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Synthesis, characterization and antimicrobial activity of 2,3-disubstituted thiazolidin-4-one derivatives

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ABSTRACT

In the present investigation an attempt has been made to develop efficient, rapid, three-component, one-pot synthesis of 2,3-disubstituted thiazolidin-4-one derivatives under different reaction conditions like reflux, stirring and microwave irradiation approach. All the synthesized compounds were characterized by melting point, elemental analysis, IR, ^{1}H NMR and Mass spectroscopy. Further, all the synthesized compounds were screened in vitro for their antimicrobial activities in terms of zone of inhibition and minimum inhibitory concentration.

Keywords: Thiazolidin-4-one, one-pot synthesis, DCC, microwave irradiation, antimicrobial activity

INTRODUCTION

Small heterocyclic compounds have attracted organic and medicinal chemists over the years as they have been reported to possess a wide range of structural and therapeutic diversity. There has been significant interest in thiazolidin-4-one derivatives, a pharmacophore in various synthetic compounds, which exhibits diversified biological activities [1] such as antibacterial [2], antifungal [2], antimicrobial [3-4], antimycobacterial [5,6], antitumor [7], anti-histaminic [8], anticonvulsant [9], antihyperglycemic [10], anti-oxidant [11], anti-inflammatory [12] and analgesic [12] activities. Thiazolidin-4-ones derivatives also reported as effective inhibitors of COX-1 enzyme [13], bacterial enzyme MurB [14] and non-nucleoside inhibitors of HIV-RT [15].

Various protocols are reported for the synthesis of thiazolidin-4-ones [1]. Basically it involves three component reactions of an amine, a carbonyl compound and a mercapto acid. These methods can be either a two-step synthesis or one-pot three-component condensation reaction. The reaction mechanism involves the formation of imine followed by addition of sulphur nucleophile to the imine carbon and finally attack of the nitrogen on the carboxylic moiety with the elimination of water affords cyclic thiazolidin-4-one derivatives. The final step is rate-determining step. If this could be improved, higher yields could be obtained in shorter reaction time. Therefore, many different protocols have been reported for the continuous removal of water during the cyclization [11,16-18]. However, these methods have certain drawbacks like prolonged heating with continuous removal of water (use of Dean Stark apparatus), reaction should be performed in sealed vessels in the presence of a desiccant like anhydrous $ZnCl_2$ or sodium sulphate or trimethylorthoformate or molecular sieves.

Initially introduced in 1986 [19], microwave-assisted reactions have become a well-known method for the efficient and rapid synthesis of new chemical entities (NCEs). The advantages of microwave-assisted synthesis include higher yields, shorter reaction times and easy work up procedure. Few reports also indicate the use of microwave in the synthesis of thiazolidin-4-one. [20,21] However, this method requires separate preparation of a hydrazine, high power microwave irradiation and in some cases produces lower yield.

Therefore, simple and efficient method for rapid synthesis of thiazolidine-4-ones would be greatly advantageous. So herewith, we are reporting the multi-component one-pot synthesis of thiazolidin-4-one in three different reaction conditions as possible antimicrobial agent.

EXPERIMENTAL SECTION

Chemicals and reagents

All the commercially available chemicals and solvents used for the syntheses were of LR grade (Loba, India) and used without further purification.

Physical measurements

Melting points of all the newly synthesized compounds were determined in open capillaries and are uncorrected. IR spectra were recorded in KBr on FT-IR 8400S Shimadzu spectrophotometer. ^{1}H NMR spectra were obtained in $CDCl_3$ on Bruker advance II at 300 MHz and chemical shifts are measured as ppm downfield from Tetramethylsilane (TMS) used as a reference standard. Mass spectra were recorded on Shimadzu LCMS 2010EV mass spectrometer. The reaction progress and purity of compounds was checked by TLC using aluminum plates precoated with silica gel 60G F_{254}, as adsorbent and visualization was accomplished under UV light. The solvent systems used were benzene:ethylacetate (7:3, v/v). All the compounds were subjected to elemental analysis (CHN) and the measured values was found within ±0.4 % with the calculated values.

General procedure for synthesis of thiazolidin-4-one derivatives:

Reflux method

A mixture of appropriate amine (0.01 M), aldehyde (0.02 M) and tetrahydrofuran (10 mL) was taken in a round bottom flask followed by addition of thioglycolic acid (0.03 M) and dicyclohexyl carbodimide (0.012 M) with constant stirring. The mixture was then refluxed for 1 h on a boiling water bath. The precipitated dicyclohexylurea (DCU) was removed by filtration and filtrate was concentrated under reduced pressure. The residue thus obtained was suspended in water and extracted with dichloromethane. The organic layer then washed with saturated sodium bicarbonate solution and dried over anhydrous sodium sulfate. The crude solid obtained on evaporation of the solvent was recrystallized from dimethyl formamide to give desired compounds in moderate yield.

Stirring method

A mixture of appropriate amine (10 mM), aldehyde (20 mM) and tetrahydrofuran (10 mL) was taken in a 100 mL flat bottom flask and stirred vigorously at 0-5º C for 5 minutes, followed by drop wise addition of thioglycolic acid (30 mM). After 5 min dicyclohexyl carbodimide (12 mM) was added to the reaction mixture maintaining the temperature 0° C. Stirring was continued for an additional 45 minutes at room temperature. Using the above workup procedure, pure products were isolated in good yield.

Microwave assisted method

A mixture of various substituted amines (1 mM) and substituted aromatic aldehyde (2 mM) were transferred in a 25 mL conical flask. To this thioglycolic acid (3 mM) and dimethyl formamide (5 mL) were added and mixed well. The mixture was then irradiated in a microwave oven at 20% power level (100 W) for 3 minutes. After completion of the reaction, the reaction mixture kept at room temperature and poured onto ice cold water with constant stirring. The precipitated crude product filtered, washed with saturated sodium bicarbonate solution, dried and recrystallized from dimethyl formamide.

2,3-diphenylthiazolidin-4-one (3a):

mp 157-159 °C; R_f : 0.63; IR (KBr, cm^{-1}): 3324, 2928, 2851, 1670, 1508, 701; ^{1}H NMR ($CDCl_3$, δ): 3.86 (d, 1H, thiazolidine CH_2), 4.01 (d, 1H, thiazolidine CH_2), 6.09 (s, 1H, thiazolidine CH), 7.13-7.40 (m, 10H, Ar-H); MS: *m/z* 255 (M^+); analysis for $C_{15}H_{13}NOS$ (255.33). calcd: C, 70.56; H, 5.13; N, 5.49. Found: C, 70.70; H, 5.25; N, 5.41.

3-benzyl-2-phenylthiazolidin-4-one (3b):

mp 152-154 °C; R_f : 0.57; IR (KBr, cm^{-1}): 3328, 2929, 2858, 1678, 1512, 710; ^{1}H NMR ($CDCl_3$, δ): 3.54 (d, 1H, CH_2Ph), 3.75 (d, 1H, thiazolidine CH_2), 3.91 (d, 1H, thiazolidine CH_2), 5.16 (d, 1H, CH_2Ph), 5.99 (s, 1H, thiazolidine CH), 7.10-7.42 (m, 10H, Ar-H); MS: *m/z* 269 (M^+); analysis for $C_{16}H_{15}NOS$ (269.36). calcd: C, 71.34; H, 5.61; N, 5.20 Found: C, 71.80; H, 5.38; N, 5.09.

2-(4-chlorophenyl)-3-phenylthiazolidin-4-one (3c):

mp 124-127 °C; Rf : 0.48; IR (KBr, cm^{-1}): 3331, 2931, 2868, 1675, 1510, 711; ^{1}H NMR ($CDCl_3$, δ): 3.87 (d, 1H, thiazolidine CH_2), 3.98 (d, 1H, thiazolidine CH_2), 6.07 (s, 1H, thiazolidine CH), 7.10-7.34 (m, 9H, Ar-H); MS: *m/z* 289 (M^+), 291 (M^{+2}); analysis for $C_{15}H_{12}ClNOS$ (289.78). calcd: C, 62.17; H, 4.17; N, 4.83. Found: C, 61.48; H,

4.28; N, 4.90.

3-benzyl-2-(4-chlorophenyl)thiazolidin-4-one (3d):
mp 132-134 °C; R_f : 0.57; IR (KBr, cm^{-1}): 3321, 2924, 2849, 1676, 1502, 708; ^1H NMR ($CDCl_3$, δ): 3.53 (d, 1H, CH_2Ph), 3.76 (d, 1H, thiazolidine CH_2), 3.91 (d, 1H, thiazolidine CH_2), 5.15 (d, 1H, CH_2Ph), 5.96 (s, 1H, thiazolidine CH), 7.05-8.04 (m, 9H, Ar-H); MS: *m/z* 303 (M^+), 305 (M^{+2}); analysis for $C_{16}H_{14}ClNOS$ (303.81). calcd: C, 63.25; H, 4.64; N, 4.61. Found: C, 63.46; H, 4.68; N, 4.45.

3-benzyl-2-(2-methoxyphenyl)thiazolidin-4-one (3e):
mp 123-124 °C; R_f : 0.41; IR (KBr, cm^{-1}): 3321, 2926, 2850, 1672, 1506, 702; ^1H NMR ($CDCl_3$, δ): 3.63 (d, 1H, CH_2Ph), 3.75 (d, 1H, thiazolidine CH_2), 3.78 (s, 3H, OCH_3), 3.83 (d, 1H, thiazolidine CH_2), 5.15 (d, 1H, CH_2Ph), 5.95 (s, 1H, thiazolidine CH), 6.86-7.34 (m, 9H, Ar-H); MS: *m/z* 299 (M^+); analysis for $C_{17}H_{17}NO_2S$ (299.39). calcd: C, 68.20; H, 5.72; N, 4.68. Found: C, 68.40; H, 5.79; N, 4.54.

3-(4-chlorophenyl)-2-(4-(dimethylamino)phenyl)thiazolidin-4-one (3f):
mp 123-125 °C; R_f : 0.53; IR (KBr, cm^{-1}): 3329, 2930, 2859, 1671, 1512, 712; ^1H NMR ($CDCl_3$, δ): 3.11 (s, 6H, $N(CH_3)_2$), 3.76 (d, 1H, thiazolidine CH_2), 3.91 (d, 1H, thiazolidine CH_2), 5.94 (s, 1H, thiazolidine CH), 6.64 (d, 2H, Ar-H), 7.03 (d, 2H, Ar-H), 7.30 (d, 2H, Ar-H), 7.57 (d, 2H, Ar-H); MS: *m/z* 332 (M^+), 334 (M^{+2}); analysis for $C_{17}H_{17}ClN_2OS$ (332.85). calcd: C, 61.34; H, 5.15; N, 8.42. Found: C, 61.30; H, 5.17; N, 8.39.

3-((2,4-dinitrophenyl)amino)-2-(4-hydroxy-3-methoxyphenyl)thiazolidin-4-one (3g):
mp 119-121° c; R_f : 0.58; IR (KBr, cm^{-1}): 3319, 2921, 2846, 1679, 1501, 704; ^1H NMR ($CDCl_3$, δ): 3.76 (d, 1H, thiazolidine CH_2), 3.84 (s, 3H, OCH_3), 3.89 (d, 1H, thiazolidine CH_2), 4.23 (s, 1H, NH), 5.31 (s, 1H, OH), 5.90 (s, 1H, thiazolidine CH), 6.92-7.43 (m, 4H, Ar-H), 8.53 (d, 1H, Ar-H), 8.98 (s, 1H, Ar-H); MS: *m/z* 406 (M^+); analysis for $C_{16}H_{14}N_4O_7S$ (406.37); calcd: C, 47.29; H, 3.47; N, 13.79. Found: C, 47.22; H, 3.41; N, 13.81.

2-(4-(dimethylamino)phenyl)-3-((2,4-dinitrophenyl)amino)thiazolidin-4-one (3h):
mp 184-185 °c; R_f : 0.42; IR (KBr, cm^{-1}): 3328, 2926, 2849, 1673, 1505, 705; ^1H NMR ($CDCl_3$, δ): 3.12 (s, 6H, $N(CH_3)_2$), 3.76 (d, 1H, thiazolidine CH_2), 3.90 (d, 1H, thiazolidine CH_2), 4.23 (s, 1H, NH), 5.90 (s, 1H, thiazolidine CH), 6.54-7.23 (m, 5H, Ar-H), 8.53 (d, 1H, Ar-H), 8.98 (s, 1H, Ar-H); MS (ESI): *m/z* 403 (M^+); analysis for $C_{17}H_{17}N_5O_5S$ (403.41). calcd: C, 50.61; H, 4.25; N, 17.36. Found: C, 50.58; H, 4.27; N, 17.31.

3-(2-aminophenyl)-2-(4-hydroxy-3-methoxyphenyl)thiazolidin-4-one (3i):
mp 176-177° c; R_f : 0.54; IR (KBr, cm^{-1}): 3336, 2934, 2853, 1680, 1510, 704; ^1H NMR ($CDCl_3$, δ): 3.76 (d, 1H, thiazolidine CH_2), 3.84 (s, 3H, OCH_3), 3.94 (d, 1H, thiazolidine CH_2), 5.31 (s, 1H, OH), 5.97 (s, 1H, thiazolidine CH), 6.13 (s, 2H, NH_2), 6.95-7.53 (m, 7H, Ar-H); MS: *m/z* 316 (M^+); analysis for $C_{16}H_{16}N_2O_3S$ (316.37). calcd: C, 60.74; H, 5.10; N, 8.85. Found: C, 60.69; H, 5.04; N, 8.89.

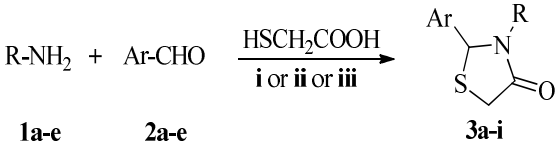
Antimicrobial activity
The disc diffusion method [22] was used to determine antimicrobial activities of synthesized compounds. The bacterial cultures were inoculated on Mueller Hinton Agar and fungal cultures on Potato Dextrose Agar. Media were prepared and transferred into sterile petri plates aseptically. Media then inoculated homogeneously with 0.2 mL suspension of microorganisms by spread plate method. The sample impregnated discs were placed on the inoculated agar medium. The plates inoculated with bacterial and fungal cultures were incubated at 37 °C for 24 h and at 25 °C for 72 h respectively. After the incubation diameter of zone of inhibition produced by the sample were measured in mm. The standards ciprofloxacin (5 µg/disc) for the antibacterial and clotrimoxazole (5 µg/disc) for the antifungal assays were used as the positive controls. Each disc contained 5 µg of synthesized compounds. Paper disc with DMSO were used as negative controls.

The broth micro dilution method [23] was followed to determine the minimum inhibitory concentration (MIC) of all the synthesized compounds. Serial dilutions of compounds were made in Muller Hinton broth, after which a standardized bacterial/fungal suspension containing 10^6-10^8 colony forming unit (CFU) microorganisms was added to the test tubes containing 1 mL of Muller Hinton broth media. Then 50 µL samples were added to all the test tubes and incubated at 37 °C for 24 h. One control test tube was prepared without sample which serves as growth control. In primary screening 500, 250 and 125 µg/mL concentrations of the synthesized drugs were used. The active drugs found in this primary screening were further diluted to obtain 62.5, 31.25, 15.6 and 7.8 µg/mL concentrations and tested for antimicrobial activity. MIC is expressed as the lowest dilution, which inhibited growth judged by lack of turbidity in the tube. The highest dilution showing at least 99% inhibition was taken as MIC.

RESULT AND DISCUSSION

Synthetic approach

A range of various amines and aldehydes were condensed with thioglycolic acid in the presence of DCC using THF as solvent under reflux and stirring condition. The synthetic route is illustrated in Scheme 1. A preliminary experiment was carried out using aniline, benzaldehyde and thioglycolic acid. To optimize the reaction conditions and amount of reactants, experiments were carried out using different ratios of the reactants. It was observed that amine, aldehyde and thioglycolic acid in ratios 1:2:3 give maximum yields. We have also observed that stirring in presence of 1.2 equivalent amount of DCC gives better yields as compared with the reaction carried out at reflux temperature.



1a-e **2a-e** **3a-i**

1a: R = Phenyl-
1b: R = Benzyl-
1c: R = *p*-chlorophenyl-
1d: R = (2,4-dinitrophenyl)-NH-
1e: R = *o*-aminophenyl-

2a: Ar = Phenyl-
2b: Ar = *p*-chlorophenyl-
2c: Ar = *o*-methoxyphenyl-
2d: Ar = *p*-dimethylaminophenyl-
2e: Ar = *p*-hydroxy-*m*-methoxyphenyl-

3a: R = Phenyl-, Ar = Phenyl-
3b: R = Benzyl-, Ar = Phenyl-
3c: R = Phenyl-, Ar = *p*-chlorophenyl-
3d: R = Benzyl-, Ar = *p*-chlorophenyl-
3e: R = Benzyl-, Ar = *o*-methoxyphenyl-
3f: R = *p*-chlorophenyl-, Ar = *p*-dimethylaminophenyl-
3g: R = (2,4-dinitrophenyl)-NH-, Ar = *p*-hydroxy-*m*-methoxyphenyl-
3h: R = (2,4-dinitrophenyl)-NH-, Ar = *p*-dimethylaminophenyl-
3i: R = *o*-aminophenyl-, Ar = *p*-hydroxy-*m*-methoxyphenyl-

Reaction conditions: **i** = THF, DCC, reflux, 1 h, (43-76%)
ii = THF, DCC, stirring, rt, 50 min, (64-85%)
iii = DMF, MW irradiation, 20% (100 W), 3 min, (75-94%)

Scheme 1 Synthetic route of compounds **3a-i**

To optimize microwave irradiation method, at first we inspected the condensation in benzene which produces lower yield. Moreover, all our attempts to improve the yield by using higher microwave power, higher temperature and longer reaction time were unsuccessful. To increase the efficiency we decided to perform the condensation in a high microwave absorbing and more polar solvent like DMF. To check the feasibility of condensation, a trial was carried out using aniline, benzaldehyde and thioglycolic acid in DMF and observed that the reaction proceeded efficiently forming the desired product in excellent yield. All the methods are compared in terms of reaction time and % yield. The results are summarized in Table 1.

Table 1 Comparison of different methods for synthesis of thiazolidin-4-one derivatives (3a-i)

Compounds	Reflux method		Stirring Method		Microwave assisted Method	
	Reaction Time (min)	%Yield[#]	Reaction Time (min)	%Yield[#]	Reaction Time (min)	%Yield[#]
3a	60	71	50	83	3	92
3b	60	65	50	78	3	89
3c	60	76	50	85	3	94
3d	60	72	50	82	3	90
3e	60	70	50	81	3	92
3f	60	65	50	78	3	88
3g	60	59	50	73	3	85
3h	60	60	50	74	3	87
3i	60	43	50	64	3	75

[#] *Yield refers to pure isolated product*

Characterization

The characterization of synthesized compounds have been achieved satisfactory with the help of physical and spectral (IR, ^{1}H NMR and mass) data. The IR absorption spectrum of all the compounds shows the characteristic stretching of the cyclic C=O and C-S-C at 1680-1670 and 712-701 cm^{-1} respectively. ^{1}H NMR spectral analysis of all synthesized compounds shows two doublets of thiazolidinone methylene (-CH_2) protons at δ 3.75-4.01 ppm and a singlet of methine (-CH) proton at δ 5.90-6.09 ppm indicating the formation of cyclic thiazolidin-4-one derivatives. All other protons were identified by their specific δ values and were found in accordance with proposed structures. The mass spectrum of all the compounds shows fairly intense molecular ion (M^+) peak, confirming the proposed molecular formula of the synthesized compound. Compounds containing chlorine atom on aryl rings shows M^+ and M^{+2} peaks in ratio of 3:1 due to isotopic abundance.

Antimicrobial activity

The *in vitro* antimicrobial screening of all the synthesized compounds were performed in the concentration of 5 µg/disc in dimethyl formamide using disc diffusion method. Various gram positive bacteria such as *Bacillus lentus,*

Bacillus cerus, *Micrococcus luteus* and *Staphylococcus albus* and gram negative bacteria such as *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris* and *Salmonella paratyphi* were selected to evaluate antibacterial activity. The *Candida albicans* was selected for evaluation of antifungal activity. The results are shown in the Table 2. The results of antimicrobial screening revealed that all the tested compounds showed weak to moderate inhibitory activity. Among the screened compounds, compounds 3g and 3i emerged as active against all the tested bacterial strains and exhibited good antibacterial activity comparable to that of standard. The compounds 3a and 3g showed good antifungal activity against *Candida albicans.*

Table 2 Antimicrobial activity of newly synthesized thiazolidin-4-one derivatives (3a-i)

Micro-organisms	Zone of inhibition (in mm)									
	Std. drug	3a	3b	3c	3d	3e	3f	3g	3h	3i
Klebsiella aerogenes	18	12	13	10	14	10	11	12	13	15
Escherichia coli	27	15	12	11	16	13	15	20	10	16
Proteus vulgaris	14	10	10	12	9	12	10	12	10	12
Salmonella paratyphi	10	8	11	8	8	8	9	11	9	8
Bacillus lentus	13	10	11	11	9	9	11	12	9	11
Staphylococcus albus	13	10	11	9	9	9	13	10	10	11
Micrococcus luteus	13	10	9	9	9	9	8	12	9	11
Bacillus cereus	12	10	8	7	7	7	11	10	8	9
Candida albicans	18	15	13	12	8	12	12	15	14	12

Further, the MIC value of all compounds was determined against each microorganism. The results are reported in Table 3. From the results, it can be concluded that compound 3g shows lower MIC values against all tested microbial strains except *Klebsiella aerogenes* and *Staphylococcus albus* where compound 3d and 3f shows lower MIC value against respective bacterial strain.

Table 3 MIC values of newly synthesized thiazolidin-4-one derivatives (3a-i)

Micro-organisms	Minimum Inhibitory Concentration (µg/mL)								
	3a	3b	3c	3d	3e	3f	3g	3h	3i
Klebsiella aerogenes	125	125	250	62.5	250	250	125	125	62.5
Escherichia coli	31.25	62.5	125	31.25	62.5	62.5	15.6	125	31.25
Proteus vulgaris	125	125	62.5	125	62.5	125	62.5	125	62.5
Salmonella paratyphi	125	62.5	125	125	125	125	62.5	125	125
Bacillus lentus	125	62.5	62.5	125	125	62.5	62.5	125	62.5
Staphylococcus albus	62.5	62.5	125	125	125	31.25	62.5	125	62.5
Micrococcus luteus	62.5	125	125	125	125	125	62.5	125	62.5
Bacillus cereus	125	250	250	250	250	125	125	250	125
Candida albicans	62.5	125	125	250	125	125	62.5	62.5	125

CONCLUSION

A straight forward protocol for three-component, one-pot, conventional solution-phase and microwave assisted synthesis described here has many unique features like rapid, simple reaction conditions, high yields and easy works up procedure. It is thus concluded that microwave assisted method is convenient, rapid and more efficient. All the synthesized compounds were screened for their efficacy as antimicrobials *in vitro* by the disc diffusion and micro dilution method against pathogenic strains. All the synthesized compounds show weak to moderate activity against all tested microorganisms.

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