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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDIES OF NEW THIAZOLIDINONES

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ABSTRACT

A new series of acetophenone appended thiazolidinones (**5-8**) were synthesized by the reaction of acetophenone and its derivatives (**1-4**) with thiosemicarbazide and merceptoacetic acid in absolute ethanol in one pot manner. The striking feature of this reaction is the formation of thiosemicarbazine *in situ* which in turn undergoes the cyclization with mercepto acetic acid, leading to the formation of new thiazolidinones. Thus the thiazolidinone ring closes at carbonyl carbon, by the attack of sulfur of mercaptoacetic acid moiety, preferentially from the front (β, axial) so that the nitrogen has an equatorial orientation (α, equatorial) to avoid steric repulsion, giving minimum steric hindrance. The new compounds were characterized by spectral (IR, ^{1}H NMR, ^{13}C NMR, MS) and analytical methods. The new compounds were screened for antimicrobial activity against various strains of bacteria and fungi, during which the new compounds depicted potential antimicrobial behaviour.

KEYWORDS: Thiosemicarbazide and merceptoacetic.

INTRODUCTION

Heterocycles have been accredited with a great amount of attention over the years by medicinal chemists for drug discovery. The interesting structural and stereochemical features of the heterocyclic compounds provide additional fascination to the researchers and thereby alterations in the skeleton have been envisaged to discover new chemical entities with a potential to afford some promising drugs of the future. The incorporation of a heterocyclic ring like thiazolidinone, thiazole or a heteroatom in the heterocyclic backbone affects the chemical properties of a heterocycle and often results in useful alterations in its biological activities.[1] Therefore, researchers are on a continuous pursuit to design and produce better heterocyclic derivatives, by following natural models. The discovery of several biologically active heterocyclic derivatives with their wide applications in therapy has also brought about an interesting interest.[1]

Thiazolidinones which is an important class of heterocyclic compounds are classified as doubly unsaturated five membered heterocyclic compounds contain one nitrogen, one sulphur and three carbon atoms including a carbonyl group. Thiazolidinones have been reported to show versatile pharmacological activities. They have been reported as COX-1 inhibitor[2], anti-inflammatory[3], antiproliferative,[4, 5] antihistaminic[6], anti-HIV[7,8], hypnotic[9], anaesthetic[10], antifungal[11], anthelmintic[12] and antiviral[13] agents as well as CNS[14] stimulants. 4-thiazolidinones and their derivatives[15] exhibit unusually high activity against *Mycobacterium tuberculosis*. Recently, a number of 4-thiazolidinones derivatives found to exhibit highly potent and selective anti-Platelet activating factor activity both *in vitro* and *in vivo*.[16] 2-Arylimino-4-thiazolidinone derivatives have also showed antibacterial,[17, 18] antifungal,[19] and anticonvulsant activities.[20,21] Keeping in view the applications of heterocycles and in continuation of previous work[22] we herein report the synthesis and antimicrobial studies of aromatic appended thiazolidinone derivatives.

EXPERIMENTAL

Chemistry

All the melting points were determined in degrees Celsius on a Kofler apparatus and are uncorrected. The IR spectra were recorded on KBr pellets with Perkin Elmer RXI Spectrophotometer and values are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were run in $CDCl_3$ on a JEOL Eclipse (400 MHz) instrument with TMS as internal standard and values are given in ppm (δ). Mass spectra were recorded on a JEOL SX 102/DA-6000 Mass Spectrometer. Thin layer chromatography (TLC) plates were coated with silica gel G and exposed to iodine vapors to check the homogeneity as well as the progress of reaction. Petroleum ether refers to a fraction of boiling point 60-80 °C. Sodium sulfate (anhydrous) was used as a drying agent. All the chemicals were purchased from Merck India and were used after distillation.

General procedure for the synthesis of 2-methyl-2-phenyl-3-thioureaylthiazolidin-4-one derivatives (339-342)

Acetophenone and its derivatives (**1-4**) (1 mmol), thiosemicarbazide (1 mmol), mercaptoacetic acid (3.0 mmol) and few drops of conc. HCl were reflux in ethanol for 5 h. The progress and completion of the reaction was monitored by TLC. After completion of reaction, the excess solvent was reduced to three fourths of the original volume under reduced pressure. The reaction mixture was then taken in ether, washed with water and dried over anhydrous sodium sulfate. Evaporation of solvents and crystallization from methanol afforded the corresponding thiazolidinones (**5-8**).

2-Methyl-2-phenyl-3-thioureaylthiazolidin-4-one (5)

Yield (70 %); Mp: 164 °C; Anal. Calcd for $C_{11}H_{13}N_3OS_2$: C, 49.41; H, 4.90; N, 15.72; found; C, 49.37, H, 4.78, N, 15.64; IR (KBr) *v* cm^{-1} 3343 (NH), 3215 (NH_2), 1645 (C=O), 1625 (CH=CH arom.), 1347 (C=S), 1229 (C-N), 647 (C-S); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.35 (s, 2H, NH_2, exchangeable with D_2O), δ 6.9 (s, 1H, NH, exchangeable with D_2O), δ 4.2 (s, 3H, C*H*$_3$), 3.36 (s, 2H, CH_2), 6.4-6.9 (m, 5H, aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 183 (C=S), 173 (*C*=O), 121-130 (6C, aromatic), 48 (C-N), 38 (CH_2). MS (EI): *(m/z)* 267 [$M^{+\cdot}$].

2-Methyl-2-(3'-hydroxy) phenyl-3-thioureayl thiazolidin-4-one (6)

Yield (72 %); Mp: 158 °C; Anal. Calcd for $C_{11}H_{13}N_3O_2S_2$: C, 46.62, H, 4.62, N, 14.83; found; C, 46.57; H, 4.58; N, 14.75; IR (KBr) *v* cm^{-1} 3358 (OH), 3353 (NH), 3218 (NH_2), 1678 (C=O), 1622 (CH=CH arom.), 1345 (C=S), 1234 (C-N), 1080 (C-O), 680 (C-S); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.2 (s, 1H, O*H*, exchangeable with D_2O), δ 7.5 (s, 1H, N*H*$_2$, exchangeable with D_2O), δ 6.7 (s, 1H, NH, exchangeable with D_2O), δ 4.2 (s, 3H, C*H*$_3$), 3.38 (s, 2H, CH_2), 6.4-6.6 (m, 4H, aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 183 (C=S), 176 (*C*=O), 121-134 (6C, aromatic), 49 (C-N), 40 (CH_2); MS (EI): *(m/z)* 283 [$M^{+\cdot}$].

2-Methyl-2-(4'-hydroxy) phenyl-3-thioureayl thiazolidin-4-one (7)

Yield (67 %); Mp: 152 °C; Anal. Calcd for $C_{11}H_{13}N_3O_2S_2$: C, 46.62, H, 4.62, N, 14.83; found; C, 46.57; H, 4.58; N, 14.75; IR (KBr) *v* cm^{-1} 3336 (OH), 3350 (NH), 3220 (NH_2), 1671 (C=O), 1620 (CH=CH arom.), 1347 (C=S), 1229 (C-N), 1080 (C-O), 667 (C-S); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.3 (s, 1H, O*H*, exchangeable with D_2O), δ 7.3 (s, 2H, N*H*$_2$, exchangeable with D_2O), δ 6.8 (s, 1H, N*H*, exchangeable with D_2O), δ 4.2 (s, 3H, C*H*$_3$), 3.35 (s, 2H, CH_2), 6.1-6.3 (m, 4H, aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 184 (C=S), 172 (*C*=O), 121-129 (6C, aromatic), 47 (C-N), 44 (CH_2); MS (EI): *(m/z)* 283 [$M^{+\cdot}$].

2-Methyl-2-(2', 4'-dihydroxy) phenyl-3-thioureayl thiazolidin-4-one (8)

Yield (71%); Mp: 160 °C; Anal. Calcd for $C_{11}H_{13}N_3O_3S_2$: C, 44.13; H, 4.38; N, 14.04; found; C, 44.07, H, 4.26, N, 13.97; IR (KBr) *v* cm^{-1} 3350 (NH), 3321, 3318 (OH), 3236 (NH_2), 1679 (C=O), 1620 (CH=CH arom.), 1354 (C=S), 1229 (C-N), 1080 (C-O), 667 (C-S); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.2, 8.0 (s, 1H, 2×O*H*, exchangeable with D_2O), δ 7.5 (s, 2H, N*H*$_2$, exchangeable with D_2O), δ 7.0 (s, 1H, N*H*, exchangeable with D_2O), δ 4.2 (s, 3H, C*H*$_3$), 3.44 (s, 2H, CH_2), 6.2-6.4 (m, 3H, aromatic); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 183 (C=S), 174 (*C*=O), 121-131 (6C, aromatic), 50 (C-N), 43 (CH_2); MS (EI): *(m/z)* 299 [$M^{+\cdot}$].

***In vitro* Antimicrobial activity**

1. Antibacterial activity

The compounds (**5-8**) were screened for their *in vitro* antibacterial activity against cultures of *Streptococcus pyogenes* (ATCC-29213), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Escherichia coli* (ATCC-25922) and *Kliebsiella pneumoniae* (Clinical isolate) by disc diffusion method.[23] Standard inoculums 1 ×10^7 - 2 ×10^7 c.f.u. ml^{-1} (0.5 McFarland standards) were introduced on to the surface of sterile agar plates and a sterile glass spreader was used for even distribution of the inoculums. The disks measuring 6 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile disks previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were also kept. Ciprofloxacin was used as positive control while the disk poured in DMSO was used as negative control. The plates were inverted and incubated for 24 h at 37 °C. Ciprofloxacin was used as positive control while the disk poured in DMSO was used as negative control. In bacterial strains the susceptibility was assessed on the basis of diameters of zone of inhibition which were measured and compared with standard drug. The bacterial zones of inhibition values are given in **Table 1**. Minimum inhibitory concentrations (MICs) and minimum bacterial concentrations (MBCs) as shown in **Table 2** were determined by broth dilution technique.

2. Antifungal activity

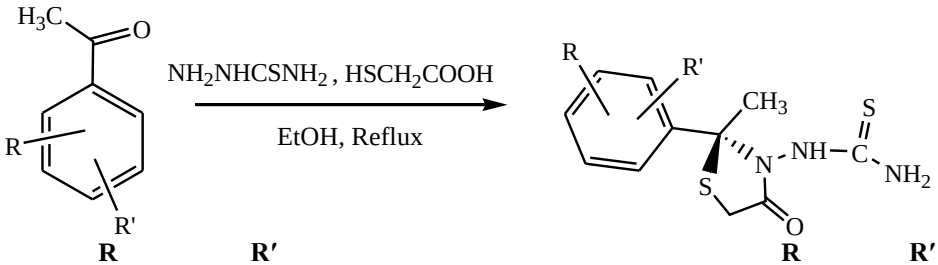
For assaying antifungal activity, different fungal strains like *Candida albicans*, *Asperergillus fumigatus*, *Pencillium marneffei* and *Trichophyton mentagrophytes* (recultured) in DMSO by agar diffusion method.[24] Sabourand agar media was prepared by dissolving peptone (1g), D-glucose (4g) and agar (2g) in distilled water (100 ml) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. 20 ml of agar media was poured into each petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37

°C for 1 h. using an agar punch, wells were made and each well was labelled. A control was also prepared in triplicate and maintained at 37 °C for 3-4 days. **Greseofulvin** was used as positive control while the disk poured in DMSO was used as negative control. The fungal zones of inhibition values were measured and compared with the standards and are given in **Table 3**. The minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) of compound **5-8** are given in **Table 4**.

RESULTS AND DISCUSSION

Chemistry

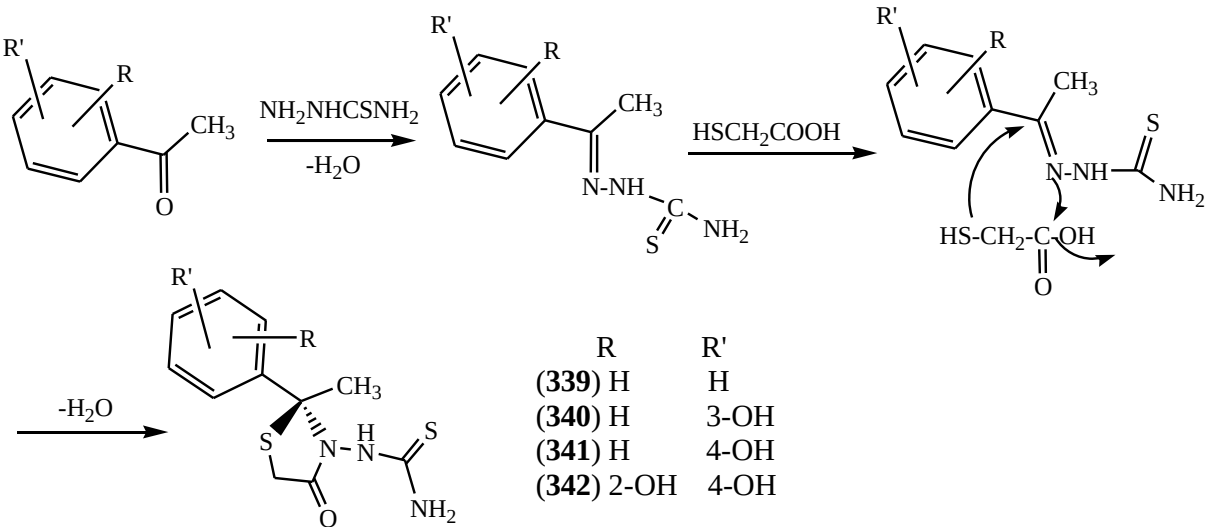
Development of highly functional molecules from simple building blocks has always been the curiosity of synthetic chemists. So we here in report the convenient route for the synthesis of new thiazolidinones (**5-8**) by reacting acetophenone and its derivatives (**1-4**) with thiosemicarbazide and merceptoacetic acid in absolute ethanol for the period of about 5 h under reflux conditions (**Scheme 1**) and on the completion of reaction, products were obtained in better yields (67-72%).



R	R′	R	R′
(**1**) H	H	(**5**) H	H
(**2**) H	3-OH	(**6**) H	3-OH
(**3**) H	4-OH	(**7**) H	4-OH
(**4**) 2-OH	4-OH	(**8**) 2-OH	4-OH

Scheme 1. Schematic representation for the formation of thiazolidinones (5-8).

The tentative mechanism for the formation of thiazolidinones (**5-8**) has been proposed in **scheme 1I**. The mechanism depicts clearly the formation of thiosemicarbazone *in situ* first by simple condensation which later undergo cyclization with merceptoacetic acid hence leads to the formation of the products (**5-8**). The structures of these compounds were characterized by spectral (IR, ^{1}H NMR, ^{13}C NMR, MS) and analytical methods.



R	R'
(**339**) H	H
(**340**) H	3-OH
(**341**) H	4-OH
(**342**) 2-OH	4-OH

Scheme 1I. Mechanism of formation 2-methyl-2-phenyl-3-thioureayl thiazolidin-4-one (5-8).

Stereochemistry

The stereoselectivity of these thiazolidinones can be explained by considering that there is a considerable amount of steric hindrance to ring-closure from one side of the ring at Carbonyl carbon which might be explained on the basis that the sulfur atom is more bulky than nitrogen during cyclization. Thus the thiazolidinone ring closes at carbonyl carbon, by the attack of sulfur of mercaptoacetic acid moiety, preferentially from the front (β, axial) so that the nitrogen has an equatorial orientation (α, equatorial) to avoid steric repulsion, giving minimum steric hindrance and maximum stability. This is further supported by the fact that during cyclization the nitrogen already attached to carbonyl

carbon is moved towards the back (α, equatorial) side to reduce the steric hindrance, and leaving the front (β, axial) side for the attack of nucleophile to close the thiazolidinone ring. Therefore the only product of this reaction with *R* stereochemistry was selectively obtained. The **dreiding models** also suggest the attack of sulfur from the β-side which pushes the nitrogen to the less hindered α-side. Hence the formulation of the compound as *R* is preferred over its isomer *S*.

The characterization studies showed good agreement with proposed structures of thiazolidinones[25] (**5-8**) (**Scheme 1**). In their IR spectra the presence of absorption bands in the range 3343-3353 shows the presence of NH and as the absorption bands at 3215-3236, 1645-1679 and 1620-1625 cm^{-1} confirm the presence of NH_2, C=O & CH=CH (arom.) groups, respectively in the compounds (**5-8**). The absorption bands at 1345-1354 and 3318-3358 cm^{-1} were ascribed to C=S and O-H groups, respectively. The presence of bands at 1227-1234 and 647-680 cm^{-1} were ascribed to C-N and C-S groups, respectively. In ^1H NMR study of the compounds (**5-8**), the three downfield singlets at δ 8.2-8.0, 7.3-7.5, 6.7-7.0 were ascribed to OH, NH_2 and NH while as the presence of two proton and thee proton singlets at δ 3.36-3.38 and δ 4.2 were assigned to methylene and methyl protons of thiazolidinone ring. The presence of broad multiplet at δ 6.1-6.9 revealed the presence of aromatic protons. In ^{13}C NMR study, the signals at δ 183-184, 172-176, 121-134, 47-50 confirm the presence of C=S, C=O, C=C (arom.) and C-N groups respectively in the products (**5-8**). Finally the presence of distinct molecular ion peak [$M^{+\cdot}$] at m/z: 267, 283, 283 and 299 in the MS after following the nitrogen rule, also proved the formation of compounds (**5-8**). The strategy can also be applied to diverse ketones, in that way thiazolidinones may also allow further modifications on the substituted heterocyclic systems.

Antimicrobial activity

The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. Compounds **5** and **6** showed good inhibition against all Gram-positive and Gram-negative bacterial strains. The compound **5** was found to be almost equally potent as the reference drug, Ciproflaxin, in case of *S. pyogenes*. The MBC of all the compounds was found was two or three folds higher than the corresponding MIC results.

Table 1. Showing zone of inhibitions with Positive control (standard); Ciprofloxacin and negative control (DMSO) measured by the Halo Zone Test (Unit, mm).

	Diameter of zone of inhibition (mm)				
Compounds	**Gram-positive bacteria**			**Gram-negative bacteria**	
	S. pyogenes	***S. aureus***	***P. aeruginosa***	***E-coli***	***K. pneumoniae***
5	22.5 ± 0.2	18.3 ± 0.7	26.1 ± 0.5	23.8 ± 0.4	14.8 ± 0.5
6	20.2 ± 0.2	18.2 ± 0.5	23.2 ± 0.6	22.8 ± 0.2	15.2 ± 0.3
7	19.4 ± 0.4	18.1 ± 0.5	23.0 ± 0.2	21.6 ± 0.1	12.4 ± 0.1
8	17.7 ± 0.2	18.2 ± 0.3	20.0 ± 0.1	18.4 ± 0.3	10.7 ± 0.3
Standard	23.1 ± 0.2	22.1 ± 0.2	32.1 ± 0.5	27.1 ± 0.4	19.1 ± 0.2
DMSO	-	-	-	-	-

Table 2. Showing MIC/MBC (µg/ml) of the compound 5-8 against bacterial strains.

	Gram-positive bacteria				**Gram-negative bacteria**					
Comp.	***S. pyogenes***		***S. aureus***		***P. aeruginosa***		***E-coli***		***K. pneumoniae***	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
5	12.5	25	12.5	50	25.0	50	12.5	25	12. 5	25
6	12.5	25	12.5	25	25.0	50	25.0	50	25.0	50
7	25.0	50	25.0	100	50.0	100	25.0	50	12.5	50
8	12.5	25	12.5	50	25.0	50	12.5	25	12. 5	25
Standard	12.5	25	6.25	12.5	12.5	25	6.25	25	6.25	12.5
DMSO	-		-		-		-		-	

Table 3. Showing zone of inhibitions with Positive control (standard); Greseofulvin and negative control (DMSO) measured by Halo Zone Test (Unit, mm).

	Diameter of zone of inhibition (mm)			
Compounds	***C. albicans***	***A. fumigatus***	***T. mentagrophytes***	***P. marneffei***
5	18.0 ± 0.1	24.5 ± 0.1	17.4 ± 0.2	17.8 ± 0.3
6	17.4 ± 0.3	21.8 ± 0.1	17.3 ± 0.3	14.1 ± 0.1
7	15.1 ± 0.1	19.1 ± 0.1	16.0 ± 0.1	14.6 ± 0.2
8	14.1 ± 0.2	17.1 ± 0.5	15.7 ± 0.4	13.1 ± 0.5
Standard	29.1 ± 0.1	26.1 ± 0.1	23.0 ± 0.2	19.5 ± 0.4
DMSO	-	-	-	-

The antifungal screening data showed moderate to good fungal inhibition. Among the screened compounds, **5** and **6** were found to have good zones of inhibition. The compound **5** showed maximum inhibition against *A. fumigatus* and *P. marneffei* strains. The compound **6** was also active against *A. fumigatus*, *P. marneffei*, and *T. mentagrophytes* but the compound **5** is more effective by showing maximum inhibition against *A. fumigatus* strain. The MFC of all the compounds was two or three folds higher than the corresponding MIC results.

Table 4. Showing MIC and MBC (µg/ml) of the compound 5-8 against fungal strains.

Compounds	*C. albicans*		*A. fumigatus*		*T. mentagrophytes*		*P. marneffei*	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
5	12.5	25	12.5	25	12.5	25	12.5	25
6	12.5	25	12.5	25	12.5	25	12.5	100
7	12.5	50	25	100	12.5	25	12.5	100
8	12.5	25	12.5	25	12.5	25	12.5	25
Standard	6.25	25	12.5	25	6.25	25	12.5	25
DMSO	-		-		-		-	

CONCLUSION

In conclusion, the convenient and operationally simple reaction for better synthesis of thiazolidinones was successfully developed. The reaction got completed in almost 5 h and on completion; potential yields were obtained. This strategy offered a very straight forward, one-pot and efficient method for access to new thiazolidinones. During the antimicrobial screening, all the newly synthesized compounds (**5-8**) were found active against different bacterial strains. The compound **5** was found to be almost equally potent as the reference drug, Ciproflaxin, in case of *S. pyogenes* strain and the compound **5** is also more effective antifungal in nature which was proved after it showed maximum inhibition against *A. fumigatus* strain The reason for this increase in the antimicrobial activity in the synthesized compounds may be due to the presence of aromatic functionality present in the product molecule because most of the heterocyclic compounds adjoined with the aromatic group, often show potential antimicrobial activity. In conclusion, the present study showed that these one pot synthesized compounds can be used as a template for future development through modification and derivatization to design more potent and selective anticancer as well as antimicrobial agents.

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