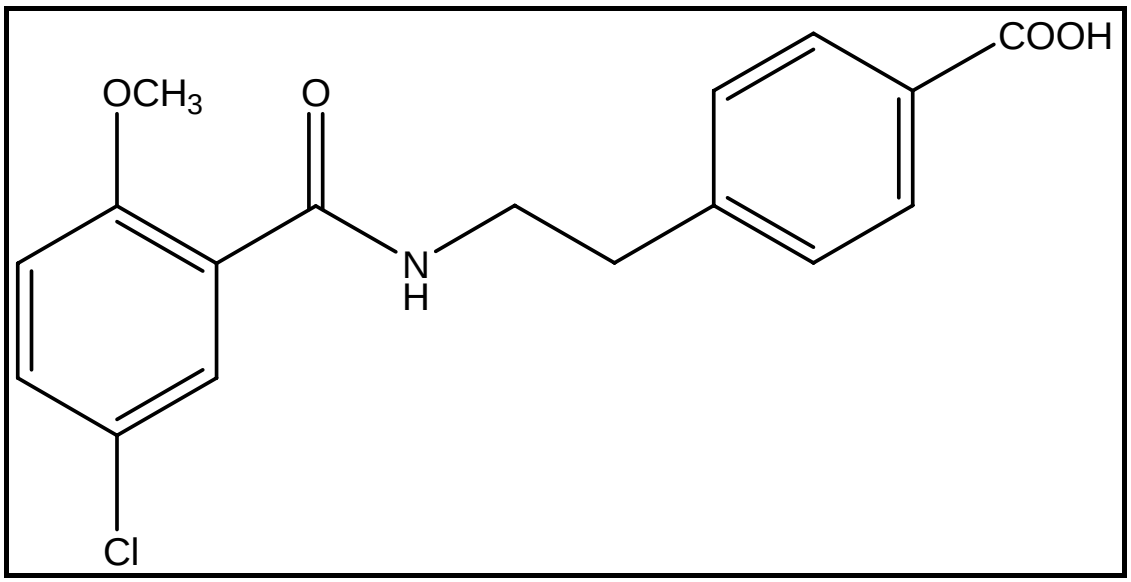


Figure 2.7: Bioisosterism concept examples of some hypoglycemic agents.

The concept of bioisosterism has been exploited fully and justified in the synthesis of thiophene analogs of established drug molecules (Martin and Reid 1959). Medicinal chemistry is rich with examples where this concept has been fully exploited for finding better leads and in this approach the replacement of a phenyl group by thiophene moiety has provided rich dividends. The replacement of a phenyl moiety in clozapine, a drug with a high risk of agranulocytosis, by a thiophene moiety has given rise to a block buster anti-psychotic drug, olanzapine with a very much improved therapeutic profile (Ross and Frances 1991, Maio 1972)**.** The replacement of aryl by thiophene moiety resulted in pyrantel, which showed a three-fold increment in its anthelmentic activity (McFarland 1969)**.**

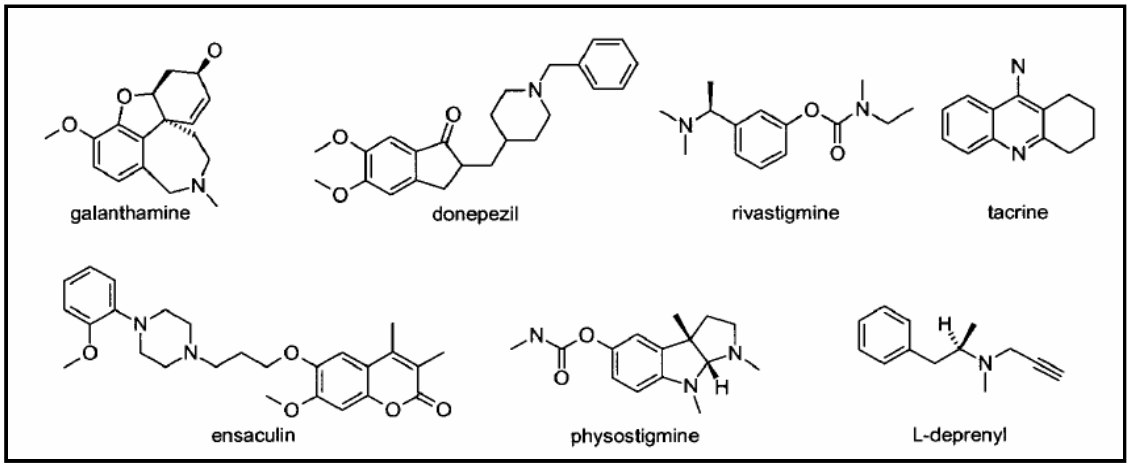


Figure 2.8: Bioisosterism concept examples inhibit the activity of AchE and MAO.

Ensaculin inhibits the activity of AChE in vitro (IC50- 0.36 μM) (Hilgert *et al.,* 1999). Furthermore, the hybrids of an AChE inhibitor and an irreversible MAO inhibitor such as L-deprenyl (Figure 2.8) showed dual AChE and irreversible MAO inhibitors (Fink *et al.,* 1996).

2.3 Coumarin:

Coumarin (2H-1-benzopyran-2-one) CAS No 91-64-5, is a crystalline white solid when seen pure, with a hay-like, sweet aromatic creamy odour with certain nutty shadings.

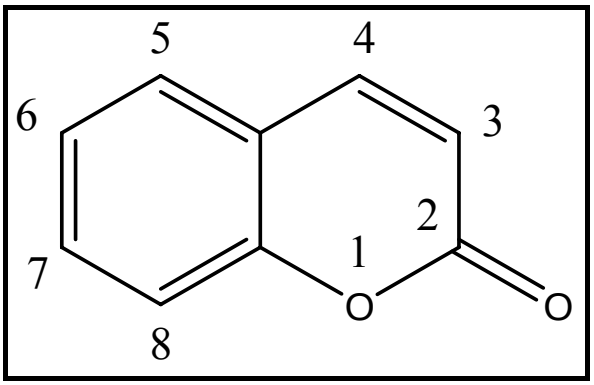


Figure 2.9: Structure of coumarin.

2.3.1 Coumarin-containing Natural Products:

Coumarin occurs widely in natural products, generally being liberated from the corresponding glycoside (melilotoside) on drying coumarin-containing herb material. Dicoumarol is a microbiological biotransformation product in spoiled *Melilotus Clover* and other hay products, and its presence in fodder at >10ppm is cause for concern.

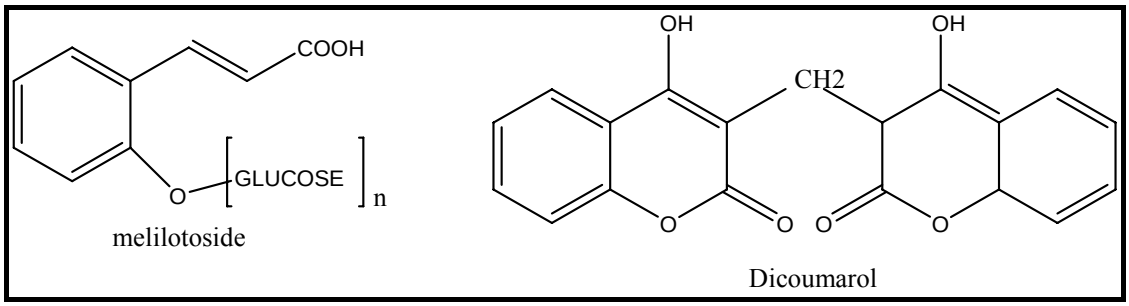


Figure 2.10: Structure of melilotoside and dicoumarol.

Coumarin occurs widely in natural products (Table 2.1).

Table 2.1: The occurrence of coumarin in some common herbs & natural products.

Some Natural Coumarin Sources	Notes
Anthoxanthum odoratum L. Flaouve oil	Both essential oil and absolute produced.
Carphephorus odoratissmus (J.F. Gemel) syn *Liatris odoratissima* Mich. syn. *Trilisia odoratissima* (J.F. Gmel.) Cass. Deer tongue or Liatris	1.6 % coumarin. Ratio of coumarin: dihydrocouamarin: 2, 3 benzofuarinin in volatile fraction of extract 1:3:20 (Appleton & Enzell 1971).
Cinnamomum cassia J. Presyl. Cassia oil	Coumarin 4-11% Coumarin to 8.73% (TNO 1996) Eu Pharm V (2) allows 1.5 to 4.0% coumarin in cassia oil monograph.
Cinnamomum zeylanicum Cinnamon bark & leaf oils	To 0.3%; rarely to 0.7%.
Galium odoratum L. syn. *Asperula odorata* Woodruff absolute & concrete	Variable; coumarin content develops on drying herb, although headspace of freshly cut woodruff found to be 80% coumarin. Used in alcoholic beverage flavourings (e.g. vodka).
Hierochloe odorata (L.) Beauv Sweet grass	Use to flavour vodka in Russia.
Lavandula spp. Lavender & Lavandin qualities.	Lavender absolute to 8.0% coumarin; lavandin absolute to 5.0% coumarin. Spike lavender oil to 0.3% coumarin.
Lolium perenne L. & other spp. Incl. *Phleum pretense* (Timothy grass), *Poa pratensis* L. (Meadow grass), *Cynosurus cristatus* (Crested Dog's-Tail), *Anthoxylum odoratum* L. and *Melilotus* spp. Foin oil.	Essential oil and absolute produced Foin essential oil contains some 8% coumarin.
Melilotus alba Medik. Bokhara Clover, or White Sweet Clover	Less used than Common Melilot (q.v.)
Melilotus officinalis L. (Pallas) Common Melilot or Yellow Sweet Clover	0.9% coumarin on dry weight basis. Wagner (1996) says 0.6.25-0.45% coumarin in herb together with umberlliferone scopolin etc.
Mentha spp. Peppermint oil	20 ppm (TNO 1996).

Coumarin also occurs in trace amounts in the oils of;

- Billy Goat Weed *Ageratum conyzoides* L.,
- Sweet wormwood *Artemisia annua* L.,
- Mugwort *Artemisia vulgaris* L.,
- Carrot Seed oil *Daucus carota* L. ssp *sativus* (Hoffm.) Arcang,
- Champaca *Michelia champaca* L.,
- *Narcissus* spp.,
- Clary sage *Salvia sclarea* L.

2.3.2 History:

In the early 20th century, North American prairie farmers imported sweet clover plants (Melilotus alba, M. officinalis) from Europe as cattle feed. At about the same time, a new hemorrhagic disease in cattle was described. In 1922, Schofield, a veterinary pathologist, reported that afflicted cattle had eaten spoiled sweet clover. In 1929, Dam reported a similar hemorrhagic disease in chicks eating feed that was vitamin K (sterol) free (Mueller 1994). Dam and others proposed, then isolated, vitamin K in 1935 (Dam 1935), leading to a 1943 Nobel Prize for Dam. A young University of Wisconsin agriculturist, Karl Link, began work on isolating the hemorrhagic agent after being confronted by a distraught farmer with a dead cow. 3, 30-methylenebis-[4-hydroxycoumarin] or dicoumarol was identified and synthesized (Campbell *et al.,* 1940). Dicoumarol was formed by the action of Aspergillus hay mold on the inactive coumarin, and had been prepared as early as 1903 but its importance went unrecognized. Link found that vitamin K completely reversed dicoumarol's actions, and assigned patent rights to the Wisconsin Alumni Research Foundation, thus the origin of the name 'warfarin', given to a more potent synthetic coumarin, 3-phenylacetyl ethyl, and 4-hydroxycoumarin. Warfarin was introduced as a highly effective rodenticide in 1948. To this day, it has been the pre-eminent oral anti-coagulant. Meyer first gave dicoumarol to human volunteers in 1941, and I. S. Wright, who had earlier used heparin, became the first to treat patients with dicoumarol in the same year (Prandoni and Wright 1942). Dicoumarol became available commercially in 1944, while Wright first used it to treat myocardial infarction in 1943, and published a randomized clinical trial in 1948 (Wright *et al.,* 1948). Link advocated warfarin as an anti-coagulant in 1950, and it was introduced

into clinical practice in 1954. President Dwight Eisenhower received Warfarin for his myocardial infarction in 1955. Not until 1978 was Warfarin's mechanism of action described, Whitlon and Bell independently describing how toxic cattle and chick feed blocked the effect of vitamin K (Mueller and Scheidt 1994).

2.3.3 Mechanism:

Warfarin, the predominant agent used in the USA, and three other coumarins, dicoumarol (also called bishydroxycoumarin), phenprocoumon and acenocoumarol, are synthetic derivatives of 4-hydroxycoumarin. Phenindione and anisindione are synthetic derivatives of indan-1, 3-dione. Vitamin K antagonists are indirect anticoagulants, functioning in hepatocytes by interfering with the reduction of vitamin K 2, 3-epoxide to vitamin KH2 by epoxide reductase. Vitamin K1 reverses the effect of warfarin by bypassing the blocked enzyme, leading to the reconstitution of a pool of vitamin KH2 (Hirsh *et al.,* 2001). Vitamin KH2 is a cofactor for carboxylase that catalyses gammacarboxylation of glutamate residues at the N-terminal portion of Factors II, VII, IX and X, leading to a required structure for binding of calcium ions. The calcium moieties mediate the interaction of the clotting factors with negatively charged phospholipids surfaces, for example, on platelets, thereby greatly enhancing the coagulation cascade and fibrin formation. As Proteins C and S are also vitamin K-dependent, coumarin also exert a potentially hypercoagulable effect. The net effect of approximately parallel reductions in the activities of Factors II, VII, and IX and X, and Proteins C and S is anti-coagulation, but dysequilibrium conditions may exist early during coumarin administration, due to disproportionate reductions of Factor VII and Protein C, owing to their relatively short half-lives. Coumarins also inhibit gamma-carboxylation of certain bone proteins, which is the cause of cartilage abnormalities in the fetal warfarin syndrome. Pleiotropic effects also occur indirectly with coumarins, as various cytokines, chemokines and adhesion molecules may be down regulated (Becker 2002).

2.3.4 Pharmacology:

Warfarin is nearly always administered orally, although a parenteral preparation is available. Warfarin is absorbed rapidly, with peak blood levels attained after 90 minutes. Bioavailability by the oral route is very high, and intravenous administration does not produce faster or greater effects. Warfarin is highly bound to albumin, accumulates in and is metabolized by the liver, excreted in the bile, enterohepatically recycled, and excreted in hydroxylated, inactive form in the urine. Warfarin is a racemic mixture; the two enantiomers (S-isomer and R-isomer) are both metabolized by cytochrome P450 isoenzymes, but have different half-lives, potencies, and rates and routes of metabolism. Plasma half-life is 36–42 hours; plasma concentrations do not correlate well with anticoagulant effect, implying variable hepatic metabolism in different individuals (Prandoni and Wright 1942). Unlike the heparins, coumarins and indandiones cross the placenta and are teratogenic in up to 30% of fetuses. The first trimester (more specifically, Weeks 6–12 of gestation) is the period of highest risk for 'warfarin embryopathy' (nasal hypoplasia and/or stippled epiphyses), while use in the second or third trimesters occasionally results in central nervous system and eye abnormalities. The fetus may also develop a bleeding disorder that may manifest as cerebral hemorrhage at labor and delivery. Whether warfarin also increases the incidence of stillbirths, pre-maturity and spontaneous abortion (Bonow *et al.,* 1998, Hung and Rahimtoola 2003) is controversial. Dicoumarol and anisindione are distributed into milk; however, warfarin is not, so it may be used safely by breastfeeding mothers. Neonates are particularly sensitive to vitamin K antagonists due to the vitamin K deficiency typically present at birth (Bethesda 2000). Causes of variable response-genetic and acquired hepatic dysfunction augments drug response via impaired synthesis of active coagulation factors, while hypermetabolism due to fever or hyperthyroidism may augment sensitivity by accelerating factor catabolism. There is marked genetic and environmental variability in the metabolism and dose-response relationship of warfarin, leading to a wide range of doses needed to provide a target level of anticoagulation (Hirsh 2001, Bathesda 2000, Cropp and Bussey 1997, Wells *et al.,* 1994). The chief, but not sole, genetic polymorphism is in the activity of CYP 2C9. More marked, autosomal-dominant, genetic resistance is due to reduced responsiveness of a target vitamin K receptor site for warfarin. Conversely, mutant Factor IX propeptides mediate sensitivity to warfarin. Acquired causes include patient non-compliance, errors in drug dosing and communication of laboratory results,

inaccuracies of testing, variable dietary vitamin K intake, altered drug metabolism, drug interactions that alter warfarin's absorption or metabolism, and pharmacodynamic interactions with drugs with anti-thrombotic or vitamin-K-dependent factor effects. Inhibition of metabolism by other drugs can be selective for specific warfarin isomers. Aspirin and heparin both potentiate warfarin, with aspirin having a direct warfarin-like effect. Alcohol can potentiate, inhibit or have neutral effects on warfarin response (Hirsh 2001).

2.3.5 Adverse effects:

Bleeding is the major side-effect associated with warfarin. Other adverse effects include skin and adipose tissue necrosis ('coumadin-induced skin necrosis'), cholesterol embolization/purple toes syndrome, alopecia, allergic/hypersensitivity reactions, dermatitis, urticaria, pruritis, paresthesias, hepatic abnormalities, gastrointestinal disturbances, cold intolerance, vasculitis, jaundice, edema, tracheal calcification, agranulocytosis, priapism, adrenal insufficiency due to hemorrhage, and fetal abnormalities (Bethesda 2000). Due to its slow onset of action, an immediately acting anti-coagulant such as heparin is always co-administered with warfarin for treatment of active thrombosis. Warfarin alone may be considered when the indication is thromboembolism prophylaxis and the indication to treat is not urgent. Warfarin should be used with caution in those with known Protein C or S deficiency because of the greater risk of Protein C or S depletion to dangerously low levels. In such patients, initial warfarin dosing must be low in order to avoid acute Protein C depletion. Intramuscular injections should be avoided when possible due to the risk of hematoma. Bleeding is directly related to the intensity of warfarin anti-coagulation. Additional risk factors include, but are not limited to, concomitant anti-thrombotic or potentiating medications, age greater than 65 years, renal insufficiency, history of stroke or gastrointestinal bleeding, untreated hypertension, malignancy and duration of therapy (Bathesda 2000, Levine *et al.,* 2001). In placebo-controlled trials, excess major bleeding and intracranial bleeding occurred in approximately 1 and 0.2% of patients/year, respectively. Intracranial hemorrhage during long-term therapy has been reported in 0.2–3.7% patients/year compared with 0.1% patient/year in non-anti-coagulated patients. In patients with prosthetic heart valves, rates of major and fatal bleeding vary from 1.2% to 5.6% patients/year and 0.2% to 0.9% patients/year,

respectively, and across all patient groups, estimates for major and fatal bleeding are approximately 3% and 0.6% patients/year, respectively (Hirsh *et al.,* 2003). Patients older than 75 years treated with high-intensity anti-coagulation were at greatest risk of bleeding (Lerine *et al.,* 2001). Warfarin embryopathy has been reported to occur over a wide incidence range of 5–67% (Bonow *et al.,* 1998), but probably occurs in 30% of patients with exposure in the first 9 weeks. While apparently safe during the first few weeks of gestation (Bonow *et al.,* 1998, Hung and Rahimtoola 2003, Ginsberg 2003), warfarin manufacturers state that the drug is contra-indicated in women who are or may become pregnant (Bathesda 2000).

2.3.6 Biological importance of coumarin:

Coumarins are well-known natural products displaying a broad range of biological activities. Most of the synthetic coumarin derivatives have been found to possess diverse biological activities namely anticoagulant and anti-inflammatory activities (Ghate *et al.,* 2005, 2003, Kontogiorgis *et al.,* 2003).

2.4 Aim of present work:

Platelets provide the initial haemostatic plug at sites of vascular injury. They also participate in reaction that leads to atherosclerosis and pathological thrombosis in numerous animal studies. Antagonists of platelet function have thus been used in attempts to prevent thrombosis and to alter the natural history of atherosclerotic vascular disease. As mentioned earlier one of the cyclooxygenase product is thromboxane A_2. Since, aspirin have been found as dual inhibitor as it block production of thromboxane A_2 by covalently acetylating a serine residue near the active site of cyclooxygenase, it is used as antiplatelet agent (Ryn *et al.,* 2000).

There are several recent reports in literature which indicate that the coumarin compounds have good anti-inflammatory activity (Thamotharan et al 2004). Coumarin compounds having significant bleeding tendency. Coumarin compound cannot use as alone therapy due to its slow onset of action. An immediately acting anti-coagulant such as heparin is always co-administered with coumarin derivative for treatment of active thrombosis.

Some 4-(3-coumarinyl)-3-benzyl-4-thiazoline-2-one compounds have reported to possess anti-inflammatory activity (Gujarati and Bhalla). Substitution at 3rd position of coumarin nucleus modulated AChE as well as MAO-B inhibitory activity. Substitution with methyl groups at 3rd and 4th position led to more active compounds toward both AChE and MOA-B enzymes (Corinne *et al.,* 2001).

Pillai A. (Pillai *et al.,* 2003) and Molvi K. I. (Movli *et al.,* 2008) have reported novel tetrasubstiuted thiophene as dual inhibitor enzymes in inflammation pathway (COX and LOX). Similarly novel substituted thiazole derivatives also reported by Franclin (Franclin *et al.,* 2007). Substituted coumarin derivatives inhibiting more than one pathway was found to be effective as an anti-inflammatory chemical entity is also reported by above workers.

The number of antibacterial compounds available in market are belongs to penicillin derivatives and sulpha drugs, which belongs to sulphur containing heterocyclic ring (thiazole and thiazolidine).

Multi-drug treatment of inflammatory conditions associated with microbial infections poses a unique problem especially for patients with impaired liver or kidney functions. Husain A and coworker has reported anti-inflammatory and analgesic compound with antimicrobial activity (Husain *et al.,* 2008, 2005, 2004).

Therefore from the pharmacoeconomic and patient compliance points of view, the mono therapy with a drug having anti-inflammatory and antimicrobial activities is highly desirable (Bekhit *et al.,* 2004).

In view of above reports new alternative and more effective coumarin and five membered sulphur containing heterocyclic compounds (thaizole and thiophene) having anti-inflammatory, anti-platelet and antibacterial activity has been designed, the tetrasubsituted thiophene as anti-inflammatory moiety and considering the 3-substituted coumarin which is liked at 5th position to thiophene and thiazole. In anticipation that coumarin moiety will be acting as anti-platelet and five membered sulphur containing heterocyclic compounds (thaizole and thiophene) acting as anti-inflammatory and antibacterial. (Figure 2.11).

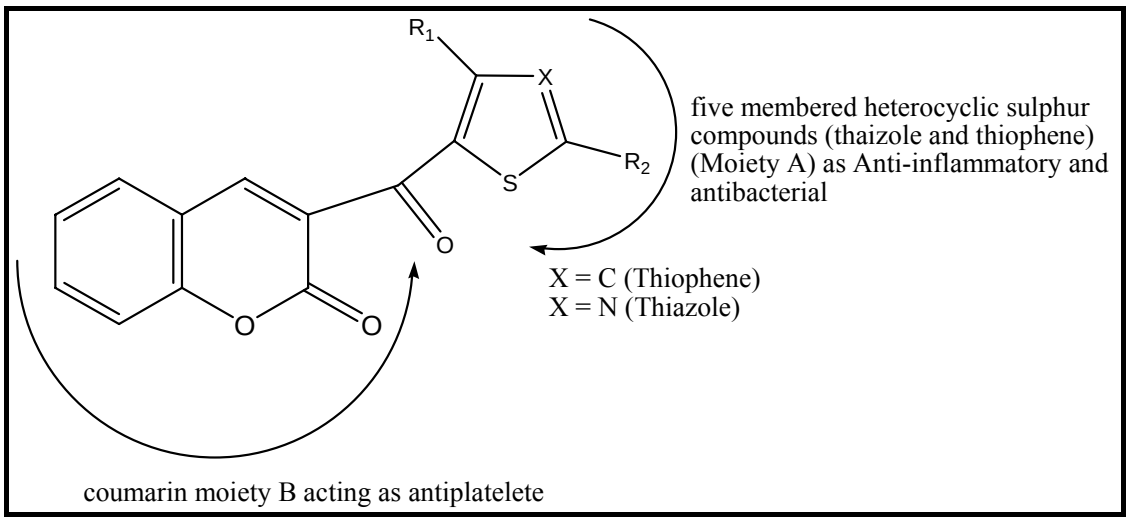
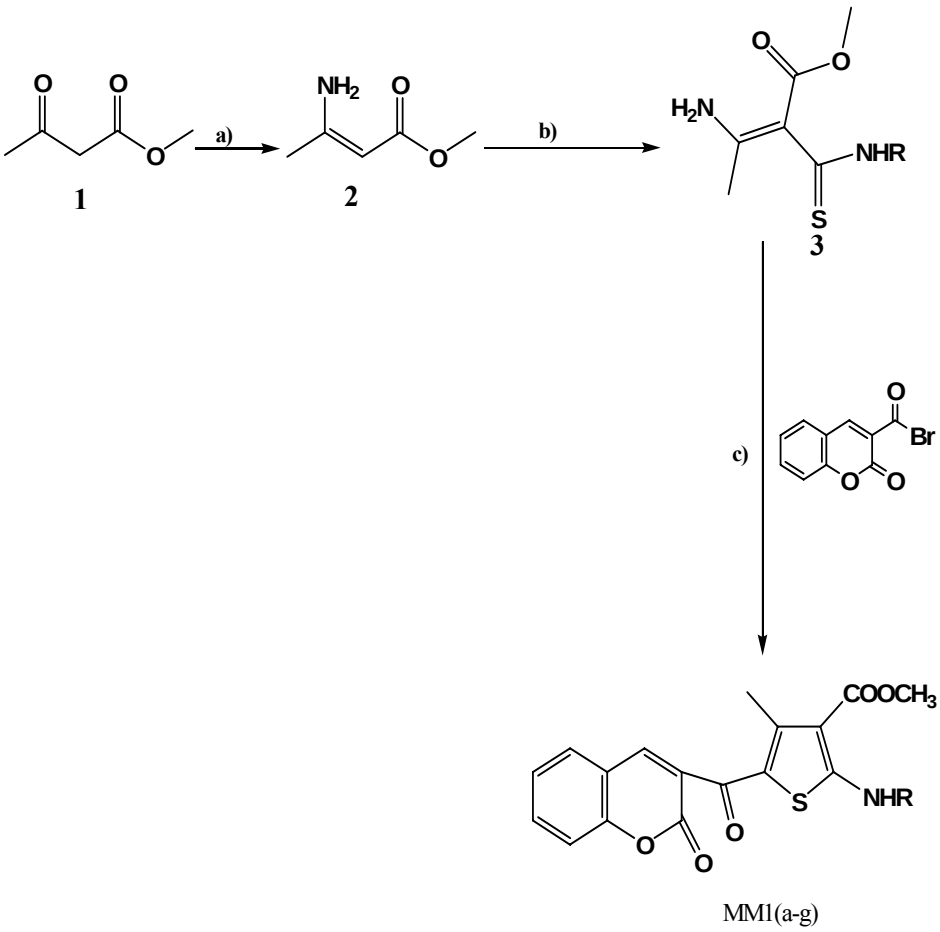


Figure 2.11: Basic structure of targeted compounds.

2.4.1 Plan of work:

1. Design and synthesis of novel heterocyclic compounds with 3-substituted coumarin which is liked at 5th position to thiophene and thiazole.

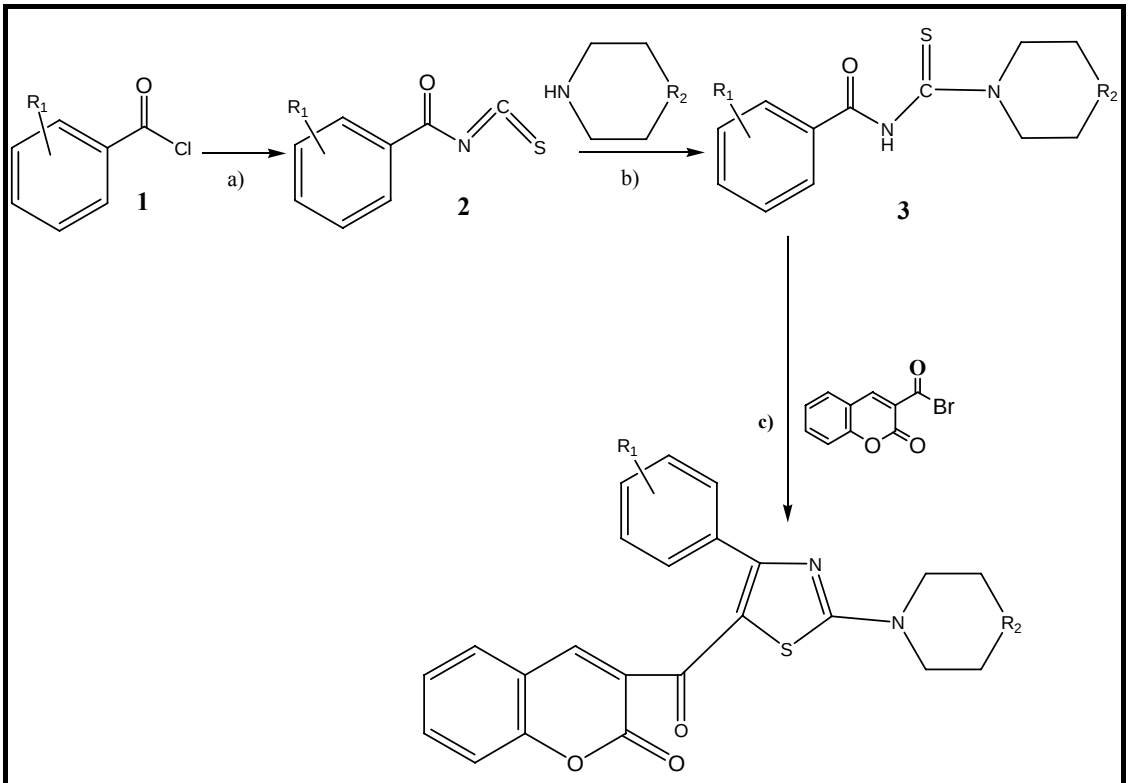
Scheme 1: Synthetic scheme for "tetrasubstituted thiophene-coumarin" derivatives



MM1(a-g)

Scheme 1. Synthesis of tetrasubstituted thiophene-coumarine derivatives:
Reagents and conditions: a) ammonia (25%), diethylether, 0-15°C, 1 h; b) ArNCS/ ArCONCS, diethylether, 0°C-r.t., 5 h; c) acetonitrile, r.t., 12 h.

Scheme 2: Synthetic scheme for coumarin- thiazole derivatives:



Scheme 2. Synthesis of Coumarin-thiazole derivatives:
Reagents and conditions: a) NH4SCN, Acetone, Reflux 25 min; b) reflux for 15 min, pour reaction mixture to crushed ice; c) dimethylformamide, stirr at 70°C to 80°C for 2 hour, pour to crushed ice.

2. Characterization of the compounds spectrally.
3. Screening of the tetrasubstituted thiophene compounds for their anti-inflammatory activity, which is performed because it has been proved that thiophene ring is acting as bioisosteric moiety of Fenamates (Molvi *et al.,* 2007, 2006).
4. Screening of thiazole compounds for their antiplatelet and antibacterial activity.

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CHAPTER III

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME 2-SUBSTITUTED-AMINO-5-(3-COUMARIN-CARBONYL)-4-METHYL-THIOPHENE-3-CARBOXYLIC ACID METHYL ESTERS

3.1 Introduction:

Thiophene derivatives represent an important class of compounds with diverse biological activities. Substituted thiophenes are also present in natural products. Various tri and tetrasubstituted thiophene derivatives and their anti-inflammatory activity are well documented in literature (Gans *et al.,* 1990, Pillai *et al.,* 2003).

According to previously reported by Molvi (Molvi *et al.,* 2008, Molvi *et al.,* 2007, Molvi *et al.,* 2006), the anti-inflammatory activity of tetrasubstituted thiophene ester/acid molecules having the features of (a) COX-1 inhibitor and 5-LOX inhibitor (acid/ester) of the anthranilic acid type (fenamates), (b) p38 MAP kinase inhibitor, can be significantly modified by using substituents at R1 (both electron releasing and electron withdrawing) in anilino moiety and R2 (electron releasing and electron withdrawing) in benzoyl moiety (Figure 3.1).

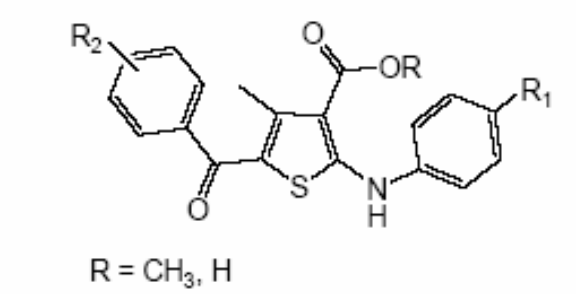


Figure 3.1: Structure of tetra substituted thiophene ester/acid molecule.

The pharmacological evaluation of tetrasubstituted thiophene esters having carbonyl spacer as aroylamino at the second position of the thiophene ring which has a proton acceptor (=C=O) and a proton donor (-NH) features in adjacent position were also reported (Figure 3.2).

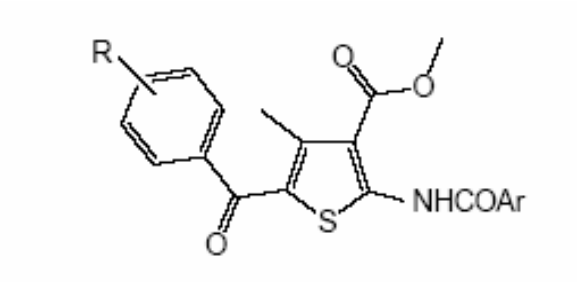
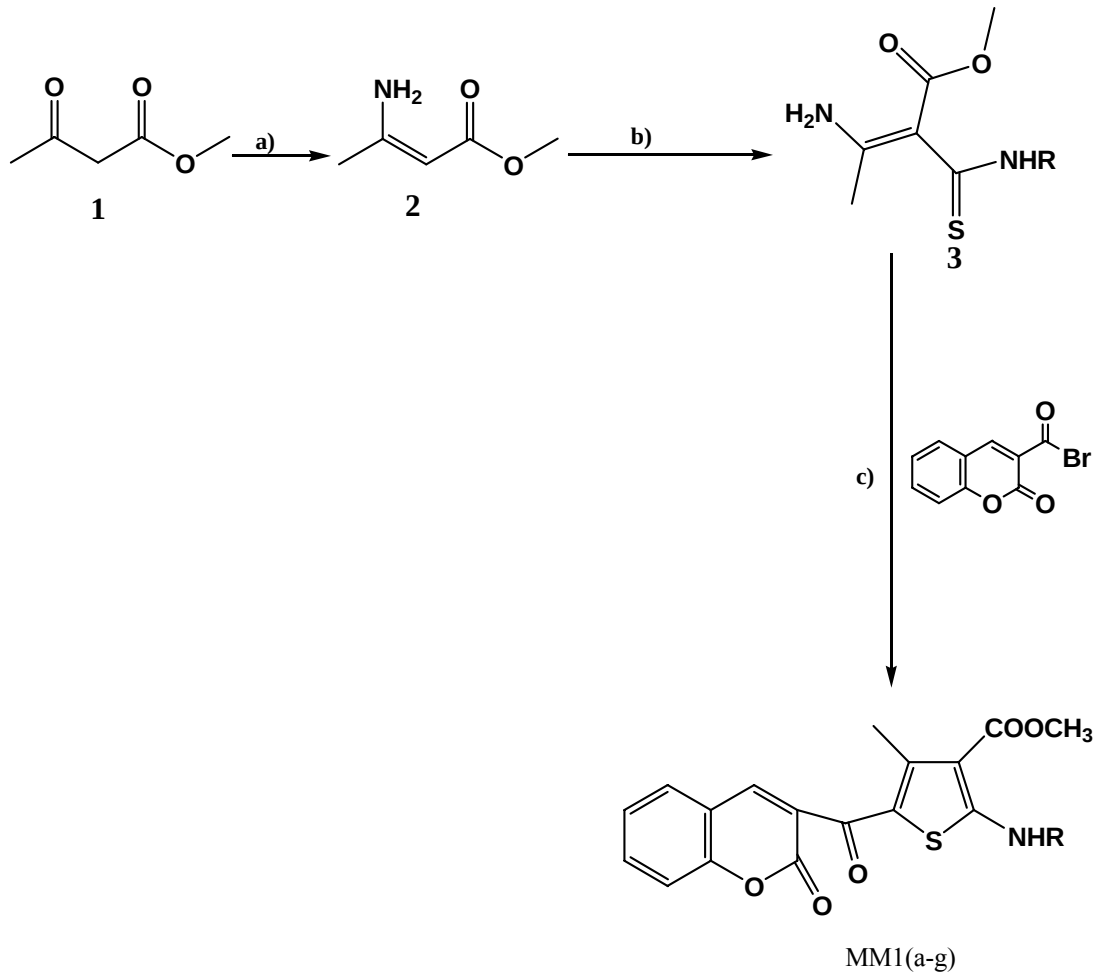


Figure 3.2: Structure of tetrasubstituted thiophene esters having carbonyl spacer as aroylamino at the second position of the thiophene ring.

In continuation to previous reports in designing and synthesizing new tetrasubstituted thiophenes with good anti-inflammatory activity and selectivity, we report here synthesis, in vivo anti-inflammatory, analgesic activity of a new series of designed tetrasubstituted thiophene ester molecules (Scheme 3.1)



MM1(a-g)

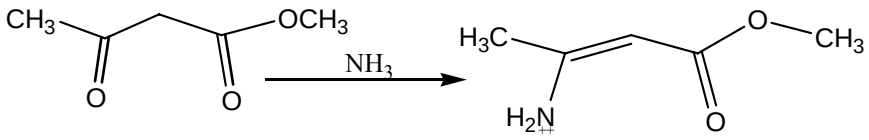
Scheme 3.1: Synthesis of tetrasubstituted thiophene-coumarine derivatives:
Reagents and conditions: a) ammonia (25%), diethylether, 0-15^{o}C, 1 h; b) ArNCS/ ArCONCS, diethylether, 0^{o}C-r.t., 5 h; c) acetonitrile, r.t., 12 h.

3.2 Synthesis of intermediates:

3.2.1 Synthesis of methyl-β-amino crotonate (ENA-1):

Reaction:

The reaction was presumed to follow by the nucleophilic attack of the amine nitrogen (NH_3), to the β carbonyl carbon of methyl acetoacetate (I) with concomitant loss of a water molecule, to yield methyl-β-amino crotonate (ENA-1).



I **ENA-1**

Requirements:

Chemicals	Molecular formula (MW)	Quantity used (Mole)
Methyl aceto acetate(I)	$C_5H_8O_2$ (100)	21.6 (0.21)
Liquor ammonia (25%)	NH_3 (17)	35.6 (1.90)

Procedure:

A neat solution of methyl acetoacetate (21.6 ml, 0.2 mole) was added 35.6 ml, 1.9 mole) of liquor ammonia (25%) at 0^oC with vigorous stirring over one hour. The solution was stirred for another three hours at 0-15^oC. To the reaction mixture, 40 ml. of chilled water was added and stirring continued for 1 hour at room temperature. White crystalline solid separated from the reaction mixture, which was filtered and washed successively with ample amount of water till removal of smell of ammonia. The solid was dried and weighed to yield 15.5 gm (62.39 %). This was coded as ENA-1.

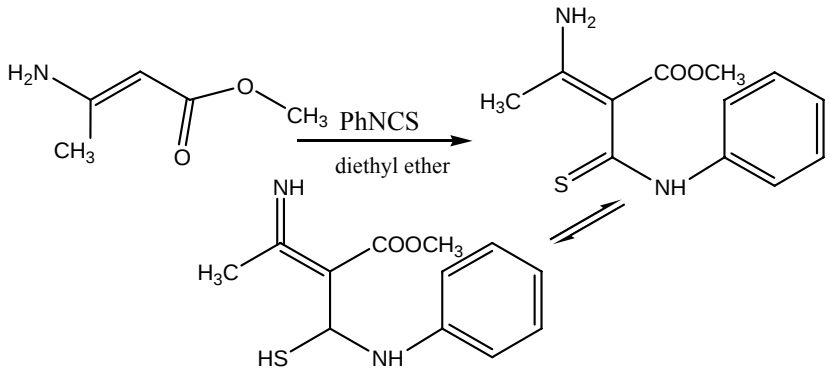
M.P.: 82-83^oC.

TLC: Mobile Phase Toluene:acetonitrile 8:2,R_f- 0.75.

Molecular formula: $C_5H_9NO_2$ (115 MW).

3.2.2 Synthesis of 1-(α-Carbomethoxy-β-amino-thiocrotonoyl)-aniline (Add-MMa) (Goerdeler 1961).

Reaction:



Requirements:

Chemicals	Molecular formula (MW)	Quantity used (Mole)
ENA-1	$C_5H_9NO_2$ (115)	0.92gm (0.008)
Phenyl isothiocyanate	C_7H_5NS (135)	0.90 ml (0.008)

Procedure:

To a stirred solution of ENA-1 (0.92 gm, 0.008 mole) in 15 ml of diethyl ether was added of phenyl isothiocyanate (0.9 ml, 0.008 mole) in 10 ml. ether at 0^0C over a period of 10 minutes and the solution was stirred at room temperature for 5 hour, and then refluxed for 1 hour. Reaction mixture was cooled to room temperature. The yellow crystalline product which separated from the reaction mixture was filtered, washed with 25 parts of warm petroleum ether (40^0C-60^0C) and dried, yielding 1.1 gm (55%) a product. This was coded as Add-MMa

M.P.: 147-148^{o}C.

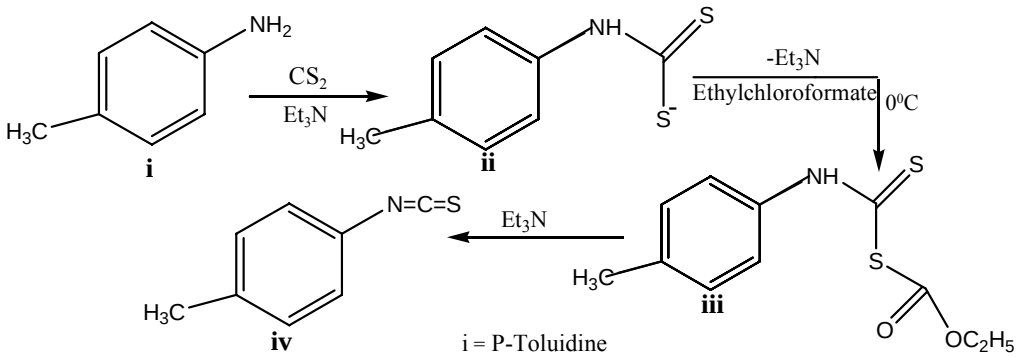
TLC: Mobile Phase Toluene:acetonitrile 8:2,R_f- 0.47.

Molecular formula: $C_{12}H_{14}N_2O_2S$ (250 MW).

3.2.3 Synthesis of Add-MMb.

3.2.3.1 Synthesis of 4-Methylphenyl isothiocyanate (Intermediate for Synthesis of Add-MMb)

Reaction:



i = P-Toluidine
ii =Triethylammonium dithiocarbamate
iii =Carboethoxy dithiocarbamate
iv = 4-methyl-phenyl-isothiocynate

Requirements:

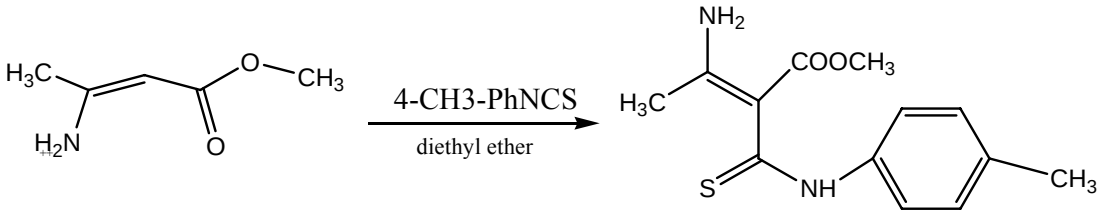
Chemicals	Molecular formula (MW)	Quantity used (Mole)
p-Toluidine	C_7H_9N (107)	10.00 ml (0.1)
Carbon disulfide	CS_2 (76)	14.00 ml (018)
Ethyl chloroformate	$C_3H_5ClO_2$ (106.5)	10.20 ml (0.1)
Triethylamine	$C_6H_{15}N$ (101)	28.00 ml (0.2)

Procedure:

p-Toluidine (10.00 ml, 0.1 mole) was dissolved in 50 ml benzene and treated with carbon disulfide (14.00 ml,0.18 mole) and triethylamine (14.00 ml, 0.1 mole) and the solution was cooled to 0^0C. The precipitated salt of triethylammonium dithiocarbamate was filtered and washed with 30 ml diethyl ether and air dried for 10 min. The salt was then dissolved in 75 ml chloroform, treated with triethylamine (14.00 ml, 01 mole) and cooled again to 0^0C.To this solution ethylchloroformate (10.20 ml, 0.1 mole) was added dropwise over a period of 15 min. period with stirring. The resulting solution was stirred at 0^0C for 10 min. and allowed to stand at room temperature for 1 hour period. The chloroform solution was then washed with 100 ml, 3M HCl solution and 2x75 ml water and was dried over sodium sulfate. The chloroform was evaporated in vacuum and the p-Methylphenyl isothiocyanate (4.5 gm.) which was dissolved in ether and taken for the preparation of adduct without purification.

3.2.3.2 Synthesis of 1-(α-Carbomethoxy-β-amino-thiocrotonoyl)-p-methyl- aniline (Add-MMb)

Reaction:



Requirements:

Chemicals	Molecular formula (MW)	Quantity used (Mole)
ENA-1	$C_5H_9NO_2$ (115)	11.5gm. (0.1)
4-methylphenyl -isothiocyanate	C_8H_7NS (149)	14.5 gm (0.1)

Procedure:

To a stirred solution of ENA-1 (11.5gm, 0.1 mole) in 35 ml of diethyl ether was added 4-methylphenyl isothiocyanate (14.9 gm,.0 mole) in 30 ml. ether at 0^0C over a period of 10 minutes and the solution stirred at room temperature for 15 hour, then refluxed for 2 hours. Reaction mixture was cooled to room temperature. The yellow crystalline product that separated from the reaction mixture was filtered, washed with 25 parts of warm petroleum ether (40^0C-60^0C fraction) and dried, yielding 7 gm product. This was coded as Add-MMb.

Molecular formula: $C_{13}H_{16}N_2O_2S$ (264 MW).

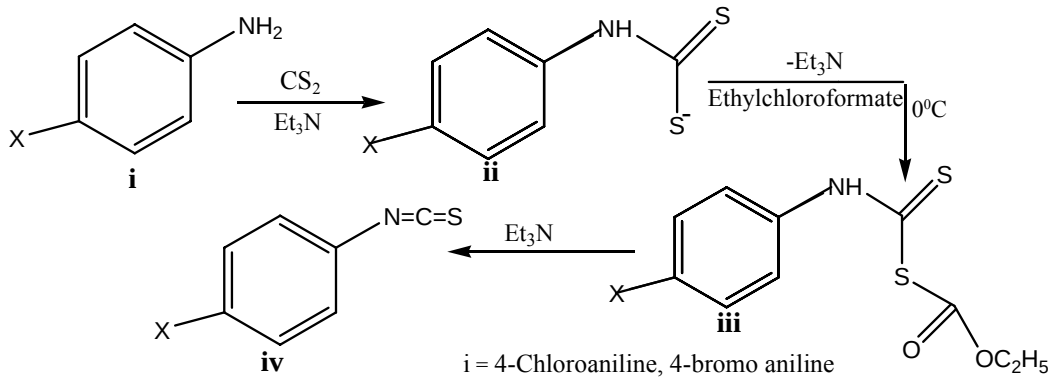
M.P.: 110^0C.

3.2.4 Synthesis of Add-MMc and Add-MMd.

3.2.4.1 Synthesis of 4-chloro and bromo-phenyl isothiocyanate (Intermediate for synthesis of Add-MMc and Add-MMd)

(Hodgkins 1964).

Reaction:



i = 4-Chloroaniline, 4-bromo aniline
ii =Triethylammonium dithiocarbamate
iii =Carboethoxy dithiocarbamate
iv = 4-Chloro-phenyl-isothiocynate,4-bromo-phenyl-isothiocynate
X-Cl, Br

Requirements:

Chemicals	Molecular formula (MW)	Quantity used (Mole)
4-Chloroaniline, 4-bromoaniline	C_6H_6ClN (127.5) C_6H_6BrN (172)	12.75 gm (0.1) 17.20 gm (0.1)
Carbon disulfide	CS_2 (76)	14.00 ml (0.1)
Ethyl chloroformate	$C_3H_5ClO_2$ (106.5)	10.20 ml (0.1)
Triethyl amine	$C_6H_{15}N$ (101)	28.00 ml (0.2)

Procedure:

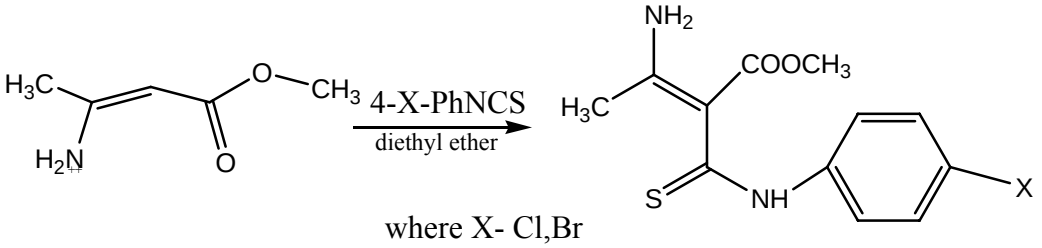
p-Chloroaniline/ p-bromoaniline (0.1 mole) was dissolved in 50 ml benzene and treated with carbon disulfide (6.6 ml, 0.1 mole) and triethylamine (14 ml, 0.1 mole) and the solution was cooled to 0^0C, the precipitated salt of triethylammonium dithiocarbamate was filtered and washed with 30 ml diethyl ether and air dried for 10 min. The salt was then dissolved in 75 ml chloroform, treated with triethylamine (14 ml, 0.1 mole) and cooled again to 0^0C.To this solution, was added ethylchloroformate (10.2 ml, 0.1 mole) dropwise over a period of 15 minutes with stirring. The resulting solution was stirred at 0^0C for 10 min. and allowed to cool at room temperature for one hour. The chloroform solution was then washed with 100 ml, 3M HCl solution

and 2x75 ml water and was dried over sodium sulfate. The chloroform was evaporated in vacuum and the isothiocyanate was recrystallised from ethanol (6.85 g 40%).

M.P.: 45-47^{0}C.

3.2.4.2 Synthesis of 1-(α-Carbomethoxy-β-amino-thiocrotonoyl)-p-chloro/bromo aniline (Add–MMc, Add-MMd)

Reaction:



where X- Cl,Br

Requirements:

Chemicals	Molecular formula (MW)	Quantity used (Mole)
ENA-1	$C_5H_9NO_2$ (115)	11.5 gm (0.1)
4-Chlorophenyl isothiocyanate	C_7H_4ClNS (169.5)	17.0 gm (0.1)
4-bromophenyl isothiocyanate	C_7H_4BrNS (214)	21.0 gm (0.1)

Procedure:

To a stirred solution of ENA-1 (11.5 gm, 0.1 mole) in 35 ml of diethyl ether was added 0.1 mole of 4-Chlorophenyl/bromophenly isothiocyanate in 30 ml ether at 0^{0}C over a period of 10 minutes, and the solution was stirred at room temperature for 15 hour, then refluxed for 2 hours. Reaction mixture was cooled to room temperature. The yellow crystalline product that separated from the reaction mixture was filtered, washed with 25 parts of warm petroleum ether and dried, yielding 0.40 gm of product. This was coded as Add-MMc, Add-MMd.

M.P.: 140-143^{o}C.

TLC: Mobile Phase Toluene:acetonitrile 8:2,R_f- 0.57.

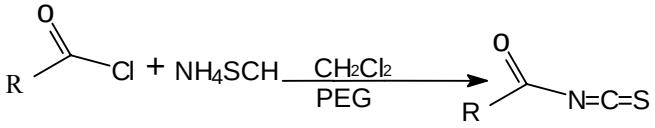
Molecular formula: $C_{12}H_{13}ClN_2O_2S$ (284.5 MW).

$C_{12}H_{13}BrN_2O_2S$ (329 MW).

3.2.5 Synthesis of Add-MMe and Add-MMf.

3.2.5.1 Synthesis of aroyl isothiocyanate (Intermediate for synthesis of Add-MMe and Add-MMf) (Pillai *et al.*, 2004,2003)

Reaction:



Requirements:

Chemicals	Molecular formula (MW)	Quantity used (Mole)
Aroyl chloride*	ArCOCl	0.1 mole
Ammonium thiocyanate	CH_4N_2S	41.0 gm
PEG-400	-	0.20 gm

*ArCOCl = Benzoyl Chloride, Furoyl Chloride

Procedure:

Powdered ammonium thiocyanate 41.00 gm. In 50.00ml dichloromethane was taken in a single necked flask, and the reaction mixture was stirred room temperature. The PEG-400 (0.20 gm.) was added to the reaction mixture, (color changes to slightly pinkish). Aroylchloride (0.1 mol) was added dropwise over a period of 15-20 min. and stirring was continued for 5 hour (until color of the reaction mixture changed to yellow or orange). Filtered off the reaction mixture and filtrate was evaporated under reduced pressure to yield crude aroyl isothiocyanate as an oil (40 %). It was used further without purification.

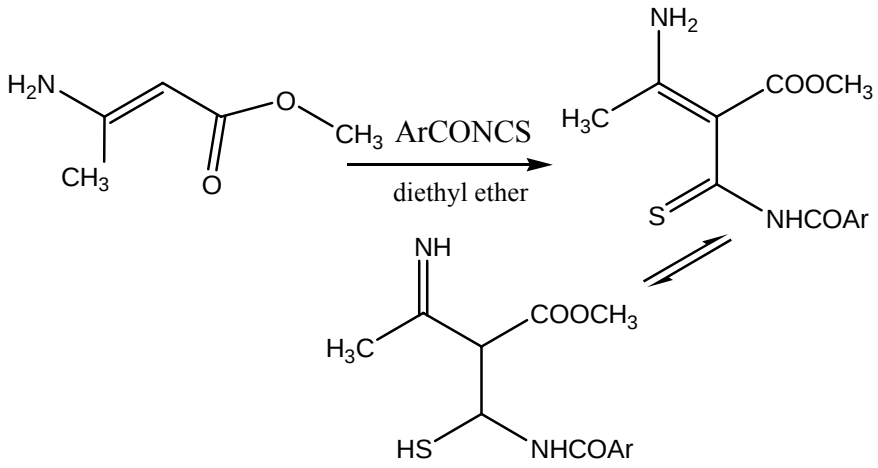
TLC: Mobile Phase Toluene:acetonitrile 8:2,R_f- 0.50.

Molecular formula: **Benzoyl Chloride:** C_8H_5NOS (163MW),

Furoyl Chloride : $C_6H_3NO_2S$ (153MW)

3.2.5.2 Synthesis of 1-(α-Carbomethoxy-β-amino-thiocrotonoyl)-aroyl amine (Add-MMe and Add-MMf).

Reaction:



Requirements:

Chemicals	Molecular formula (MW)	Quantity used (Mole)
ENA-1	$C_5H_9NO_2$ (115)	11.5 gm (0.1)
Benzoyl isothiocyanate	C_8H_5NOS (163)	16.3 gm (0.1)
Furoyl isothiocyanate	$C6H_3NO_2S$ (153)	15.3 gm (0.1)

Procedure:

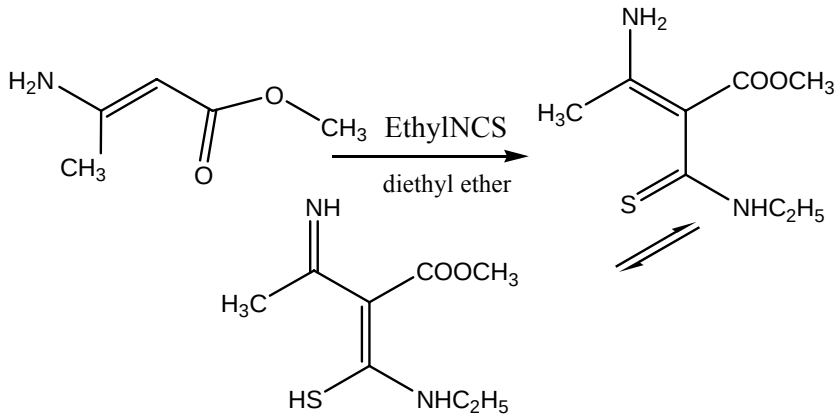
To a stirred solution of ENA-1 (11.5 gm,0.1 mole) in 35 ml of diethyl ether was added 0.1 mole of aroyl isothiocyanate in 30 ml. ether at 0^0C over a period of 10 minutes, and the solution was stirred at room temperature for 15 hour, then refluxed for 2 hours. Reaction mixture was cooled to room temperature. The orange to reddish crystalline product that separated from the reaction mixture was filtered, washed with 25 parts of warm petroleum ether (40^0C-60^0C fraction) and dried, yielding 4.00 gm of a product. This was coded as Add-MMe- Add-MMf.

Adducts	M.P	TLC* R_f	Molecular formula: (MW)
Adduct MMe	145	0.35	$C_{13}H_{14}N_2O_3S$(278)
Adduct MMf	240	0.30	$C_{11}H_{12}N_2O_4S$(268)

*Mobile Phase Toluene:acetonitrile 8:2

3.2.6 Synthesis of 1-(α-Carbomethoxy-β-amino-thiocrotonoyl)-ethyl amine (Add-MMg)

Reaction:



Requirements:

Chemicals	Molecular formula (MW)	Quantity used (Mole)
ENA-1	$C_5H_9NO_2$ (115)	1.15 gm (0.0100)
Ethyl isothiocyanate	C_3H_5NS (87)	0.90 gm (0.0103)

Procedure:

To a stirred solution of ENA-1 (1.5 gm, 0.01 mole) in 15 ml of diethyl ether was added ethyl isothiocyanate (0.6 ml, 0.0103 mole) in 10 ml. ether at 0^0C over a period of 10 minutes and the solution was stirred at room temperature for 5 hour, then refluxed on water bath for1.5 hours. Reaction mixture was cooled to room temperature. The yellow crystalline product, separated from the reaction mixture was filtered, washed with 25 parts of warm petroleum ether and chilled acetone and dried, yielding 0.8gm of a product. This was coded as Add-MMg.

M.P.: 95-96^{o}C (Pillai 1999).

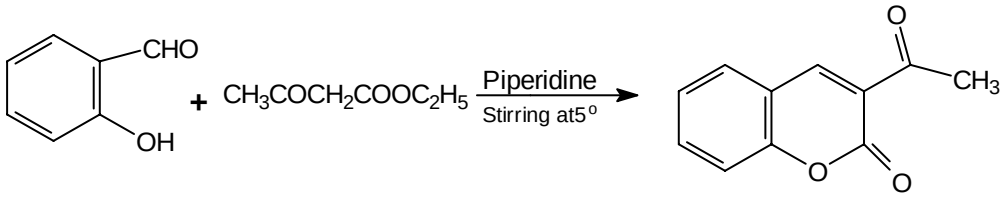
TLC: Mobile Phase Toluene:acetonitrile 8:2,R_f- 0.22.

Molecular formula: $C_8H_{14}N_2O_2S$ (202 MW).

3.2.7 Synthesis of 3-bromoacetylcoumarin:

3.2.7.1 Synthesis of 3-acetylcoumarin:

Reaction:



Requirements:

Chemicals	Molecular formula (MW)	Quantity used (Mole)
Ethyl acetoacetate	$C_6H_{10}O_3$ (130)	13.00 gm (0.1)
Salicylaldehyde	$C_7H_6O_2$ (122)	12.20 gm (0.1)

Procedure:

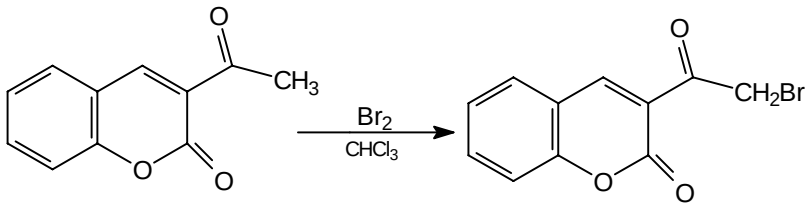
To a mixture of ethyl acetoacetate (13.00gm, 0.1 mole) and piperidine (0.5 ml) was added freshly distilled salicyladehyde (12.4 gm, 0.1 mole) dropwise, with stirring at 0-5^{o}C over a period of 30 minutes. Stirring was continued for 1 hour. The crude product that separated in the flask was boiled with water repeatedly and filtered. On cooling the filtrate a colorless crystalline mass separated. It was collected and dried, yielding 11.7 gm (66%) of product.

M.P.: 121^{o}C (122^{o}C) (Knovenagel et al 1998).

Molecular formula: $C_{11}H_8O_3$ (188 MW).

3.2.7.2 Synthesis of 3-Bromoacetylcoumarin:

Reaction:



Requirements:

Chemicals	Molecular formula (MW)	Quantity used (Mole)
3-Acetylcoumarin	$C_{11}H_8O_3$ (188 MW)	18.80 gm (0.1)
Bromine	Br_2 (160)	16.00 gm (0.1)

Procedure:

To a stirred solution of 3-acetylcoumarin (18.80 gm,0.1 mole) in chloroform (65 ml) was added bromine (16.00 gm, 0.1 mole) in chloroform (15 ml.) over a period of 20 minutes .The reaction mixture was further heated for 30 minutes on a steam bath to expel most of the hydrogen bromide. The crude bromo product that separated on cooling was filtered, washed with cold ether (15 ml) and dried. It was purified by recrystallisation from acetic acid.

M.P.: 165^o C (Koclesch *et al.,* 1950).

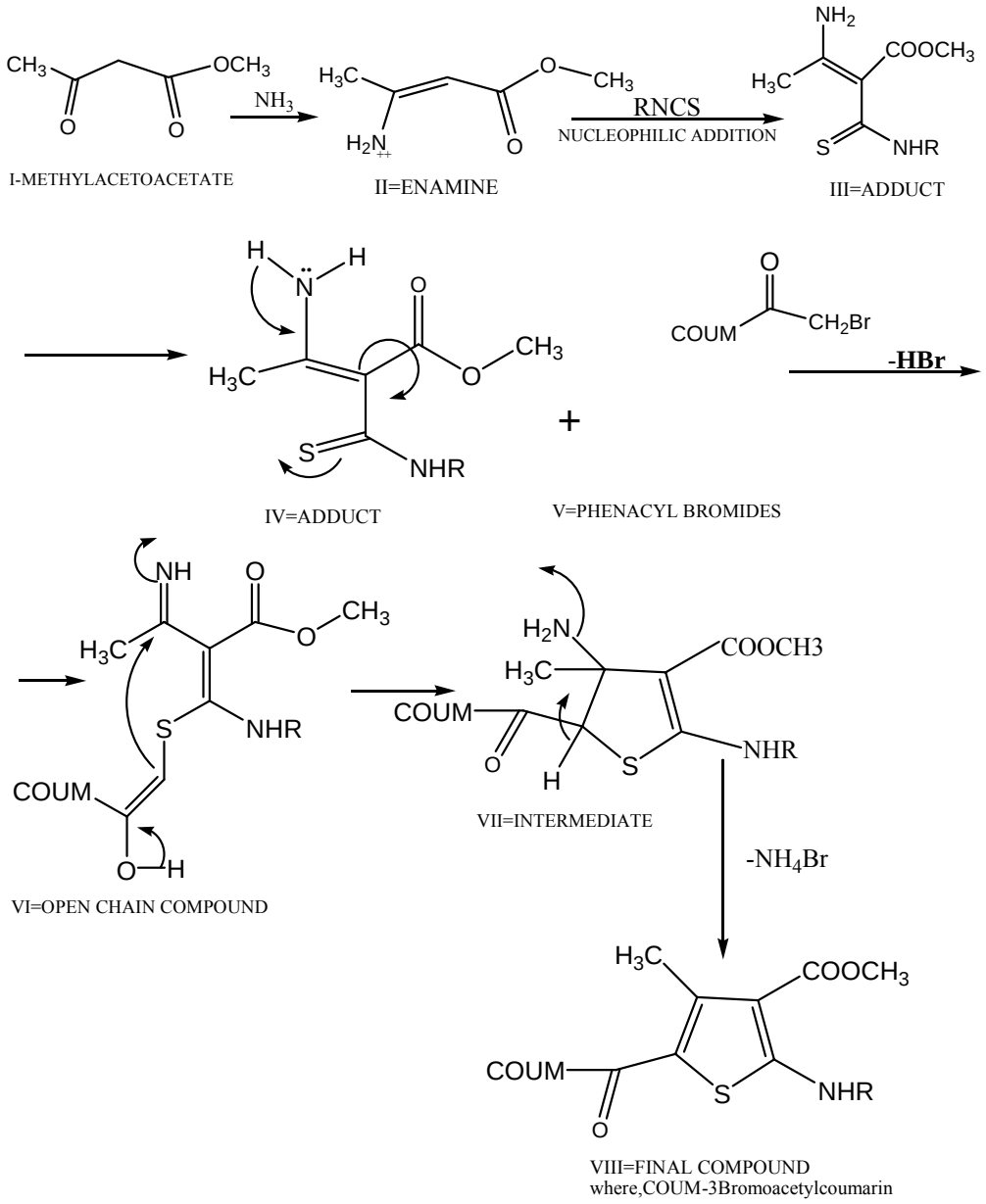
Molecular formula: $C_{11}H_7BrO_3$ (267 MW).

3.3 Synthesis of tetra substituted thiophene:

(Target compounds coded as MM1a-MM1g*

3.3.1 Mechanism of reaction:

The literature (Rajappa and Advani 1971) reports outline the usage of polar protic solvents like isopropyl alcohol, for this type of reactions without any added base and refluxing for half an hour. We utilized a polar non-protic neutral solvent like acetonitrile in many cases, without any added base. The overall mechanism of the reaction is as follows.



***** Published:-

Movli KI, Mansuri MM, Sudarsanam V, Patel MM, Syed MA, Haque N, Synthesis, anti-inflammatory, analgesic and antioxidant acitivites of some tetrasubstituted thiophenes. J Enz Inhib Med Chem. 2008;23(6):829-838.

3.3.2 General procedure for synthesis of 2-substituted-amino-5-(3-coumarin-carbonyl)-4- methyl- thiophene-3-carboxylic acid methyl esters derivatives.

As shown in Scheme 1, enamine (II) was obtained by reacting ammonia (25%) with methyl acetoacetate in equimolar quantity in diethylether at 0–158C (I). 1- (α-Carbomethoxy-β-aminothiocrotonoyl)-aryl/aroyl amines (III) were synthesized by nucleophilic addition of aryl/aroylisothiocyanate and enamine (II) as per reported procedure (Pillai 2003). Arylisothiocyanates were synthesized using modified Kaluza method (Hodkins 1964) whereas aroylisothiocyanate by previously reported procedure (Reeves *et al.,* 1981). The compounds MM1(a-g) were synthesized by adding 0.001mol of the 3-(bromoacetyl)coumarin (Koelsch 1950) to a solution of (III) (0.001mol) in 2 ml of acetonitrile without adding base at room temperature (Hodkings 1964). The reaction mixture was stirred until either the solid was separated from the reaction mixture or absence of starting materials on TLC. The solid was filtered off, washed with chilled acetonitrile, dried, recrystalized with methanol yielding coloured product corresponding to the (MM1a-g) characterized as per the analytical data.

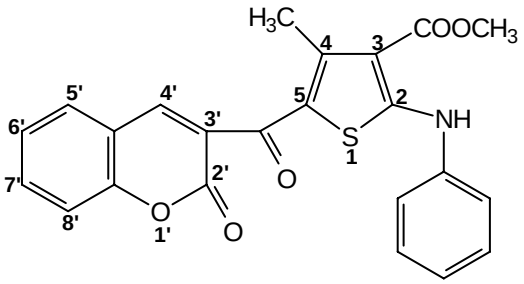
3.3.3 Characteristics of the synthesized compounds:

- 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 vapour.
- 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.

(i) MM1a

Methyl-2-anilino-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate.

Structure:



MW **:** 419

Mol.Formula **:** $C_{23}H_{17}NO_5S$

M.P. **:** 238^0C

Yield **:** 85%

TLC: Mobile Phase- Toluene**:**Acetonitrile 7**:**3**,** Rf-0**.**72.

^{1}H NMR: *($CDCl_3$, δ, ppm)* = 2**.**65 (s, 3H, CH_3 at 4th position), 3**.**90 (s, 3H, CH_3 of ester at 3rd position), 7.17-7.66 (m, 9H, (5H of aromatic protons of 2nd position and 4H of coumarin ring), 7.93 (s, 1H, aromatic proton), 10.70 (s, 1H, NH aryl at 2nd position).

IR *(KBr, cm^{-1})***:** 3036 (aryl NH stretching), 2860 (aromatic CH stretching), 1692 (C=O stretching of ester), 1606 (C=O stretching), 1454 (C=C stretching, aromatic), 1280 (N-C of aromatic), 1239, (C-C[=O]-O symmetric stretching), 975 (-C=C out of plane bending for mono substituted benzene), 674-772 (strongest of thiophene bands C-S stretching).

MASS: 419 (M^+).

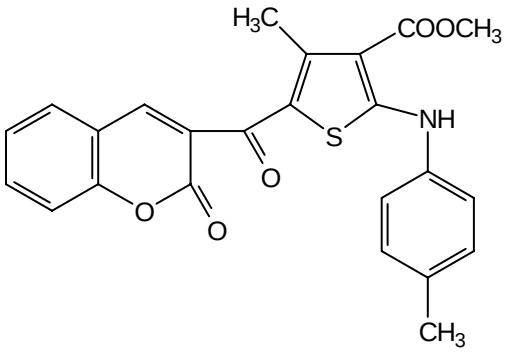
Analysis calculated : C, 65.88; H, 4.05; N, 3.33;

Found : C,66.16; H, 4.01; N, 3.47%.

(ii) MM1b

Methyl-2-(4-methylanilino)-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate.

Structure:



MW **:** 433

Mol.Formula **:** $C_{24}H_{19}NO_5S$

M.P. **:** 162^0C

Yield **:** 45 %

TLC: Mobile Phase- Toluene:Acetonitrile 7:3, Rf-0.69.

^{1}H NMR: *($CDCl_3$, δ, ppm)* = 2.27 (s, 3H, CH_3 at 4^{th} position), 2.56 (s, 3H, CH_3 at 2^{nd} position), 3.93 (s, 3H, CH_3 of ester at 3^{rd} position), 7.09 (d, 2H, J = 5.45 Hz aromatic), 7.19-7.22 (m, 3H, aromatic), 7.40 (t, 2H, aromatic), 7.72 (d, 1H, aromatic), 8.76 (s, 1H, aromatic), 10.09 (s, 1H, NH- at 2^{nd} Position)

IR *(KBr, cm^{-1})***:** 3550 (aryl NH stretching), 2950 (aromatic CH stretching), 1678 (C=O stretching of ester), 1615 (C=O stretching of ketone), 1457 (C=C stretching, aromatic), 1368 (N-C of aromatic), 1180 (C-C[=O]-O symmetric stretching of ester), 685-745 (strongest of thiophene bands C-S stretching).

MASS : m/z 433 (M^+).

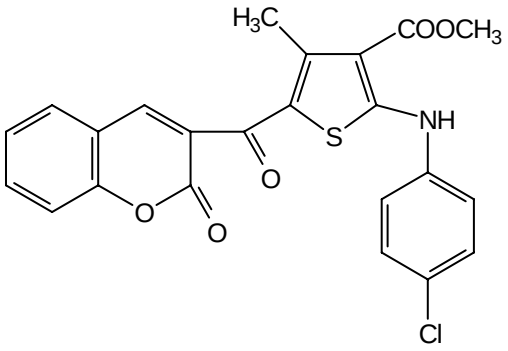
Analysis calculated. : C, 66.52; H, 4.38; N, 3.23;

Found : C, 66.70; H, 4.54; N, 3.65%.

(iii) MM1c

Methyl-2-(4-chloroanilino)-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate.

Structure:



MW **:** 453.5

Mol.Formula **:** $C_{23}H_{16}ClNO_5S$

M.P. **:** 250^0C

Yield **:** 84%

TLC: Mobile Phase- Toluene:Acetonitrile 7:3, Rf- 0.84.

^{1}H NMR: *($CDCl_3$, δ, ppm)* = 2.65 (s, 3H, CH_3 at 4th position),3.91 (s, 3H, CH_3 of ester at 3rd position), 7.31 (d, 2H, J = 5.12 Hz aromatic), 7.37-7.41 (m, 3H, aromatic), 7.61 (t, 2H, J = 4.10 Hz aromatic), 7.64 (d, 1H, aromatic), 7.94 (s, 1H, aromatic), 10.69 (s, 1H, NH at 2nd position)

IR *(KBr, cm^{-1})***:** 3440 (amine NH stretching), 3198 (aromatic CH stretching), 1710 (C=O stretching of ester), 1660 (C=O stretching), 1452 (C=C stretching, aromatic), 1358 (N-C of aromatic),690-797 (strongest of thiophene bands C-S stretching).

MASS : m/z 455 (M^+2).

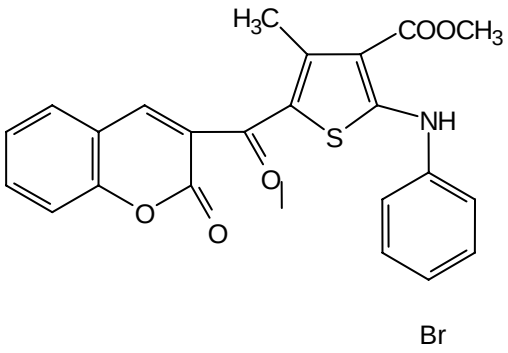
Analysis calculated. : C, 60.88;H, 3.52; N, 3.08;

Found : C, 60.89; H, 3.84; N,3.61%.

(iv) MM1d

Methyl-2-(4-bromoanilino)-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate.

Structure:



MW **:** 497

Mol.Formula **:** $C_{23}H_{16}BrNO_5S$

M.P. **:** 187^0C

Yield **:** 38%

TLC: Mobile Phase- Toluene:Acetonitrile 7:3, Rf- 0.73.

^{1}H NMR: *($CDCl_3$, δ, ppm)* = 2.66 (s, 3H, CH_3 at 4th position),3.90 (s, 3H, CH_3 of ester at 3rd position), 7.42 (d, 2H, aromatic), 7.55-7.62 (m, 3H, aromatic), 7.64 (d, 1H, aromatic), 7.93 (d, 2H, aromatic), 7.97 (s, 1H, aromatic), 10.69 (s, 1H, NH at 2nd position).

IR *(KBr, cm^{-1})***:** 3440 (amine NH stretching), 3198 (aromatic CH stretching), 1693 (C=O stretching of ester), 1648 (C=O stretching of ketone), 1452 (C=C stretching, aromatic), 1358 (N-C of aromatic),690-797 (strongest of thiophene bands C-S stretching).

MASS : m/z 498 (M^+).

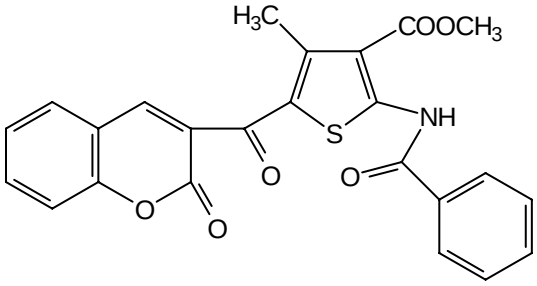
Analysis calculated. : C, 55.43; H, 3.21; N, 2.80;

Found : C,55.91; H, 3.64; N, 2.58%.

(v) MM1e

Methyl-2-benzoylamino-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate.

Structure:



MW **:** 447

Mol.Formula **:** $C_{24}H_{17}NO_6S$

M.P. **:** 182^0C

Yield **:**67%

TLC: Mobile Phase- Toluene:Acetonitrile 7:3, Rf-0.76.

^{1}H NMR: ($CDCl_3$, δ, ppm) = 2.64 (s, 3H, CH_3 at 4th position),3.96 (s, 3H, CH_3 of ester at 3rd position), 7.47-7.53 (m, 6H, aromatic), 8.01 (t, 2H, J = 6.70 Hz aromatic), 7.36 (d, 1H, J = 5.80 Hz aromatic), 8.75 (s, 1H, aromatic),12.66 (s, 1H, NH at 2nd position).

IR *(KBr, cm^{-1})***:** 3360 (amide NH stretching), 2867 (aromatic CH stretching), 1710 (C=O stretching of ester), 1589 (C=O stretching of ketone), 1549 (asym.stretching of aromatic nitro),1452 (C=C stretching, aromatic), 1358 (N-C of aromatic), 994 (-C=C out of plane bending for mono substituted benzene), 680-781 (strongest of thiophene bands C-S stretching).

MASS : m/z 447 (M^+).

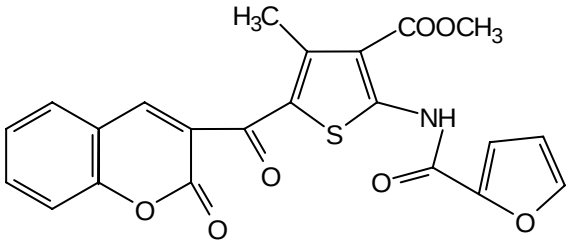
Analysis calculated. : C, 64.42; H, 3.79; N,3.12;

Found : C, 63.98; H, 3.75; N, 3.02%.

(vi) MM1f

Methyl-2-(2-furoylamino)-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate.

Structure:



MW **:** 437

Mol.Formula **:** $C_{22}H_{15}NO_7S$

M.P. **:**188^0C

Yield **:** 60%

TLC: Mobile Phase- Toluene:Acetonitrile 7:3, Rf-0.72.

^{1}H NMR: ($CDCl_3$, δ, ppm) = 2.66 (s, 3H, CH_3 at 4^{th} position), 3.88 (s, 3H, CH_3 of ester at 3^{rd} position), 6.46 (q, 1H, aromatic), 7.39-7.43 (m,3H, aromatic) 7.59 (t, 2H, J ¼ 5.80 Hz aromatic),7.70 (d, 1H, aromatic), 8.06 (s, 1H, aromatic).

IR *(KBr, cm^{-1})***:** 3398 (amide NH stretching), 3048 (aromatic CH stretching), 1724 (C=O stretching of ester), 1682 (C=O stretching of ketone), 1433 (C=C stretching, aromatic), 1350 (N-C of aromatic), 1192 (C-O-C stretching of furan), 676-779 (strongest of thiophene bands C-S stretching).

MASS : m/z 437 (M^+).

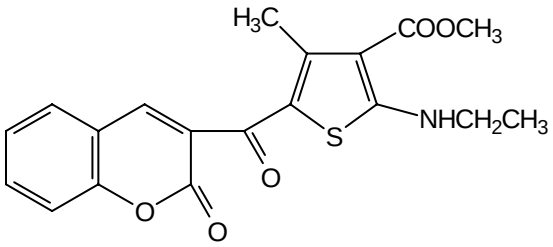
Analysis calculated. : C,60.42; H, 3.43; N, 3.20;

Found : 60.67; H, 3.61; N,3.32%.

(vii) MM1g

Methyl-2-ethylamino-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate.

Structure:



MW **:** 371

Mol.Formula **:** $C_{19}H_{17}NO_6S$

M.P. **:**205^0C

Yield **:**35%

TLC: Mobile Phase- Toluene:Acetonitrile 7:3, Rf-0.68.

^{1}H NMR: ($CDCl_3$, δ, ppm) = 1.35 (t, 3H, J ¼ 3.15 Hz CH3 at 2nd position), 2.61 (s, 3H, CH_3 at 4th position), 3.31 (q, 2H, J ¼ 3.54 Hz CH3at 2nd position), 3.82 (s, 3H, CH3 of ester), 7.30-7.37 (m, 2H, aromatic), 7.57 (t, 2H, aromatic), 7.90 (s, 1H, aromatic), 10.65 (s, 1H, NH at 2nd position).

IR *(KBr, cm^{-1})***:** 3330 (alkyl NH stretching), 2963 (aromatic CH stretching), 2934(aliphatic CH stretching), 1717 (C=O stretching of ester), 1606 (C=O stretching of ketone), 1445 (C=C stretching, aromatic), 1373 (N-C of aromatic), 1269 (C-C[=O]-O symmetric stretching of ester), 680-780 (strongest of thiophene bands C-S stretching).

MASS : m/z 371 (M^+).

Analysis calculated. : C,61.45; H, 4.57; N, 3.77;

Found : C ,61.78; H, 4.78; N,4.01%.

3.4 Pharmacological Screening:

Animals. Albino rats (150–250 g) of either sex were provided with pellet diet and water kept under standard laboratory condition at 25 ± 2 ^{0}C.

Anti-inflammatory activity.

We have used the method previously described by Winter et al (Winter *et al.,* 1962). The animals were studied for toxicity of DMSO up to 10% v/v in saline, and 5% DMSO was selected as a vehicle to suspend the standard drugs and the test compounds. Albino rats of either sex weighing between 150–250 g were starved for 18 h prior to the experiment. The animals were weighed, marked for identification and divided into groups of six. The standard drug ibuprofen (20 mg/kg body weight) and mefanamic acid (100mg/kg body weight) and the test compounds were given orally (10, 20 and 40mg/kg body weight) as a suspension using 5% DMSO as a vehicle. One hour later foot paw oedema was induced by injecting 0.1mL of 1% carrageenan subcutaneously into the planter portion of the right hind paw of each rat. Initial foot paw volume was measured immediately by mercury plethysmometer. Oedema was measured three hours after carrageenan administration. The swelling in test group animals was used to calculate the percent inhibition ±SEM of oedema achieved by the compound at the test dose compared with the vehicle control group. The percentage protection of oedema was calculated according to the formula;

% anti-inflammatory activity =100 * (1-Vt/Vc).

where Vt and Vc are the volume of oedema in test compounds and control groups respectively.

Analgesic activity (Acetic acid induced writhing response model):

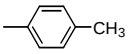
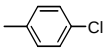
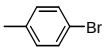
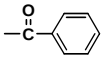
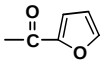
All the targeted compounds were investigated for their analgesic activity in acetic acid induced writhing response in albino mice (20–25 g) at 10 mg/kg body weight dose following the method of Siegmund (Siegmund *et al.,* 1957). Synthesized compounds, 10 mg/kg was administered intra-peritoneally to groups of mice (6 in each group) starved for 16 h. The first group received the test compounds while the groups which served as positive and negative controls received 10 mg/kg ibuprofen and 0.5 ml/100 g body weight of 1% DMSO solution respectively. One hour after treatment, the animals in each group received 0.1ml of 3% acetic acid to induce the characteristic

writhing response. The number of writhing occurring within 30min was recorded and the mean was compared with that of the control and converted into % inhibition.

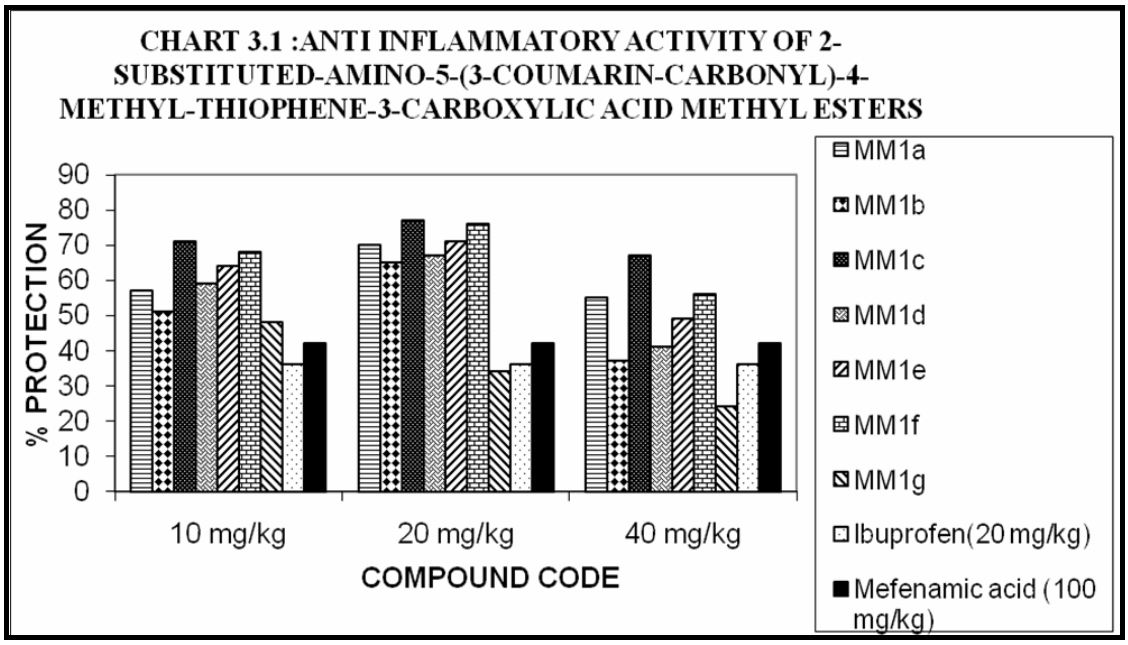
3.5 Results and Discussion:

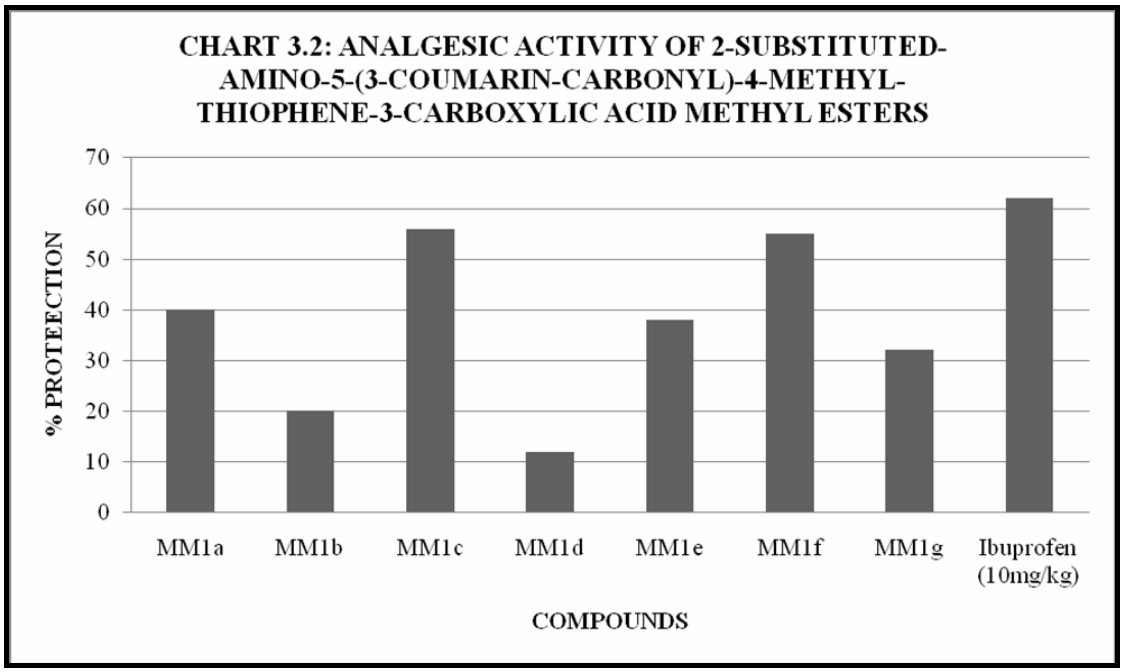
The synthesized compounds MM1(a-g) were screened by in vivo assay for their anti-inflammatory activity using carrageenan-induced rat hind paw oedema model at three graded doses employed at 10, 20 and 40 mg/kg body weight using mefanamic acid, ibuprofen as standard, and analgesic activity using acetic acid-induced writhing response in albino mice at dose of 10mg/kg using ibuprofen as standard.The results are shown in Table 3.1.

Table 3.1. Chemical Structures, anti-inflammatory and analgesic activity of tetra substituted thiophene – coumarin derivatives

		Anti-inflammatory activity Carrageenan-induced rat hind paw oedema % protection			Analgesic activity Acetic acid induced writhing test % protection
Compound no.	**R**	**10 mg/kg**	**20 mg/kg**	**40 mg/kg**	**10 mg/kg**
MM1a		57	70	55	40
MM1b		51	65	37	20
MM1c		71	77	67	56
MM1d		59	67	41	12
MM1e		64	71	49	38
MM1f		68	76	56	55
MM1g	CH_2CH_3	48	34	24	32
Ibuprofen (20 mg/kg)	-	36			-
Mefanamic acid (100mg/kg)	-	42			-
Ibuprofen (10 mg/kg)	-	-	-	-	62

Taking into account the diverse biological activities coumarin derivatives namely anticoagulant and anti-inflammatory activities (Ghate *et al.,* 2005, Ghate *et al.,* 2003, Kontogiorgis 2003), the compounds MM1(a-d) were synthesized keeping coumarin-3-yl constant at fifth position and introducing both electron releasing (-CH3) and electron withdrawing groups (-Cl,-Br) at fourth position in arylamino moiety at second position of thiophene nucleus. In order to examine the effect of introduction of carbonyl spacer attached to -NH group in the form of benzoyl and furoyl at the second position of thiophene moiety, keeping coumarin–3-yl constant substituted at fifth position of thiophene moiety MM1e and MM1f were synthesized. The compound MM1g was synthesized having ethyl group at second position to explore the effect of presence of aliphatic chain on inflammatory activity of profile of the candidate. Among the compounds MM1(a-g), MM1c showed maximum anti-inflammatory activity. It displayed 71% protection at 10mg/kg and 77% protection at 20 mg/kg to inflamed paw, however % protection decreased to 67% at 40 mg/kg as compared to the reference drugs ibuprofen which showed 36% protection at 20mg/kg and mefanamic acid which displayed 42% protection at 100 mg/kg. The compounds MM1a, MM1b, MM1d, MM1e and MM1f showed % protection of 57%, 51%, 59%, 64%, 68% at 10 mg/kg and 70%, 65%, 67%, 71%, 76% at 20 mg/kg to inflamed paw which were comparable to anti-inflammatory activity of both ibuprofen and mefanamic acid. The compounds MM1a, MM1b, MM1d, MM1e and MM1f also showed decrease in % protection to inflamed paw at 40 mg/kg dose which was similar pattern as observed for MM1c the most potent candidate among MM1(a-g). The compound MM1g showed poorer anti-inflammatory activity at all the three graded doses employed. On the basis of structure activity relationship studies of MM1a-MM1g it can be concluded that in this series of compounds the presence of -Cl group in anilino moiety at second position of the thiophene nucleus contribute in enhancing the anti-inflammatory activity profile of the candidate (MM1c). The presence of -CH3 in aniline moiety at second position of the thiophene nucleus seems to reduce the inflammatory activity profile of the candidate (MM1b). Also the presence of benzoyl (MM1e) and 2-furoyl moiety (MM1f) attached to -NH at the second position of the thiophene also contributes significantly to anti-inflammatory activity profile of the candidates.

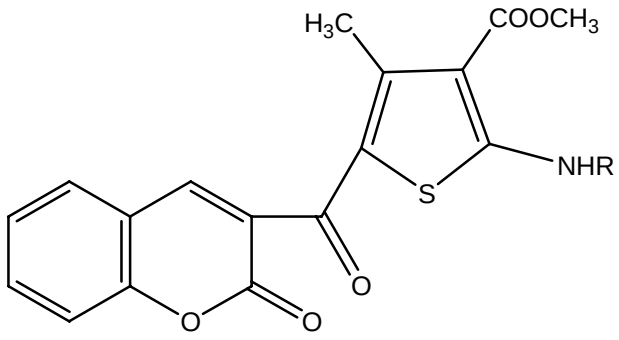




All the synthesized compounds were evaluated for their analgesic activity by in vivo assay using acetic acid induced writhing response test in albino mice at 10 mg/kg dose. Among MM1a- MM1g only MM1c and MM1f showed comparable analgesic activity of 56% and 55% inhibition as compared to reference drug ibuprofen which displayed 62% inhibition at 10 mg/kg dose. The compounds MM1a and MM1e showed moderate analgesic activity of 40% and 38% inhibition at 10 mg/kg dose. The

results of analgesic activity of compounds MM1a- MM1g showed that presence of 4-chlorophenyl and 2-furoyl attached to -NH at second position of thiophenes and coumarin-3-yl attached to -CO group at fifth position of thiophene ring contribute significantly to analgesic activity profile of the candidates MM1c and MM1f.

Table 3.2: Physical characteristics of 2-substituted-amino-5-(3-coumarin-carbonyl)-4-methyl- thiophene-3-carboxylic acid methyl esters derivatives.



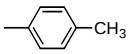
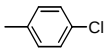
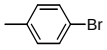
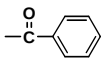
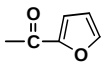
Compounds	R	M.P. (^{0}C)	Yield (%)	MW	Mol. Formula
MM1a		238	85	419	$C_{23}H_{17}NO_5S$
MM1b		162	45	433	$C_{24}H_{19}NO_5S$
MM1c		250	84	453.5	$C_{23}H_{16}ClNO_5S$
MM1d		187	38	497	$C_{23}H_{16}BrNO_5S$
MM1e		182	67	447	$C_{24}H_{17}NO_6S$
MM1f		188	60	437	$C_{22}H_{15}NO_7S$
MM1g	$-CH_2CH_3$	205	35	371	$C_{19}H_{17}NO_6S$

Table 3.3: Spectral data 2-substituted-amino-5-(3-coumarin-carbonyl)-4- methyl- thiophene-3-carboxylic acid methyl esters derivatives.

Code	Nomenclature	^{1}HNMR ($CDCl_3$-d6) (ppm)	IR (Cm^{-1})	MASS (m/z)
MM1a	*Methyl 2-anilino-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate*	2.65 (s, 3H, CH_3 at 4th position), 3.90 (s, 3H, CH_3 of ester at 3rd position), 7.17-7.66 (m, 9H, (5H of aromatic protons of 2nd position and 4H of coumarin ring), 7.93 (s, 1H, aromatic proton), 10.70 (s, 1H, NH aryl at 2nd position).	3036 (aryl NH stretching), 2860 (aromatic CH stretching), 1692 (C=O stretching of ester), 1606 (C=O stretching), 1454 (C=C stretching, aromatic), 1280 (N-C of aromatic), 1239, (C-C[=O]-O symmetric stretching), 975 (-C=C out of plane bending for mono substituted benzene), 674-772 (strongest of thiophene bands C-S stretching).	419 (M^+)
MM1b	*Methyl 2-(4-methylanilino)-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate*	2.27 (s, 3H, CH_3 at 4th position), 2.56 (s, 3H, CH_3 at 2nd position), 3.93 (s, 3H, CH_3 of ester at 3rd position), 7.09 (d, 2H, J = 5.45 Hz aromatic), 7.19-7.22 (m, 3H, aromatic), 7.40 (t, 2H, aromatic), 7.72 (d, 1H, aromatic), 8.76 (s, 1H, aromatic), 10.09 (s, 1H, NH- at 2nd Position).	3550 (aryl NH stretching), 2950 (aromatic CH stretching), 1678 (C=O stretching of ester), 1615 (C=O stretching of ketone), 1457 (C=C stretching, aromatic), 1368 (N-C of aromatic), 1180 (C-C[=O]-O symmetric stretching of ester), 685-745 (strongest of thiophene bands C-S stretching).	433 (M^+).
MM1c	*Methyl 2-(4-chloroanilino)-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate*	2.65 (s, 3H, CH_3 at 4th position),3.91 (s, 3H, CH_3 of ester at 3rd position), 7.31 (d, 2H, J = 5.12 Hz aromatic), 7.37-7.41 (m, 3H, aromatic), 7.61 (t, 2H, J = 4.10 Hz aromatic), 7.64 (d, 1H, aromatic), 7.94 (s, 1H, aromatic), 10.69 (s, 1H, NH at 2nd position).	3440 (amine NH stretching), 3198 (aromatic CH stretching), 1710 (C=O stretching of ester), 1660 (C=O stretching), 1452 (C=C stretching, aromatic), 1358 (N-C of aromatic),690-797 (strongest of thiophene bands C-S stretching).	455 (M^+2)
MM1d	*Methyl 2-(4-bromoanilino)-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate*	2.66 (s, 3H, CH_3 at 4th position),3.90 (s, 3H, CH_3 of ester at 3rd position), 7.42 (d, 2H, aromatic), 7.55-7.62 (m, 3H, aromatic), 7.64 (d, 1H, aromatic), 7.93 (d, 2H, aromatic), 7.97 (s, 1H, aromatic), 10.69 (s, 1H, NH at 2nd position).	3440 (amine NH stretching), 3198 (aromatic CH stretching), 1693 (C=O stretching of ester), 1648 (C=O stretching of ketone), 1452 (C=C stretching, aromatic), 1358 (N-C of aromatic),690-797 (strongest of thiophene bands C-S stretching).	498 (M^+)
MM1e	*Methyl 2-benzoylamino-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate*	2.64 (s, 3H, CH_3 at 4th position),3.96 (s, 3H, CH_3 of ester at 3rd position), 7.47-7.53 (m, 6H, aromatic), 8.01 (t, 2H, J = 6.70 Hz aromatic), 7.36 (d, 1H, J = 5.80 Hz aromatic), 8.75 (s, 1H, aromatic),12.66 (s, 1H, NH at 2nd position).	3360 (amide NH stretching), 2867 (aromatic CH stretching), 1710 (C=O stretching of ester), 1589 (C=O stretching of ketone), 1549 (asym.stretching of aromatic nitro),1452 (C=C stretching, aromatic), 1358 (N-C of aromatic), 994 (-C=C out of plane bending for mono substituted benzene), 680-781 (strongest of thiophene bands C-S stretching).	447 (M^+).
MM1f	*Methyl 2-(2-furoylamino)-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate*	2.66 (s, 3H, CH_3 at 4th position), 3.88 (s, 3H, CH_3 of ester at 3rd position), 6.46 (q, 1H, aromatic), 7.39-7.43 (m,3H, aromatic) 7.59 (t, 2H, J ¼ 5.80 Hz aromatic),7.70 (d, 1H, aromatic), 8.06 (s, 1H, aromatic).	3398 (amide NH stretching), 3048 (aromatic CH stretching), 1724 (C=O stretching of ester), 1682 (C=O stretching of ketone), 1433 (C=C stretching, aromatic), 1350 (N-C of aromatic), 1192 (C-O-C stretching of furan), 676-779 (strongest of thiophene bands C-S stretching).	437 (M^+).
MM1g	*Methyl 2-ethylamino-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate*	1.35 (t, 3H, J ¼ 3.15 Hz CH3 at 2nd position), 2.61 (s, 3H, CH_3 at 4th position), 3.31 (q, 2H, J ¼ 3.54 Hz CH3at 2nd position), 3.82 (s, 3H, CH3 of ester), 7.30-7.37 (m, 2H, aromatic), 7.57 (t, 2H, aromatic), 7.90 (s, 1H, aromatic), 10.65 (s, 1H, NH at 2nd position).	3330 (alkyl NH stretching), 2963 (aromatic CH stretching), 2934(aliphatic CH stretching), 1717 (C=O stretching of ester), 1606 (C=O stretching of ketone), 1445 (C=C stretching, aromatic), 1373 (N-C of aromatic), 1269 (C-C[=O]-O symmetric stretching of ester), 680-780 (strongest of thiophene bands C-S stretching).	371 (M^+)

3.6 Conclusion

The synthesized targeted compounds (MM1a- MM1g) were evaluated for their in vivo anti-inflammatory and analgesic activities. On the basis of structure-activity relationship studies of MM1a- MM1g, it can be concluded that presence of –Cl phenyl moiety (MM1c), benzoyl (MM1e) and 2-furoyl moiety (MM1f) attached to -NH at the second position of the thiophene contributes significantly to anti-inflammatory and analgesic activity profile of the candidates.

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CHAPTER IV

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME 3-(4-(SUBSTITUTED) 2-MORPHOLINO-4-YL-4-PHENYL-THIAZOLE-5-CARBONYL)-1-BENZOPYRAN-2-ONE

4.1 Introduction:

Thiazole moiety as optimal molecular scaffolding:

Thiazole having nitrogen and sulphur as two heteroatoms with an aromatic character is reported to be an important scaffold for designing drugs of various therapeutic categories. Thiazole moiety is present in Vitamin-B1. The utility of the of the structure status of the privilege structure status of thiazole is immediately apparent from the number of drug candidates that have been designed and discovered in the different therapeutic areas as given in figure 4.1 (Muller 2003).

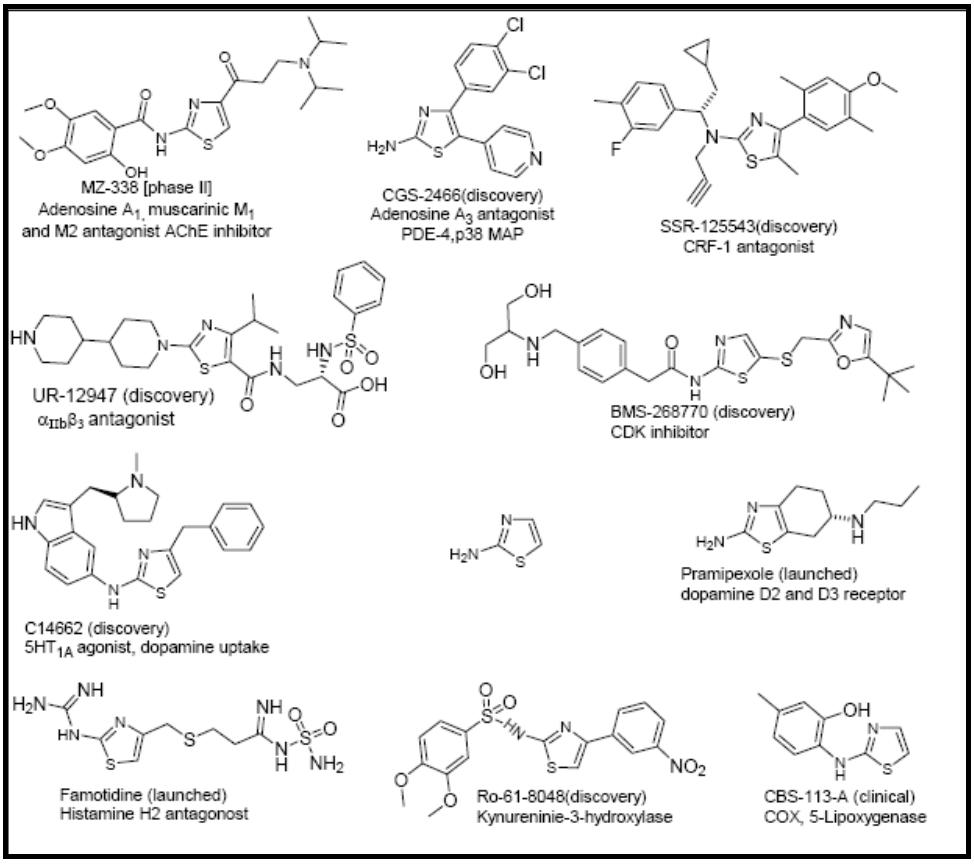


Figure 4.1: Privilege structure status of thiazole.

Thiazoles linked to other heterocycles have been reported to produce various therapeutic effects. Thiazole attached quinoline (Figure 4.2) by amide linkage produce anti-arthritic and analgesic activity (Clemmence 1988).

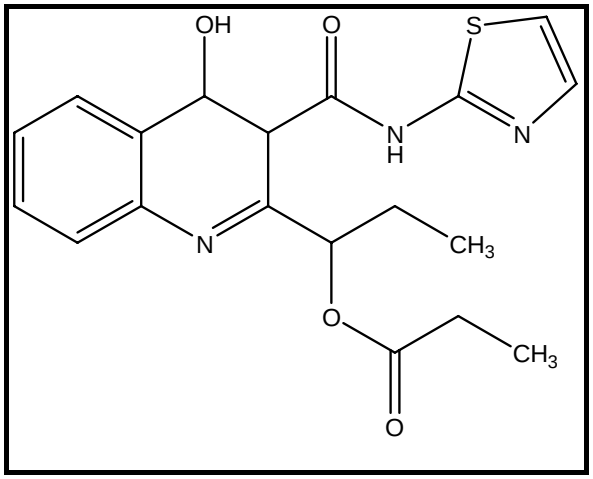


Figure 4.2: Thiazole attached to quinoline.

Meloxicam with significant Cox-2 activity was obtained by replacing pyridine in Piroxicam and isoxazole in Isoxicam (Figure 4.3) (Carty 1988).

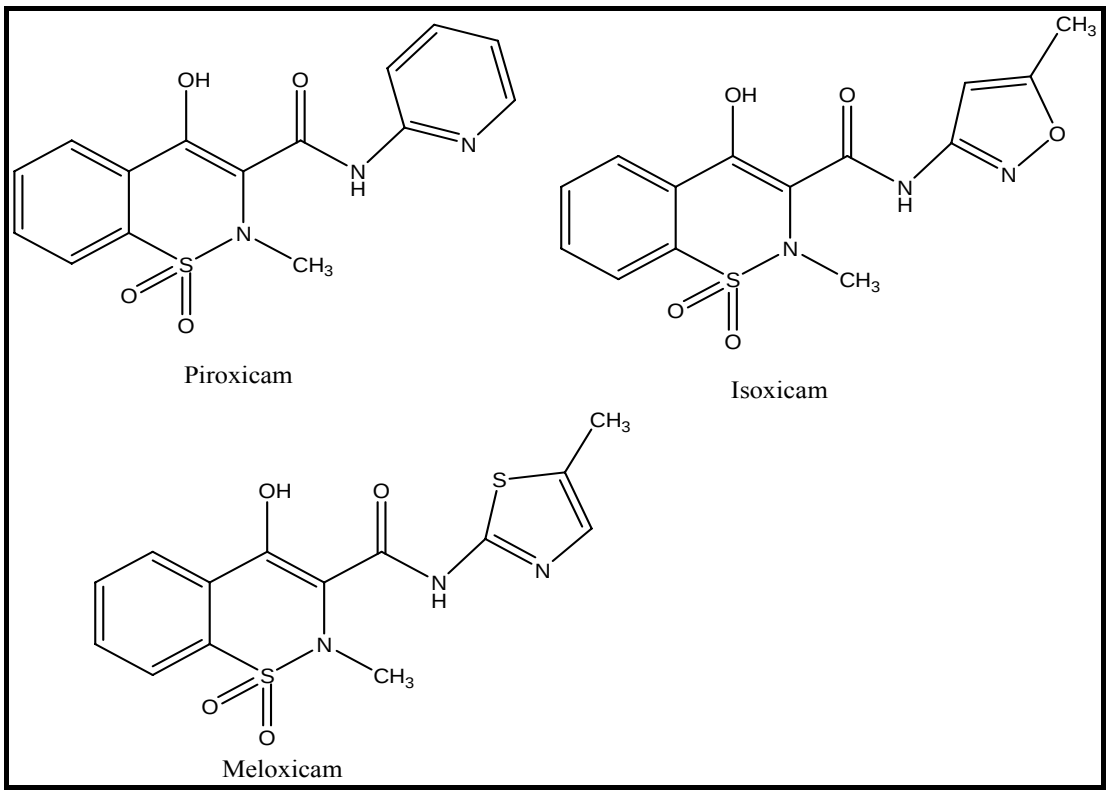


Figure 4.3: Structure of Piroxicam, Isoxicam, Meloxicam.

Selectivity of drugs towards 5-lipoxygenase enzyme in comparison to cyclooxygenase was achieved by novel methoxyalkayl-thiazole derivatives (Figure 4.4) (Bird 1991).

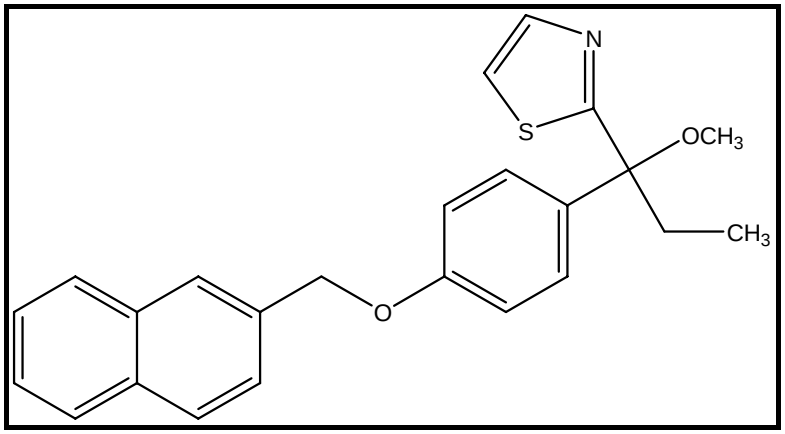


Figure 4.4: Methoxyalkayl-thiazole derivatives.

Kerdesky found that hydroxy thiazoles (Figure 4.5) were potent and selective inhibitors of 5-lipoxygenase enzyme in vitro (Kerdesky 1991).

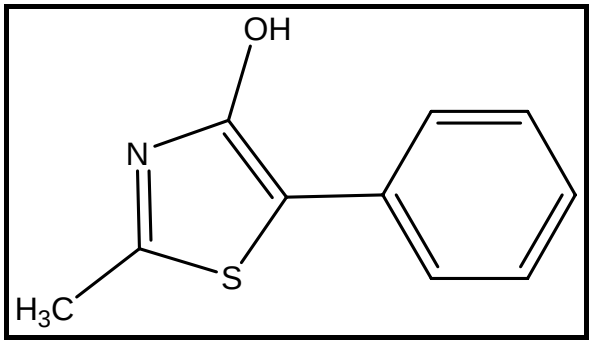


Figure 4.5: Hydroxy thiazoles derivatives.

Fenclozic acid [2-(4-chlorophenyl)-4-thiazole acetic acid] (Figure 4.6), and Fentiazac (Figure 4.7) are thiazole derivatives reported to have anti-inflammatory activity.

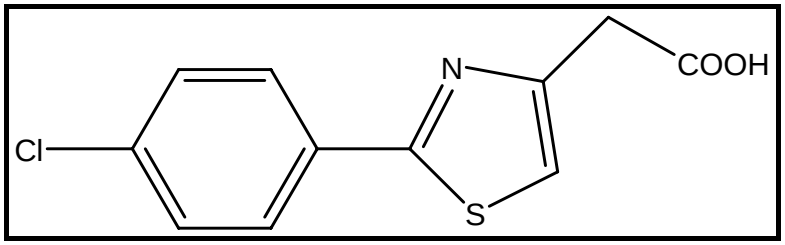


Figure 4.6: Thiazole containing compound – Fenclozic acid.

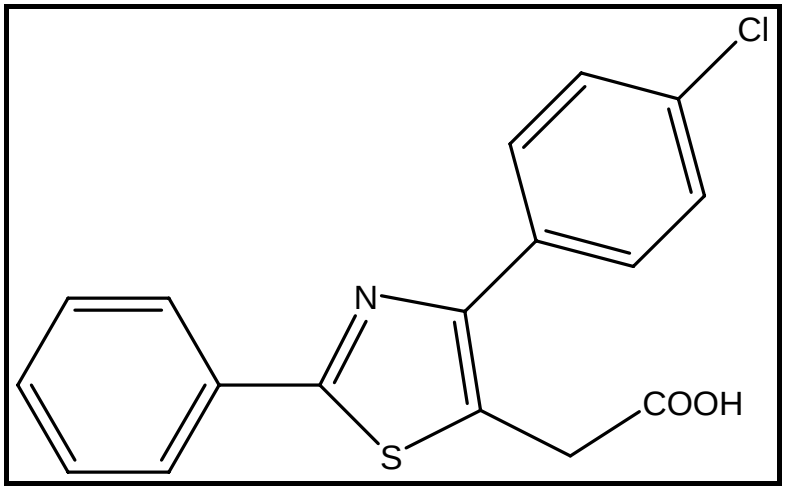


Figure 4.7: Thiazole containing compound – Fentiazac.

2-aryl amino -4-trifluromethyl-5-aminomethyl thiazole (Figure 4.8) represents a novel series of high affinity corticotrophin releasing factor-1 receptor (CRF1R) antagonists (Gene 2003).

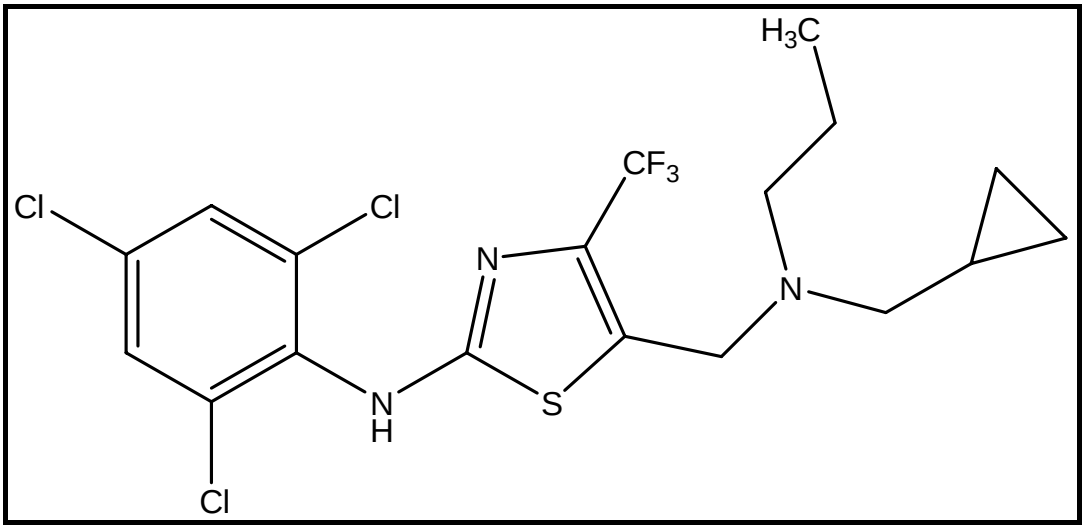


Figure 4.8: 2-Aryl amino -4-trifluromethyl-5-aminomethyl thiazole.

Thiazoles derivatives are identified as inhibitors of macrophage migration inhibitory factor (MIF) a pro-inflammatory mediator produced by immune and endocrine cells and inhibits anti- inflammatory activities of glucocorticoids (Masaya 2003).

AR-A008055 [±-1-(4-methyl-5-thiazolyl-1-phenylmethylamine] (Figure 4.8) a novel compound structurally related to clomethiazole was found to effective as neuroprotective agents (Nelson 2001).

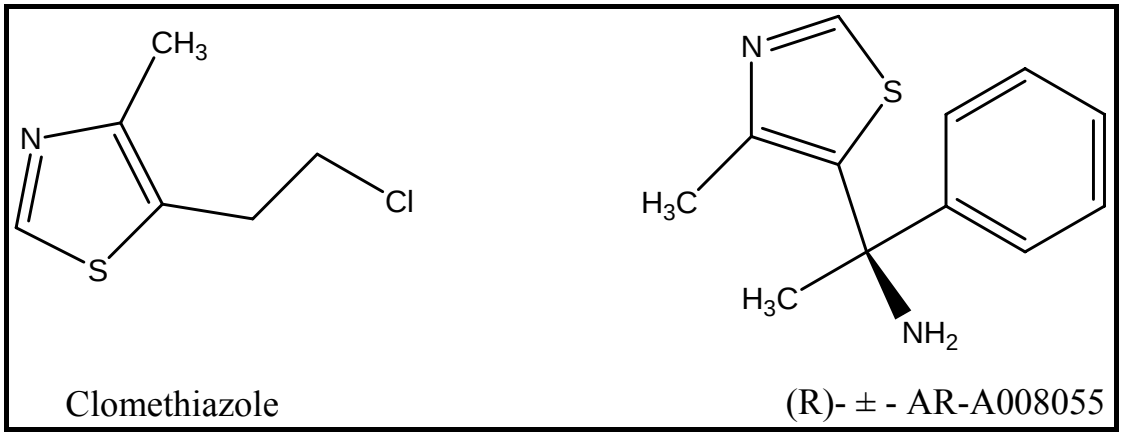


Figure 4.9: Structure of clomethiazole and AR-A008055.

Substituted thiazoles recently found to be cyclin dependent kinase inhibitors which are useful in the treatment of cancer. BMS-419437 and BMS-387032 (Figure 4.10) showed in-vivo antitumor activity in the human ovarian carcinoma A2780 xenograft model in mice (Misra 2002).

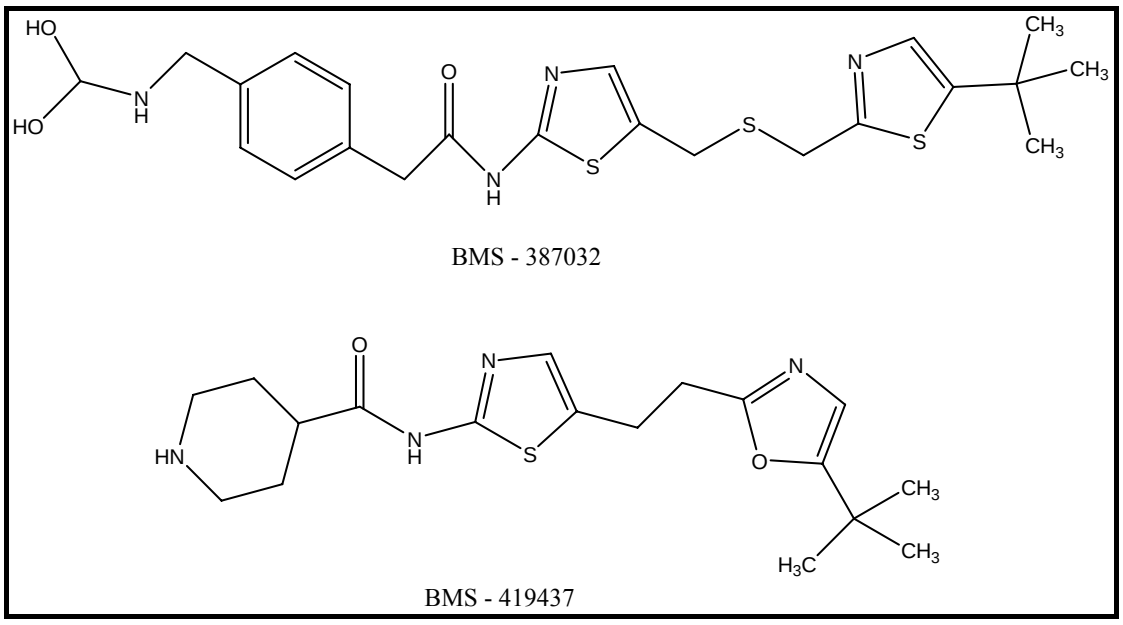


Figure 4.10: Thaizole derivatives-BMS-419437 and BMS-387032.

2-aminothiazole derivatives (Figure 4.11a and Figure 4.11b) with potent antiproliferative activity in the human tumor cell lines inhibits Rb phosphorylation, blocks cells in the G1/s phase and induces apoptosis in A549 cells, consistent with its CDK2/CDK4 inhibition (DePinto *et al.,* 2003).

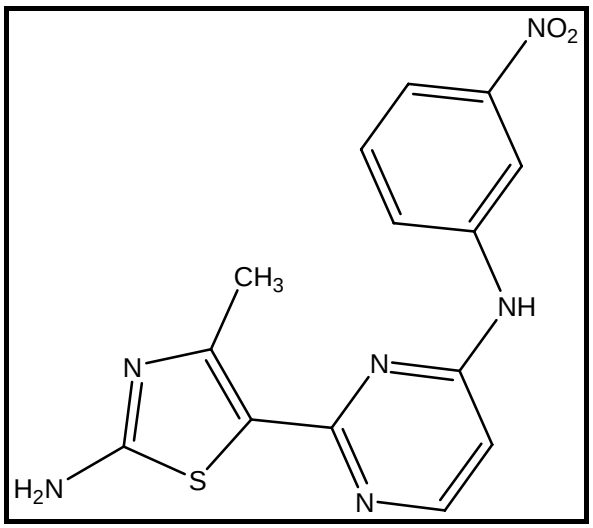


Figure 4.11: 2-Aminothiazole derivatives CDK2 inhibitor.

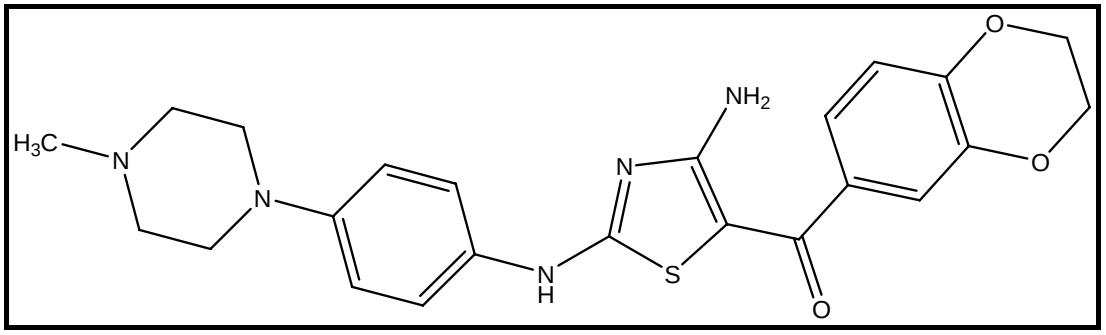


Figure 4.12: 2-Aminothiazole derivatives CDK4 inhibitor.

Thiazole α-ketoamides as HDAC inhibitors (Figure 4.13), showed antiproliferative activity in vitro, as .well as efficacy in anti-tumor model in vivo (Frey 2002).

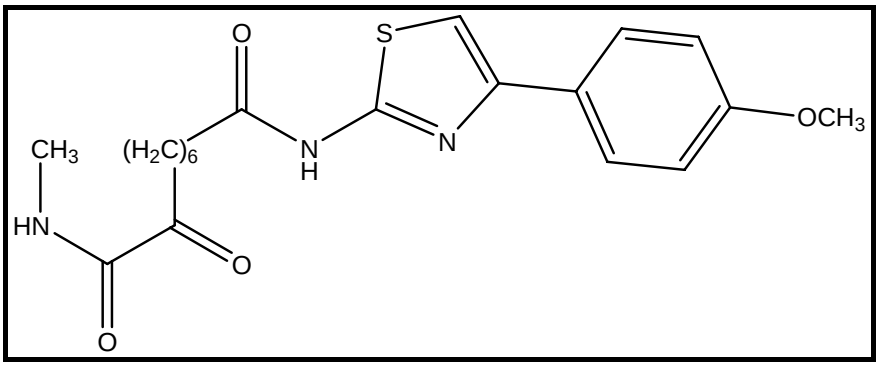


Figure 4.13: Thiazole α-ketoamides derivative.

Pheny thiazolyl urea and carbamate derivatives (Figure 4.14) as new inhibitors of bacterial cell wall biosynthesis (Gerard 2004).

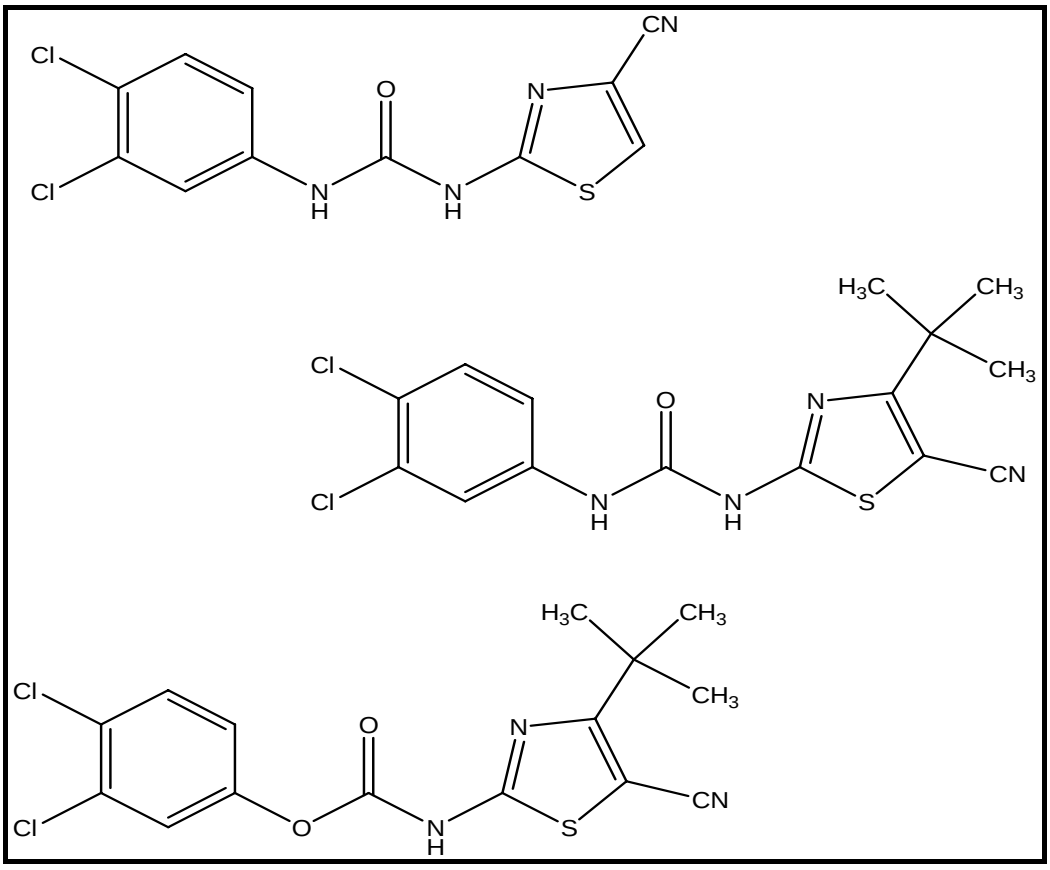


Figure 4.14: Pheny thiazolyl urea and carbamate derivatives.

2-amino thiazole (Figure 4.15) emerged as lead compounds from a high- throughput screening assay, inhibited the growth of staphylococcus aures (Kane et 2003).

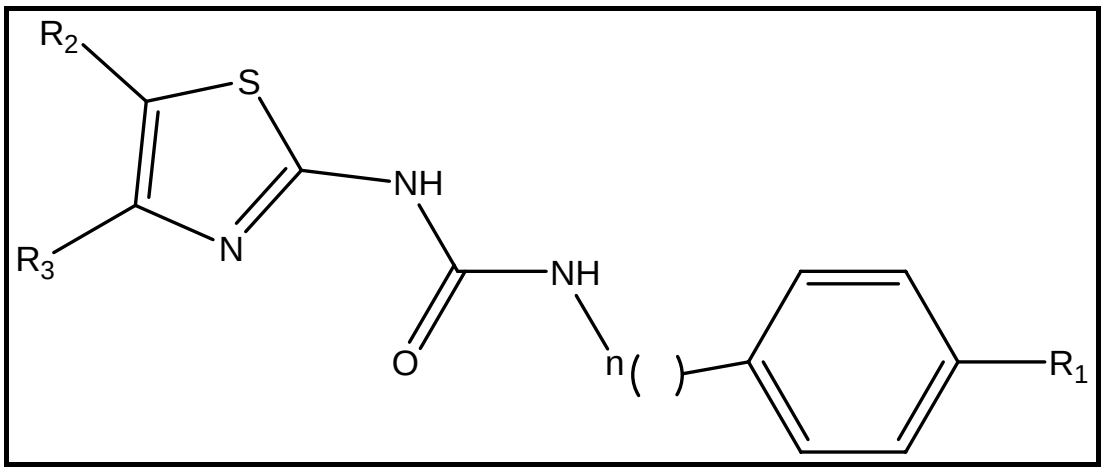


Figure 4.15: 2-Amino thiazole- as lead compounds from a high- throughput screening.

Novel phenolic thiazole (Figure 4.16) with potent antioxidant properties had shown activity in vivo neuro-protection in mitochondrial toxin models (Jeremiah 2004).

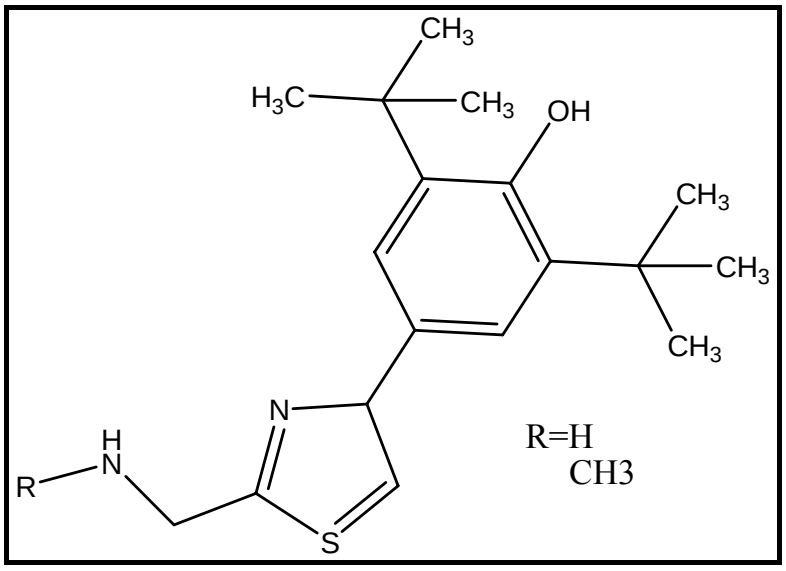


Figure 4.16: Novel phenolic thiazole derivatives.

2-Acetylamino thiazole derivatives (Figure 4.17) have shown hypotensive activity (Grant 1972).

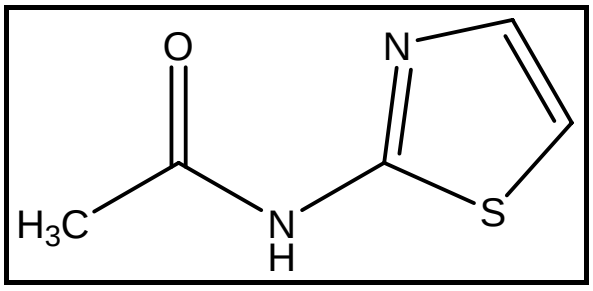


Figure 4.17: 2-Acetylamino thiazole derivatives.

5-(2-aminoethyl)-2-aminothiazole derivatives (Figure 4.18) elicited the histamine H2 receptor agonistic activity. It was hypothesized that the acceptance of a proton by a thiazole nucleus from the receptor site stimulates the histamine H2 receptor (Eriks 1992).

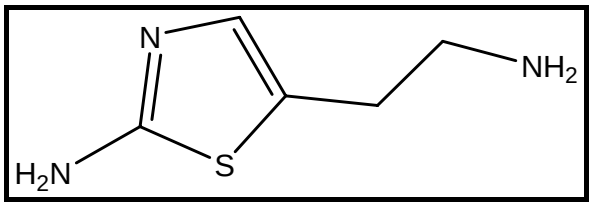


Figure 4.18: 5-(2-aminoethyl)-2-aminothiazole derivatives.

N-(2-phenylethyl)-N-(2-thiazolyl) thiourea (LY 73497) (Figure 4.19) inhibits HIV-1 reverse transcriptase enzyme useful as anti-AIDS molecule (Bell *et al.,* 1995).

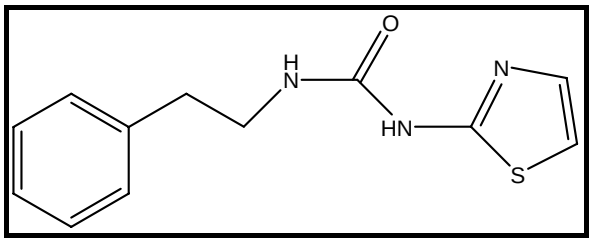


Figure 4.19: N-(2-phenylethyl)-N-(2-thiazolyl) thiourea derivative.

Thiazoles and their derivatives have attracted continuing interest over the years because of their varied biological activities in hypertension (Patt et al 1992), inflammation, schizophrenia (Jaen *et al.,* 1990), bacterial (Tsuji and Ishikawa 1994), HIV infections (Bell *et al.,* 1995), hypnotics (Ergenc *et al.,* 1999), treatment of pain (Carter *et al.,* 1999), fibrinogen receptor antagonists with antithrombotic activity (Badorc 1997) and as new inhibitors of bacterial DNA gyrase B (Rudolph 2001).

These illustrate the utility and importance of having thiazole moeity as molecular scaffolding in our target structures to which optimal pharmacophoric features can be attached for good biological activity.

Thus attempts have been made to attach a thiazole side chain at C-3 position of coumarin. In addition, to expand the structural diversity of synthetic courmarins for biological functions, the morpholine fusion at 2^{nd} position of thiazole was also attempted. The Scheme of proposed work is represented in **Scheme 4.1.**

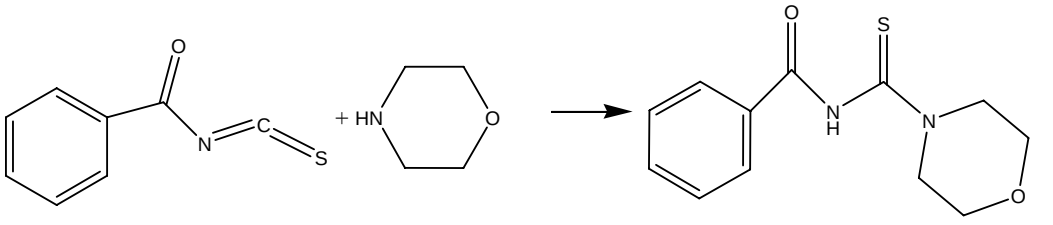
4.2 Synthesis of intermediate:

4.2.1 Synthesis of 3-bromoacetylcoumarin: As per previous chapter 3.2.7

4.2.2 Synthesis of phenyl isothiocyanate and substituted chloro phenyl isothiocyanate: As per previous chapter 3.2.5

4.2.3 Synthesis of substituted-(morpholine-4-thioyl) benzamide (Preparation of Add-M1 to Add-M4)

4.2.3.1 Synthesis of N-(morpholine-4-thioyl) benzamide (Add-M1):



Benzyl isothiocyanate Morpholine *Add-M1: N*-(morpholine-4-thioyl)benzamide

Requirements:

Chemicals	Molecular formula	Quantity used (Mole)
Ammonium thiocyanate	CH_4N_2S	10.48 gm (0.1379)
Benzoyl chloride	C_7H_5ClO	17.76 gm (0.1263)
Morpholine	C_4H_9NO	10.00 gm (0.1149)

Procedure (Rasmussen *et al.*, 1988) :

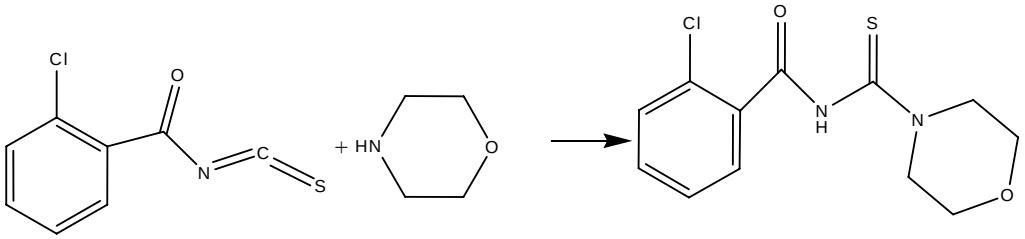
To a stirred solution of ammonium thiocyanate (10.48gm, 0.1379 mole) in 100 ml acetone at room temperature, was added benzoyl chloride (17.76gm, 0.1263 mole) in 5 minutes. The reaction mixture was refluxed for 15 minutes. After reflux, morpholine (10gm, 0.1149 mole) was added into reaction mixture at reflux temperature in 3 minutes and mixture was further refluxed for 30 minutes. Product was isolated by pouring reaction mixture into crushed ice, and separated solid was filtered, washed with acetone and dried. This was coded as Add-M1.

M.P.: 125-127^oC.

TLC: Mobile Phase Toluene:acetonitrile 8:2,R_f- 0.80.

Molecular formula: $C_{12}H_{14}N_2O_2S$ (250 MW).

4.2.3.2 Synthesis of 2-chloro-N-(Morpholine carbothioly)-benzamaide (Add-M2):



2-chlorobenzoyl isothiocyanate Morpholine Add-M2 : 2-chloro-*N*-(morpholine-4-thioyl)benzamide

Requirements:

Chemicals	Molecular formula	Quantity used (Mole)
Ammonium thiocyanate	CH_4N_2S	10.48 gm (0.1379)
2-chlorobenzolychloride	$C_7H_4Cl_2O$	21.97 gm (0.1263)
Morpholine	C_4H_9NO	10.00 gm (0.1149)

Procedure (Rasmussen *et al.,* 1988) :

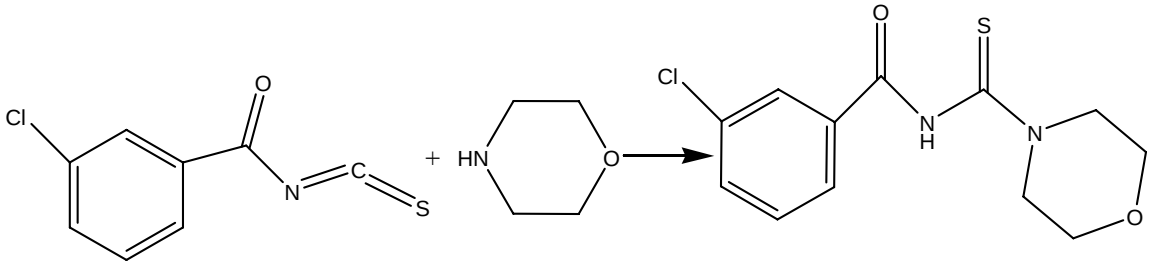
To a stirred solution of ammonium thiocyanate (10.48gm, 0.1379 mole) in 100 ml acetone at room temperature, was added 2-chloro-benzoyl chloride (21.97gm, 0.1263 mole) in 5 minutes. The reaction mixture was refluxed for 15 minutes. After reflux, morpholine (10gm, 0.1149 mole) was added into reaction mixture at reflux temperature in 3 minutes and mixture was further refluxed for 30 minutes. Product was isolated by pouring reaction mixture into crushed ice, and separated solid was filtered, washed with acetone and dried. This was coded as Add-M2.

M.P.: 160-162^oC.

TLC: Mobile Phase Toluene:acetonitrile 8:2,R_f- 0.64.

Molecular formula: $C_{12}H_{13}ClN_2O_2S$ (284 MW).

4.2.3.3 Synthesis of 3-chloro-N-(Morpholine carbothioly)-benzamaide (Add-M3):



3-chlorobenzoyl isothiocyanate Morpholine Add-M3 : 3-chloro-*N*-(morpholine-4-thioyl)benzamide

Requirements:

Chemicals	Molecular formula	Quantity used (Mole)
Ammonium thiocyanate	CH_4N_2S	10.48 gm (0.1379)
3-chlorobenzolychloride	$C_7H_4Cl_2O$	21.97 gm (0.1263)
Morpholine	C_4H_9NO	10.00 gm (0.1149)

Procedure (Rasmussen *et al.,* 1988):

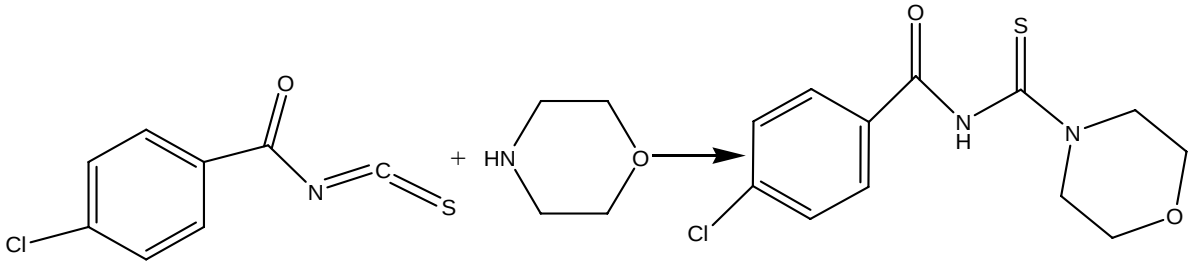
To a stirred solution of ammonium thiocyanate (10.48gm, 0.1379 mole) in 100 ml acetone at room temperature, was added 3-chloro-benzoyl chloride (21.97gm, 0.1263 mole) in 5 minutes. The reaction mixture was refluxed for 15 minutes. After reflux, morpholine (10gm, 0.1149 mole) was added into reaction mixture at reflux temperature in 3 minutes and mixture was further refluxed for 30 minutes. Product was isolated by pouring reaction mixture into crushed ice, and separated solid was filtered, washed with acetone and dried. This was coded as Add-M3.

M.P.: 156-158^oC.

TLC: Mobile Phase Toluene:acetonitrile 8:2,R_f- 0.53.

Molecular formula: $C_{12}H_{13}ClN_2O_2S$ (284 MW).

4.2.3.4 Synthesis of 4-chloro-N-(Morpholine carbothioly)-benzamaide (Add-M4):



4-chlorobenzoyl isothiocyanate Morpholine Add-M4 : 4-chloro-*N*-(morpholine-4-thioyl)benzamide

Requirements:

Chemicals	Molecular formula	Quantity used (Mole)
Ammonium thiocyanate	CH_4N_2S	10.48 gm (0.1379)
4-chlorobenzolychloride	$C_7H_4Cl_2O$	21.97gm (0.1263)
Morpholine	C_4H_9NO	10.00 gm (0.1149)

Procedure: (Rasmussen *et al.*, 1988)

To a stirred solution of ammonium thiocyanate (10.48gm, 0.1379 mole) in 100 ml acetone at room temperature, was added 4-chloro-benzoyl chloride (21.97gm, 0.1263 mole) in 5 minutes. The reaction mixture was refluxed for 15 minutes. After reflux, morpholine (10gm, 0.1149 mole) was added into reaction mixture at reflux temperature in 3 minutes and mixture was further refluxed for 30 minutes. Product was isolated by pouring reaction mixture into crushed ice, and separated solid was filtered, washed with acetone and dried. This was coded as Add-M4.

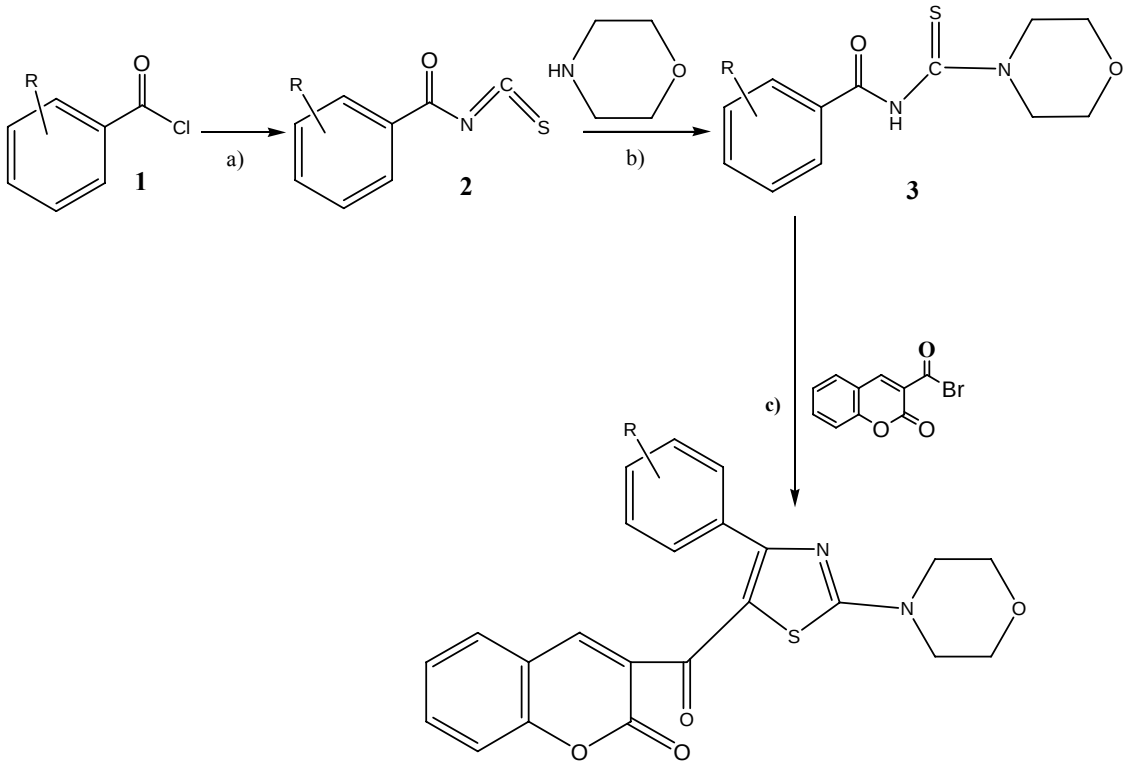
M.P.: 110-112^oC.

TLC: Mobile Phase Toluene:acetonitrile 8:2,R_f- 0.46.

Molecular formula: $C_{12}H_{13}ClN_2O_2S$ (284 MW).

4.3 Synthesis of Morpholino-thiazole-coumarin derivatives (Targeted Compounds coded as MM3M1 –MM3M4)*

4.3.1 Scheme for synthesis of 3-(4-(substituted)-2-morpholino-4-yl-4-phenyl-thiazole-5-carbonyl)-1-benzopyran-2-one:



SCHEME 4.1: Synthesis of 3-(4-(substituted)-2-morpholino-4-yl-4-phenyl-thiazole-5-carbonyl)-1-benzopyran-2-one.
Reagents and conditions: a) NH_4SCN, Acetone, Reflux 25 min; b) reflux for 15 min, pour reaction mixture to crushed ice; c) dimethylformamide, stirr at 70^oC to 80^oC for 2 hour, pour to crushed ice.

4.3.2 General Procedure for Synthesis of 3-(4-(substituted) 2-Morpholino-4-yl-4-Phenyl-Thiazole-5-Carbonyl)-1-Benzopyran-2-One (Reji *et al.,* 2008):

To a solution of the adduct M (0.0025 mmol) in N,N-dimethylformamide (5 ml), 3-bromoacetylcoumarin (0.0025 mmol) was added. The reaction mixture was warmed on a water bath at 80-85 ^{o} C for 5 min. To this, triethylamine (0.0025 mmol, 0.3 ml) was added and heating was continued for another 15 min. The above mixture was cooled and poured into ice-cold water with stirring. A yellow precipitate thus obtained was filtered, wash with water and air-dried. The crude material was purified by preparative TLC (Hexane : Ethyl acetate 3:7).

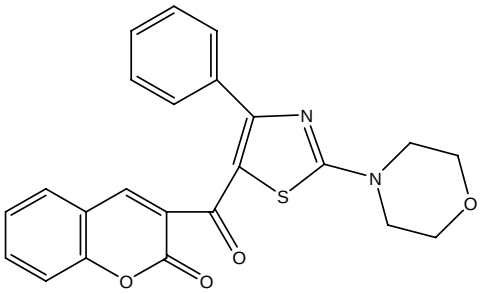
*Published online: Mansuri MM, Seth AK, Molvi KI, Prajapati BR, Desai DG. Synthesis And Pharmacological Evaluation Of Some 3-(4-(Substituted) 2-Morpholino-4-Yl-4-Phenyl-Thiazole-5-Carbonyl)-1-Benzopyran-2-One. Pharma Sci Monitor- Online Published 2011;1490-1500.

4.3.3 Characteristics of synthesized compounds:

(i) MM3M1

3-(2-Morpholin-4-yl-4-phenyl-thiazole-5-carbonyl)-chromen-2-one.

Structure:



MW **:** 419

Mol.Formula **:** $C_{23}H_{18}N_2O_4S$

M.P. **:** 166-168^{0}C

Yield **:** 85%

TLC: Mobile Phase- Haxane **:**Ethyl acetate 3**:7,** Rf-0**.**79.

^{1}H NMR: *(DMSO, δ, ppm)* = 3.60-3.62 (t, 4H, 3rd and 5th morpholin CH_2 at 2nd position of thiazole ring), 3.75-3.78 2nd and 6th morpholin CH_2 at 2nd position of thiazole ring), 6.91-7.5 (m, 9H, (5H of aromatic protons of 4th position and 4H of coumarin ring), 7.6 (s, 1H, aromatic proton).

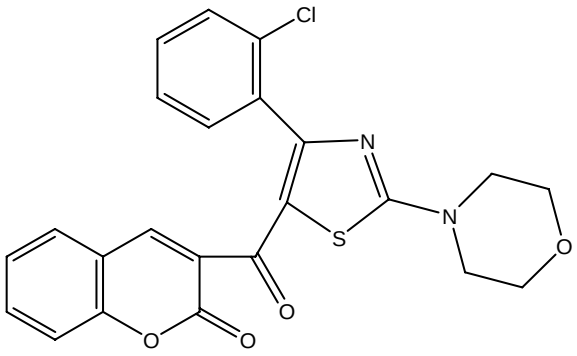
IR *(KBr, cm^{-1})***:** 3100-3000(-CH_2 stretching), 1735 (strong band of -C=O stretching), 1458-1539 (C=C stretching, aromatic), 1374(C-N) 1245, (C-C[=O]-O symmetric stretching).

MASS: 419 ($M^{+\cdot}$).

(ii) MM3M2

3-(4-(2-Chloro-phenyl)-(2-Morpholin—4-phenyl-thiazole-5-carbonyl)-chromen-2-one.

Structure:



MW **:** 453

Mol.Formula **:** $C_{23}H_{17}ClN_2O_4S$

M.P. **:** 212-214^{0}C

Yield **:** 87%

TLC: Mobile Phase- Toluene : Methanol 9**:**1**,** Rf-0**.**60.

^{1}H NMR: *(DMSO, δ, ppm)* = 3.64-3.68 (t, 4H, 3rd and 5th morpholin CH_2 at 2nd position of thiazole ring,J=5Hz),3.8117-3.862 2nd and 6th morpholin CH_2 at 2nd position of thiazole ring,J=5Hz), 6.96-7.56 (m, 8H, (4H of aromatic protons of 4th position and 4H of coumarin ring), 7.72 (s, 1H, aromatic proton).

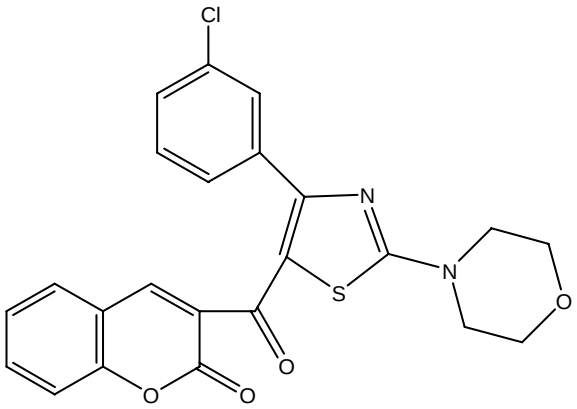
IR *(KBr, cm^{-1})***:** 3100-3000(medium band indicate CH_2 stretching), 1728 (strong band of -C=O stretching), 1450-1530 (C=C stretching, aromatic), 1374(C-N) 1245, (C-C[=O]-O symmetric stretching), 788.89 (Ar-Cl stretching).

MASS m/z: 453 ($M^{+\cdot}$), 454(M^{+1}), 455(M^{+2}).

(iii) MM3M3

3-(4-(3-Chloro-phenyl)-(2-Morpholin—4-phenyl-thiazole-5-carbonyl)-chromen-2-one.

Structure:



MW **:** 453

Mol.Formula **:** $C_{23}H_{17}ClN_2O_4S$

M.P. **:** 182-184^{0}C

Yield **:** 80%

TLC: Mobile Phase- Toluene : Methanol 9**:**1**,** Rf-0**.**65.

^{1}H NMR: *(DMSO, δ, ppm)* = 3.60-3.63 (t, 4H, 3rd and 5th morpholin CH_2 at 2nd position of thiazole ring),3.75-3.77 2nd and 6th morpholin CH_2 at 2nd position of thiazole ring), 6.92-7.51 (m, 8H, (4H of aromatic protons of 4th position and 4H of coumarin ring), 7.68 (s, 1H, aromatic proton).

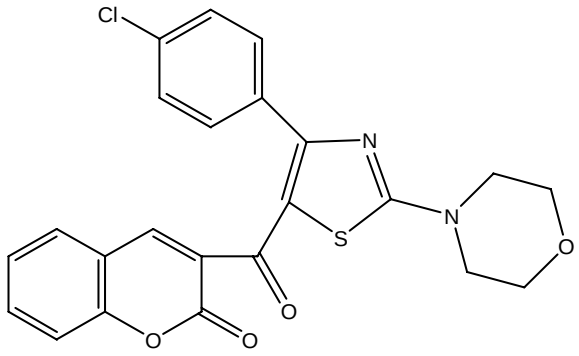
IR *(KBr, cm^{-1})***:** 3100-3000 (medium band indicate CH2 stretching), 1730 (strong band of -C=O stretching), 1448-1533 (C=C stretching, aromatic), 1368 (C-N) 1246 (C-C[=O]-O symmetric stretching), 794 (Ar-Cl stretching).

MASS m/z: 453 ($M^{+\cdot}$), 454(M^{+1}), 455(M^{+2}).

(iv) MM3M4

3-(4-(4-Chloro-phenyl)-(2-Morpholin—4-phenyl-thiazole-5-carbonyl)-chromen-2-one.

Structure:



MW **:** 453

Mol.Formula **:** $C_{23}H_{17}ClN_2O_4S$

M.P. **:** 196-198^{0}C

Yield **:** 78 %

TLC: Mobile Phase- Toluene : Methanol 9**:**1**,** Rf-0**.**64.

^{1}H NMR: *(DMSO, δ, ppm)* = 3.61-3.64 (t, 4H, 3rd and 5th morpholin CH_2 at 2nd position of thiazole ring),3.83-3.86 2nd and 6th morpholin CH_2 at 2nd position of thiazole ring), 7.23-7.63 (m, 8H, (4H of aromatic protons of 4th position and 4H of coumarin ring), 7.65 (s, 1H, aromatic proton).

IR *(KBr, cm^{-1})***:** 3100-3000(medium band indicate CH2 stretching), 1723 (strong band of -C=O stretching), 1448-1533 (C=C stretching, aromatic), 1369 (C-N) 1246 (C-C[=O]-O symmetric stretching), 764 (Ar-Cl stretching).

MASS m/z: 453 ($M^{+\cdot}$), 454(M^{+1}), 455(M^{+2}).

4.4 Pharmacological Screening:

4. 4.1 Back Ground of Antibacterial Research

Before the introduction of antibiotics in 1940's and 1950's patients who had bacteraemia for example, with streptococcus pneumoniae had low chance of survival, and the mortality from tuberculosis was 50% (Austrain 1964, Dineen *et al.,* 1976). Antibiotics radically changed this bleak prognosis, and new class of antibiotics rapidly entered the market in the 1950's and 1960's. Unfortunately this led to over-confidence that Infectious disease would be eradicated. In addition, the large cost of research in discovering new, broad spectrum antimicrobial drugs with previously unexploited modes of action, discouraged pharmaceutical companies from th is area, and many left the field. No new classes of antibiotics were produced in 37 years between the introduction of Nalidixic acid in 1962 and Linezolid in 2000; all of the antibacterial agents that entered the market during this period were modifications of existing molecules (Anthony 2002). Majority of currently used antibiotics acts against a limited number of antibacterial targets. Resistance to this current armory of antibacterials is increasing rapidly, challenging our ability to treat infections caused by common bacterial pathogens. Consequently, both the medical community and government organizations recognize the need for antibiotics that act via inhibition of novel antibacterial targets. Bacterial genomics has reshaped antibacterial drug discovery. The interplay of genomics, bioinformatics and genomic technologies has enabled an in depth analysis of the component enzymes of the bacterial fatty-acid biosynthesis pathway as a source of novel anti-bacterial targets. This evaluation has revealed that many of the enzymes are potentially selective, broad-spectrum antibacterial targets. Fatty acid biosynthesis in bacteria is essential to the production of a number of lipid-containing components including the cell membrane. The bacterial fatty acid synthase system (FASII) utilizes discrete mono-functional enzymes that operate in conjunction with acyl carrier protein (ACP) associated substrates (Figure 4.20). Mammalian fatty acid synthase (FASI) differs from FASII in that lipid synthesis is mediated by single multifunctional enzyme-ACP complex. The differences in prokaryote and eukaryote fatty acid biosynthesis offer an attractive opportunity for selective FASII inhibition. FabI is an enoyl- ACP reducate that catalyses the ultimate and rate–limiting step of the chain elongation process of FASII. The reaction involves the conjugation of an enoyl-ACP to the corresponding acyl-

ACP using the cofactor NAD(P)H as a hydride source. Reports describing the antibacterial agents isoniazid, diazaboranes, and triclosan (Figure 4.21) as inhibitors of bacterial enoyl-ACP reductase support a Fab-I-targeted approach to antibacterial drug therapy (David 2001).

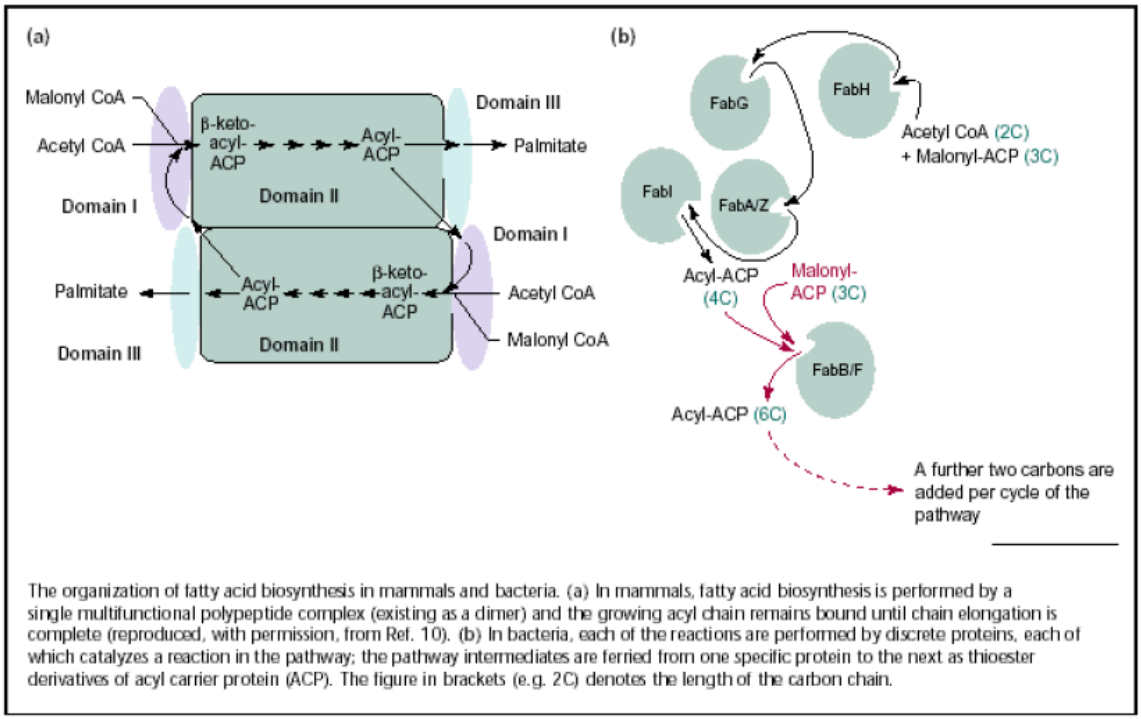


Figure 4.20: The organization of fattry acid biosynthesis in mammals and bacteria.

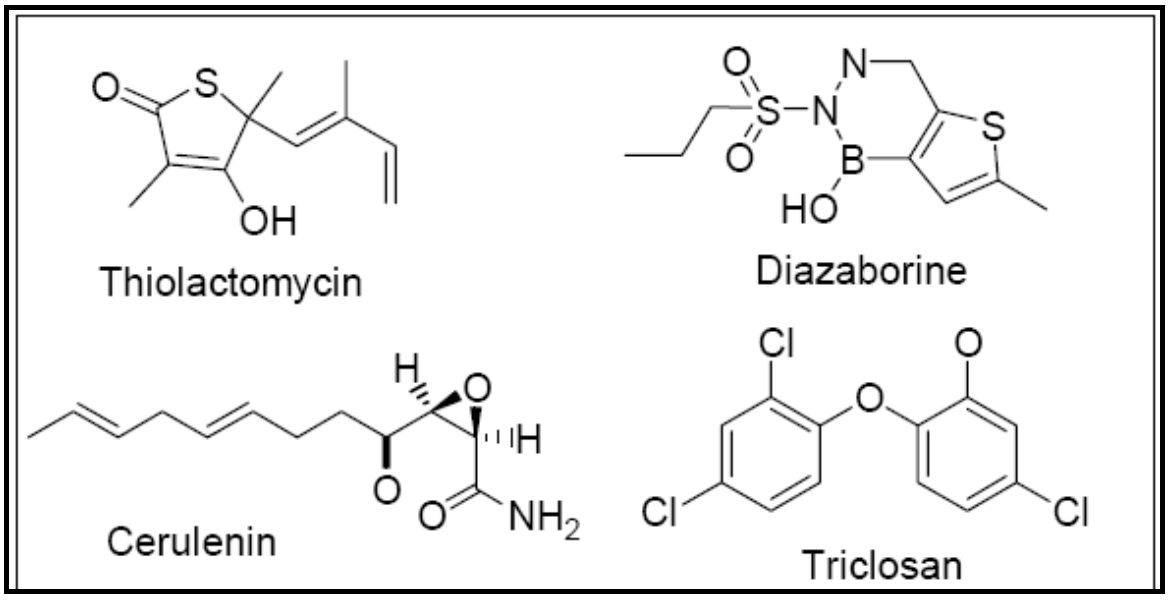


Figure 4.21: Inhibitors of bacterial fatty acid biosynthetic enzyme.

Imidazole is a bioisostere of pyridine, and thaizole is a bioisosteric with imidazole. By a process of extended biosteric concept we have proposed the following frame work as possible candidate's for antibacterial evaluation.

4. 4.2 Antibacterial activity:

The newly synthesized compounds were screened for their antibacterial screening using agar well diffusion method (Perez *et al.*,, 1990). The antibacterial activity of the test compounds was evaluated against two Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis* and Gram-negative bacteria *Escherichia coli.* Ciprofloxacin was used as standard drug. Dimethylsulfoxide was used as solvent control. The microorganisms were activated by inoculation a loopful of the strain in the nutrient broth (25ml) and incubating at room temperature in a rotary shaker. The test organisms (0.2 ml; 10^8 cells/ml as per McFarland standard) were then inoculated into the molten Mueller Hinton agar media. After proper homogenization it was poured into sterile 100 mm petri dishes (Hi-media) and allowed to solidify. A well was made in the seeded plates with the help of a sterile cork borer (8.5 mm). The test solution (0.05 ml) in dimethylsulfoxide (100 µg/ml) was introduced into the well and all the plates were incubated at 37^0C for 24 hours. The experiment was performed in triplicate under aseptic conditions. The control was also maintained with 0.05 ml of DMSO under similar conditions and the zone of inhibition of the bacteral growth were measured and recorded. Priliminary screening was conducted for all compounds at 100 µg/mL concentration, against the above-mentioned microorganisms. Different series of dilutions of compounds were made (1.56 to 100 µg/mL) to determine the MIC.

4.4.3 Anti-platelet activity:

ADP-induced platelet aggregation of platelet-rich plasma (PRP), quantitated using optical density filter at 405 nm as a measurement point in kinetic mode. To obtain PRP, a citrated tube of blood was inverted 3 to 5 times for gentle mixing and centrifuged at room temperature for 10 minutes at 200g. After centrifugation, the upper turbid layer of PRP was removed, and the residual blood was centrifuged for 5 minutes at 2000 g to obtain platelet-poor plasma (PPP). The PPP was used as the baseline optical density for platelet aggregation. A total of 180 µL of PRP containing about 3 x 10^8 platelets/ml was incubated at 37°C in the 96 well plate for 3-5 minutes, Then 10 µL of test compounds were added in PRP containing wells and incubated for the period of 15 minutes with intermittent shaking. ADP (10 µL) at a final concentration of 10,20 and 40 µmol/L was added in above wells with intermittent shaking mode. Optical density readings were measured at every one minute with

intermittent shaking up to 5 minutes. Platelet aggregation was expressed as the change in optical density at 5 minutes, compared with PPP as a reference and converted to % aggregation (O'Brien 1962). Aspirin was used as a positive control.

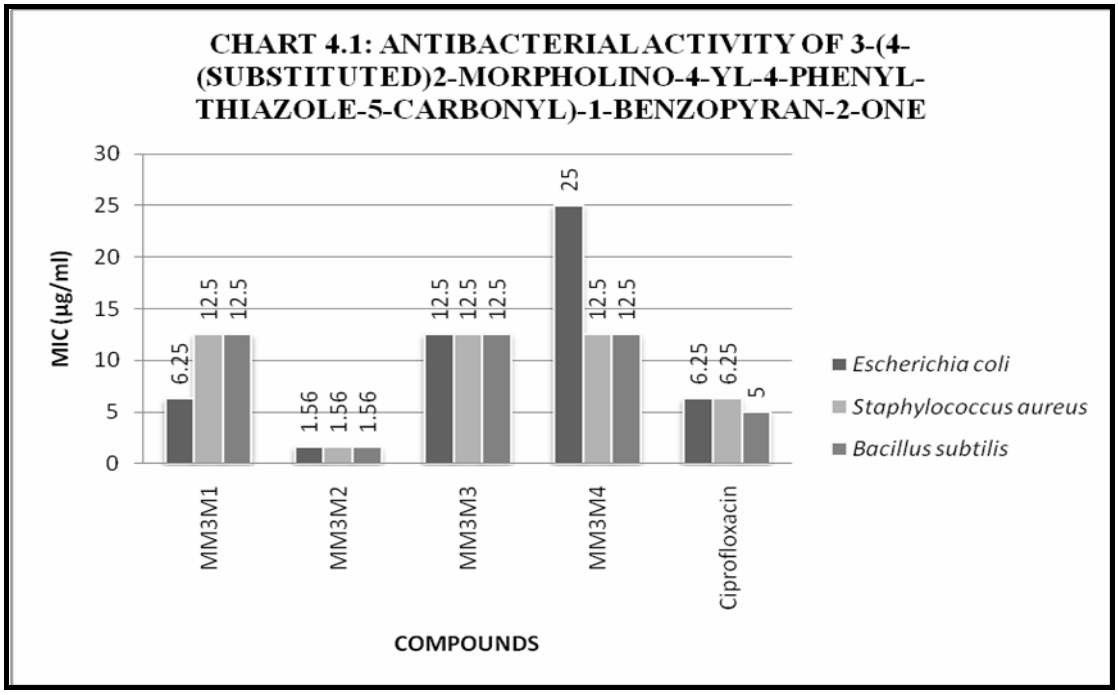
4.5 Results and Discussion:

Taking in to consideration of diverse biological activities of coumarine derivatives as antiplatelet and antibacterial (Rafat M. Mohareb *et al.,,* 2009) and the chemistry and pharmacology of thiazole derivatives has been of great interest to medicinal chemists lately (Nora de Souza 2005). Attempts have also been made to attach a thiazole side chain at C-3 position of coumarin. In addition, to expand the structural diversity of synthetic courmarins for biological functions, the morpholine fusion at 2nd position of thiazole was also attempted. The compounds MM3M1 was synthesized attaching coumarin-3-yl at fifth position, morpholine at 2nd position and phenyl moiety at 4th position of thiazole nucleus. The compounds MM3M2 to MM3M4 were synthesized keeping coumarin-3-yl constant at fifth position, morpholine at 2nd position and introducing electron withdrawing groups (-Cl) at ortho, meta and para position in phenyl moiety at 4th position of thiazole nucleus. The results are given in Table 4.1.

Table 4.1 : Chemical Structure, anti-bacterial and anti-platelet activity data of synthesized 3-(4-(substituted)2-morpholino-4-yl-4-phenyl-thiazole-5-carbonyl)-1-benzopyran-2-one

Compound Code	R	Anti-bacterial activity			Platelet aggregation inhibition (%)
		MIC* in µg/ml (zone of inhibition in mm)			
		Gram –ve Becteria	Gram +ve Bacteria		
		Escherichia coli	*Staphylococcus aureus*	*Bacillus subtilis*	
MM3M1	-H	6.25(18)	12.5(13)	12.5(13)	34.85
MM3M2	2-Chloro	1.56 (18)	1.56 (18)	1.56 (18)	76.02
MM3M3	3-Chloro	12.5(13)	12.5(13)	12.5(13)	7.25
MM3M4	4-Chloro	25(08)	12.5(13)	12.5(13)	4.34
Ciprofloxacin		6.25(18)	6.25(18)	5(21)	-
Aspirin		-	-	-	90.00

*MIC values were evaluated at concentration range 1.56 to 100 µg/ml. The values in the table shows the MIC values and the corresponding zone of inhibition (in mm).



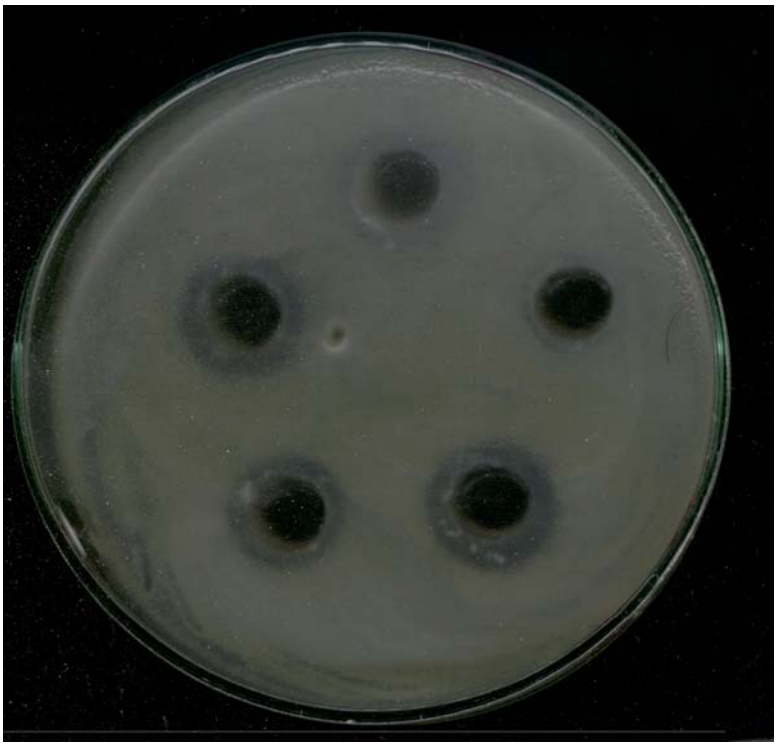


Figure 4.22: Photograph of antibacterial activity.

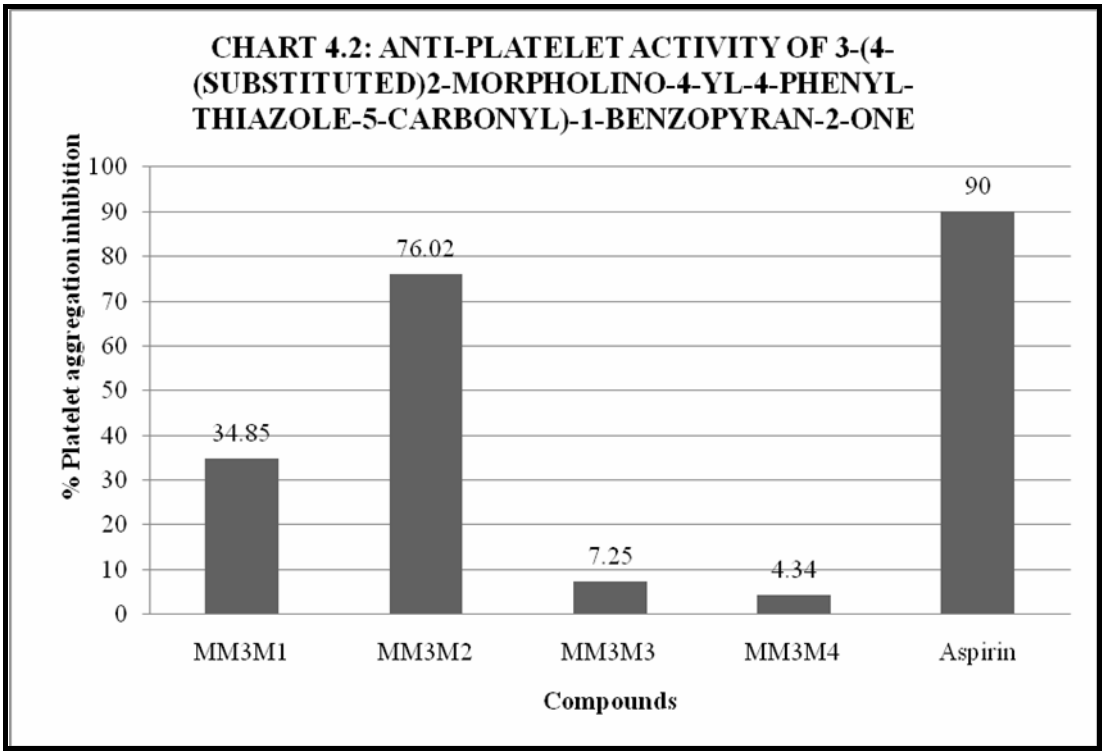
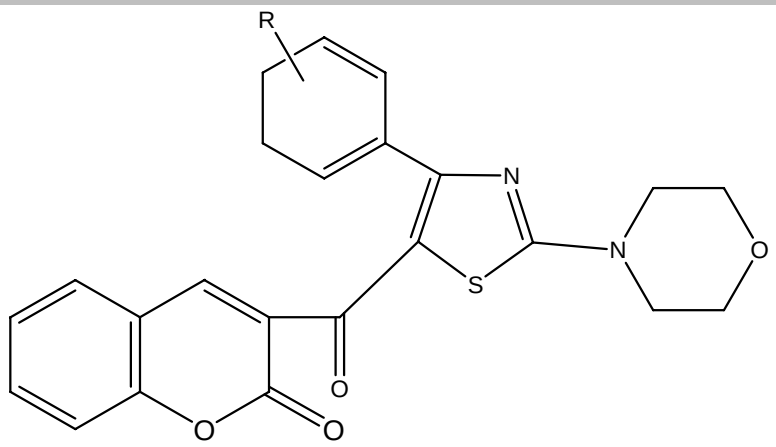


Table 4.2: Physical properties of synthesized 3-(4-(substituted)2-morpholino-4-yl-4-phenyl-thiazole-5-carbonyl)-1-benzopyran-2-one



Compound Code	R	M.P. ^{0}C	Yield (%)	MW	Mol. Formula
MM3M1	-H	166-168	85	419	$C_{23}H_{18}N_2O_4S$
MM3M2	2-Chloro	212-214	87	453	$C_{23}H_{17}ClN_2O_4S$
MM3M3	3-Chloro	156-158	80	453	$C_{23}H_{17}ClN_2O_4S$
MM3M4	4-Chloro	110-112	78	453	$C_{23}H_{17}ClN_2O_4S$

Table 4.3: Spectral data 3-(4-(substituted)-2-morpholino-4-yl-4-phenyl-thiazole-5-carbonyl)-1-benzopyran-2-one

Code	Nomenclature	^{1}HNMR (DMSO-d6) (ppm)	IR (Cm^{-1})	MASS (m/z)
MM3M1	*3-(2-Morpholin-4-yl-4-phenyl-thiazole-5-carbonyl)-chromen-2-one*	3.60-3.62 (t, 4H, 3rd and 5th morpholin CH_2 at 2nd position of thiazole ring), 3.75-3.78 2nd and 6th morpholin CH_2 at 2nd position of thiazole ring), 6.91-7.5 (m, 9H, (5H of aromatic protons of 4th position and 4H of coumarin ring), 7.6 (s, 1H, aromatic proton)	3100-3000(-CH_2 stretching), 1735 (strong band of -C=O stretching), 1458-1539 (C=C stretching, aromatic), 1374(C-N) 1245, (C-C[=O]-O symmetric stretching)	419 (M^+)
MM3M2	*3-(4-(2-Chloro-phenyl)-(2-Morpholin—4-phenyl-thiazole-5-carbonyl)-chromen-2-one*	3.64-3.68 (t, 4H, 3rd and 5th morpholin CH_2 at 2nd position of thiazole ring,J=5Hz),3.8117-3.862 2nd and 6th morpholin CH_2 at 2nd position of thiazole ring,J=5Hz), 6.96-7.56 (m, 8H, (4H of aromatic protons of 4th position and 4H of coumarin ring), 7.72 (s, 1H, aromatic proton)	3100-3000(medium band indicate CH_2 stretching), 1728 (strong band of -C=O stretching), 1450-1530 (C=C stretching, aromatic), 1374(C-N) 1245, (C-C[=O]-O symmetric stretching), 788.89 (Ar-Cl stretching)	453 ($M^{+\cdot}$), 454(M^{+1}), 455(M^{+2}).
MM3M3	*3-(4-(3-Chloro-phenyl)-(2-Morpholin—4-phenyl-thiazole-5-carbonyl)-chromen-2-one*	3.60-3.63 (t, 4H, 3rd and 5th morpholin CH2 at 2nd position of thiazole ring),3.75-3.77 2nd and 6th morpholin CH2 at 2nd position of thiazole ring), 6.92-7.51 (m, 8H, (4H of aromatic protons of 4th position and 4H of coumarin ring), 7.68 (s, 1H, aromatic proton)	3100-3000 (medium band indicate CH2 stretching), 1730 (strong band of -C=O stretching), 1448-1533 (C=C stretching, aromatic), 1368 (C-N) 1246 (C-C[=O]-O symmetric stretching), 794 (Ar-Cl stretching)	453 ($M^{+\cdot}$), 454(M^{+1}), 455(M^{+2}).
MM3M4	*3-(4-(4-Chloro-phenyl)-(2-Morpholin—4-phenyl-thiazole-5-carbonyl)-chromen-2-one*	3.61-3.64 (t, 4H, 3rd and 5th morpholin CH2 at 2nd position of thiazole ring),3.83-3.86 2nd and 6th morpholin CH2 at 2nd position of thiazole ring), 7.23-7.63 (m, 8H, (4H of aromatic protons of 4th position and 4H of coumarin ring), 7.65 (s, 1H, aromatic proton)	3100-3000(medium band indicate CH2 stretching), 1723 (strong band of -C=O stretching), 1448-1533 (C=C stretching, aromatic), 1369 (C-N) 1246 (C-C[=O]-O symmetric stretching), 764 (Ar-Cl stretching)	453 ($M^{+\cdot}$), 454(M^{+1}), 455(M^{+2}).

The investigation of antibacterial screening revealed that the tested compounds showed moderate to good bacterial inhibition. Compounds MM3M1,MM3M3, and MM3M4 shows moderate activity against Gram-positive microorganisms, i.e. *Staphylococcus aureus* and Bacillus subtillis. However compounds MM3M1 and MM3M2 are highly active against *Escherichia coli* when compared to Ciprofloxacin. Amongst all synthesized compounds, MM3M2 has exhibited very good activity against all the bacterial strains.

All the synthesized compounds were also evaluated for anti-platelet activity. The compounds MM3M1, MM3M2, MM3M3, MM3M4 showed 34.85% , 76.02%, 7.25% and 4.35 % platelet aggregation inhibition respectively. Aspirin showed 90 % platelet aggregation inhibition. Compound MM3M3 and MM3M4 shows poor antiplatelet activity, while compound MM3M1 shows moderate anti-platelet activity. Compound MM3M2 exhibited very good anti-platelet activity when compared with Aspirin.

4.6 Conclusion:

The synthesized targeted compounds (MM3M1- MM3M4) were evaluated for their in vitro antibacterial and anti-platelet activities. On the basis of structure-activity relationship studies of MM3M1- MM3M4 it can be concluded that presence of 2-chlorophenyl group at the 4^{th} position of the thiazole contributes significantly to antibacterial and anti-platelet activity profile of the candidates.

4.7 References:

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CHAPTER V

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME 3-(4-(SUBSTITUTED) PHENYL-2-PIPERIDIN-1-YL-4-THIAZOLE-5-CARBONYL)-1-CHROMEN-2-ONE

5.1 Introduction:

As seen in the previous chapter IV of morpholino-thiazole-coumarin derivatives showed antibacterial and antiplatelet activity. To explore overall profile of thiazole substituion functionality at the 3rd position of coumarin, we proposed the incorporation of bioisosteric replacement “oxygen” with the “carbon” i.e. morpholine with piperidine, looking the hypothesis that this incorporation may reduce the polarity of molecule and increase lipophilicity, which may enhance penetration of drug. The Scheme of proposed work is represented in **Scheme 5.1**

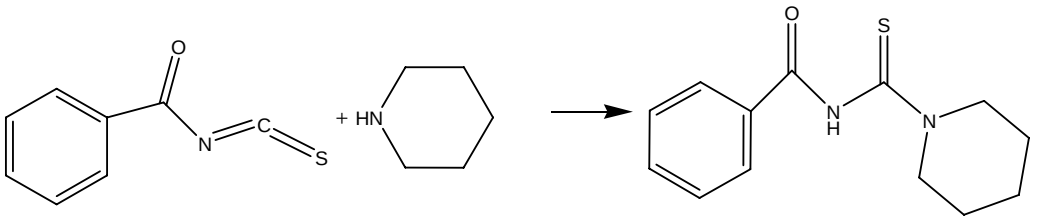
5.2 Synthesis of intermediate:

5.2.1 Synthesis of 3-bromoacetylcoumarin: As per previous chapter 3.2.7

5.2.2 Synthesis of phenyl isothiocyanate and substituted chloro phenyl isothiocyanate: As per previous chapter 3.2.5

5.2.3 Synthesis of substituted -N-(piperidin-1-thioly)-benzamaide (Preparation of Add-P1 to Add-P4)

5.2.3.1 Synthesis of N-(piperidin-1-thioyl) benzamide (Add-P1):



Benzyl isothiocyanate Piperidine Add-P1: *N*-(piperidine-1-thioyl)benzamide

Requirements:

Chemicals	Molecular formula	Quantity used (Mole)
Ammonium thiocyanate	CH_4N_2S	10.48 gm (0.1379)
Benzoyl chloride	C_7H_5ClO	17.76 gm (0.1263)
Piperidin	$C_5H_{11}N$	09.76 gm (0.1149)

Procedure (Rasmussen *et al.*, 1988):

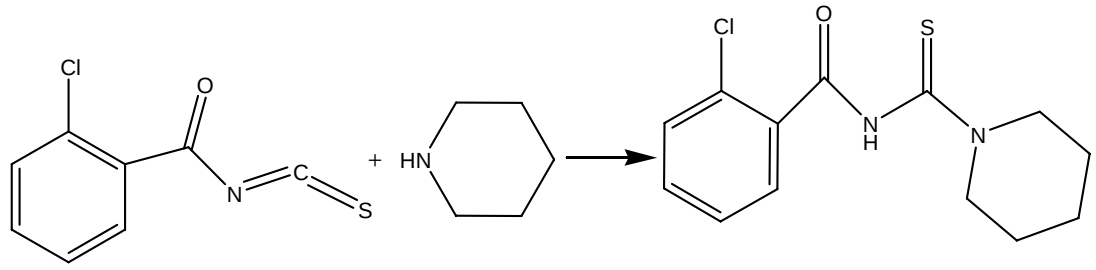
To a stirred solution of ammonium thiocyanate (10.48 gm, 0.1379 mole) in 100 ml acetone at room temperature, was added benzoyl chloride (17.76 gm, 0.1263 mole) in 5 minutes. The reaction mixture was refluxed for 15 minutes. After reflux, piperidin (9.76 gm, 0.1149 mole) was added into reaction mixture at reflux temperature in 3 minutes and mixture was further refluxed for 30 minutes. Reaction mixture was poured into crushed ice, and separated solid was filtered, washed with acetone and dried. This was coded as Add-P1.

M.P.: 110-112^oC.

TLC: Mobile Phase Toluene:methanol 9:1,R_f- 0.72.

Molecular formula: $C_{13}H_{16}N_2OS$ (248 MW).

5.2.3.2 Synthesis of 2-chloro-N-(piperidin-1-thioly)-benzamaide (Add-P2):



2-chlorobenzoyl isothiocyanate Piperidine Add-P2: 2-chloro-*N*-(piperidine-1-thioyl)benzamide

Requirements:

Chemicals	Molecular formula	Quantity used (Mole)
Ammonium thiocyanate	CH_4N_2S	10.48 gm (0.1379)
2-chlorobenzolychloride	$C_7H_4Cl_2O$	21.97 gm (0.1263)
Piperidin	$C_5H_{11}N$	09.76 gm (0.1149)

Procedure (Rasmussen *et al.*, 1988):

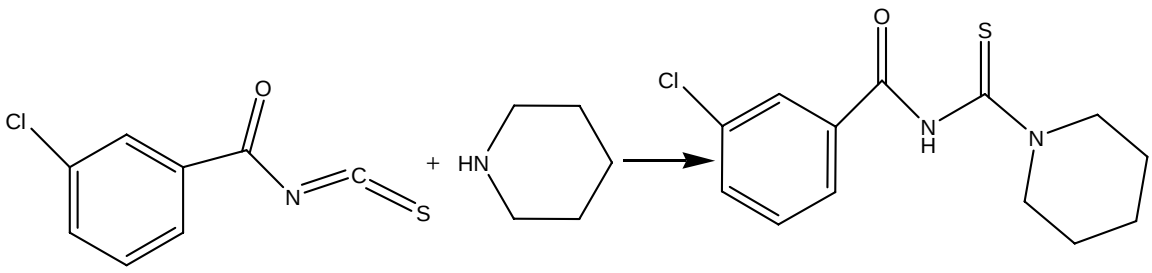
To a stirred solution of ammonium thiocyanate (10.48 gm, 0.1379 mole) in 100 ml acetone at room temperature, was added 2-chloro-benzoyl chloride (21.97gm, 0.1263 mole) in 5 minutes. The reaction mixture was refluxed for 15 minutes. After reflux, piperidin (9.76 gm, 0.1149 mole) was added into reaction mixture at reflux temperature in 3 minutes and mixture was further refluxed for 30 minutes. Product was isolated by pouring reaction mixture into crushed ice, and separated solid was filtered, washed with acetone and dried. This was coded as Add-P2.

M.P.: 108-110^oC.

TLC: Mobile Phase Toluene : methanol 9:1,R_f- 0.55.

Molecular formula: $C_{13}H_{15}ClN_2OS$ (283 MW).

5.2.3.3 Synthesis of 3-chloro-N-(piperidin 1-thioly)-benzamaide (Add-P3):



3-chlorobenzoyl isothiocyanate Piperidine Add-P3 : 3-chloro-*N*-(piperidine-1-thioyl)benzamide

Requirements:

Chemicals	Molecular formula	Quantity used (Mole)
Ammonium thiocyanate	CH_4N_2S	10.48 gm (0.1379)
3-chlorobenzolychloride	$C_7H_4Cl_2O$	21.97 gm (0.1263)
Piperidin	$C_5H_{11}N$	09.76 gm (0.1149)

Procedure (Rasmussen *et al.*, 1988):

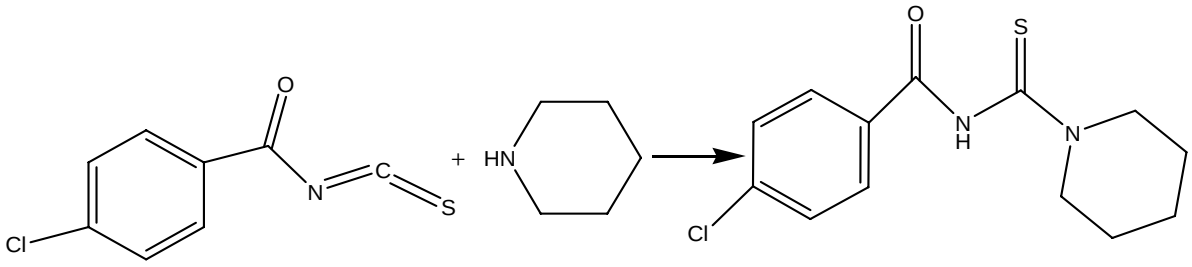
To a stirred solution of ammonium thiocyanate (10.48gm, 0.1379 mole) in 100 ml acetone at room temperature, was added 3-chloro-benzoyl chloride (21.97gm, 0.1263 mole) in 5 minutes. The reaction mixture was refluxed for 15 minutes. After reflux, piperidin (9.76 gm, 0.1149 mole) was added into reaction mixture at reflux temperature in 3 minutes and mixture was further refluxed for 30 minutes. Product was isolated by pouring reaction mixture into crushed ice, and separated solid was filtered, washed with acetone and dried. This was coded as Add-P3.

M.P.: 120-122^oC.

TLC: Mobile Phase Toluene:methanol 9:1,R_f- 0.55.

Molecular formula: $C_{13}H_{15}ClN_2OS$ (283 MW).

5.2.3.4 Synthesis of 4-chloro-N-(piperidin-1-thioly)-benzamaide (Add-P4):



4-chlorobenzoyl isothiocyanate Piperidine Add-P4: 4-chloro-*N*-(piperidine-1-thioyl)benzamide

Requirements:

Chemicals	Molecular formula	Quantity used (Mole)
Ammonium thiocyanate	CH_4N_2S	10.48 gm (0.1379)
4-chlorobenzolychloride	$C_7H_4Cl_2O$	21.97 gm (0.1263)
Piperidin	$C_5H_{11}N$	09.76 gm (0.1149)

Procedure (Rasmussen *et al.*, 1988):

To a stirred solution of ammonium thiocyanate (10.48gm, 0.1379 mole) in 100 ml acetone at room temperature, was added 4-chloro-benzoyl chloride (21.97gm, 0.1263 mole) in 5 minutes. The reaction mixture was refluxed for 15 minutes. After reflux, piperidin (9.76 gm, 0.1149 mole) was added into reaction mixture at reflux temperature in 3 minutes and mixture was further refluxed for 30 minutes. Product was isolated by pouring reaction mixture into crushed ice, and separated solid was filtered, washed with acetone and dried. This was coded as Add-P4.

M.P.: 115-118^oC

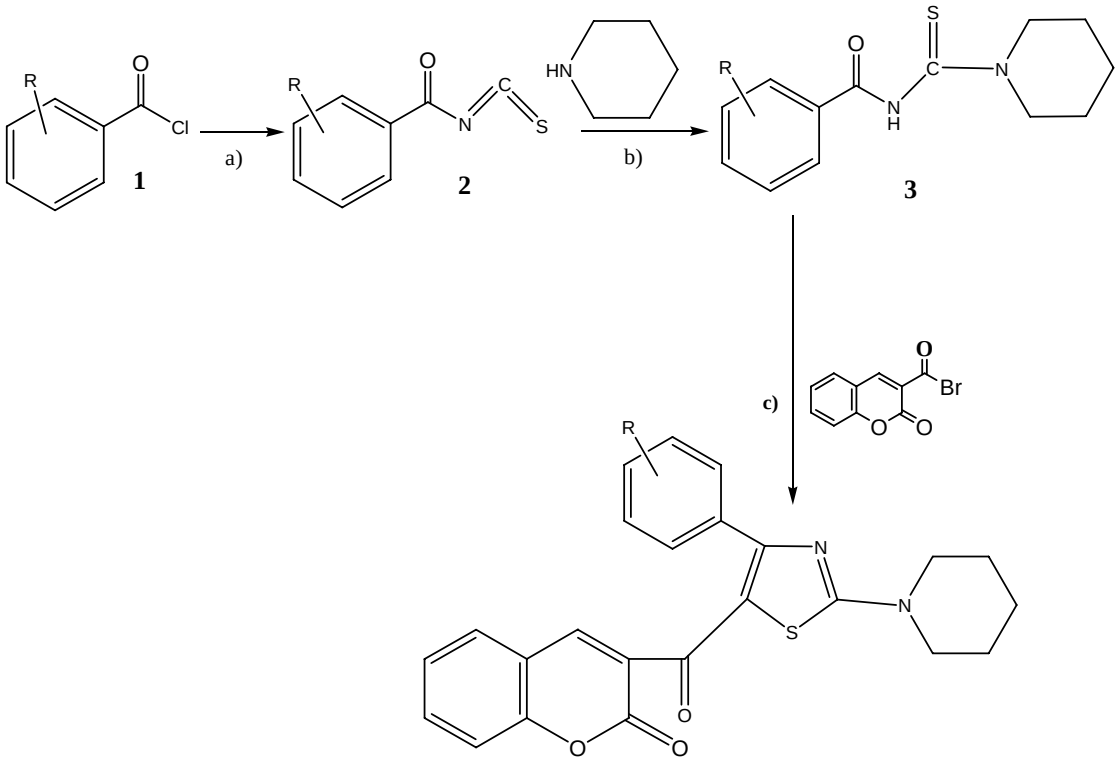
TLC: Mobile Phase Toluene:methanol 9:1,R_f- 0.91

Molecular formula: $C_{13}H_{15}ClN_2OS$ (283 MW)

5.3 Synthesis of 3-(4-(substituted)-phenyl-2-piperidin-1-yl-4-thiazole-5-carbonyl)-1-chromen-2-one.

(Targeted compounds coded as MM3P1 to MM3P4).

5.3.1 Synthesis of 3-(4-(substituted)-phenyl-2-piperidin-1-yl-4-thiazole-5-carbonyl)-1-chromen-2-one.



Scheme 5.1. Synthesis of 3-(4-(substituted) phenyl- 2-piperidin-1-yl-4-thiazole-5-carbonyl)-1-chromen-2-one.

Reagents and conditions: a) NH4SCN, Acetone, Reflux 25 min; b) reflux for 15 min, pour reaction mixture to crushed ice; c) dimethylformamide, stirr at 70^{o}C to 80^{o}C for 2 hour, pour to crushed ice.

5.3.2 General Procedure for synthesis of 3-(4-(substituted) phenyl- 2-piperidin-1-yl-4-thiazole-5-carbonyl)-1-chromen-2-one (Reji *et al.*, 2008):

To a solution of the Add-P (0.0025 mmol) in N,N-dimethylformamide (5 ml), 3-bromoacetylcoumarin (0.0025 mmol) was added. The reaction mixture was warmed on a water bath at 80-85 ^{o} C for 5 min. To this, triethylamine (0.0025 mmol, 0.3 ml) was added and heating was continued for another 15 min or till reaction mixture shows absent of starting material. The above mixture was cooled and poured into ice-cold water with stirring. A yellow precipitate thus obtained was filtered, wash with water and air-dried. The crude product was purified by preparative TLC

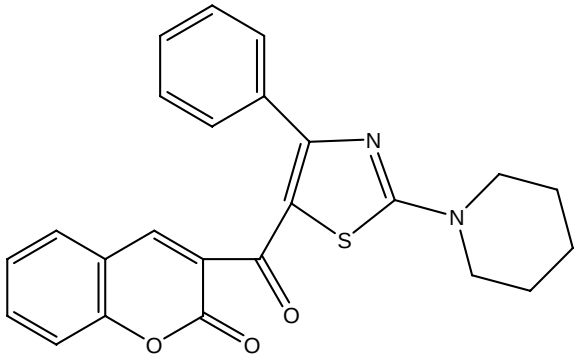
(Hexane:Ethyl acetate, 3:7) corresponding to the (MM3P1-MM3P4) characterized as per the analytical data.

5.3.3 Characteristics of synthesized compounds:

(i) MM3P1

3-(4-Phenyl-2-piperidin--1-yl-thiazole-5-carbonyl)-chromen-2-one.

Structure:



MW **:** 416

Mol.Formula **:** $C_{24}H_{20}N_2O_3S$

M.P. **:** 160-162^{0}C

Yield **:** 70%

TLC: Mobile Phase- Toluene : methanol 9**:**1, Rf-0**.**70.

^{1}H NMR: *(DMSO, δ, ppm)* = 1.67 (t, 6H, 3^{rd,} 4th and 5th piperidin CH2 at 2nd position of thiazole ring), 2.51-2.52(t,4H,2nd and 6th piperidin CH2 at 2nd position of thiazole ring), 6.95-7.55 (m, 9H, (5H of aromatic protons of 4th position and 4H of coumarin ring), 7.7 (s, 1H, aromatic proton).

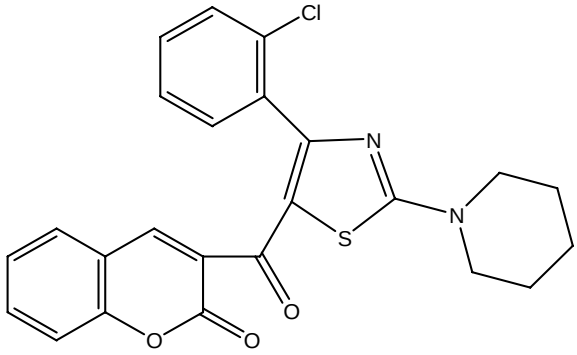
IR *(KBr, cm^{-1})***:** 3100-3000(-CH2 stretching), 1734 (strong band of -C=O stretching), 1456-1539 (C=C stretching, aromatic), 1375(C-N) 1249, (C-C[=O]-O symmetric stretching).

MASS: 416 ($M^{+\cdot}$)

(ii) MM3P2

3-(4-(2-Chloro-phenyl)-(2-Piperidin-1-yl-thiazole-5-carbonyl)-chromen-2-one.

Structure:



MW **:** 451

Mol.Formula **:** $C_{24}H_{19}ClN_2O_3S$

M.P. **:** 232-234^{0}C

Yield **:** 77%

TLC: Mobile Phase- Toluene : Methanol 9**:**1**,** Rf-0**.**65.

^{1}H NMR: *(DMSO, δ, ppm)* = 2.16 (t, 6H, 3^{rd,} 4th and 5th piperidin CH2 at 2nd position of thiazole ring), 2.58-2.59 (t,4H,2nd and 6th piperidin CH2 at 2nd position of thiazole ring), 6.90-7.49 (m, 8H, (5H of aromatic protons of 4th position and 4H of coumarin ring), 7.7 (s, 1H, aromatic proton).

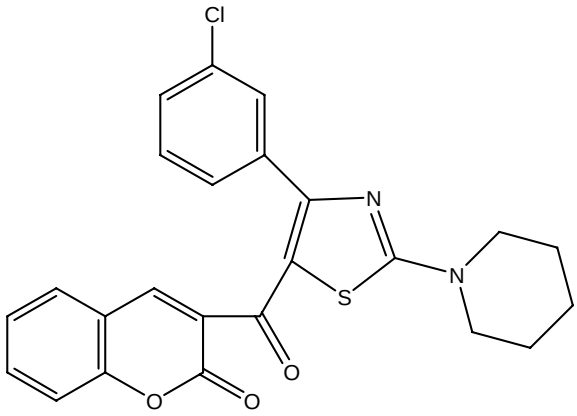
IR *(KBr, cm^{-1})***:** 3100-3000(-CH2 stretching), 1735 (strong band of -C=O stretching), 1456-1539 (C=C stretching, aromatic), 1375(C-N) 1249, (C-C[=O]-O symmetric stretching).

MASS m/z: 451 ($M^{+\cdot}$), 452(M^{+1}), 453(M^{+2}).

(iii) MM3P3

3-(4-(3-Chloro-phenyl)-(2-Piperidin-1-yl-thiazole-5-carbonyl)-chromen-2-one.

Structure:



MW **:** 451

Mol.Formula **:** $C_{24}H_{19}ClN_2O_3S$

M.P. **:** 165-167^{0}C

Yield **:** 65%

TLC: Mobile Phase- Hexane : Ethyl acetate 3**:**7**,** Rf-0**.**62.

^{1}H NMR: *(DMSO, δ, ppm)* = 2.16 (t, 6H, 3^{rd,} 4th and 5th piperidin CH2 at 2nd position of thiazole ring), 2.58-2.59 (t,4H,2nd and 6th piperidin CH2 at 2nd position of thiazole ring), 6.90-7.49 (m, 8H, (5H of aromatic protons of 4th position and 4H of coumarin ring), 7.7 (s, 1H, aromatic proton).

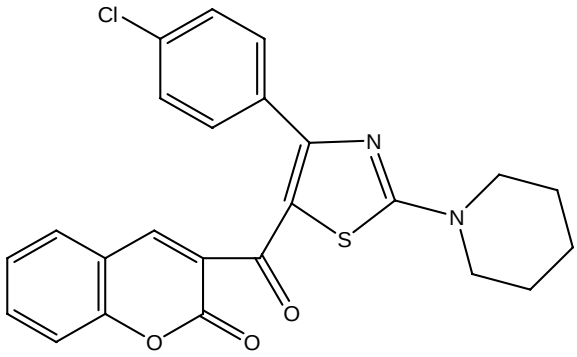
IR *(KBr, cm^{-1})***:** 3100-3000(-CH2 stretching), 1735 (strong band of -C=O stretching), 1456-1539 (C=C stretching, aromatic), 1375(C-N) 1249, (C-C[=O]-O symmetric stretching).

MASS m/z: 451 ($M^{+\cdot}$), 452(M^{+1}), 453(M^{+2}).

(iv) MM3P4

3-(4-(4-Chloro-phenyl)-(2-Piperidin-1-yl-thiazole-5-carbonyl)-chromen-2-one.

Structure:



MW **:** 451

Mol.Formula **:** $C_{24}H_{19}ClN_2O_3S$

M.P. **:** 162-164^{0}C

Yield **:** 60 %

TLC: Mobile Phase- Hexane : Ethyl acetate 3**:**7**,** Rf-0**.**60.

^{1}H NMR: *(DMSO, δ, ppm)* = 2.1 (t, 6H, 3^{rd,} 4th and 5th piperidin CH2 at 2nd position of thiazole ring), 2.51-2.52 (t,4H,2nd and 6th piperidin CH2 at 2nd position of thiazole ring), 6.90-7.49 (m, 8H, (5H of aromatic protons of 4th position and 4H of coumarin ring), 7.8 (s, 1H, aromatic proton).

IR *(KBr, cm^{-1})***:** 3100-3000(-CH2 stretching), 1735 (strong band of -C=O stretching), 1456-1539 (C=C stretching, aromatic), 1375(C-N) 1249, (C-C[=O]-O symmetric stretching).

MASS m/z: 451 ($M^{+\cdot}$), 452(M^{+1}), 453(M^{+2}).

5. 4 Pharmacological Screening:

Antibacterial activity and antiplatelet activity

The newly synthesized compounds were screened for their antibacterial activity and anti-platelet activity as per methods explained in previous chapter 4.3.4.2 and 4.3.4.3.

5.5 Results and Discussion:

In this chapter the compounds were synthesized by attaching a thiazole side chain at C-3 position of coumarin. In addition, to expand profile of synthetic courmarins for biological functions, the piperidine fusion at 2nd position of thiazole was also attempted to enhance the lipophilicity. The compounds MM3P1 was synthesized keeping coumarin-3-yl at fifth position, piperidine at 2nd position and phenyl moiety at 4th position of thiazole nucleus. The compounds MM3P2 to MM3P4 were synthesized keeping coumarin-3-yl constant at fifth position, piperidine at 2nd position and introducing electron withdrawing groups (-Cl) at ortho, meta and para position in phenyl moiety at 4th position of thiazole nucleus. The results are given in Table 5.1.

Table 5.1: Chemical Structure, anti-bacterial and anti-platelet activity data of synthesized 3-(4-(substituted) phenyl-2-piperidin-1-yl-4-thiazole-5-carbonyl)-1-chromen-2-one.

Compound Code	R	Anti-bacterial activity			Platelet aggregation inhibition (%)
		MIC in µg/ml (zone of inhibition in mm)			
		Gram -ve Bacteria	Gram +ve Bacteria		
		Escherichia coli	*Staphylococcus aureus*	*Bacillus subtilis*	
MM3P1	-H	6.25(18)	12.5(13)	12.5(13)	2.39
MM3P2	2-Chloro	6.25(18)	12.5(13)	25 (08)	89.26
MM3P3	3-Chloro	6.25(18)	12.5(13)	25 (08)	75.36
MM3P4	4-Chloro	12.5(13)	12.5(13)	12.5(13)	49.27
Ciprofloxacin	-	6.25(18)	6.25(18)	5(21)	-
Aspirin	-	-	-	-	90.00

Note: the MIC values were evaluated at concentration range 1.56 to 100 µg/ml. The values in the table show the MIC values and the corresponding zone of inhibition (in mm).

CHART 5.1: ANTIBACTERIAL ACTIVITY OF 3-(4-(SUBSTITUTED) PHENYL- 2-PIPERIDIN-1-YL-4-THIAZOLE-5-CARBONYL)-1-CHROMEN-2-ONE

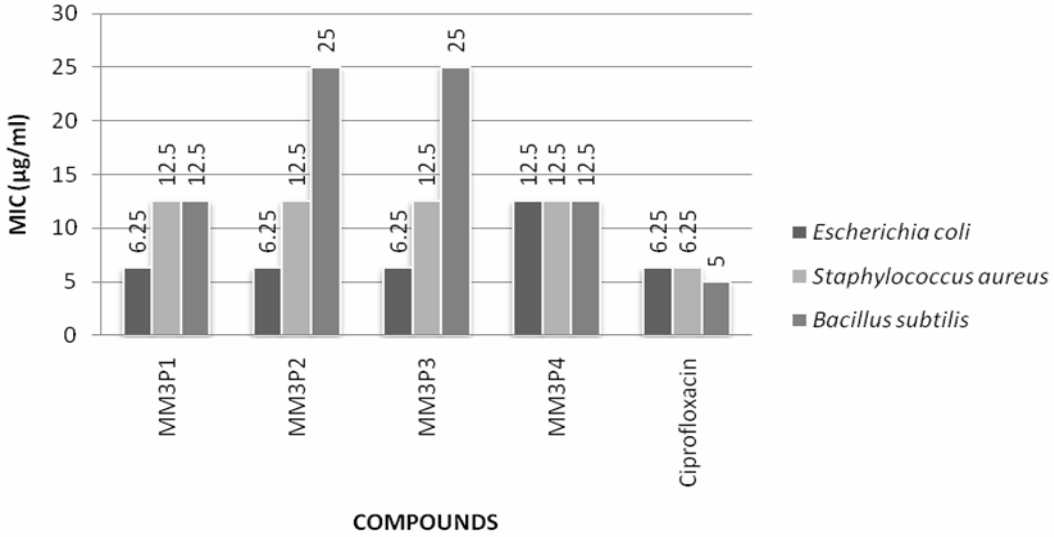


CHART 5.2: ANTI-PLATELET ACTIVITY OF 3-(4-(SUBSTITUTED) PHENYL- 2-PIPERIDIN-1-YL-4-THIAZOLE-5-CARBONYL)-1-CHROMEN-2-ONE

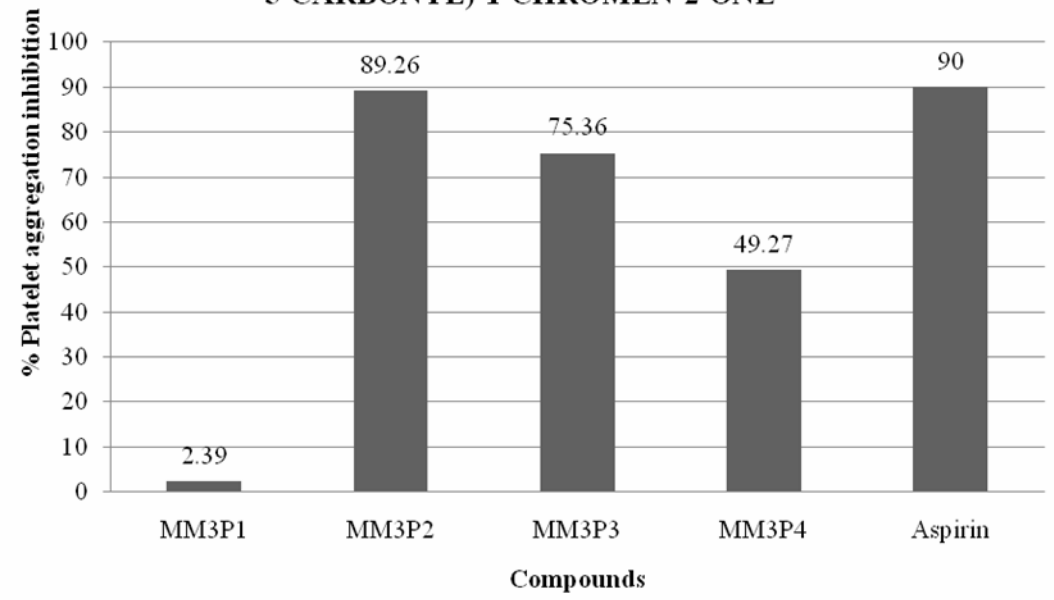
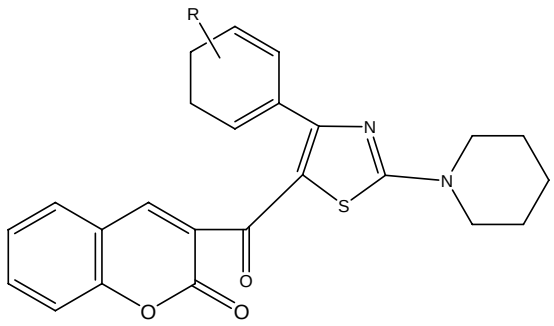


Table 5.2: Physical properties of synthesized 3-(4-(substituted)-phenyl-2-piperidin-1-yl-4-thiazole-5-carbonyl)-1-chromen-2-one



Compound Code	R	M.P. (^{0}C)	Yield (%)	MW	Mol.Formula
MM3P1	-H	166-168	70	416	$C_{24}H_{20}N_2O_3S$
MM3P2	2-Chloro	212-214	77	451	$C_{24}H_{19}ClN_2O_3S$
MM3P3	3-Chloro	156-158	65	451	$C_{24}H_{19}ClN_2O_3S$
MM3P4	4-Chloro	110-112	60	451	$C_{24}H_{19}ClN_2O_3S$

The results depicted in Table 5.1, revealed that tested compounds displayed variable inhibitory effects on the growth of the tested Gram-positive and Gram-negative bacterial strains. All the compounds showed moderate activity against *Staphylococcus aureus*, while compounds MM3P2 and MM3P4 exhibit poor activity against *Bacillus subtilis*. However compounds MM3P1, MM3P2 and MM3P3 are highly active against *Escherichia coli*- when compared to Ciprofloxacin. In general, most of the tested compounds revealed better activity against the Gram-negative rather than the Gram-positive bacteria.

All the synthesized compounds were evaluated for anti-platelet activity. The compounds MM3P1, MM3P2, MM3P3, MM3P4 showed 2.39%, 89.26%, 75.36 % and 49.27 % platelet aggregation inhibition respectively. Aspirin showed 90 % platelet aggregation inhibition. Compound MM3P1 showed poor antiplatelet activity, while compound MM3M4 shows moderate anti-platelet activity. Compound MM3P2 and MM3P3 exhibited very good anti-platelet activity when compared with aspirin.

Table 5.3: Spectral data synthesized 3-(4-(substituted) phenyl-2-piperidin-1-yl-4-thiazole-5-carbonyl)-1-chromen-2-one

Code	Nomenclature	^{1}HNMR (DMSO-d6) (ppm)	IR (Cm^{-1})	MASS (m/z)
MM3P1	*3-(4-Phenyl-2-piperidin-1-yl-thiazole-5-carbonyl)-chromen-2-one*	1.67 (t, 6H, 3rd, 4th and 5th piperidin CH2 at 2nd position of thiazole ring), 2.51-2.52(t,4H,2nd and 6th piperidin CH2 at 2nd position of thiazole ring), 6.95-7.55 (m, 9H, (5H of aromatic protons of 4th position and 4H of coumarin ring), 7.7 (s, 1H, aromatic proton)	3100-3000(-CH2 stretching), 1734 (strong band of -C=O stretching), 1456-1539 (C=C stretching, aromatic), 1375(C-N) 1249, (C-C[=O]-O symmetric stretching).	416 ($M^{+\cdot}$)
MM3P2	*3-(4-(2-Chloro-phenyl)-(2-Piperidin-1-yl-thiazole-5-carbonyl)-chromen-2-one*	2.16 (t, 6H, 3rd, 4th and 5th piperidin CH2 at 2nd position of thiazole ring), 2.58-2.59 (t,4H,2nd and 6th piperidin CH2 at 2nd position of thiazole ring), 6.90-7.49 (m, 8H, (5H of aromatic protons of 4th position and 4H of coumarin ring), 7.7 (s, 1H, aromatic proton).	3100-3000(-CH2 stretching), 1735 (strong band of -C=O stretching), 1456-1539 (C=C stretching, aromatic), 1375(C-N) 1249, (C-C[=O]-O symmetric stretching).	451 ($M^{+\cdot}$), 452(M^{+1}), 453(M^{+2}).
MM3P3	*3-(4-(3-Chloro-phenyl)-(2-Piperidin-1-yl-thiazole-5-carbonyl)-chromen-2-one*	2.16 (t, 6H, 3rd, 4th and 5th piperidin CH2 at 2nd position of thiazole ring), 2.58-2.59 (t,4H,2nd and 6th piperidin CH2 at 2nd position of thiazole ring), 6.90-7.49 (m, 8H, (5H of aromatic protons of 4th position and 4H of coumarin ring), 7.7 (s, 1H, aromatic proton).	3100-3000(-CH2 stretching), 1735 (strong band of -C=O stretching), 1456-1539 (C=C stretching, aromatic), 1375(C-N) 1249, (C-C[=O]-O symmetric stretching).	451 ($M^{+\cdot}$), 452(M^{+1}), 453(M^{+2}).
MM3P4	*3-(4-(4-Chloro-phenyl)-(2-Piperidin-1-yl-thiazole-5-carbonyl)-chromen-2-one*	2.1 (t, 6H, 3rd, 4th and 5th piperidin CH2 at 2nd position of thiazole ring), 2.51-2.52 (t,4H,2nd and 6th piperidin CH2 at 2nd position of thiazole ring), 6.90-7.49 (m, 8H, (5H of aromatic protons of 4th position and 4H of coumarin ring), 7.8 (s, 1H, aromatic proton).	3100-3000(-CH2 stretching), 1735 (strong band of -C=O stretching), 1456-1539 (C=C stretching, aromatic), 1375(C-N) 1249, (C-C[=O]-O symmetric stretching).	451 ($M^{+\cdot}$), 452(M^{+1}), 453(M^{+2}).

It would be also noticed that compounds belonging to the piperidino-thiazole series exhibited better antiplatelet activity potential than members of morpholino-thiazole one, which support the hypothesis of incorporation of bioisosteric replacement “oxygen” with the “carbon” i.e. morpholine with piperidine. Here reduction in the polarity of molecule leads to increase lipophilicity, thus enhance penetration of drug and better activity.

5.6 Conclusions:

The synthesized targeted compounds (MM3P1- MM3P4) were evaluated for their in vitro antibacterial and anti-platelet activities. On the basis of structure-activity relationship studies of MM3P1- MM3P4 it can be concluded presence of electron withdrawing chlorophenyl group at 4^{th} position and piperidine fusion at 2^{nd} position of thiazole ring contributes to enhance antibacterial activity against Gram-negative and anti-platelet activity profile of the candidates.

5.7 References:

Rasmussen CR, Villani FJ, Weaner Jr. LE, Reynolds BE, Hood AR, Hecker LR, Nortey SO, Hanslin A, Costanzo MJ, Powell ET, Molinar AJ. Improved procedures for the preparation of cycloalkyl-arylalkyl and arylthioureasil. Synthesis.1988;6:456-459.

Reji TF, Devi SK, Thomas KK, Shreejalekshmi KG, Manji SL, Franscis M, Philip SK, Bharathan A, Rajasekharan KN. Synthesis and cytotoxixity studies of thiazole analogs of the anticancer marine alkaloid dendrodoine. Indian J Chem2008;47B: 1145-1150.

CHAPTER VI

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME 3-[4-(SUBSTITUTED PHENYL)-2-PIPERAZIN-1-YL-THIAZOLE-5-CARBONYL]-CHROMEN-2-ONE

6.1 Introduction:

As discussed in earlier chapters, the compounds of 3-(4-(substituted) phenyl-2-piperidin-1-yl-4-thiazole-5-carbonyl)-1-chromen-2-one series had shown increased activity as compared to 3-(4-(substituted)-2-morpholino-4-yl-4-phenyl-thiazole-5-carbonyl)-1-benzopyran-2-one. The increase in activity may be due to the reduction of polarity of the molecule in piperidine series. In this chapter we proposed the incorporation of bioisosteric replacement “oxygen” with the “Nitrogen” looking the hypothesis that the incorporation of two nitrogen may change polarity of molecule. Moreover nitrogen has better ability to form hydrogen bond with the targets proteins and enzymes so that better activity can be predicted. The Scheme of proposed work is represented in **Scheme 6.1**.

6.2 Synthesis of intermediate:

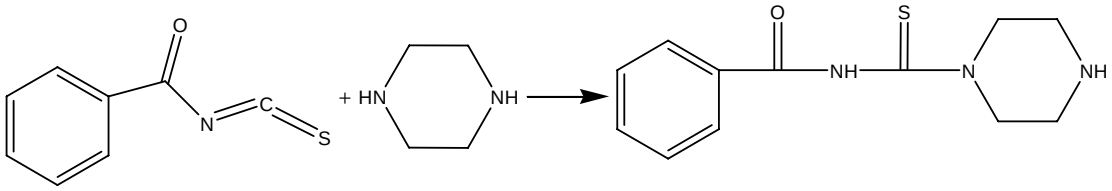
6.2.1 Synthesis of 3-bromoacetylcoumarin: As per previous chapter 3.2.7

6.2.2 Synthesis of phenyl isothiocyanate and substituted chloro phenyl isothiocyanate: As per previous chapter 3.2.5

6.2.3 Synthesis of substituted-(piperazine-1-thioyl)-benzamide:

(Preparation of Add-Pz1 to Add-Pz4)

6.2.3.1 Synthesis of N-(piperazine-1-thioyl)-benzamide (Add-Pz1):



Benzyl isothiocyanate piperazine Add-Pz1: *N*-(piperazine-1-thioyl)-benzamide

Requirements:

Chemicals	Molecular formula	Quantity used (Mole)
Ammonium thiocyanate	CH_4N_2S	10.58 gm (0.139)
Benzoyl chloride	C_7H_5ClO	17.93 gm (0.127)
Piperazine	$C_4H_{10}N_2$	05.00 gm (0.058)

Procedure (Dhavan *et al.*, 1983):

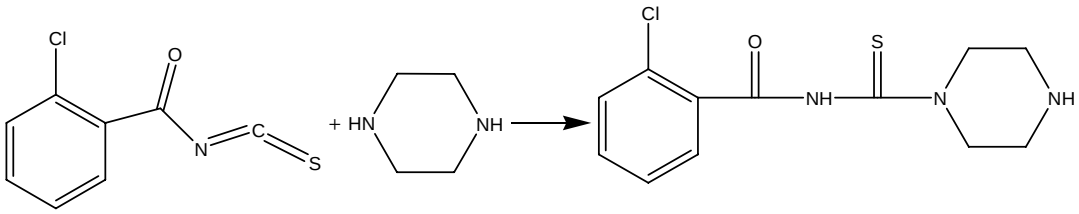
To a stirred solution of ammonium thiocyanate (10.58 gm, 0.1392 mole) in 70 ml acetone at room temperature, was added benzoyl chloride (17.93 gm, 0.127 mole) in 20 minutes. The reaction mixture was refluxed for 15 minutes. After reflux, piperazine (5.0 gm, 0.058 mole) dissolved in 70 ml acetone was added into reaction mixture at reflux temperature in 10 minutes and mixture was further refluxed for 40 minutes. The reaction mixture was poured in to 400 gm crushed ice with stirring and separated solid was filtered, washed with 500 ml water and 210 ml methanol and dried. This was coded as Add-Pz1.

M.P.: 176-178 ^oC.

TLC: Mobile Phase Hexane;Ethyl-acetate 3:7,R_f- 0.66.

Molecular formula: $C_{12}H_{15}N_3OS$ (249 MW).

6.2.3.2 Synthesis of 2-chloro-N-(piperazine-1-thioyl)-benzamide(Add-Pz2):



2-chlorobenzyl isothiocyanate piperazine Add-Pz2: 2-chloro-*N*-(piperazine-1-thioyl)-benzamide

Requirements:

Chemicals	Molecular formula	Quantity used (Mole)
Ammonium thiocyanate	CH_4N_2S	10.58 gm (0.139)
2-chlorobenzolychloride	$C_7H_4Cl_2O$	22.22 gm (0.127)
Piperazine	$C_4H_{10}N_2$	05.00 gm (0.058)

Procedure (Dhavan *et al.*, 1983):

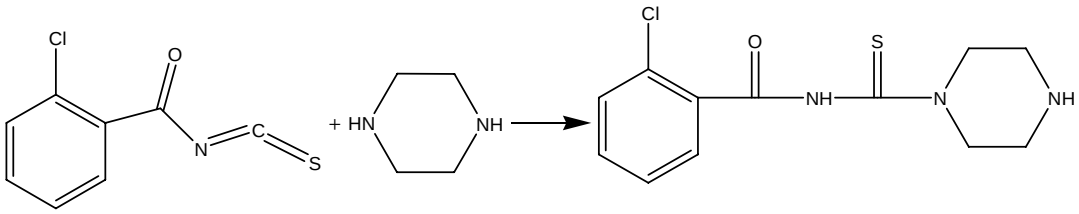
To a stirred solution of ammonium thiocyanate (10.58 gm, 0.1392 mole) in 70 ml acetone at room temperature, was added 2-chlorobenzoyl chloride (22.22 gm, 0.127 mole) in 20 minutes. The reaction mixture was refluxed for 15 minutes. After reflux, piperazine (5.0 gm, 0.058 mole) dissolved in 70 ml acetone was added into reaction mixture at reflux temperature in 10 minutes and mixture was further refluxed for 40 minutes. The reaction mixture was poured in to 400 gm crushed ice with stirring and separated solid was filtered, washed with 500 ml water and 210 ml methanol and dried. This was coded as Add-Pz2.

M.P.: 150-152 ^oC.

TLC: Mobile Phase Hexane;Ethyl-acetate 3:7,R_f- 0.78.

Molecular formula: $C_{12}H_{14}ClN_3OS$ (284 MW).

6.2.3.3 Synthesis of 3-chloro-N-(piperazine-1-thioyl)-benzamide(Add-Pz3):



3-chlorobenzyl isothiocyanate piperazine Add-Pz3: 3-chloro-*N*-(piperazine-1-thioyl)-benzamide

Requirements:

Chemicals	**Molecular formula**	**Quantity used (Mole)**
Ammonium thiocyanate	CH_4N_2S	10.58 gm (0.139)
3-chlorobenzolychloride	$C_7H_4Cl_2O$	22.22 gm (0.127)
Piperazine	$C_4H_{10}N_2$	05.00 gm (0.058)

Procedure (Dhavan *et al.*, 1983):

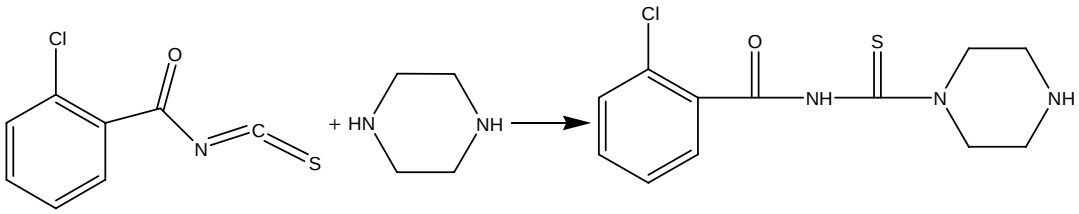
To a stirred solution of ammonium thiocyanate (10.58 gm, 0.1392 mole) in 70 ml acetone at room temperature, was added 3-chlorobenzoyl chloride (22.22 gm, 0.127 mole) in 20 minutes. The reaction mixture was refluxed for 15 minutes. After reflux, piperazine (5.0 gm, 0.058 mole) dissolved in 70 ml acetone was added into reaction mixture at reflux temperature in 10 minutes and mixture was further refluxed for 40 minutes. The reaction mixture was poured in to 400 gm crushed ice with stirring and separated solid was filtered, washed with 500 ml water and 210 ml methanol and dried. This was coded as Add-Pz3.

M.P.: 132-134 ^oC.

TLC: Mobile Phase Hexane;Ethyl-acetate 3:7,R_f- 0.65.

Molecular formula: $C_{12}H_{14}ClN_3OS$ (284 MW).

6.2.3.4 Synthesis of 4-chloro-N-(piperazine-1-thioyl)-benzamide(Add-Pz4):



4-chlorobenzyl isothiocyanate piperazine Add-Pz4: 4-chloro-*N*-(piperazine-1-thioyl)-benzamide

Requirements:

Chemicals	**Molecular formula**	**Quantity used (Mole)**
Ammonium thiocyanate	CH_4N_2S	10.58 gm (0.139)
4-chlorobenzolychloride	$C_7H_4Cl_2O$	22.22 gm (0.127)
Piperazine	$C_4H_{10}N_2$	05.00 gm (0.058)

Procedure (Dhavan *et al.*, 1983):

To a stirred solution of ammonium thiocyanate (10.58 gm, 0.1392 mole) in 70 ml acetone at room temperature, was added 4-chlorobenzoyl chloride (22.22 gm, 0.127 mole) in 20 minutes. The reaction mixture was refluxed for 15 minutes. After reflux, piperazine (5.0 gm, 0.058 mole) dissolved in 70 ml acetone was added into reaction mixture at reflux temperature in 10 minutes and mixture was further refluxed for 40 minutes. The reaction mixture was poured in to 400 gm crushed ice with stirring and separated solid was filtered, washed with 500 ml water and 210 ml methanol and dried. This was coded as Add-Pz4.

M.P.: 138-140 ^oC.

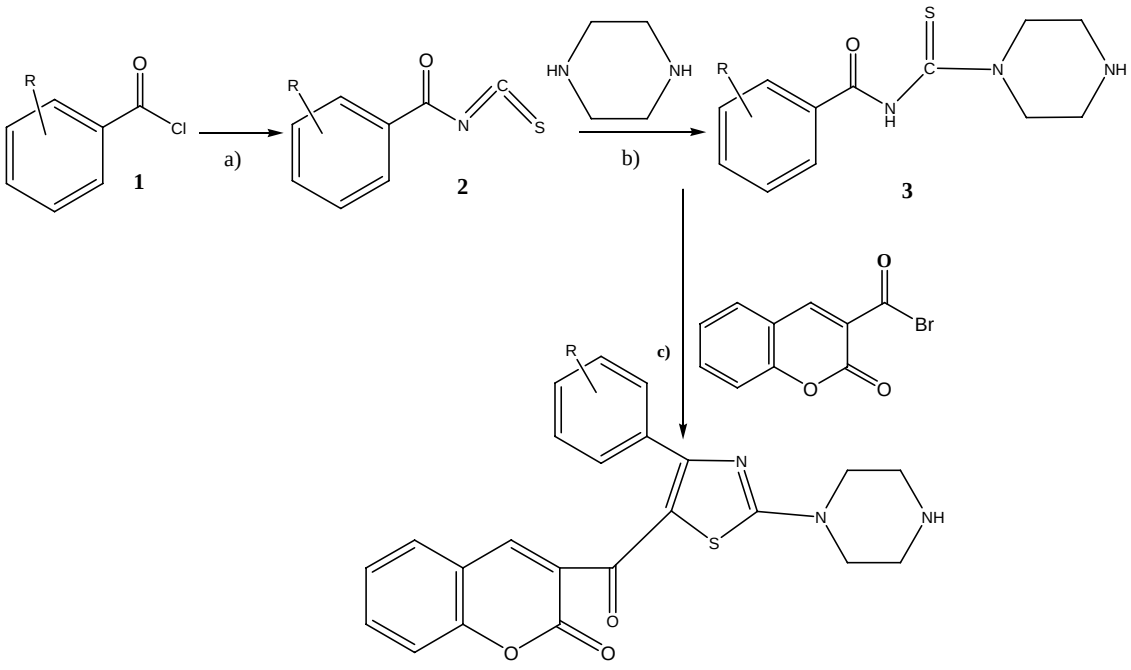
TLC: Mobile Phase Hexane;Ethyl-acetate 3:7,R_f- 0.80.

Molecular formula: $C_{20}H_{18}Cl_2N_4O_2S_2$ (481 MW).

6.3 Synthesis of 3-[4-(substituted)-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one

(Targeted compounds coded as MM3Pz1 to MM3Pz4)

6.3.1 Scheme for synthesis of 3-[4-(substituted-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one.



Scheme 6.1. Synthesis of 3-[4-(substituted)-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one

Reagents and conditions: a) NH4SCN, Acetone, Reflux 25 min; b) reflux for 15 min, pour reaction mixture to crushed ice; c) dimethylformamide, stirr at 70^{o}C to 80^{o}C for 2 hour, pour to crushed ice.

6.3.2 General procedure for synthesis of 3-[4-(substituted-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one (Reji *et al.*, 2008):

To a solution of the adduct Pz (0.0025 mmol) in N,N-dimethylformamide (5 ml), 3-bromoacetylcoumarin (0.0025 mmol) was added. The reaction mixture was warmed on a water bath at 80-85 ^{o} C for 5 min. To this, triethylamine (0.0025 mmol, 0.3 ml) was added and heating was continued for another 15 min or till reaction mixture shows absent of starting material. The above mixture was cooled and poured into ice-cold water with stirring. A yellow precipitate thus obtained was filtered, wash with water and air-dried. The crude product was purified by preparative TLC (Hexane :

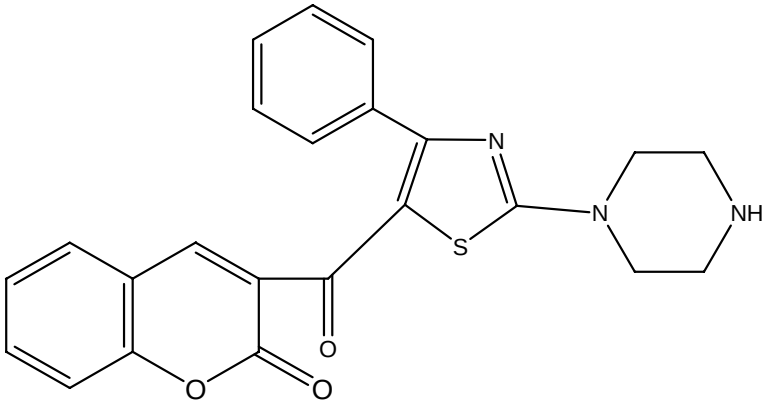
Ethyl acetate 3:7) corresponding to the (MM3Pz1-MM3Pz4) characterized as per the analytical data.

6.3.3 Characteristics of synthesized compounds:

(i) MM3Pz1

3-(4-Phenyl-2-piperazin-1-yl-thiazole-5-carbonyl)-chromen-2-one.

Structure:



MW **:** 418

Mol.Formula **:** $C_{23}H_{19}N_3O_3S$

M.P. **:** 158-160^{0}C

Yield **:** 80%

TLC: Mobile Phase Hexane;Ethyl-acetate 3:7,R_f- 0.95.

^{1}H NMR: *(DMSO, δ, ppm)* = 3.85-3.90 (t,9H, piperazine protons at 2nd position of thiazole ring), 6.9-7.46 (m, 9H, (5H of aromatic protons of 4th position and 4H of coumarin ring), 8.17 (s, 1H, aromatic proton).

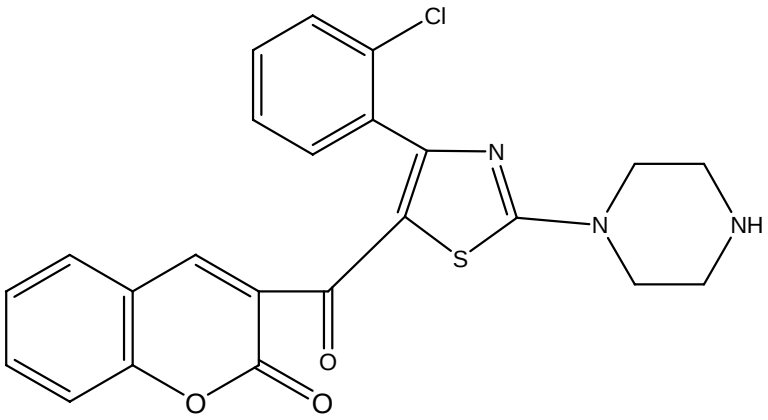
IR *(KBr, cm^{-1})***:** 3500 (-CH2 stretching-piperazine ring), 1748 (strong band of -C=O stretching), 1457-1533 (C=C stretching, aromatic), 1374(C-N) 1238, (C-C[=O]-O symmetric stretching)

MASS: 418 ($M^{+\cdot}$)

(ii) MM3Pz2

3-[4-(2-Chloro-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one.

Structure:



MW **:** 452

Mol.Formula **:** $C_{23}H_{18}ClN_3O_3S$

M.P. **:** 138-140^{0}C

Yield **:** 87%

TLC: Mobile Phase Hexane;Ethyl-acetate 3:7,R_f- 0.65.

^{1}H NMR: *(DMSO, δ, ppm)* = 3.18-3.50 (t,9H, piperazine protons at 2nd position of thiazole ring), 7.0-7.7 (m, 8H, (4H of aromatic protons of 4th position and 4H of coumarin ring), 9.6 (s, 1H, aromatic proton).

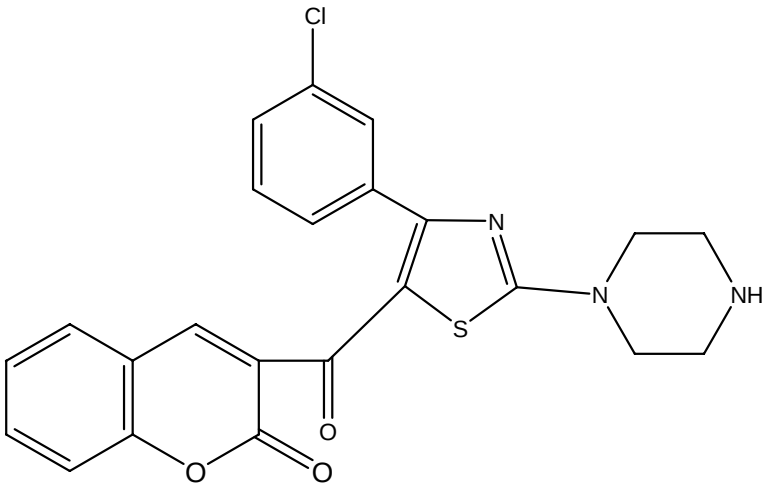
IR *(KBr, cm^{-1})***:** 3032 (-CH2 stretching-piperazine ring), 1733 (strong band of -C=O stretching), 1457-1533 (C=C stretching, aromatic), 1374(C-N) 1239, (C-C[=O]-O symmetric stretching).

MASS m/z: 452 ($M^{+\cdot}$), 453(M^{+1}), 454(M^{+2}).

(iii) MM3Pz3

3-[4-(3-Chloro-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one.

Structure:



MW **:** 452

Mol.Formula **:** $C_{23}H_{18}ClN_3O_3S$

M.P. **:** 118-120^{0}C

Yield **:** 85%

TLC: Mobile Phase Hexane;Ethyl-acetate 3:7,R_f- 0.97.

^{1}H NMR: *(DMSO, δ, ppm)* = 2.51-3.86 (9H, piperazine protons at 2nd position of thiazole ring), 6.98-7.22 (m, 8H, (4H of aromatic protons of 4th position and 4H of coumarin ring), 8.64 (s, 1H, aromatic proton).

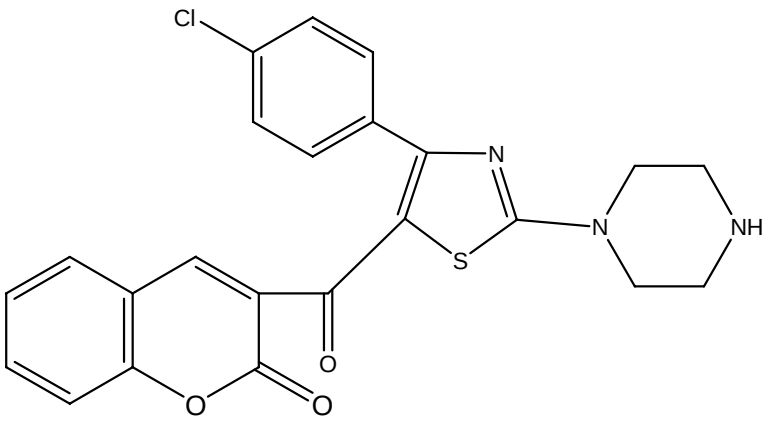
IR *(KBr, cm^{-1})***:** 3032 (-CH2 stretching-piperazine ring), 1730 (strong band of -C=O stretching), 1456-1533 (C=C stretching, aromatic), 1374(C-N) 1243, (C-C[=O]-O symmetric stretching).

MASS m/z: 452 ($M^{+\cdot}$), 453(M^{+1}), 454(M^{+2}).

(iv) MM3Pz4

3-[4-(4-Chloro-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one.

Structure:



MW **:** 452

Mol.Formula **:** $C_{23}H_{18}ClN_3O_3S$

M.P. **:** 122-124^{0}C

Yield **:** 80 %

TLC: Mobile Phase Hexane;Ethyl-acetate 3:7,R_f- 0.93.

^{1}H NMR: *(DMSO, δ, ppm)* = 2.51-2.52 (9H, piperazine protons at 2nd position of thiazole ring), 7.21-7.57 (m, 8H, (4H of aromatic protons of 4th position and 4H of coumarin ring), 7.69 (s, 1H, aromatic proton).

IR *(KBr, cm^{-1})***:** 3041 (-CH2 stretching-piperazine ring), 1740 (strong band of -C=O stretching), 1456-1533 (C=C stretching, aromatic), 1374(C-N) 1241, (C-C[=O]-O symmetric stretching).

MASS m/z: 452 ($M^{+\cdot}$), 453(M^{+1}), 454(M^{+2}).

6.4 Pharmacological Screening:

Antibacterial activity and antiplatelet activity

The synthesized compounds (MMPz1-MMPz4) were screened for their antibacterial activity and anti-platelet activity as per methods explained in previous chapter 4.3.4.2 and 4.3.4.3.

6.5 Results and Discussion:

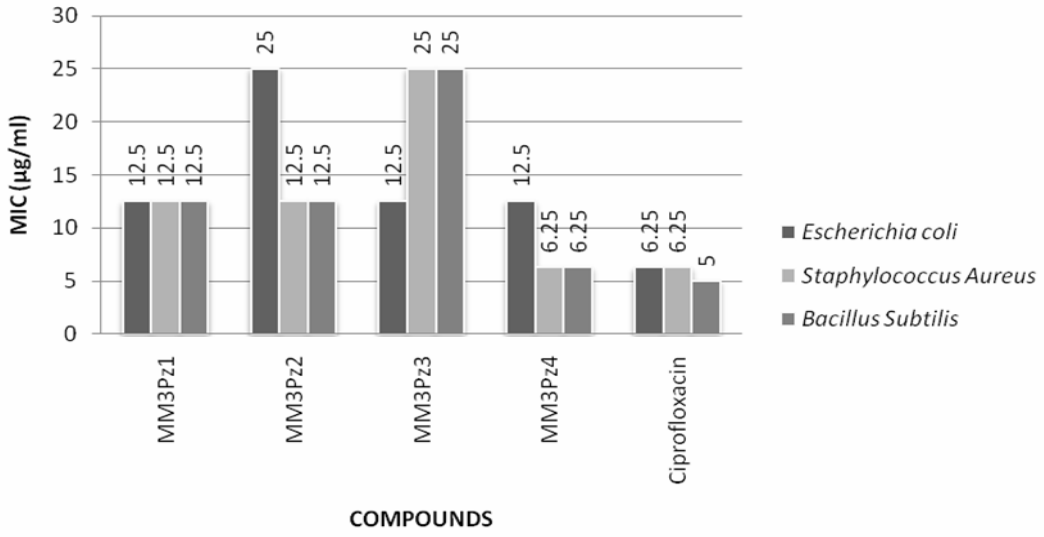
In continuation of earlier research of incorporating the piperidine ring instead of morpholine as 3-substituted coumarin derivatives, in this chapter the compounds were synthesized by keeping a thiazole side chain at C-3 position of coumarin. In addition, to expand profile of synthetic courmarins for biological functions, the piperazine fusion at 2^{nd} position of thiazole was also attempted to check the effect of change in the lipophilicity. The compounds MM3Pz1 was synthesized by fusion of coumarin-3-yl at fifth position, piperazine at 2^{nd} position and phenyl moiety at 4^{th} position of thiazole nucleus. The compounds MM3Pz2 to MM3Pz4 were synthesized keeping coumarin-3-yl constant at fifth position, piperazine at 2^{nd} position and introducing electron withdrawing group (-Cl) at ortho, meta and para position in phenyl moiety at 4^{th} position of thiazole nucleus. The results are depicted in Table 6.1.

Table 6.1 : Chemical Structure, anti-bacterial and anti-platelet activity data of synthesized 3-[4-(2-Chloro-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one

Compound Code	R	Anti-bacterial activity			Platelet aggregation inhibition (%)
		MIC in µg/ml (zone of inhibition in mm)			
		Gm –ve Becteria	Gm +ve Bacteria		
		Escherichia coli	*Staphylococcus aureus*	*Bacillus subtilis*	
MM3Pz1	-H	12.5(18)	12.5(18)	12.5(18)	27.17
MM3Pz2	2-Chloro	25 (08)	12.5(18)	12.5(18)	31.88
MM3Pz3	3-Chloro	12.5(18)	25 (08)	25 (08)	3.76
MM3Pz4	4-Chloro	6.25(18)	6.25(18)	6.25(18)	24.13
Ciprofloxacin		6.25(18)	6.25(18)	5(21)	-
Aspirin		-	-	-	90.00

Note: the MIC values were evaluated at concentration range 1.56 to 100 µg/ml. The values in the table show the MIC values and the corresponding zone of inhibition (in mm).

CHART 6.1: ANTIBACTERIAL ACTIVITY OF 3-[4-(SUBSTITUTED PHENYL)-2-PIPERAZIN-1-YL-THIAZOLE-5-CARBONYL]-CHROMEN-2-ONE



CHAPTER 6.2: ANTI-PLATELET ACTIVITY OF 3-[4-(SUBSTITUTED PHENYL)-2-PIPERAZIN-1-YL-THIAZOLE-5-CARBONYL]-CHROMEN-2-ONE

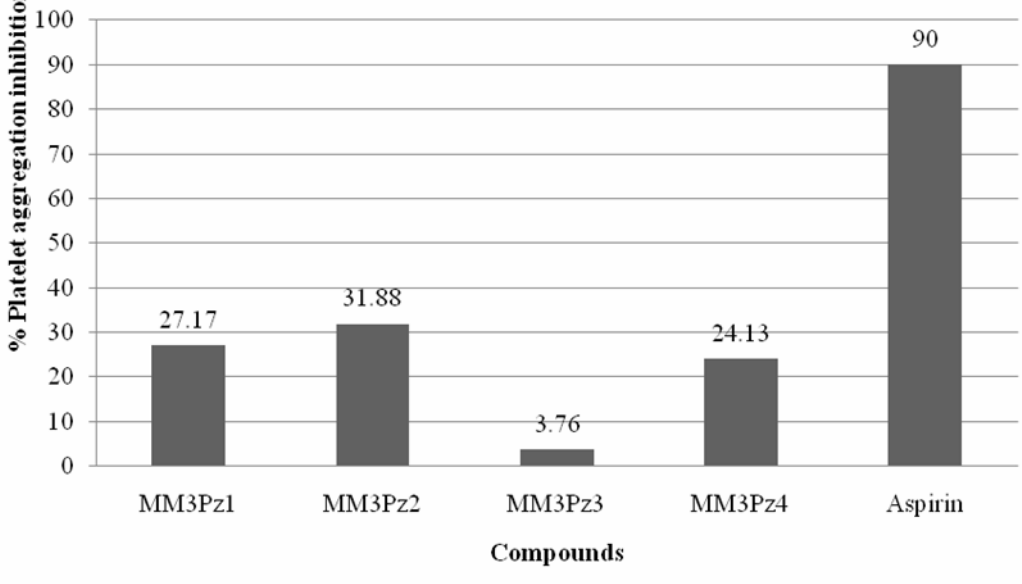
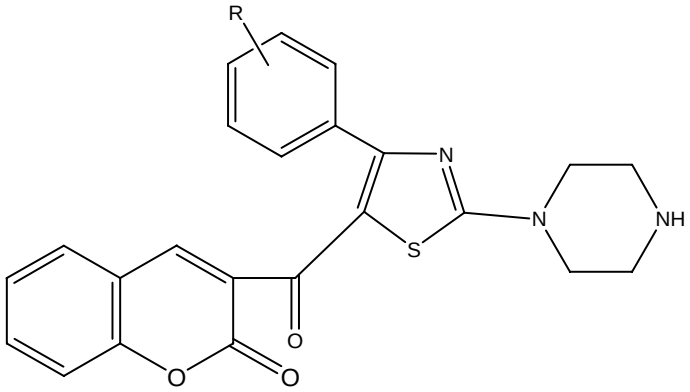


Table 6.2: Physical characteristics of synthesized 3-[4-(2-Chloro-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one



Compound Code	R	M.P. (^{0}C)	Yield (%)	MW	Mol.Formula
MM3Pz1	-H	158-160	80	418	$C_{23}H_{19}N_3O_3S$
MM3Pz2	2-Chloro	138-140	87	452	$C_{23}H_{18}ClN_3O_3S$
MM3Pz3	3-Chloro	118-120	85	452	$C_{23}H_{18}ClN_3O_3S$
MM3Pz4	4-Chloro	122-124	80	452	$C_{23}H_{18}ClN_3O_3S$

The investigation of antibacterial screening revealed that some of the tested compounds showed moderate to good bacterial inhibition. Particularly compound MM3Pz1 showed moderate activity against *Bacillus subtilis* and *Escherichia coli*. Compound MM3Pz4 exhibited very good activity against all the bacterial strains when compared to Ciprofloxacin. The high activity attributed in MM3Pz4 may be due to presence of electron withdrawing 4-chloro functional group in phenyl ring at 4th position of thiazole. While in other compounds like MM3Pz2, MM3Pz3 decrease in activity may be due to stearic hinderance.

The compounds MM3Pz1, MM3Pz2, MM3Pz3, MM3Pz4 shows 27.17 % , 31.88 %,3.76 % and 24.13 % platelet aggregation inhibition respectively. Aspirin showed 90 % platelet aggregation inhibition. Compound MM3Pz3 showed poor antiplatelet activity, while other compounds MM3Pz1, MM3Pz2 and MM3Pz4 exhibited moderate anti-platelet activity when compared with aspirin.

Table 6.3: Spectral data synthesized 3-[4-(2-chloro-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one

Code	Nomenclature	^{1}HNMR (DMSO-d6) (ppm)	IR (Cm^{-1})	MASS (m/z)
MM3Pz1	*3-(4-Phenyl-2-piperazin-1-yl-thiazole-5-carbonyl)-chromen-2-one*	3.85-3.90 (t,9H, piperazine protons at 2nd position of thiazole ring), 6.9-7.46 (m, 9H, (5H of aromatic protons of 4th position and 4H of coumarin ring), 8.17 (s, 1H, aromatic proton).	3500 (-CH2 stretching-piperazine ring), 1748 (strong band of -C=O stretching), 1457-1533 (C=C stretching, aromatic), 1374(C-N) 1238, (C-C[=O]-O symmetric stretching).	418 ($M^{+\cdot}$)
MM3Pz2	*3-[4-(2-Chloro-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one*	3.18-3.50 (t,9H, piperazine protons at 2nd position of thiazole ring), 7.0-7.7 (m, 8H, (4H of aromatic protons of 4th position and 4H of coumarin ring), 9.6 (s, 1H, aromatic proton).	3032 (-CH2 stretching-piperazine ring), 1733 (strong band of -C=O stretching), 1457-1533 (C=C stretching, aromatic), 1374(C-N) 1239, (C-C[=O]-O symmetric stretching).	452 ($M^{+\cdot}$), 453(M^{+1}), 454(M^{+2}).
MM3Pz3	*3-[4-(3-Chloro-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one*	2.51-3.86 (9H, piperazine protons at 2nd position of thiazole ring), 6.98-7.22 (m, 8H, (4H of aromatic protons of 4th position and 4H of coumarin ring), 8.64 (s, 1H, aromatic proton).	3032 (-CH2 stretching-piperazine ring), 1730 (strong band of -C=O stretching), 1456-1533 (C=C stretching, aromatic), 1374(C-N) 1243, (C-C[=O]-O symmetric stretching).	452 ($M^{+\cdot}$), 453(M^{+1}), 454(M^{+2}).
MM3Pz4	*3-[4-(4-Chloro-phenyl)-2-piperazin-1-yl-thiazole-5-carbonyl]-chromen-2-one*	2.51-2.52 (9H, piperazine protons at 2nd position of thiazole ring), 7.21-7.57 (m, 8H, (4H of aromatic protons of 4th position and 4H of coumarin ring), 7.69 (s, 1H, aromatic proton).	3041 (-CH2 stretching-piperazine ring), 1740 (strong band of -C=O stretching), 1456-1533 (C=C stretching, aromatic), 1374(C-N) 1241, (C-C[=O]-O symmetric stretching).	452 ($M^{+\cdot}$), 453(M^{+1}), 454(M^{+2}).

6.6 Conclusions:

The synthesized targeted compounds (MM3Pz1- MM3Pz4) were evaluated for their in vitro antibacterial and anti-platelet activities. On the basis of structure-activity relationship studies of MM3P1- MM3P4 it can be concluded that presence of 4-Chlorophenyl group at the 4^{th} position and piperazine at 2^{nd} position of thiazole ring contributes to enhance antibacterial activity activity profile of the candidates. But surprisingly moderate antiplatelet activity was found at this position.

6.7 References:

Dhavan B, Southwick PL. Thiocarbamoyl derivatives of N-acylpiperazines. J Heterocyclic Chemistry.1983;20:244.

Ketcham R, Lam G, Shah VP. Synthesis and biological study of a series of S-substituted -mercaptohippuramides and nitriles. J Med Chem.1971;14:743-777.

Reji TF, Devi SK, Thomas KK, Shreejalekshmi KG, Manji SL, Franscis M, Philip SK, Bharathan A, Rajasekharan KN. Synthesis and cytotoxixity studies of thiazole analogs of the anticancer marine alkaloid dendrodoine. Indian J Chem2008;47B: 1145-1150.

CHAPTER VII

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF 3-[2-(4-METHYL-PIPERAZIN-1-YL)-4-PHENYL-THIAZOLE-5-CARBONYL]-CHROMEN-2-ONE

7.1 Introduction

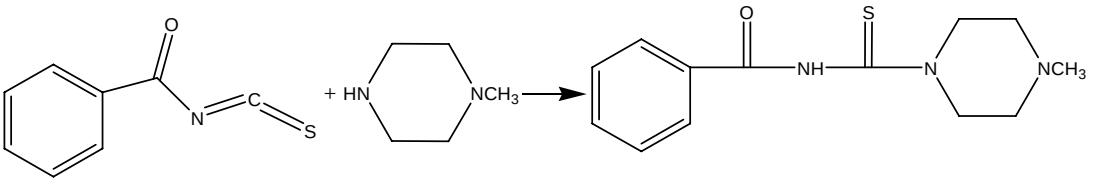
In the previous chapters, compounds were studied by fusion of thiazole and coumarin, with change at 4^{th} position i.e. electron withdrawing group (chloro group attached to aromatic ring) and 2^{nd} position i.e morpholine, piparidine, piperazine of thiazole was studied, which modulate the activity. In this chapter the compound was synthesized to study the incorporation of electron donating group –CH3 i.e. replacement of piperazine ring with N-methylpiperazine at 2^{nd} position of thiazole ring. The Scheme of proposed work is represented in **Scheme 6.1**

7.2 Synthesis of intermediate:

7.2.1 Synthesis of 3-bromoacetylcoumarin: As per previous chapter 3.2.7

7.2.2 Synthesis of phenyl isothiocyanate: As per previous chapter 3.2.5

7.2.3 Synthesis of N-(4-methylpiperazine-1-thioyl)-benzamide (Add-mPz1):



Benzyl isothiocyanate 1-methylpiperazine Add-mPz1: *N*-(4-methylpiperazine-1-thioyl)-benzamide

Requirements:

Chemicals	Molecular formula	Quantity used (Mole)
Ammonium thiocyanate	CH_4N_2S	10.58 gm (0.139)
Benzoyl chloride	C_7H_5ClO	17.93 gm (0.127)
1-methylpiperazine	$C_5H_{12}N_2$	05.80 gm (0.058)

Procedure: (Dhavan *et al.*, 1983)

To a stirred solution of ammonium thiocyanate (10.58 gm, 0.1392 mole) in 70 ml acetone at room temperature, was added benzoyl chloride (17.93 gm, 0.127 mole) over a period of 20 minutes. The reaction mixture was refluxed for 15 minutes. After reflux, 1-methylpiperazine (5.8 gm, 0.058 mole) dissolved in 70 ml acetone was added into reaction mixture at reflux temperature in 10 minutes and mixture was further refluxed for 40 minutes. The reaction mixture was poured in to 400 gm crushed ice with stirring and separated solid was filtered, washed with 500 ml water and 210 ml methanol and dried. This was coded as Add-mPz1.

M.P.: 144-146^oC.

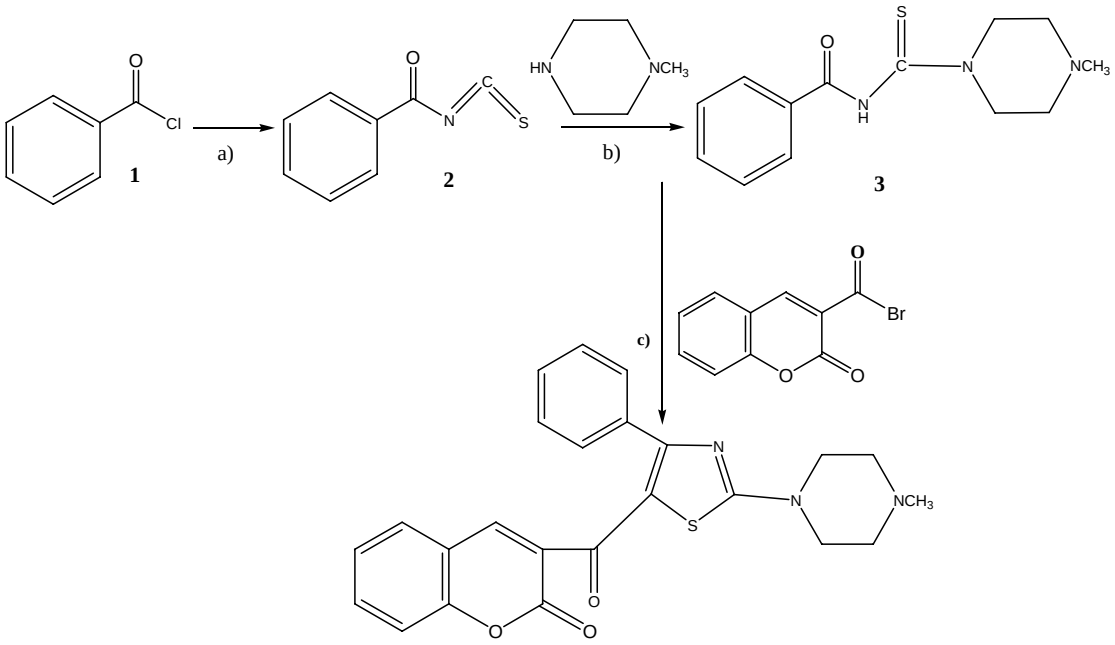
TLC: Mobile Phase Hexane:Ethyl acetate 3:7,R_f- 0.69.

Molecular formula: $C_{13}H_{17}N_3OS$ (263 MW).

7.3 Synthesis of 3-[2-(4-methyl-piperazin-1-yl)-4-phenyl-thiazole-5-carbonyl]-chromen-2-one

(Targeted compound coded as MM3mPz1)

7.3.1 Scheme for synthesis of 3-[2-(4-methyl-piperazin-1-yl)-4-phenyl-thiazole-5-carbonyl]-chromen-2-one



Scheme 7.1. Synthesis of 3-[2-(4-methyl-piperazin-1-yl)-4-phenyl-thiazole-5-carbonyl]-chromen-2-one

Reagents and conditions: a) NH_4SCN, Acetone, Reflux 25 min; b) reflux for 15 min, pour reaction mixture to crushed ice; c) dimethylformamide, stirr at 70^{o}C to 80^{o}C for 2 hour, pour to crushed ice.

7.3.2 Procedure for synthesis of 3-[2-(4-Methyl-piperazin-1-yl)-4-phenyl-thiazole-5-carbonyl]-chromen-2-one (Reji *et al.*, 2008):

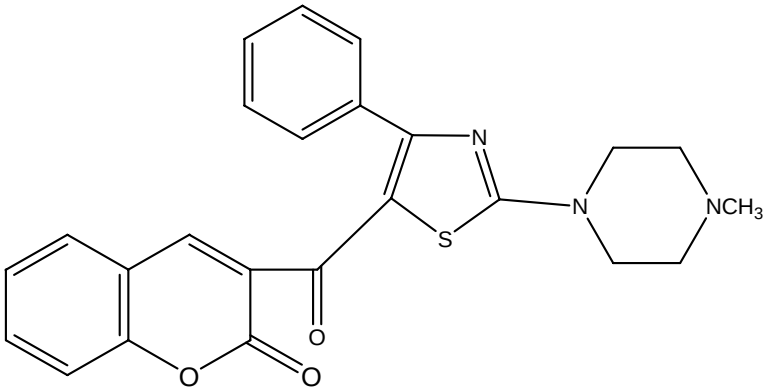
To a solution of the adduct mPz1 (0.0025 mmol) in N,N-dimethylformamide (5 ml), 3-bromoacetylcoumarin (0.0025 mmol) was added. The reaction mixture was warmed on a water bath at 80-85 ^{o} C for 5 min. To this, triethylamine (0.0025 mmol, 0.3 ml) was added and heating was continued for another 15 min or till reaction mixture showed absent of starting material. The above mixture was cooled and poured into ice-cold water with stirring. A yellow precipitate thus obtained was filtered, wash with water and air-dried. The crude material was purified by preparative TLC (Hexane : Ethyl acetate 3:7).

7.3.3 Characteristics of synthesized compound:

(i) MM3mPz1

3-[2-(4-Methyl-piperazin-1-yl)-4-phenyl-thiazole-5-carbonyl]-chromen-2-one.

Structure:



MW **:** 432

Mol.Formula **:** $C_{24}H_{21}N_3O_3S$

M.P. **:** 126-128^{0}C

Yield **:** 65%

TLC: Mobile Phase- Hexane : Ethyl acetate 3**:**7**,** Rf-0**.**98.

^{1}H NMR: *(DMSO, δ, ppm)* = 2.56 (s,3H, CH3 at 4th position of methyl piperazine), 2.57-2.58(t,4H, CH2 at 3rd and 5th position of methyl piperazine), 2.94-2.96 (t,4H, CH2 at 2rd and 6th position of methyl piperazine) 7.57-8.15 (m, 9H, (5H of aromatic protons of 4th position and 4H of coumarin ring), 8.70 (s, 1H, aromatic proton).

IR *(KBr, cm^{-1})***:** 3100-3000 (-CH2 stretching-),1750 (strong band of -C=O stretching), 1457-1533 (C=C stretching, aromatic), 1374(C-N) 1248, (C-C[=O]-O symmetric stretching).

MASS: 432 ($M^{+\cdot}$), 433(M+1).

7.4 Pharmacological Screening:

Antibacterial activity and antiplatelet activity

The newly synthesized compound was screened for antibacterial activity and anti-platelet activity as per methods explained in previous chapter 4.3.4.2 and 4.3.4.3.

7.5 Results and Discussion:

In this chapter the compound was synthesized by attaching a thiazole side chain at C-3 position of coumarin. In addition, to expand profile of synthetic courmarins for biological functions, the N-methylpiperazine fusion at 2nd position of thiazole was also attempted to study the effect of electrone donating group. The compounds MM3MPz1 was synthesized by fusion of coumarin-3-yl constant at fifth position, N-methylpiperazine at 2nd position and phenyl moiety at 4th position of thiazole nucleus. The results are given in Table 7.1.

Table 7.1 : Chemical Structure, anti-bacterial and anti-platelet activity data of synthesised 3-[2-(4-Methyl-piperazin-1-yl)-4-phenyl-thiazole-5-carbonyl]-chromen-2-one

Compound Code	R	Anti-bacterial activity			Platelet aggregation inhibition (%)
		MIC in µg/ml (zone of inhibition in mm)			
		Gm –ve Becteria	Gm +ve Bacteria		
		Escherichia coli	*Staphylococcus Aureus*	*Bacillus Subtilis*	
MM3mPz1	-H	6.25(18)	6.25(18)	6.25(18)	17.17
Ciprofloxacin		6.25(18)	6.25(18)	5(21)	-
Aspirin		-	-	-	90.00

Note: the MIC values were evaluated at concentration range 25 to 100 µg/ml. The values in the table show the MIC values and the corresponding zone of inhibition (in mm).

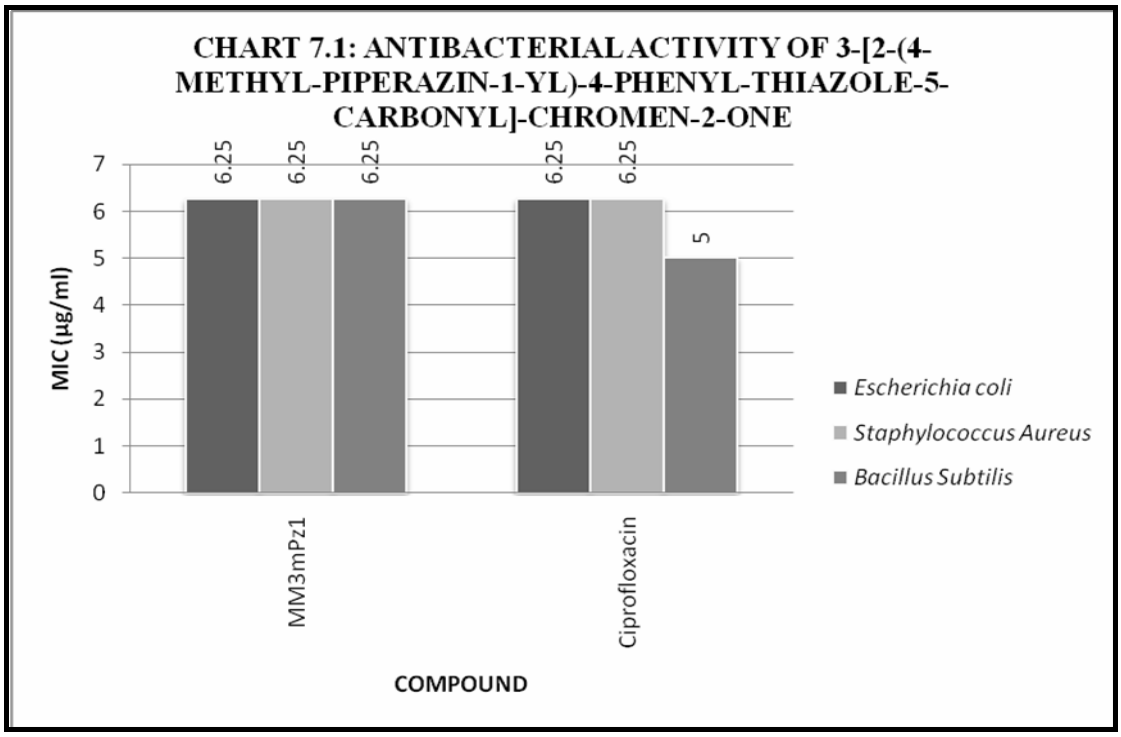


CHART 7.1: ANTIBACTERIAL ACTIVITY OF 3-[2-(4-METHYL-PIPERAZIN-1-YL)-4-PHENYL-THIAZOLE-5-CARBONYL]-CHROMEN-2-ONE
MIC (µg/ml)
7
6
5
4
3
2
1
0
6.25
6.25
6.25
6.25
6.25
5
MM3mPz1
Ciprofloxacin
COMPOUND
Escherichia coli
Staphylococcus Aureus
Bacillus Subtilis

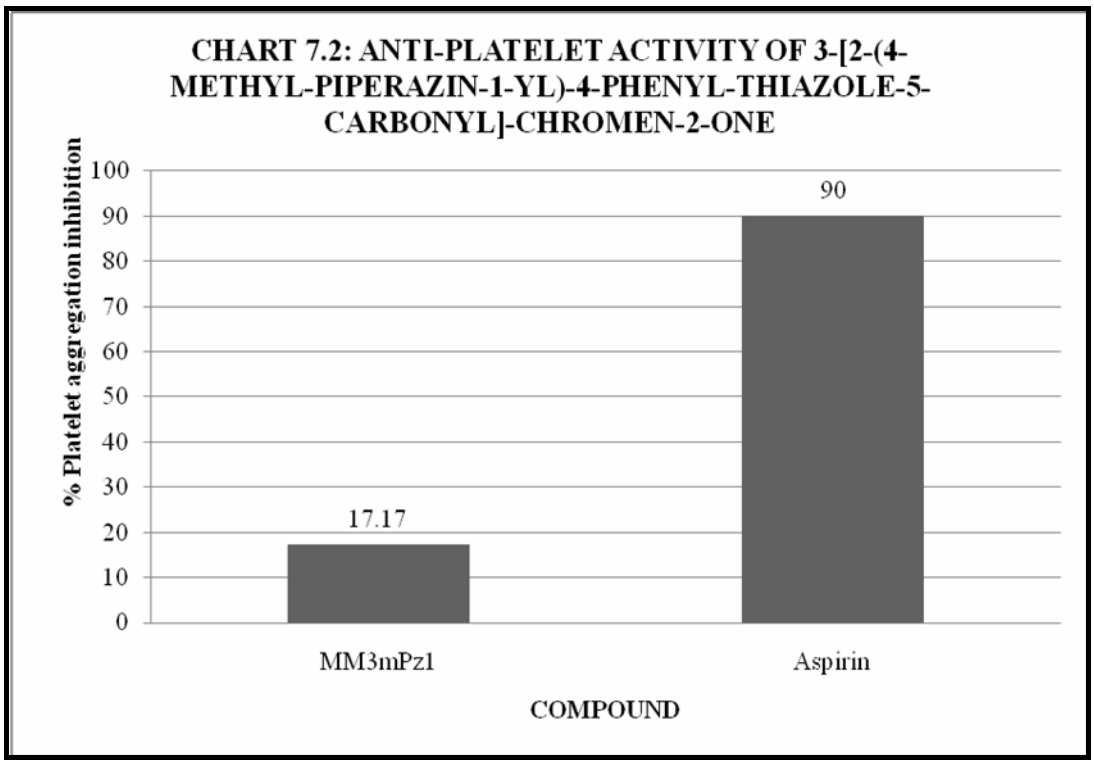
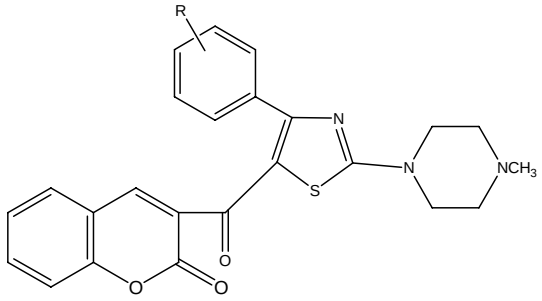


CHART 7.2: ANTI-PLATELET ACTIVITY OF 3-[2-(4-METHYL-PIPERAZIN-1-YL)-4-PHENYL-THIAZOLE-5-CARBONYL]-CHROMEN-2-ONE
% Platelet aggregation inhibition
100
90
80
70
60
50
40
30
20
10
0
17.17
90
MM3mPz1
Aspirin
COMPOUND

Table 7.2: Physical Characteristcs of synthesised 3-[2-(4-Methyl-piperazin-1-yl)-4-phenyl-thiazole-5-carbonyl]-chromen-2-one



Compound Code	R	M.P. (^{0}C)	Yield (%)	MW	Mol.Formula
MM3mPz1	-H	126-128	65	432	$C_{24}H_{21}N_3O_3S$

Tested compound, MM3MPz1exhibited very good antibacterial activity against Gram-positive and Gram-negative bacteria, when compared to Ciprofloxacin. While it shows 17.17 % platelet aggregation inhibition.

7.6 Conclusion:

The synthesized targeted compound (MM3MPz1) was evaluated for antibacterial and anti-platelet activities. On the basis of which it can be concluded that presence of electron donating group at 2nd position of thiazole ring contributes to enhance antibacterial activity activity profile of the candidates. But it also shows poor antiplatelet activity at this position. It is, therefore, suggested that these compounds to be studied further with other substitution to explore its full potential and detailed structure activity relationship

7.7 References:

Dhavan B, Southwick PL. Thiocarbamoyl derivatives of N-acylpiperazines. J Heterocyclic Chemistry.1983;20:244.

Reji TF, Devi SK, Thomas KK, Shreejalekshmi KG, Manji SL, Franscis M, Philip SK, Bharathan A, Rajasekharan KN. Synthesis and cytotoxixity studies of thiazole analogs of the anticancer marine alkaloid dendrodoine. Indian J Chem2008;47B: 1145-1150.

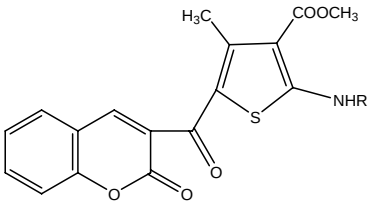
CHAPTER VIII

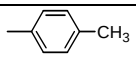
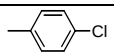
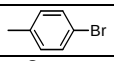
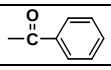
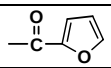
SUMMARY

8.1 TETRASUBSTITUTED THIOPHENE -COUMARIN DERIVATIEVES

All the synthesized tetra-substituted thiophene-coumarin compounds (MM1a- MM1g) were evaluated for their invivo anti-inflammatory and analgesic activity. On the basis of structure-activity relationship studies of synthesized thiophene compounds it can be concluded that presence of -Cl group in anilino moiety (MM1c), benzoyl (MM1e) and 2-furoyl moiety (MM1f) attached to -NH at the second position of the thiophene contributes to anti-inflammatory and analgesic activity profile of the candidates.

Table 8.1: Chemical Structures, anti-inflammatory and analgesic activity of tetra substituted thiophene – coumarin derivatives

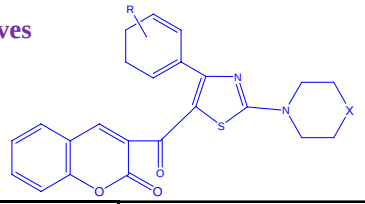


		Anti-inflammatory activity Carrageenin-induced rat hind paw oedema % protection			Analgesic activity Acetic acid induced writhing test % protection
Compound no.	**R**	**10 mg/kg**	**20 mg/kg**	**40 mg/kg**	**10 mg/kg**
MM1a		57	70	55	40
MM1b		51	65	37	20
MM1c		71	77	67	56
MM1d		59	67	41	12
MM1e		64	71	49	38
MM1f		68	76	56	55
MM1g	CH_2CH_3	48	34	24	32
Ibuprofen (20 mg/kg)	-	36			-
Mefanamic acid (100mg/kg)	-	42			-
Ibuprofen (10 mg/kg)	-	-	-	-	62

8.2 Thiazole-coumarin derivatives

Among all the synthesized coumarin-thiazole derivatives, basic structural difference is at 2nd position namely morpholine, piperidine, piparazine and N-methyl piperazine. Antibacterial activity in all piperidino compounds against gram negative microorganism i.e. *Escherichia coli* was found comparable and consistent. Whereas, almost similar results were observed in all other compounds against gram positive microorganism i.e. *Staphylococcus aureus* and *Bacillus subtilis*. The anti-platelet activity was found consistent and comparably greater in all the phenyl substituted piperidino compounds. Whereas it was moderate to poor in morpholino, piperazino,N-methyl-piperazino compounds. Based on this results it is concluded that an increase in activity in piperidino compounds against gram negative bacteria and anti-platelet activity may be because of lipophilic nature of piperidine ring.

Table 8.2: Chemical Structures, antibacterial and anti-platelet activity of coumarin-thiazole derivatives



COMPOUNDS	X=O (MORPHOLINE)				X=C (PIPERIDINE)				X=NH (PIPERAZINE)				X=NCH$_3$ (METHYL PIPERAZINE)			
R=	Anti-bacterial activity MIC in µg/ml (zone of inhibition in mm)			Platelet aggregation inhibition (%)	Anti-bacterial activity MIC in µg/ml (zone of inhibition in mm)			Platelet aggregation inhibition (%)	Anti-bacterial activity MIC in µg/ml (zone of inhibition in mm)			Platelet aggregation inhibition (%)	Anti-bacterial activity MIC in µg/ml (zone of inhibition in mm)			Platelet aggregation inhibition (%)
	Gm –ve Becteria	Gm +ve Bacteria			Gm –ve Becteria	Gm +ve Bacteria			Gm –ve Becteria	Gm +ve Bacteria			Gm –ve Becteria	Gm +ve Bacteria		
	E coli	*S. aureus*	*B. subtilis*		*E coli*	*S. aureus*	*B. subtilis*		*E coli*	*S. aureus*	*B. subtilis*		*E coli*	*S. aureus*	*B. subtilis*	
-H	+++	++	++	++	+++	++	++	+	++	++	++	++	+++	+++	+++	+
2-Chloro	++++	++++	++++	+++	+++	++	++	+++	+	++	++	++	-	-	-	-
3-Chloro	++	++	++	+	+++	++	+	+++	++	+	+	+	-	-	-	-
4-Chloro	+	++	++	+	++	++	++	+++	+++	+++	+++	++	-	-	-	-

+: Poor, ++: moderate, +++:good and +++:very good.

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Synthesis, anti-inflammatory, analgesic and antioxidant activities of some tetrasubstituted thiophenes

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Synthesis, anti-inflammatory, analgesic and antioxidant activities of some tetrasubstituted thiophenes

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Abstract

Sets of tetrasubstituted thiophene esters ***4a-4g, 5a-5f*** and ***6a-6e*** were synthesized by reaction of 1-(α-Carbomethoxy-β-aminothiocrotonoyl)-aryl/aroyl amines (***3***) with 3-(bromoacetyl)coumarin, 1,4-dibromodiacetyl and chloroacetone respectively. The compound ***3*** were synthesized by nucleophilic addition of aryl/aroylisothiocyanate and enamine (***2***). The synthesized targeted compounds (***4a-4g***, ***5a-5f*** and ***6a-6e***) were evaluated for their *in vivo* anti-inflammatory activity in carrageenin-induced rat hind paw oedema model at three graded doses employed at 10, 20 and 40 mg/kg body weight using mefanamic acid, ibuprofen and *in vivo* analgesic activity in acetic acid induced writhing response model at 10 mg/kg dose using ibuprofen as standard drug. The compounds ***4a-4f***, ***5c, 5f, 6c*** and ***6e*** were evaluated for their *in vitro* antioxidant nitric oxide radical scavenging assay at the concentrations of 5, 10, 15, 20, 25, 30 and 35 μg/mL using ascorbic acid as standard drug. Among all the targeted compounds ***4c*** showed maximum anti-inflammatory activity of 71% protection at 10 mg/kg and 77% protection at 20 mg/kg to inflamed paw and analgesic activity of 56% inhibition and also maximum *in vitro* nitric oxide radical scavenging activity having IC_{50} value 31.59 μg/mL.

Keywords: *Tetrasubstituted thiophenes, coumarin, anti-inflammatory activity, analgesic activity, antioxidant activity*

Introduction

Inflammation occurs as a defensive response which induces physiological adaptations to limit tissue damage and remove the pathogenic infections. Diseases caused by inflammation are an important factor of morbidity and mortality in humans. Inflammatory disorders include rheumatoid arthritis, osteoarthritis, inflammatory bowl diseases, retinitis, multiple sclerosis, psoriasis and atherosclerosis [1]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of acute and chronic inflammation, pain and fever. Most of NSAIDs that are available in market are known to inhibit isoforms, a constitutive form, COX-1 and an inducible form COX-2, to offer therapeutic effect. However, long-term clinical usage of NSAIDs is associated with significant side effects of gastrointestinal lesions, bleeding and nephrotoxicity [2–4]. Therefore, the discovery of new safer anti-inflammatory drugs represents a challenging goal for such a research area.

Thiophene derivatives represent an important class of compounds with diverse biological activities. Substituted thiophenes are also present in natural products. Various tri and tetrasubstituted thiophene derivatives and their anti-inflammatory activity are well documented in literature [5,6]. According to our previous reports [7–9], the anti-inflammatory activity of tetrasubstituted thiophene ester/acid molecules

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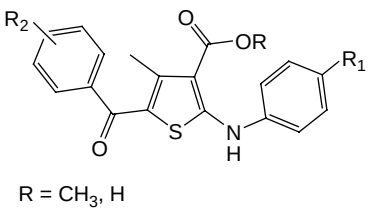
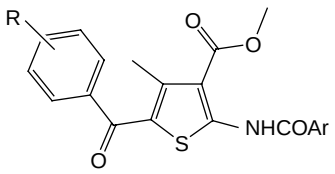


Structure 1.



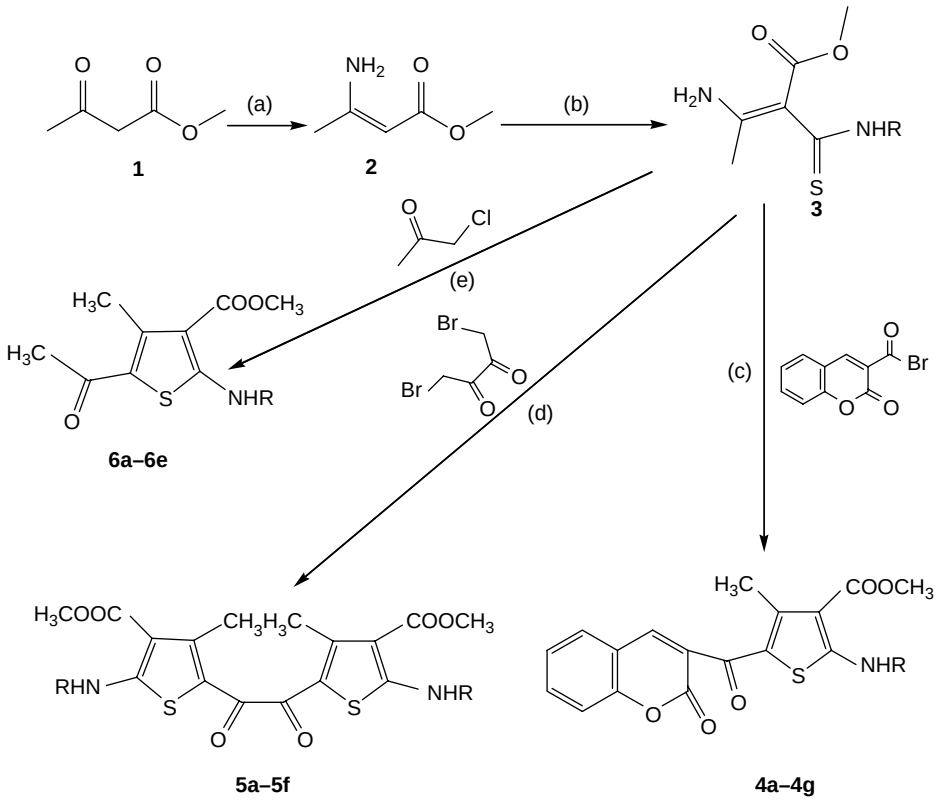
Structure 2.

having the features of (a) COX-1 inhibitor and 5-LOX inhibitor (acid/ester) of the anthranilic acid type (fenamates), (b) p38 MAP kinase inhibitor, can be significantly modified by using substituents at R_1 (both electron releasing and electron withdrawing) in anilino moiety and R_2 (electron releasing and electron withdrawing) in benzoyl moiety (Structure 1). The pharmacological evaluation of tetrasubstituted thiophene esters having carbonyl spacer as aroylamino at the second position of the thiophene ring which has a proton acceptor (—C═O) and a proton donor (—NH) features in adjacent position were also reported (Structure 2). In continuation to our previous efforts in designing and synthesizing new tetrasubstituted thiophenes with good anti-inflammatory/antioxidant activity and selectivity, we report here synthesis, *in vivo* anti-inflammatory, analgesic and *in vitro* antioxidant nitric oxide radical scavenging activity of a new series of designed tetrasubstituted thiophene ester molecules (Scheme 1).

Materials and methods

Chemistry

All reagents and solvents were used as obtained from the supplier or recrystallized/redistilled as necessary. Thin-layer chromatography was performed using glass plates coated with silica gel G and toluene:acetonitrile as a mobile phase. The spots were developed using iodine. Melting points were recorded on capillary melting point apparatus and are uncorrected. Infrared spectra (KBr discs) were recorded with a Buck Scientific M-500 Infrared spectrophotometer.[1]H-NMR spectra were recorded in $CDCl_3$ and DMSO-d6 with 300/200 MHz Bruker FT-NMR (Advance DPX200) spectrometer using



Scheme 1. Synthesis of compound **4**, **5**, & **6** Reagents and conditions: a) ammonia (25%), diethylether, 0–15°C, 1 h; b)ArNCS/ArCONCS, diethylether, 0°C-r.t., 5 h; c), d) & e)acetonitrile, r.t., 12 h.

tetramethylsilane as internal standard and the chemical shifts (δ) are reported in ppm, coupling constants ($\mathcal{J}$) are given in Hz. Mass spectra of the compounds were recorded on Perkin-Elmer Sciex atmospheric pressure ionization liquid chromatography mass instrument (LCMS) and Electron impact (EI) mass spectra were recorded on a Jeol JMS-D-300 spectrometer with the ionization potential of 70 eV. Elemental analysis data were determined using a Carlo-Erba 1108 instrument or Elementar's Vario EL III micro-analyzer. UV spectra were recorded in Shimadzu 1601 UV-Visible spectrophotometer.

General method for synthesis of compounds ***(4a)–(4g)***. As shown in Scheme 1, enamine *(2)* was obtained by reacting ammonia (25%) with methyl acetoacetate in equimolar quantity in diethylether at 0–15°C *(1)*. 1-(α-Carbomethoxy-β-aminothiocrotonoyl)-aryl/aroyl amines *(3)* were synthesized by nucleophilic addition of aryl/aroylisothiocyanate and enamine *(2)* as per reported procedure [6]. Arylisothiocyanates were synthesized using modified Kaluza method [10] whereas aroylisothiocyanate by previously reported procedure [11]. The compounds ***4a-4g*** were synthesized by adding 0.001 mol of the 3-(bromoacetyl)coumarin [12] to a solution of *(3)* (0.001 mol) in 2 mL of acetonitrile without adding base at room temperature [6]. The solution was stirred until the solid was separated from the reaction mixture or until no more of the starting materials could be detected on TLC. The solid was filtered off, washed with chilled acetonitrile, dried, recrystalized with methanol yielding coloured product corresponding to the *(**4a-4g**)* characterized as per the analytical data.

General method for synthesis of compounds ***(5a) - (5f)***. The compounds ***5a-5f*** were synthesized by adding 0.001 mol of 1,4-dibromodiacetyl to a solution of *(3)* (0.002 mol) in 5 mL of acetonitrile without adding base at room temperature. The solution was stirred until the solid was separated from the reaction mixture or until no more of the starting materials could be detected on TLC. The solid that separated was filtered off, washed with chilled acetonitrile, dried, recrystalized with DMSO yielding coloured product corresponding to the ***(5a-5f)*** characterized as per the analytical data.

General method for synthesis of compounds ***(6a)–(6e)***. The compounds ***6a-6e*** were synthesized by adding 0.001 mol of chloroacetone to a solution of *(3)* (0.001 mol) in 4 mL of acetonitrile without adding base at room temperature. The solution was stirred until the solid was separated from the reaction mixture or until no more of the starting materials could be detected on TLC. The solid that separated was filtered off, washed with chilled acetonitrile, dried, recrystalized with methanol yielding coloured product corresponding to the ***(6a-6e)*** characterized as per the analytical data.

Methyl 2-anilino-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate ***(4a)***. Yield: 85%; m.p.: 238°C; R_f: 0.72 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 1692 (C=O stretching of ester), 1606 (C=O stretching of ketone), 772, 674; ^{1}H-NMR: (200 MHz, $CDCl_3$) δ (ppm): 2.65 (s, 3H, CH_3-4), 3.90 (s, 3H, CH_3 of ester), 7.30-7.44 (m, 6H, aromatic), 7.58 (t, 2H, $\mathcal{J} = 6.70$ Hz aromatic), 7.65 (d, 1H, aromatic), 7.93 (s, 1H, aromatic), 10.70 (s 1H, NH-2); MS: *m/z* 419 (M^+); Anal. calcd. for $C_{23}H_{17}NO_5S$: C, 65.88; H, 4.05; N, 3.33; Found: C, 66.16; H, 4.01; N, 3.47%.

Methyl 2-(4-methylanilino)-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate ***(4b)***. Yield: 45%; m.p.: 162°C; R_f: 0.69 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 1678 (C=O stretching of ester), 1615 (C=O stretching of ketone), 745, 685; ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm): 2.27 (s, 3H, CH_3-2), 2.56 (s, 3H, CH_3-4), 3.92 (s, 3H, CH_3 of ester), 7.09 (d, 2H, $\mathcal{J} = 5.45$ Hz aromatic), 7.19-7.22 (m, 3H, aromatic), 7.40 (t, 2H, aromatic), 7.72 (d, 1H, aromatic), 8.76 (s, 1H, aromatic), 10.09 (s, 1H, NH-2); MS: *m/z* 433 (M^+); Anal. calcd. for $C_{24}H_{19}NO_5S$: C, 66.52; H, 4.38; N, 3.23; Found: C, 66.70; H, 4.54; N, 3.65%.

Methyl 2-(4-chloroanilino)-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate ***(4c)***. Yield: 84%; m.p.: 250°C; R_f: 0.84 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 1710 (C=O stretching of ester), 1660 (C=O stretching of ketone), 797, 690; ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm): 2.65 (s, 3H, CH_3-4), 3.91 (s, 3H, CH_3 of ester), 7.31 (d, 2H, $\mathcal{J} = 5.12$ Hz aromatic), 7.37-7.41 (m, 3H, aromatic), 7.61 (t, 2H, $\mathcal{J} = 4.10$ Hz aromatic), 7.64 (d, 1H, aromatic), 7.94 (s, 1H, aromatic), 10.69 (s, 1H, NH-2); MS: *m/z* 455 ($M^+ + 2$); Anal. calcd. for $C_{23}H_{16}ClNO_5S$: C, 60.88; H, 3.52; N, 3.08; Found: C, 60.89; H, 3.84; N, 3.61%.

Methyl 2-(4-bromoanilino)-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate ***(4d)***. Yield: 38%; m.p.: 187°C; R_f: 0.73 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 1693 (C=O stretching of ester), 1648 (C=O stretching of ketone), 785, 656; ^{1}H-NMR: (200 MHz, $CDCl_3$) δ (ppm): 2.66 (s, 3H, CH_3-4), 3.90 (s, 3H, CH_3 of ester), 7.42 (d, 2H, aromatic), 7.55-7.62 (m, 3H, aromatic), 7.64 (d, 1H, aromatic), 7.93 (d, 2H, aromatic), 7.97 (s, 1H, aromatic), 10.64 (s, 1H, NH-2); MS: *m/z* 498 (M^+); Anal. calcd. for $C_{23}H_{16}BrNO_5S$: C, 55.43; H, 3.21; N, 2.80; Found: C, 55.91; H, 3.64; N, 2.58%.

Methyl 2-benzoylamino-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate ***(4e)***. Yield: 67%; m.p.: 182°C; R_f: 0.76 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 1710 (C═O stretching of ester), 1589 (C═O stretching of ketone), 781, 680; ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm): 2.64 (s, 3H, CH_3-4), 3.96 (s, 3H, CH_3 of ester), 7.47-7.53 (m, 6H, aromatic), 8.01 (t, 2H, $\mathcal{J} = 6.70$ Hz aromatic), 7.36 (d, 1H, $\mathcal{J} = 5.80$ Hz aromatic), 8.75 (s, 1H, aromatic), 12.66 (s, 1H, NH-2); MS: *m/z* 447 (M^+); Anal. calcd. for $C_{24}H_{17}NO_6S$: C, 64.42; H, 3.79; N, 3.12; Found: C, 63.98; H, 3.75; N, 3.02%.

Methyl 2-(2-furoylamino)-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate ***(4f)***. Yield: 60%; m.p.: 188°C; R_f: 0.72 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 1724 (C═O stretching of ester), 1582 (C═O stretching of ketone), 779, 676; ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm): 2.66 (s, 3H, CH_3-4), 3.88 (s, 3H, CH_3 of ester), 6.46 (q, 1H, aromatic), 7.39-7.43 (m, 3H, aromatic), 7.59 (t, 2H, $\mathcal{J} = 5.80$ Hz aromatic), 7.70 (d, 1H, aromatic), 8.06 (s, 1H, aromatic); MS: *m/z* 437 (M^+); Anal. calcd. for $C_{22}H_{15}NO_7S$: C, 60.42; H, 3.43; N, 3.20; Found: C, 60.67; H, 3.61; N, 3.32%.

Methyl 2-ethylamino-5-(3-coumarinoyl)-4-methylthiophene-3-carboxylate ***(4g)***. Yield: 35%; m.p.: 205°C; R_f: 0.68 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 3330 (alkyl NH stretching), 1717 (C═O stretching of ester), 1605 (C═O stretching of ketone), 780, 680; ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm): 1.35 (t, 3H, $\mathcal{J} = 3.15$ Hz CH_3-2), 2.61 (s, 3H, CH_3-4), 3.31 (q, 2H, $\mathcal{J} = 3.54$ Hz CH_3-2), 3.82 (s, 3H, CH_3 of ester), 7.30-7.37 (m, 2H, aromatic), 7.57 (t, 2H, aromatic), 7.90 (s, 1H, aromatic), 10.65 (s, 1H, NH-2); MS: *m/z* 371 (M^+); Anal. calcd. for $C_{19}H_{17}NO_5S$: C, 61.45; H, 4.57; N, 3.77; Found: C, 61.78; H, 4.78; N, 4.01%.

Bis-(2-anilino-3-methoxycarbonyl-4-methyl-5-thienyl)ethane-1,2-dione ***(5a)***. Yield: 80%; m.p.: 220°C; R_f: 0.68 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 3320 (aryl NH stretching), 1648 (C═O stretching of ester), 1600 (C═O stretching of ketone), 757, 693; ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm): 2.05 (s, 6H, CH_3-4), 3.78 (s, 6H, CH_3 of ester), 7.22-7.61 (m, 10H, aromatic), 10.72 (s, 2H, NH-2); MS: *m/z* 548 (M^+); Anal. calcd. for $C_{28}H_{24}N_2O_6S_2$: C, 61.30; H, 4.37; N, 5.10; Found: C, 61.63; H, 4.80; N, 5.45%.

Bis-[2-(4-methylanilino)-3-methoxycarbonyl-4-methyl-5-thienyl]ethane-1,2-dione ***(5b)***. Yield: 35%; m.p.: 245°C; R_f: 0.75 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 3368 (aryl NH stretching), 1656 (C═O stretching of ester), 1612 (C═O stretching of ketone), 760, 689; MS: *m/z* 576 (M^+); Anal. calcd. for $C_{30}H_{28}N_2O_6S_2$: C, 62.51; H, 4.85; N, 4.85; Found: C, 62.65; H, 4.65; N, 5.11%.

Bis-[2-(4-chloroanilino)-3-methoxycarbonyl-4-methyl-5-thienyl]ethane-1,2-dione ***(5c)***. Yield: 90%; m.p.: 252°C; R_f: 0.75 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 3383 (aryl NH stretching), 1666 (C═O stretching of ester), 1614 (C═O stretching of ketone), 785, 698; ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm): 2.68 (s, 6H, CH_3-4), 4.00 (s, 6H, CH_3 of ester), 7.98 (d, 4H, aromatic), 8.13 (d, 4H, aromatic), 10.90 (s, 2H, NH-2); MS: *m/z* (rel. abund. %) 617 (M^+, 40), 307 (52), 232 (48), 157 (100), 137 (45), 107 (19); Anal. calcd. for $C_{28}H_{22}Cl_2N_2O_6S_2$: C, 54.47; H, 3.56; N, 4.53; Found: C, 54.44; H, 3.94; N, 4.47%.

Bis-[2-(4-bromoanilino)-3-methoxycarbonyl-4-methyl-5-thienyl]ethane-1,2-dione ***(5d)***. Yield: 56%; m.p.: 262°C; R_f: 0.67 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 3390 (aryl NH stretching), 1673 (C═O stretching of ester), 1620 (C═O stretching of ketone), 786, 686; MS: *m/z* 706 (M^+); Anal. calcd. For $C_{28}H_{22}Br_2N_2O_6S_2$: C, 47.61; H, 3.21; N, 3.96; Found: C, 47.53; H, 3.48; N, 4.08%.

Bis-(2-benzoylamino-3-methoxycarbonyl-4-methyl-5-thienyl)ethane-1,2-dione ***(5e)***. Yield: 85%; m.p.: 239°C; R_f: 0.70 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 3416 (aryl NH stretching), 1711 (C═O stretching of ester), 1594 (C═O stretching of ketone), 779, 687; ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm): 2.77 (s, 6H, CH_3-4), 4.01 (s, 6H, CH_3 of ester), 7.18-7.61 (m, 10H, aromatic), 12.25 (s, 2H, NH-2); MS: *m/z* (rel. abund. %) 604 (M^+, 20), 301 (45), 207 (18), 149 (60); Anal. calcd. for $C_{30}H_{24}N_2O_8S_2$: C, 59.59; H, 3.96; N, 4.63; Found: C, 59.37; H, 4.22; N, 4.92%.

Bis-[2-(2-furoylamino)-3-methoxycarbonyl-4-methyl-5-thienyl]ethane-1,2-dione ***(5f)***. Yield: 68%; m.p.: 208°C; R_f: 0. 80 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 3446 (aryl NH stretching), 1724 (C═O stretching of ester), 1687 (C═O stretching of ketone), 799, 699; ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm): 2.76 (s, 6H, CH_3-4), 4.07 (s, 6H, CH_3 of ester), 7.19-7.38 (m, 6H, aromatic), 12.13 (s, 2H, NH-2); MS: *m/z* (rel. abund. %) 584 (M^+, 22), 289 (38), 232 (16), 154 (100), 136 (85), 107 (38); Anal. calcd. for $C_{26}H_{20}N_2O_{10}S_2$: C, 53.42; H, 3.42; N, 4.79; Found: C, 53.12; H, 3.29; N, 4.67%.

Methyl 2-anilino-5-acetyl-4-methylthiophene-3-carboxylate ***(6a)***. Yield: 48%; m.p.: 125°C; R_f: 0.65 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 1665 (C═O stretching of ester), 1621 (C═O stretching of ketone), 754, 690; ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm): 2.47 (s, 3H, CH_3-4), 2.74 (s, 3H, CH_3-5), 3.91 (s, 3H, CH_3 of ester), 7.17-7.44 (m, 5H, aromatic), 10.57 (s 1H, NH-2); MS: *m/z* 290 ($M^+ + 1$); Anal. calcd. for $C_{15}H_{15}NO_3S$: C, 62.29; H, 5.18; N, 4.84; Found: C, 62.54; H, 5.31; N, 4.67%.

Methyl 2-(4-methylanilino)-5-acetyl-4-methylthiophene-3-carboxylate ***(6b)***. Yield: 38%; m.p.: 175°C; R_f: 0.88 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 1665 (C═O stretching of ester), 1614 (C═O stretching of ketone), 750, 699; ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm): 2.28 (s, 3H, CH_3-2), 2.35 (s, 3H, CH_3-4), 2.56 (s, 3H, CH_3-5), 3.92 (s, 3H, CH_3 of ester), 7.13 (d, 2H, $\mathcal{J} = 7.11$ Hz aromatic), 7.21 (d, 2H, aromatic), 10.09 (s 1H, NH-2); MS: *m/z* 323 ($M^+ + 23$), 303 (M^+); Anal. calcd. for $C_{16}H_{17}NO_3S$: C, 63.37; H, 5.27; N, 4.61; Found: C, 63.19; H, 5.58; N, 4.37%.

Methyl 2-(4-chloroanilino)-5-acetyl-4-methylthiophene-3-carboxylate ***(6c)***. Yield: 78%; m.p.: 162°C; R_f: 0.80 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 1720 (C═O stretching of ester), 1661 (C═O stretching of ketone), 785, 690; ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm): 2.47 (s, 3H, CH_3-4), 2.74 (s, 3H, CH_3-5), 3.91 (s, 3H, CH_3 of ester), 7.29 (d, 2H, $\mathcal{J} = 5.33$ Hz aromatic), 7.36 (d, 2H, aromatic), 10.58 (s 1H, NH-2); MS: *m/z* 346 ($M^+ + 23$), 324 ($M^+ + 1$); Anal. calcd. for $C_{15}H_{14}ClNO_3S$: C, 55.64; H, 4.32; N, 4.32; Found: C, 55.87; H, 4.48; N, 4.31%.

Methyl 2-(4-bromoanilino)-5-acetyl-4-methylthiophene-3-carboxylate ***(6d)***. Yield: 35%; m.p.: 129°C; R_f: 0.75 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 1700 (C═O stretching of ester), 1645 (C═O stretching of ketone), 770, 658; ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm): 2.57 (s, 3H, CH_3-4), 2.61 (s, 3H, CH_3-5), 3.93 (s, 3H, CH_3 of ester), 7.07 (d, 2H, $\mathcal{J} = 4.80$ Hz aromatic), 7.51 (d, 2H, aromatic), 10.28 (s 1H, NH-2); MS: *m/z* 369 ($M^+ + 1$); Anal. calcd. for $C_{15}H_{14}BrNO_3S$: C, 48.92; H, 3.80; N, 3.80; Found: C, 48.73; H, 4.22; N, 3.68%.

Methyl 2-(2-furoylamino)-5-acetyl-4-methylthiophene-3-carboxylate ***(6e)***. Yield: 56%; m.p.: 238°C; R_f: 0.72 (7:3, toluene/acetonitrile); IR (KBr, cm^{-1}): 3251 (amide NH stretching), 1729 (C═O stretching of ester), 1600 (C═O stretching of ketone), 764, 670; ^{1}H-NMR: (300 MHz, $CDCl_3$) δ (ppm): 2.36 (s, 3H, CH_3-4), 2.72 (s, 3H, CH_3-5), 3.96 (s, 3H, CH_3 of ester), 6.34 (d, 1H, aromatic), 6.58 (t, 1H, aromatic), 7.35 (d, 1H, aromatic); MS: *m/z* 307 (M^+); Anal. calcd. for $C_{14}H_{13}NO_5S$: C, 54.72; H, 4.23; N, 5.55; Found: C, 54.59; H, 4.12; N, 4.32%.

Pharmacological screening

Animals. Albino rats (150–250 g) of either sex were provided with pellet diet (Lipton, India) and water *ad libitum* and kept under standard laboratory condition at 25 ± 2°C. The experimental protocol was approved by the Institutional Ethics Committee constituted by the Ministry of Social Justice and Empowerment, (Government of India).

Anti-inflammatory activity. We have used the method previously described by Winter *et al* [13]. The animals were studied for toxicity of DMSO up to 10% v/v in saline, and 5% DMSO was selected as a vehicle to suspend the standard drugs and the test compounds. Albino rats of either sex weighing between 150–250 g were starved for 18 h prior to the experiment. The animals were weighed, marked for identification and divided into groups of six. The standard drug ibuprofen (20 mg/kg body weight) and mefanamic acid (100 mg/kg body weight) and the test compounds were given orally (10, 20 and 40 mg/kg body weight) as a suspension using 5% DMSO as a vehicle. One hour later foot paw oedema was induced by injecting 0.1 mL of 1% carrageenin subcutaneously into the planter portion of the right hind paw of each rat. Initial foot paw volume was measured immediately by mercury plethysmometer. Oedema was measured three hours after carrageenin administration. The swelling in test group animals was used to calculate the percent inhibition ± SEM of oedema achieved by the compound at the test dose compared with the vehicle control group. The percentage protection of oedema was calculated according to the formula, % anti-inflammatory activity $= 100 \times (1 - Vt/Vc)$ where Vt and Vc are the volume of oedema in test compounds and control groups respectively.

Analgesic activity: Acetic acid induced writhing response model. All the targeted compounds were investigated for their analgesic activity in acetic acid induced writhing response in albino mice (20–25 g) at 10 mg/kg body weight dose following the method of Siegmund *et al*. [14]. 10 mg/kg of the selected compounds was administered intra-peritoneally to groups of mice (6 in each group) starved for 16 h. The first group received the test compounds while the groups which served as positive and negative controls received 10 mg/kg ibuprofen and 0.5 mL/100 g body weight of 1% DMSO solution respectively. One hour after treatment, the animals in each group received 0.1 mL of 3% acetic acid to induce the characteristic writhing response. The number of writhing occurring within 30 min was recorded and the mean was compared with that of the control and converted into % inhibition.

Antioxidant activity: Nitric oxide radical scavenging assay. Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which can be measured by Griess reagent [15]. The reaction mixture (3 mL) containing sodium nitroprusside (10 mmol) in phosphate buffered saline (PBS) and test compounds (***4a–4f***, ***5c***, ***5f***, ***6c*** and ***6e***) at different concentrations (5, 10, 15, 20,

25, 30, and 35 μg/mL) were incubated at 25°C for 150 minutes. Each 30 min, 0.5 mL of the incubated sample was removed. 0.5 mL of Griess reagent (1% sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H_3PO_4) was added to the 0.5 mL aliquot of the sample removed. The absorbance of the chromophore formed was measured at 546 nm. The experiment was performed (in triplicate) and % scavenging activity was calculated using the formula 100 − [100/blank absorbance × sample absorbance]. The activity was compared with ascorbic acid at concentration 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 μg/mL, which was used as a standard antioxidant.

Result and discussion

The synthesized compounds (***4a-4g***, ***5a-5f*** and ***6a-6e***) were screened by *in vivo* assay for their anti-inflammatory activity using carrageenin-induced rat hind paw oedema model at three graded doses employed at 10, 20 and 40 mg/kg body weight using mefanamic acid, ibuprofen and analgesic activity using acetic acid-induced writhing response in albino mice at dose of 10 mg/kg using ibuprofen and the results are shown in Table I. In order to arrive at possible mechanism of the anti-inflammatory activity of the compounds (***4a-4f***, ***5c***, ***5f***, ***6c*** and ***6e***) which gave more than 50% protection to the inflamed paw, were selected for investigating their *in vitro* antioxidant nitric oxide radical scavenging assay at the concentrations of 5, 10, 15, 20, 25, 30 and 35 μg/mL using standard drug ascorbic acid and the results are shown in Table II.

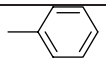
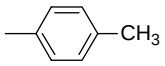
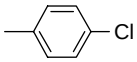
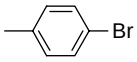
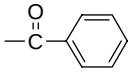
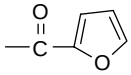
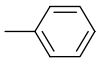
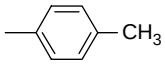
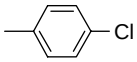
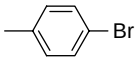
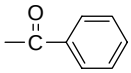
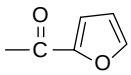
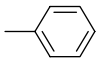
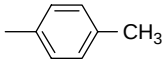
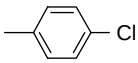
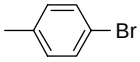
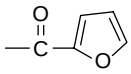
Taking into account the diverse biological activities coumarin derivatives namely anticoagulant and anti-inflammatory activities [16–18] the compounds ***4a-4d*** were synthesized keeping coumarin-3-yl constant at fifth position and introducing both electron releasing (—CH_3) and electron withdrawing groups (—Cl, Br) at fourth position in arylamino moiety at second position of thiophene nucleus. In order to examine the effect of introduction of carbonyl spacer attached to —NH group in the form of benzoyl and furoyl at the second position of thiophene moiety, keeping coumarin–**3**-yl constant substituted at fifth position of thiophene moiety ***4e*** and ***4f*** were synthesized. The compound ***4g*** was synthesized having ethyl group at second position to explore the effect of presence of aliphatic chain on inflammatory activity of profile of the candidate.

Among the compounds ***4a-4g***, ***4c*** showed maximum anti-inflammatory activity. It displayed 71% protection at 10 mg/kg and 77% protection at 20 mg/kg to inflamed paw, however % protection decreased to 67% at 40 mg/kg as compared to the reference drugs ibuprofen which showed 36% protection at 20 mg/kg and mefanamic acid which displayed 42% protection at 100 mg/kg. The compounds ***4a***, ***4b***, ***4d***, ***4e*** and ***4f*** showed % protection of 57%, 51%, 59%, 64%, 68% at 10 mg/kg and 70%, 65%, 67%, 71%, 76% at 20 mg/kg to inflamed paw which were comparable to anti-inflammatory activity of both ibuprofen and mefanamic acid. The compounds ***4a***, ***4b***, ***4d***, ***4e*** and ***4f*** also showed decrease in % protection to inflamed paw at 40 mg/kg dose which was similar pattern as observed for ***4c*** the most potent candidate among ***4a-4g***. The compound ***4g*** showed poorer anti-inflammatory activity at all the three graded doses employed. On the basis of structure-activity relationship studies of ***4a-4g*** it can be concluded that in this series of compounds the presence of —Cl group in anilino moiety at second position of the thiophene nucleus contribute in enhancing the anti-inflammatory activity profile of the candidate (***4c***). The presence of —CH_3 in anilino moiety at second position of the thiophene nucleus seems to reduce the inflammatory activity profile of the candidate (***4b***). Also the presence of benzoyl (***4e***) and 2-furoyl moiety (***4f***) attached to —NH at the second position of the thiophene also contributes significantly to anti-inflammatory activity profile of the candidates.

There are several reports in literature which indicate that incorporation of more than one minimum structural feature essential for activity in a single molecule can lead to significant enhancement in the activity profile of the targeted compounds [19,20]. The compounds ***5a-5f*** were synthesized incorporating two minimum structural features (as in fig I) essential for the anti-inflammatory activity on the basis of structure-activity relationship studies of tetrasubstituted thiophenes and their pharmacological profile reported in our earlier work [8–11] in targeted compounds in the assumption that an additional =CO in (***5a-5f***) will perhaps provides one more hydrogen bond acceptor feature to facilitates better binding and superior signal transduction on the macromolecule target or targets.

In case of ***5a-5f***, the difference of anti-inflammatory activity of the compounds was observed too small at the three-graded doses employed at 10, 20 and 40 mg/kg. The compounds ***5c***, ***5e*** and ***5f*** showed % protection of 62%, 44% and 61% respectively at 10 mg/kg dose and nearly similar % protection at both 20 and 40 mg/kg dose. The compounds ***5a***, ***5b*** and ***5d*** showed poorer anti-inflammatory activity at all the employed three-graded dose. On the basis of structure-activity relationship studies of ***5a-5f*** it is evident that in their cases also the presence of —Cl group in anilino moiety at second position of the thiophene nucleus contribute in enhancing the anti-inflammatory activity profile of the candidate. The presence of 2-furoyl moiety attached to —NH at the second position of the thiophene contribute to

Table I. Chemical structures, anti-inflammatory and analgesic activity of tetrasubstituted thiophenes.

		Anti-inflammatory activity* Carrageenin-induced rat hind paw oedema % protection			Analgesic activity** Acetic acid induced writhing test % inhibition
Compound no.	R	10 mg/kg	20 mg/kg	40 mg/kg	10 mg/kg
4a		57	70	55	40
4b		51	65	37	20
4c		71	77	67	56
4d		59	67	41	12
4e		64	71	49	38
4f		68	76	56	55
4g	—CH_2CH_3	48	34	24	32
5a		37	38	39	14
5b		36	33	37	16
5c		62	60	61	10
5d		38	42	44	17
5e		44	41	42	08
5f		61	64	64	18
6a		31	28	19	15
6b		49	47	32	23
6c		51	39	28	38
6d		34	30	31	19
6e		58	43	31	32

* Oral administration for all test compounds, $P < 0.05$, Student's t-test versus controls, the standard drugs, (dose and % protection) were: ibuprofen (20 mg/kg, 36%) and mefanamic acid (100 mg/kg, 42%).

** Intra-peritoneal administration for all test compounds, $P < 0.05$, Student's t-test versus controls, the standard drug, (dose and % inhibition) was: ibuprofen (10 mg/kg, 62%).

anti-inflammatory activity profile of the candidate in same scale.

The compounds ***6a-6d*** were synthesized in which keeping acetyl at fifth position, substituents at R_1 in anilino moiety were modified by using both electron releasing and electron withdrawing groups. The compound ***6e*** was synthesized in which keeping acetyl at fifth position, 2-furoylamino moiety were introduced at second position which has a proton acceptor (—C═O) and a proton donor (—NH) features in adjacent position. In case of ***6a-6e*** compounds ***6c, 6e*** and ***6b*** showed comparable anti-inflammatory activity of 51%, 58% and 49% protection at 10 mg/kg dose. The anti-inflammatory activity of ***6a-6e*** compounds significantly decreases at 20 and 40 mg/kg doses. For ***6a-6e*** our attempt to correlate biological result with variation of substituents attached to —NH at second position of thiophene was unsuccessful.

Table II. Antioxidant activity of tetrasubstituted thiophenes.

Compound no.	% Scavenging (Mean ± SEM) of triplicates							[‡]IC_{50} μg/mL	r**
	5 μg/mL	10 μg/mL	15 μg/mL	20 μg/mL	25 μg/mL	30 μg/mL	35 μg/mL		
4a	03.25 ± 0.001*	08.40 ± 0.002*	18.24 ± 0.002*	26.27 ± 0.001*	30.41 ± 0.001*	33.17 ± 0.003*	34.01 ± 0.001*	na	0.93
4b	12.25 ± 0.003*	15.05 ± 0.002*	20.05 ± 0.001*	27.25 ± 0.001*	32.41 ± 0.003*	37.04 ± 0.001*	41.24 ± 0.003*	na	0.99
4c	08.62 ± 0.002*	14.57 ± 0.003*	25.24 ± 0.003*	39.51 ± 0.001*	44.23 ± 0.002*	45.54 ± 0.002*	51.25 ± 0.001*	31.59	0.94
4d	–	–	–	–	–	–	–	–	–
4e	03.25 ± 0.002*	06.95 ± 0.003*	10.21 ± 0.003*	17.31 ± 0.001*	20.23 ± 0.002*	27.26 ± 0.002*	34.25 ± 0.001*	na	0.98
4f	05.24 ± 0.001*	10.27 ± 0.003*	19.23 ± 0.001*	26.29 ± 0.002*	38.89 ± 0.001*	49.14 ± 0.001*	58.25 ± 0.003*	31.12	0.98
5c	18.27 ± 0.002*	25.24 ± 0.003*	30.51 ± 0.003*	34.21 ± 0.001*	38.83 ± 0.002*	40.63 ± 0.002*	44.84 ± 0.001*	na	0.97
5f	10.42 ± 0.001*	14.54 ± 0.003*	22.20 ± 0.001*	28.40 ± 0.002*	36.64 ± 0.001*	46.85 ± 0.001*	50.21 ± 0.003*	34.18	0.98
6c	–	–	–	–	–	–	–	–	–
6e	02.32 ± 0.002*	04.47 ± 0.003*	05.98 ± 0.003*	10.31 ± 0.001*	14.25 ± 0.002*	16.20 ± 0.002*	20.54 ± 0.001*	na	0.98
[†]**Ascorbic acid**	06.25 ± 0.002*	18.43 ± 0.001*	27.88 ± 0.001*	36.21 ± 0.003*	46.27 ± 0.002*	53.17 ± 0.002*	67.21 ± 0.001*	00.88	0.98

*$P < 0.001$ compared to reagent blank. ** Regression analysis, [‡]$IC_{50} = 50\%$ Inhibitory concentration, na = $IC_{50} > 35$ μg/mL, — showed no scavenging activity.
[†] Ascorbic acid tested at 0.1 μg/mL, 0.2 μg/mL, 0.4 μg/mL, 0.6 μg/mL, 0.8 μg/mL, 1.0 μg/mL, 1.2 μg/mL.

Among the ***4a***, ***5a*** and ***6a*** the substituents attached to carbonyl group at the fifth position of thiophene nucleus are coumarin-**3**-yl, tetra substituted thiophene and methyl respectively. Similar is the case with ***4b***, ***5b*** and ***6b***; ***4c***, ***5c*** and ***6c*** and ***4f***, ***5f*** and ***6e***. On the basis of variation of substituents at fifth position of thiophene nucleus keeping other substituents (at second, third and fourth) constant; we attempted to find out structure-activity relationship of trio like ***4a***, ***5a*** and ***6a*** and similar compound groups. On the basis of these structure-activity relationship studies of trio ***4a***, ***5a***, ***6a*** and similar groups it was found that the presence of coumarin-**3**-yl (***4a***) significantly contribute to anti-inflammatory activity of the candidate as compare to both tetrasubstituted thiophene (***5a***) derivatives and methyl (***6a***) group attached to carbonyl function at fifth position of thiophene.

All the synthesized compounds were evaluated for their analgesic activity by *in vivo* assay using acetic acid induced writhing response test in albino mice at 10 mg/kg dose. Among ***4a-4g*** only ***4c*** and ***4f*** showed comparable analgesic activity of 56% and 55% inhibition as compared to reference drug ibuprofen which displayed 62% inhibition at 10 mg/kg dose. The compounds ***4a*** and ***4e*** showed moderate analgesic activity of 40% and 38% inhibition at 10 mg/kg dose. Among ***5a-5f***, all the compounds displayed very poor analgesic activity. Among ***6a-6e***, only ***6c*** and ***6e*** showed moderate analgesic activity of 38% and 32% inhibition. The results of analgesic activity of compounds ***4a-4g*** showed that presence of 4-chlorophenyl and 2-furoyl attached to —NH at second position of thiophenes and coumarin-**3**-yl attached to —CO group at fifth position of thiophene ring contribute significantly to analgesic activity profile of the candidates ***4c*** and ***4f***. In case of ***6a-6e***, for ***6c*** and ***6e*** also the presence of 4-chlorophenyl and 2-furoyl attached to —NH at second position of thiophenes contributes moderately to analgesic activity profile of the candidates ***6c*** and ***6e***.

Compounds (***4a-4f***, ***5c***, ***5f***, ***6c*** and ***6e***) which gave more than 50% protection to the inflamed paw were selected for investigating their *in vitro* antioxidant nitric oxide radical scavenging assay at the concentrations of 5, 10, 15, 20, 25, 30 and 35 μg/mL using standard drug ascorbic acid. Among these compounds ***4c*** showed maximum *in vitro* nitric oxide radical scavenging activity having IC_{50} value 31.59 μg/mL. The compounds ***4f*** and ***5f*** showed IC_{50} value of 31.12 and 34.18 μg/mL respectively. The compounds ***4a***, ***4b***, ***4e*** and ***5c*** showed *in vitro* nitric oxide radical scavenging activity of 34.01 ± 0.001, 41.24 ± 0.003, 34.25 ± 0.001 and 44.84 ± 0.001 respectively at 35 μg/mL. The compound ***6e*** was found to have poor *in vitro* antioxidant nitric oxide radical scavenging activity whereas ***4d*** and ***6c*** have showed no scavenging activity. The best candidate among whole series was *4c* however it was found to have poor *in vitro*

antioxidant nitric oxide radical scavenging activity as compared to standard drug ascorbic acid which showed IC_{50} value 0.88 μg/mL at concentration 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 μg/mL. From the IC_{50} values of ***4a-4f***, ***5c***, ***5f***, ***6c*** and ***6e***, it can be concluded that the presence of 2-furoyl attached to —NH at second position of thiophene contribute moderately to antioxidant nitric oxide radical scavenging activity profile of the candidates ***4f*** and ***5f***. When 4-chlorophenyl is attached to —NH at second position of thiophene and coumarin-**3**-yl attached to —CO group at fifth position of thiophene ring ***4c*** showed maximum *in vitro* nitric oxide radical scavenging activity having IC_{50} value 31.59 μg/mL; however when 4-chlorophenyl is attached to —NH at position 2 of thiophene and methyl group is attached to —CO group at position 5 of thiophene ring ***6c*** showed no scavenging activity. This result shows that the presence of coumarine-**3**-yl attached to —CO group at fifth position of thiophene ring contribute in enhancing *in vitro* nitric oxide radical scavenging activity of ***4c*** and in ***4a***, ***4b*** and ***4e***.

Conclusion

The synthesized targeted compounds (***4a-4g***, ***5a-5f*** and ***6a-6e***) were evaluated for their *in vivo* anti-inflammatory, analgesic activities and *in vitro* anti-oxidant activity. On the basis of structure-activity relationship studies of ***4a-4g*** it can be concluded that presence of —Cl group in anilino moiety (***4c***) and benzoyl (***4e***), 2-furoyl moiety (***4f***) attached to —NH at the second position of the thiophene contributes significantly to anti-inflammatory and analgesic activity profile of the candidates. In case of structure-activity relationship of ***5a-5f*** it is evident that in these cases the presence of —Cl group in anilino moiety and 2-furoyl moiety attached to —NH at the second position of the thiophene contribute to anti-inflammatory activity profile of the candidate in same scale. For ***6a-6e*** our attempt to correlate our biological result with variation of substituents attached to —NH at second position of thiophene was unsuccessful. On the basis of these structure-activity relationship studies of trio ***4a***, ***5a***, ***6a*** and similar groups it was found that the presence of coumarin-**3**-yl (***4a***) significantly contribute to anti-inflammatory activity of the candidate as compare to both tetrasubstituted thiophene (***5a***) derivatives and methyl (***6a***) group attached to carbonyl function at fifth position of thiophene. Among the compounds (***4a-4f***, ***5c***, ***5f***, ***6c*** and ***6e***) only ***4c***, ***4f*** and ***5f*** showed IC_{50} value of 31.59 μg/mL, 31.12 and 34.18 μg/mL respectively suggesting that the mechanism of anti-inflammatory activity of potent candidates could be mediated through inhibition of nitric oxide burst in inflammatory situation. Further studies are needed to explore the efficacy and safety of the most potent candidate ***4c***.

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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME 3-(4-(SUBSTITUTED) 2-MORPHOLINO-4-YL-4-PHENYL-THIAZOLE-5-CARBONYL)-1-BENZOPYRAN-2-ONE

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ABSTRACT

Taking in to consideration of diverse biological activities of coumarin and thiazole derivatives, attempts have been made to attach a thiazole side chain at C-3 position of coumarin. In addition, to expand the structural diversity of synthetic courmarins for biological functions, the morpholine fusion at 2nd position of thiazole was also attempted. The compounds MM3M1 was synthesized keeping coumarin-3-yl constant at fifth position, morpholine at 2nd position and phenyl moiety at 4th position of thiazole nucleus. The compounds MM3M2, MM3M3 and MM3M4 were synthesized keeping coumarin-3-yl constant at fifth position, morpholine at 2nd position and introducing electron withdrawing group (-Cl) at ortho, meta and para position respectively in phenyl moiety at 4th position of thiazole nucleus. All the synthesized compounds were evaluated for their invitro antibacterial and anti-platelet activity. On the basis of structure-activity relationship studies of synthesized MM3M1- MM3M4 it can be concluded that presence of 2-chlorophenyl group at the 4th position of the thiazole contributes significantly to antibacterial and anti-platelet activity profile of the candidates.

Keywords: Coumarin, thiazole, antibacterial activity, anti-platelet activity.

INTRODUCTION

Some 4-(3-coumarinyl)-3-benzyl-4-thiazoline-2-one compounds have reported to possess anti-inflammatoy activity [1]. Substitution at 3rd position of coumarin nucleus modulated AChE as well as MAO-B inhibitory activity. Substitution with methyl groups at 3rd and 4th position led to more active compounds toward both AChE and MOA-B enzymes [2].

Pillai A. [3] and Molvi K.I.[4] has reported novel tetrasubstiuted thiophene as dual inhibitor enzymes in inflammation pathway (COX and LOX). Similarly novel substituted thiazole derivatives also reported by Franclin [5]. Substituted coumarin derivatives

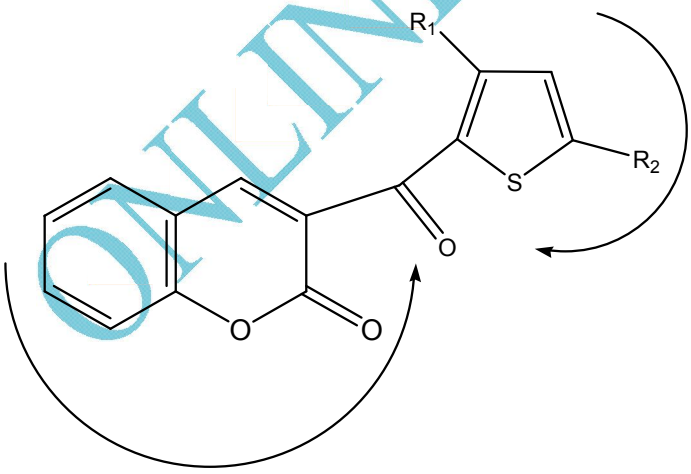
inhibiting more than one pathway was found to be effective as an anti-inflammatory chemical entity is also reported by above workers.

The number of antibacterial compounds available in market are belongs to penicillin derivatives and sulpha drugs, which belongs to sulphur containing heterocylcil ring (thiazole and thiazolidine).

Multi-drug treatment of inflammatory conditions associated with microbial infections poses a unique problem especially for patients with impaired liver or kidney functions. Husain A and coworker has reported anti-inflammatory and analgesic compound with antimicrobial activity [6], [7], [8].

Therefore from the pharmacoeconomic and patient compliance points of view, the mono therapy with a drug having anti-inflammatory and antimicrobial activities is highly desirable [9].

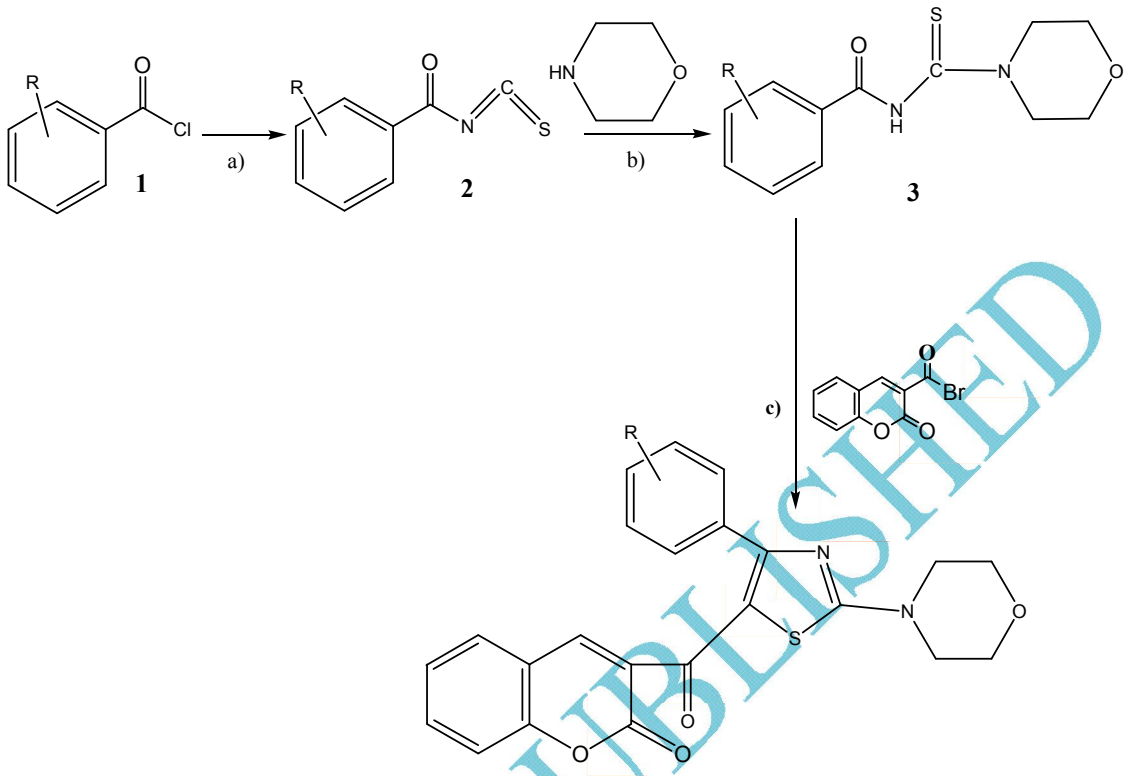
In view of above reports new alternative and more effective coumarin and five membered sulphur containing heterocyclic compounds (thaizole) having anti-platelet and antibacterial activity has been designed (scheme 1), considering the 3-substituted coumarin which is liked at 5th position to thiazole. In anticipation that coumarin moiety will be acting as anti-platelet and five membered sulphur containing heterocyclic compounds (thaizole) acting as antibacterial. (Figure 1).



five membered heterocyclic sulphur compounds (thaizole) (Moiety A) as antibacterial

coumarin moiety B acting as anti-platelet

Figure 1



SCHEME 1: SYNTHESIS OF 3-(4-(SUBSTITUTED)2-MORPHOLINO-4-YL-4-PHENYL- THIAZOLE-5-CARBONYL)-1-BENZOPYRAN-2-ONE

Reagents and conditions: a) NH4SCN, Acetone, Reflux 25 min; b) reflux for 15 min, pour reaction mixture to crushed ice; c) dimethylformamide, stirr at 70°C to 80°C for 2 hour, pour to crushed ice.

MATERIALS AND METHODS

Chemistry

All reagents and solvents were used as obtained from the supplier or recrystallized/redistilled as necessary. 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. Melting points were recorded on open capillary melting point apparatus and are uncorrected. 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 advance at 400 MHz using Tetramethyl silane (TMS) as internal standard and the chemical shift (δ) are reported in

parts per million,. Gram-positive microorganism (*S.Aureus, B.Subtilis*) and Gram-negative microorganism (*E. Coli*) were cultured at laboratory scale.

General Procedure for Synthesis of 3-(4-(substituted) 2-Morpholino-4-yl-4-Phenyl-Thiazole-5-Carbonyl)-1-Benzopyran-2-One :

As shown in Scheme 1, isothiocynate (2) was obtained by reacting ammonium thiocyanate (0.1379 mole) in 100 ml acetone at room temperature, with benzoyl chloride/ substituted benzoyl chloride (0.1263 mole) in 5 minutes. The reaction mixture was refluxed for 15 minutes. Substituted N-(morpholine-4-thioyl) benzamide (3) was synthesized by nucleophilic addition of benzoyl isothiocyanate /substituted benzoyl isothiocyanate and morpholine (0.1149 mole) at reflux temperature .The compounds MM3M1-MM3M4 were synthesized by adding 3-bromoacetylcoumarin (0.0025 mmol) to a solution of the adduct (0.0025 mmol) (3) in N,N-dimethylformamide (5 ml). The reaction mixture was warmed on a water bath at 80-85 ^{o}C for 5 min. To this, triethylamine (0.0025 mmol, 0.3 ml) was added and heating was continued for another 15 min. The above mixture was cooled and poured into ice-cold water with stirring [10]. A yellow precipitate thus obtained was filtered, wash with water and air-dried, purified by preparative TLC (Hexane : Ethyl acetate 3:7) corresponding to the (MM3M1-MM3M4) characterized as per the analytical data

3-(2-Morpholin-4-yl-4-phenyl-thiazole-5-carbonyl)-chromen-2-one **(MM3M1)**. Yield: 85%; M.P.: 166-168^{0}C; TLC: Rf-0.79 (3:7,Haxane :Ethyl acetate); ^{1}H NMR: *(DMSO, δ, ppm)* = 3.60-3.62 (t, 4H, 3rd and 5th morpholin CH2 at 2nd position of thiazole ring), 3.75-3.78 2nd and 6th morpholin CH2 at 2nd position of thiazole ring), 6.91-7.5 (m, 9H, (5H of aromatic protons of 4th position and 4H of coumarin ring), 7.6 (s, 1H, aromatic proton); IR *(KBr, cm^{-1})*: 3100-3000(-CH2 stretching), 1735 (strong band of -C=O stretching), 1458-1539 (C=C stretching, aromatic), 1374(C-N) 1245, (C-C[=O]-O symmetric stretching);MASS: 419 ($M^{+\cdot}$)

3-(4-(2-Chloro-phenyl)-(2-Morpholin—4-phenyl-thiazole-5-carbonyl)-chromen-2-one **(MM3M2)**. Yield: 87%; M.P.: 212-214^{0}C; TLC: Rf-0.60 (9:1,: Toluene : Methanol);^{1}H NMR: *(DMSO, δ, ppm)* = 3.64-3.68 (t, 4H, 3rd and 5th morpholin CH2 at 2nd position of thiazole ring,J=5Hz),3.8117-3.862 2nd and 6th morpholin CH2 at 2nd position of thiazole ring,J=5Hz), 6.96-7.56 (m, 8H, (4H of aromatic protons of 4th position and 4H of

coumarin ring), 7.72 (s, 1H, aromatic proton);IR *(KBr, cm^{-1})*: 3100-3000(medium band indicate CH2 stretching), 1728 (strong band of -C=O stretching), 1450-1530 (C=C stretching, aromatic), 1374(C-N) 1245, (C-C[=O]-O symmetric stretching), 788.89 (Ar-Cl stretching);MASS m/z: 453 ($M^{+.}$), 454(M^{+1}), 455(M^{+2}).

3-(4-(3-Chloro-phenyl)-(2-Morpholin—4-phenyl-thiazole-5-carbonyl)-chromen-2-one **(MM3M3).** Yield: 80%; M.P.: 182-184^{0}C; TLC: Rf-0.65 (9:1,: Toluene : Methanol);^{1}H NMR: *(DMSO, δ, ppm)* = 3.60-3.63 (t, 4H, 3rd and 5th morpholin CH2 at 2nd position of thiazole ring),3.75-3.77 2nd and 6th morpholin CH2 at 2nd position of thiazole ring), 6.92-7.51 (m, 8H, (4H of aromatic protons of 4th position and 4H of coumarin ring), 7.68 (s, 1H, aromatic proton);IR *(KBr, cm^{-1})*: 3100-3000 (medium band indicate CH2 stretching), 1730 (strong band of -C=O stretching), 1448-1533 (C=C stretching, aromatic), 1368 (C-N) 1246 (C-C[=O]-O symmetric stretching), 794 (Ar-Cl stretching);MASS m/z: 453 ($M^{+.}$), 454(M^{+1}), 455(M^{+2}).

3-(4-(4-Chloro-phenyl)-(2-Morpholin—4-phenyl-thiazole-5-carbonyl)-chromen-2-one **(MM3M4).** Yield: 78 %; M.P.: 196-198^{0}C; TLC: Rf-0.64 (9:1,: Toluene : Methanol); ^{1}H NMR: *(DMSO, δ, ppm)* = 3.61-3.64 (t, 4H, 3rd and 5th morpholin CH2 at 2nd position of thiazole ring),3.83-3.86 2nd and 6th morpholin CH2 at 2nd position of thiazole ring), 7.23-7.63 (m, 8H, (4H of aromatic protons of 4th position and 4H of coumarin ring), 7.65 (s, 1H, aromatic proton);IR *(KBr, cm^{-1})*: 3100-3000(medium band indicate CH2 stretching), 1723 (strong band of -C=O stretching), 1448-1533 (C=C stretching, aromatic), 1369 (C-N) 1246 (C-C[=O]-O symmetric stretching), 764 (Ar-Cl stretching);MASS m/z: 453 ($M^{+.}$), 454(M^{+1}), 455(M^{+2}).

Pharmacological Screening:

Antibacterial activity:

The newly synthesized compounds were screened for their antibacterial screening using agar well diffusion method [11]. The antibacterial activity of the test compounds was evaluated against two Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis* and Gram-negative bacteria *Escherichia coli* Ciprofloxacin was used as standard drug. Dimethylsulfoxide was used as solvent control. The microorganism was activated by inoculation a loopful of the strain in the nutrient broth (25ml) and incubating at room temperature in a rotary shaker. The test organism (0.2 ml; 10^8 cells/ml as per McFarland

standard) was then inoculated into the molten Mueller Hinton agar media. After proper homogenization it was poured into sterile 100 mm petri dishes (Hi-media) and allowed to solidify. A well was made in the seeded plates with the help of a sterile cork borer (8.5 mm). the test solution (0.05 ml) in dimethylsulfoxide (100 µg/ml) was introduced into the well and all the plates were incubated at 37^0C for 24 hours. The experiment was performed in triplicate under aseptic conditions. The control was also mainatained with 0.05 ml of DMSO under similar conditions and the zone of inhibition of the bacteral growth were measured and recorded. Priliminary screening was conducted for all compounds at 100 µg/mL concentration, against the above-mentioned microorganisms. Different series of dilutions of compounds were made (1.56 to 75 µg/mL) to determine the MIC.

Anti-platelet activity:

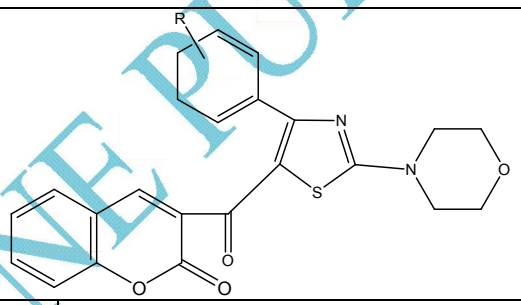
ADP-induced platelet aggregation of platelet-rich plasma (PRP), quantitated using optical density filter at 405 nm as a measurement point in kinetic mode. To obtain PRP, a citrated tube of blood was inverted 3 to 5 times for gentle mixing and centrifuged at room temperature for 10 minutes at 200g. After centrifugation, the upper turbid layer of PRP was removed, and the residual blood was centrifuged for 5 minutes at 2000 g to obtain platelet-poor plasma (PPP). The PPP was used as the baseline optical density for platelet aggregation. A total of 180 µL of PRP containing about 3 x 10^8 platelets/ml was incubated at 37°C in the 96 well plate for 3-5 minutes, Then 10 µL of test compounds were added in PRP containing wells and incubated for the period of 15 minutes with intermittent shaking. ADP (10 µL) at a final concentration of 10,20 and 40 µmol/L was added in above wells with intermittent shaking mode. Optical density readings were measured at every 1-minute with intermittent shaking up to 5 minutes. Platelet aggregation was expressed as the change in optical density at 5 minutes, compared with PPP as a reference and converted to % aggregation [12]. Aspirin was used as a positive control.

RESULTS AND DISCUSSION:

Taking in to consideration of diverse biological activities of coumarin derivatives as antipletelet and antibacterial [13]and The chemistry and pharmacology of thiazole derivatives has been of great interest to medicinal chemists lately [14]. Attempts have also

been made to attach a thiazole side chain at C-3 position of coumarin. In addition, to expand the structural diversity of synthetic courmarins for biological functions, the morpholine fusion at 2nd position of thiazole was also attempted. The compounds MM3M1 was synthesized keeping coumarin-3-yl constant at fifth position, morpholine at 2nd position and phenyl moiety at 4th position of thiazole nucleus. The compounds MM3M2, MM3M3 and MM3M4 were synthesized keeping coumarin-3-yl constant at fifth position, morpholine at 2nd position and introducing electron withdrawing group (-Cl) at ortho, meta and para position respectively in phenyl moiety at 4th position of thiazole nucleus. The chemical structure, anti-bacterial and anti-platelet activity data are given in Table 1.

TABLE 1: CHEMICAL STRUCTURE, ANTI-BACTERIAL AND ANTI-PLATELET ACTIVITY DATA OF SYNTHESIZED 3-(4-(SUBSTITUTED)2-MORPHOLINO-4-YL-4-PHENYL-THIAZOLE-5-CARBONYL)-1-BENZOPYRAN-2-ONE



Compound Code	R	Anti-bacterial activity MIC in µg/ml (zone of inhibition in mm)			Platelet aggregation inhibition (%)
		Gm –ve Becteria	Gm +ve Bacteria		
		Escherichia coli	*Staphylococcus Aureus*	*Bacillus Subtilis*	
MM3M1	-H	6.25(18)	12.5(13)	12.5(13)	34.85
MM3M2	2-Chloro	1.56 (18)	1.56 (18)	1.56 (18)	76.02
MM3M3	3-Chloro	12.5(13)	12.5(13)	12.5(13)	7.25
MM3M4	4-Chloro	25(08)	12.5(13)	12.5(13)	4.34
Ciprofloxacin		6.25(18)	6.25(18)	5(21)	-
Aspirin		-	-	-	90.00

Note: the MIC values were evaluated at concentration range 1.56 to 25 µg/ml. The values in the table show the MIC values and the corresponding zone of inhibition (in mm).

CHART 1: ANTIBACTERIAL ACTIVITY OF 3-(4-(SUBSTITUTED)2-MORPHOLINO-4-YL-4-PHENYL-THIAZOLE-5-CARBONYL)-1-BENZOPYRAN-2-ONE

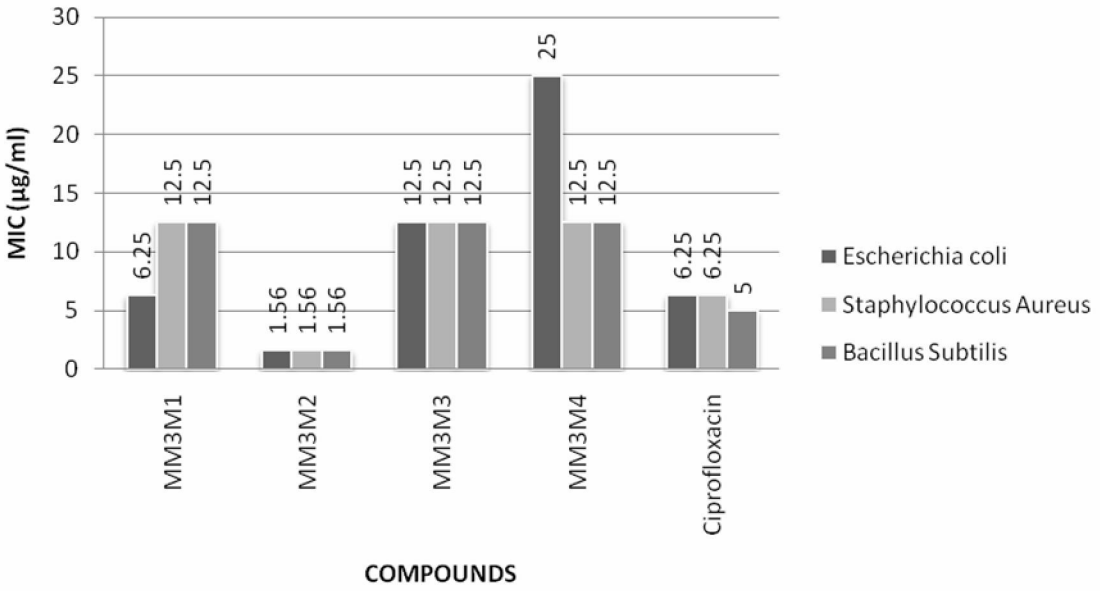


CHART 2: ANTI-PLATELET ACTIVITY OF 3-(4-(SUBSTITUTED)2-MORPHOLINO-4-YL-4-PHENYL-THIAZOLE-5-CARBONYL)-1-BENZOPYRAN-2-ONE

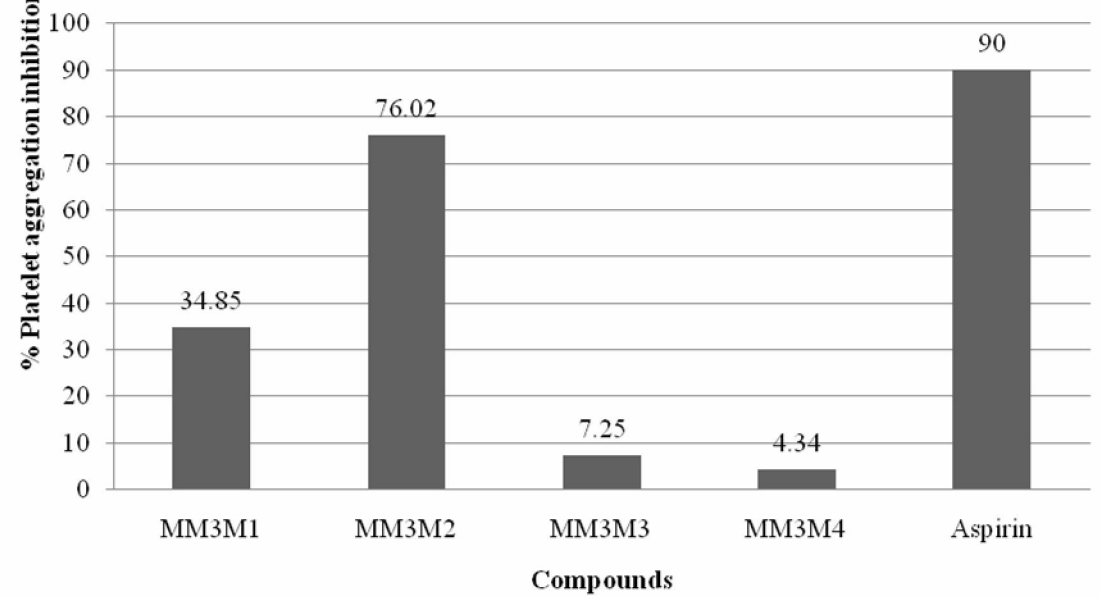


TABLE 2: CHEMICAL PROPERTIES OF SYNTHESISED 3-(4-(SUBSTITUTED)2-MORPHOLINO-4-YL-4-PHENYL-THIAZOLE-5-CARBONYL)-1-BENZOPYRAN-2-ONE

Compound Code	R	M.P. ^{0}C	Yield (%)	MW	Mol. Formula
MM3M1	-H	166-168	85	419	$C_{23}H_{18}N_2O_4S$
MM3M2	2-Chloro	212-214	87	453	$C_{23}H_{17}ClN_2O_4S$
MM3M3	3-Chloro	156-158	80	453	$C_{23}H_{17}ClN_2O_4S$
MM3M4	4-Chloro	110-112	78	453	$C_{23}H_{17}ClN_2O_4S$

The investigation of antibacterial screening revealed that the tested compounds showed moderate to good bacterial inhibition. Compounds MM3M1,MM3M3, and MM3M4 shows moderate activity against Gm^+ve microorganism i.e. *Staphylococcus aureus* and Bacillus subtillis, however compounds MM3M1 and MM3M2 are highly active against *Escherichia coli*- when compared to Ciprofloxacin. Compound MM3M2 has exhibited very good activity against all the bacterial strains.

All the synthesized compounds were evaluated for anti-platelet activity. The compounds MM3M1, MM3M2, MM3M3, MM3M4 shows 34.85% , 76.02%, 7.25% and 4.35 % platelet aggregation inhibition respectively. Aspirin shows 90 % platelet aggregation inhibition. Compound MM3M3 and MM3M4 shows poor antiplatelet activity, while compound MM3M1 shows moderate anti-platelet activity. Compound MM3M2 exhibited very good anti-platelet activity when compared with Aspirin.

CONCLUSION

The synthesized targeted compounds (MM3M1- MM3M4) were evaluated for their in vitro antibacterial and anti-platelet activities. On the basis of structure-activity relationship studies of MM3M1- MM3M4 it can be concluded that presence of 2-chlorophenyl group at the 4th position of the thiazole contributes significantly to antibacterial and anti-platelet activity profile of the candidates.

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