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Design, Synthesis and Biological Evaluation of Thiazolidinone Derivatives for Anti-Bacterial and Anti-Tubercular Activity

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ABSTRACT

Numerous pharmaceuticals and physiologically significant compounds comprise heterocyclic systems. One unique class of heterocyclic compounds with a wide range of biological activity is thiazolidinone. New series of thiazolidinone 3(a–h) have been synthesized from mercaptoacetic acid as a starting material by conventional. All the synthesized compounds 3(a–h) were screened for their antibacterial and antifungal activities against some selected bacteria and fungi and antitubercular activity screened against Mycobacterium tuberculosis. The structure of all the synthesized compounds were confirmed by chemical and spectral analyses such as IR, 1H NMR, 13C NMR and Mass.

KEYWORDS: Heterocyclic compound, Thiazolidinone, Synthesis, Antibacterial, antitubercular

1. INTRODUCTION

Currently, the treatment for tuberculosis diseases still remains an important and challenging problem because of emerging resistance to current regimen and also appearance of drug-resistant strains in tuberculosis like mycobacterium tuberculosis H37RV strain. Now, it is challenging and essential target for medicinal chemists in drug search for treatment of tuberculosis. (1) Thiazolidine derivatives are an important class of heterocyclic compounds known for their potential pharmaceutical applications. Recently, this framework containing compounds were effective against antimicrobial (2), antischistosomal activity (3), antifungal (4), antiinflammatory (5), antimalarial (6), herbicidal (7), antiviral (8), antidiabetic (9), and antioxidant (10) activities. Thiazole derivatives are heterocyclic compounds containing nitrogen and sulfur atoms in their structure and are proved to be clinically useful agents against different kinds of disease. Thiazole derivatives have been employed in the preparation of different important drugs required for treatment of antimicrobial (1), antibacterial (12, 13), antifungal (Bharti 14, 15), antiinflammatory (16), and antitubercular (17), some of the thiazole derivatives are used as antiprotozoal (18) drugs. The present work designed and developed to synthesize some new compounds with significant medicinal value. Here we reported the synthesis and characterization of various thiazolidine-2,4-diones. These compounds screened for their antitubercular activity against mycobacterium tuberculosis H37RV.

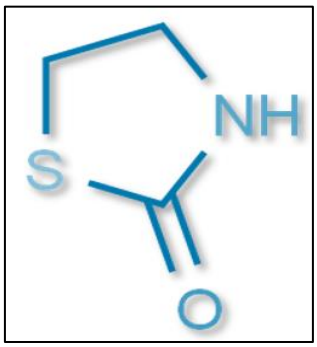

Figure 1: Thiazolidinone

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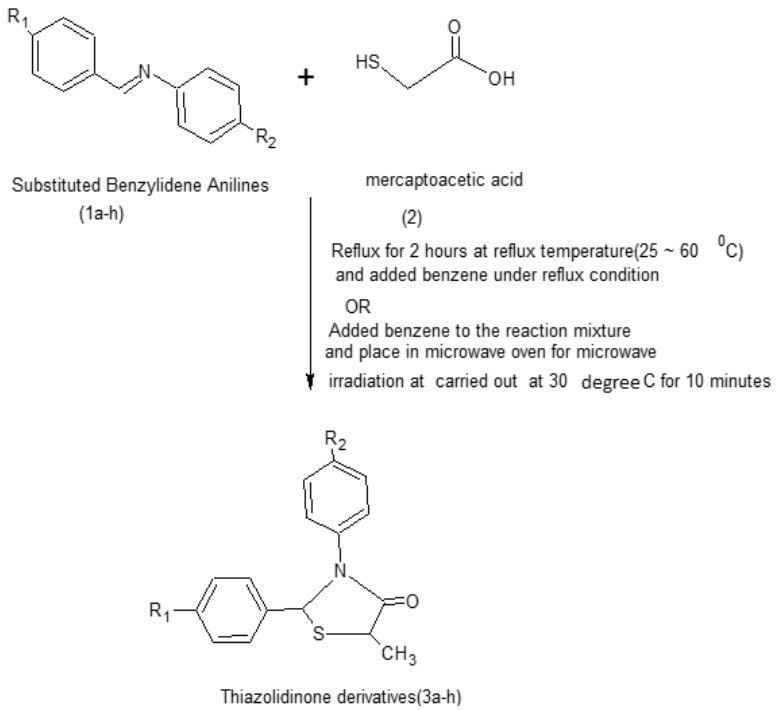
2. EXPEREMENTAL

Melting points of the synthesized compounds were determined in an open capillary tube using digital melting point apparatus and are uncorrected. The purity of the compounds were established by thin layer chromatography by using pre-coated silica gel strips, chloroform and acetone (1:2) as solvent system and iodine vapors as visualizing agent. Infrared spectra (υ cm-1) were recorded on a Shimadzu-8400 FT-IR spectrometer using KBr disks. CHNO elemental analysis carried by Perkin Elmer Series II 2400 CHNS/O Elemental analyzer. Mass spectra were obtained on LCMS-6410 fi-om Agilent Technology at 70ev using direct insertion probe method. NMR spectra were taken on Bruker Avance H-400 MHz NMR spectrometer High Resolution Multinuclear FT – NMR spectrometer by using TMS as internal standard and the solvent used was DMSO.

2.1 Synthesis of Thiazolidinone derivatives: Scheme 1 shows the yields of thiazolidin-4-ones 3a–h arising from the reaction between mercaptoacetic acid and suitable benzylideneanilines (1a–h) in benzene, after 2 h at reflux temperature, and those obtained by microwave irradiation at 30 °C after 10 min. The data indicate 3a–h compound was synthesized under microwave irradiation than with the conventional method at reflux temperature over a longer time frame (25–69%). (19)

To directly compare reaction results, the same solvent (benzene) used in the conventional method was used in the microwave-assisted method. Benzene is an apolar, aprotic solvent, not miscible with the water produced during the reaction and is characterized by low permittivity. Those latter two characteristics work in favor of the conventional reaction allowing the water removal from the reaction mixture and limit the microwave absorption only to the reacting species. (20, 21)

In fact, the results scheme 1 seem to suggest that, under microwave irradiation at low temperature (30 °C), the yield increase could be related to the polarity induced in the benzylidene–anilines 1a–h by decreasing polar substituents NO_2 > Cl > Me > H. (22)



Scheme 1: Thiazolidinone derivatives synthesis

2.2 Antibacterial Activity:

The purified products were screened for their antibacterial activity. The nutrient agar broth prepared by the usual method, was inoculated aseptically with 0.5 ml of 24 hours old subcultures of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonae* in separate conical flasks at 40-50°C and mixed well by gentle shaking. About 25 ml content of the flask were poured and evenly spreaded in a petri dish (13 cm in diameter) and allowed to set for 2 hrs. The cups (10 mm in diameter) were formed by the help of borer in agar medium and filled with 0.04 mL (40 mg) solution of sample in DMSO.

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The plates were incubated at 37°C for 24 hrs and the control was also maintained with 0.04 mol of DMSO in a similar manner and the zones of inhibition of the bacterial growth were measured in millimeter and are recorded in Table 4 and 5. (23, 24)

2.3 Anti-Tubercular activity: These are nonmotile, non-sporing and non-capsulated bacilli, arranged singly or in groups. They are acid-fast due to the presence of mycolic acid in cell-wall and weakly Gram positive. With Ziehl-Neelsen stain, M. tuberculosis look slender, straight or slightly curved rod with beaded or barred appearance and *M. bovis* appear straighter, stouter and shorter with uniform staining.

Culture: Turbercle bacilli are aerobes, grow slowly (generation time 14-15 hrs), optimum temperature 2>1 °C, pH 6.4-7.0. They grow only in specially enriched media containing egg, asparagines, potatoes, serum and meat extracts. Colonies appear in 2-6 weeks. M. tuberculosis grows more luxuriantly in culture (eugenic) than M. bovis which grows sparsely (dysgenic). The drag susceptibility test may be performed by either the direct or the indirect method. The direct drag susceptibility test is performed by using a subculture from a primary culture as the inoculum. (25, 26)

2.4 Methods used for Primary and Secondary Screening

Each synthesized drag was diluted obtaining 2000 µg/mL concentration, as a stock solution.

i) Primary screening: In primary screening 500 µg/mL, 250 µg/mL and 125 µg/mL concentrations of the synthesized drags were taken. The active synthesized drags found in this primary screening were further tested in a second set of dilution against all microorganisms.

ii) Secondary screening: The drags found active in primary screening were similarly diluted to obtain l00 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.250 µg/mL, 3.125 µg/mL and 1.5625 µg/mL concentrations.

The highest dilution showing at least 99 % inhibition is taken as MIC. The result of this test is much affected by the size of the inoculum. The test mixture should contain 10^8 organism/mL.

1. The minimum inhibitory concentration: MIC is defined as the minimum concentration of the drug required to inhibit the growth of the organisms, where growth is defined as 20 colonies or more. This definition of growth is chosen so that only a small proportion (e.g. 1%) of wild strains would be classified as resistant by its use. This method is simple and be carried out with a single drug containing slope although it is preferable to use more than one slope.

2. Resistance ratio: This consists of expression the resistance as a ratio of the MIC of a test strain to that of control strain. This procedure calls for a rigid standardization since the inherent technical errors usually make it less efficient than the MIC method in distinguishing sensitive and resistant strains. A further disadvantage of the use of RR is that there may be more variation in sensitivity of the control strain than in wild strain resulting in increase in the error. However, the Resistance Ratios are more than one slope.

3. Proportion method: This method of testing sensitivity has a high degree of precision. The inoculum suspension is standardized by weight of the bacilli and serial ten-fold dilution of the suspension are made for seeding into drug free and drug containing slopes. Two variants, a simplified variant and standard variant, are over a period of years.

We have used the minimum inhibitory concentration to evaluate the antituberculosis activity. It is one of the non-automated in vitro bacterial susceptibility tests. This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms. It is carried out in bottle. (27-30)

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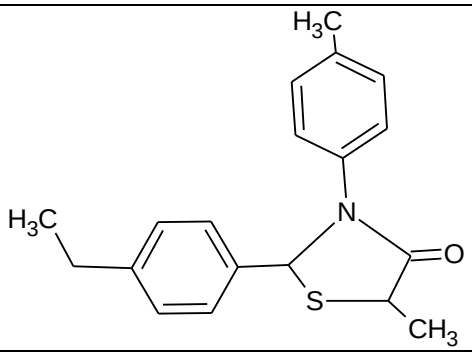
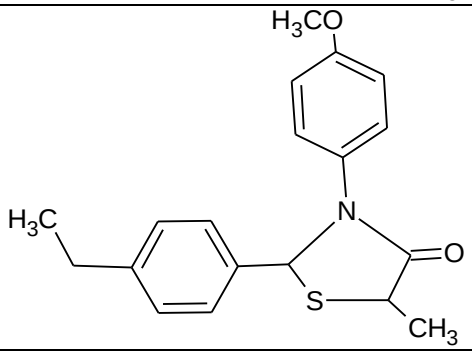
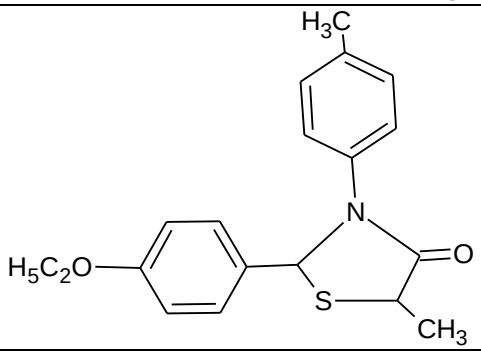
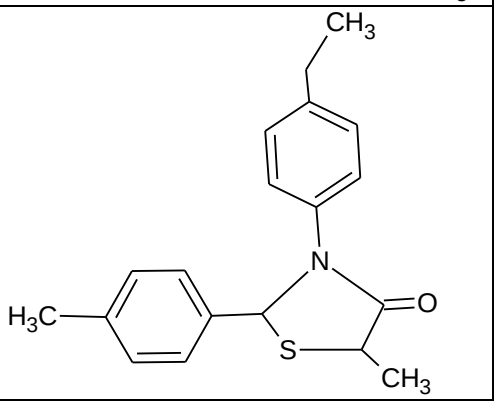
3. RESULTS AND DISCUSSION

Table 1: List of compounds synthesized

Derivatives	R1	R2	Structure
3a	C_2H_5	CH_3	
3b	C_2H_5	OCH_3	
3c	OC_2H_5	CH_3	
3d	CH_3	C_2H_5	

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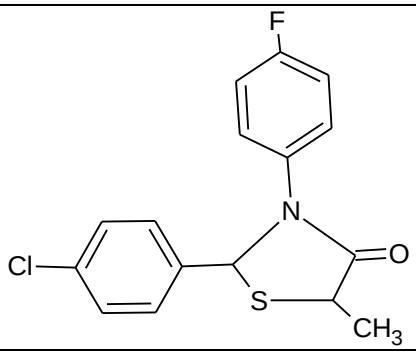
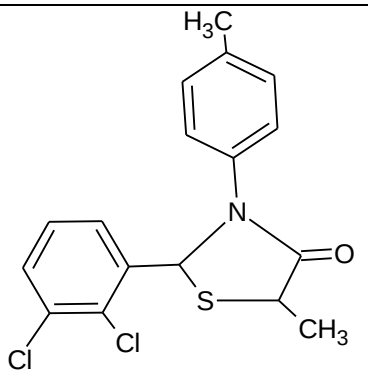
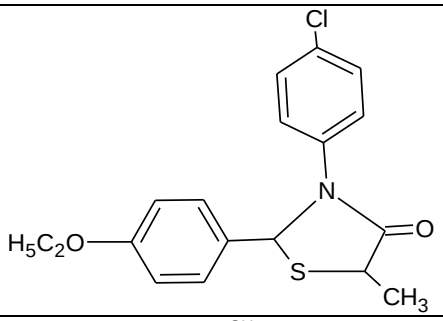
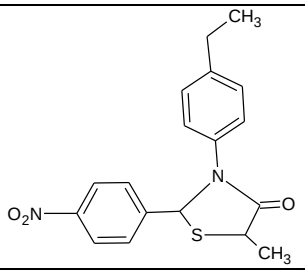
3e	Cl	F	
3f	2,3 Dichloro	CH_3	
3g	OC_2H_5	Cl	
3h	NO_2	C_2H_5	

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Table 2: Chemical Properties of synthesized compounds(3a-3h)

Derivatives	Chemical Formula	M.W	Composition C							M.P.(°C)
			C	H	N	O	S	Cl	F	
3a	$C_{19}H_{21}NOS$	311.44	73.27%	6.80%	4.50%	5.14%	10.30%		-	109°C
3b	$C_{19}H_{21}NO_2S$	327.44	69.69%	6.46%	4.28%	9.77%	9.79%	-	-	112°C
3c	$C_{19}H_{21}NO_2S$	327.44	69.69%	6.46%	4.28%	9.77%	9.79%	-	-	119°C
3d	$C_{19}H_{21}NOS$	311.44	73.27%	6.80%	4.50%	5.14%	10.30%	-	-	114°C
3e	$C_{16}H_{13}ClFNOS$	321.79	59.72%	4.07%	4.35%	4.97%	9.96%	11.02%	5.90%	128°C
3f	$C_{17}H_{15}Cl_2NO_5$	352.78.	57.96%	4.29%	3.98%	4.54%	9.10%	20.13%	-	118°C
3g	$C_{18}H_{18}ClNO_2S$	347.85	62.15%	5.22%	4.03%	9.20%	9.22%	10.19%	-	124°C
3h	$C_{18}H_{18}N_2O_3S$	342.412	63.14%	5.30%	8.18%	14.02%	9.36%	-	-	108°C

Table 3: Physical and chemical properties of synthesized compound

Code	Chemical Formula	Colour	Rf value	% yield
3a	$C_{19}H_{21}NOS$	Yellowish white solid	0.45	59.30%
3b	$C_{19}H_{21}NO_2S$	Yellowish brown solid	0.56	66.30%
3c	$C_{19}H_{21}NO_2S$	Pale yellow solid	0.52	51.40%
3d	$C_{19}H_{21}NOS$	Yellowish white solid	0.48	48.70%
3e	$C_{16}H_{13}ClFNOS$	Pale yellow solid	0.52	65.20%
3f	$C_{17}H_{15}Cl_2NO_5$	Pale yellow crystals	0.60	63.80%
3g	$C_{18}H_{18}ClNO_2S$	Yellowish crystals	0.41	57.05%
3h	$C_{18}H_{18}N_2O_3S$	Off White crystals	0.46	52.60%

3.1 Spectral Analysis

2-(4-chlorophenyl)-3-(4-fluorophenyl)-5-methyl-1, 3-thiazolidin-4-one: IR (υ cm^{-1}) spectrum- 1508-1395 (Aromatic stretching); 2831 (Methyl group); 1685 (Carbonyl group); 2950 (-CH2 Stretching); 3170 (Tertiary amine); 1H NMR (400MHz, DMSO)- 4.97 (Singlet); 8.43-8.48 (Multiplet); 9.43 (Singlet); 7.17-7.22 (Multiplet); 2.96-3.04 (Multiplet); 3.57-3.68 (Multiplet); Mass m/z-366.43 and 367.5.

2-(2,3-dichlorophenyl)-5-methyl-3-(4-methylphenyl)-1, 3-thiazolidin-4-one: IR (υ cm^{-1}) spectrum- 801-713 (C-Cl stretching); 1588-1421 (Aromatic stretching); 1667 (Carbonyl group); 2829 (-CH3 Stretching); 3076 (Tertiary amine); 1H NMR (400MHz, DMSO)- 8.65 (Singlet); 7.32-7.50 (Multiplet); 5.44 (Singlet); 3.23-3.36 (Multiplet); 2.87 (Multiplet); Mass m/z-345.24 and 346.2.

Table 4: MIC data of synthesized compounds on gram positive bacteria

S. No.	Derivatives	Gram positive bacteria µg/ml		
		S. aureus	***S. pyogenes***	***S. pneumoniae***
1	3a	125	125	250
2	3b	100	250	250
3	3c	200	100	200
4	3d	100	125	200
5	3e	62.5	62.5	125
6	3f	125	125	62.5
7	3g	100	125	125
8	3h	100	100	250
9	Ciprofloxacin	25	25	25

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Table 5: MIC data of synthesized compounds on gram negative bacteria

S. No.	Derivatives	Gram negative bacteria µg/ml		
		E. coli	***P. aeruginosa***	***K. pneumonae***
1	3a	250	125	100
2	3b	100	125	250
3	3c	100	100	125
4	3d	125	250	250
5	3e	62.5	125	62.5
6	3f	125	62.5	62.5
7	3g	100	100	100
8	3h	125	125	125
9	Ciprofloxacin	50	50	50

Table 6: MIC data of synthesized compounds on gram positive bacteria

S. No.	Derivatives	Gram positive bacteria µg/ml		
		S. aureus	***S. pyogenes***	***S. pneumoniae***
1	3a	250	100	250
2	3b	125	125	250
3	3c	100	250	250
4	3d	100	125	200
5	3e	62.5	100	100
6	3f	62.5	100	62.5
7	3g	100	125	125
8	3h	100	250	250
9	Ampicillin	100	100	100

Table 7: MIC data of synthesized compounds on gram negative bacteria

S. No.	Derivatives	Gram negative bacteria µg/ml		
		E. coli	***P. aeruginosa***	***K. pneumonae***
1	3a	250	125	125
2	3b	125	250	250
3	3c	250	100	250
4	3d	125	250	100
5	3e	100	100	125
6	3f	100	100	62.5
7	3g	250	250	250
8	3h	125	250	250
9	Ampicillin	250	250	250

3.2 Discussion

4-Thiazolidinone derivatives play a vital role in many biological processes and synthetic drugs. Diverse biological activities such as bactericidal, pesticidal, fungicidal, insecticidal, anticonvulsant, tuberculostatic, anti inflammatory, antithyroidal, etc. have been found to be associated with thiazolidinone derivatives.

Synthesis: The first step involved the reflux condensation of different substituted benzaldehyde with mercaptoacetic acid and added benzene.

Characterization: These class of compounds were characterized by 1H NMR, IR and MS spectroscopy. As evidence, these class of compounds were characterized by 1H NMR. The multiplet in the range of 3.36-3.23 5 ppm was exhibited by the methylene proton of thiazolidinone moiety. FTIR spectra, the C=O group of the amide group observed as a strong and sharp bend at 1667cm-1. Further the tertiary amine -N stretching frequency was observed at 3075cm^{-1}.

Biological activity: The most of compounds tested, exhibited considerable activities against six bacterial species, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonae. Staphylococcus aureus, Streptococcus pyogenes and Streptococcus pyogenes. Compound 3e and 3f showed excellent activity at 62.5 µg/mL and compound 3b, 3d, 3g and 3h showed a moderate activity at 100 µg/mL against S. aureus as compared to Ciprofloxacin (MIC= 25 (µg/mL). Compounds 3d, 3f, and 3h exhibited a moderate activity at 125 g/mL against Escherichia coli as compared to Ciprofloxacin (MIC= 50 µg/mL). Compounds 3e exhibited excellent activity at 62.5 µg/mL against *S. pyogenes* as compared to Ciprofloxacin (MIC= 25 µg/mL). Compounds 3a and 3g exhibited a moderate activity at 100 µg/mL against K. pneumonae as compared to Ciprofloxacin (MIC= 50 µg/mL).

The most of compounds tested, exhibited considerable activities against six bacterial species, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonae. Staphylococcus aureus, Streptococcus pyogenes and Streptococcus pyogenes. Compound 3e and 3f showed excellent activity at 62.5 and 100 µg/mL and compound 3b, 3d and 3h showed a moderate activity at 125 µg/mL against Escherichia coli as compared to Ampicillin (MIC= 250 (µg/mL). Compounds 3a, 3c, and 3g exhibited a moderate activity at 250 g/mL against Escherichia coli as compared to Ampicillin (MIC= 100 µg/mL). Compounds 3e and 3f exhibited excellent activity at 62.5 µg/mL against Staphylococcus aureus as compared to Ampicillin (MIC= 100 µg/mL). Compounds 3b, 3d and 3g exhibited a moderate activity at 100 µg/mL against Streptococcus pyogenes as compared to Ampicillin (MIC= 100 µg/mL).

4. CONCLUSION

A class of heterocyclic compounds were synthesized such as thiazolidinones, against different strains of bacteria through Minimum inhibitory concentration. Suitably substituted thiazolidinones derivatives have been screened and found active against Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonae. Staphylococcus aureus, Streptococcus pyogenes and Streptococcus pyogenes.

Finally, we were fortunate enough to synthesize suitably functionalized thiazolidinone derivatives. Some of these molecules as a result of conjugation exhibited even better antibacterial and antitubercular activity than our reference molecules ampicillin and ciprofloxacin respectively.

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