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Full Length Research Paper

Synthesis, characterization and biological activity of some novel azetidinones

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A series of seven novel azetedinones 4a-g have been synthesized by cyclocondensation of various Schiff bases of phenothiazine with chloroacetyl chloride in presence of triethylamine. Various Schiff bases of phenothiazine were synthesized by condensation of 2-hydrazinyl-1-(10H-phenothiazin-10-yl)ethanone 2 with various aryl aldehydes. Compound 2 was synthesized by reacting 2-chloro-1-(10H-phenothiazin-10-yl) ethanone 1 with hydrazine hydrate. The synthesized compounds were characterized by IR, ^{1}H-NMR and mass spectra. The titled compounds were evaluated for anti-tubercular, anti-bacterial, anti-fungal and anti-inflammatory activity by Lowenstein-Jensen medium method, cup plate method, disc diffusion method and carrageenan induced paw edema method respectively. All the seven compounds 4a-g at a concentration of 100, 10 and 1 µg/L showed inhibition against the growth of Mycobacterium tuberculosis. Compounds showed good anti-bacterial activity against Staphylococcus aureus and Bacillus subtilis. Compound 4e showed equipotent antibacterial activity with streptomycin standard and showed better anti-bacterial activity against gram positive bacteria profile than gram negative bacteria. Zone of inhibition was not found for the compounds against Escherichia coli and Pseudomonas aeruginosa. Compounds 4a-g exhibited good antifungal activity against Aspergillus species and no activity against Candida albicans. None of the reported compounds showed promising anti-inflammatory activity.

Key words: Azetidinones, anti-tubercular, anti-bacterial, anti-fungal, inflammation.

INTRODUCTION

Azetidin-2-one, a four-membered cyclic lactam (ß-lactam) skeleton has been recognized as a useful building block for the synthesis of a large number of organic molecules by exploiting the strain energy associated with it. The Staudinger reaction ([2+2] ketene-imine cycloaddition reaction) is regarded as one of the most fundamental and versatile methods for the synthesis of structurally diverse 2-azetidinone derivatives, although many synthetic methods have been developed to date (Van der steen et al., 1991; Palomo et al., 1999; Jiaxi, 2009). Azetidin-2-ones can also be synthesized by enolate-imine condensations (Maurizio et al., 2000), Kinugasa (Jose, 2004), annulations (Anthony et al, 1987) and cyclization reactions (Benito and Alberto, 1998). Also it is used in the synthesis of a variety of ß-lactam antibiotics (Deshmukh et al., 2004). Efforts have been made in exploring such new aspects of ß-lactam chemistry versatile intermediates for the synthesis of aromatic ß-amino acids and their derivatives, peptides, polyamines, polyamino alcohols, amino sugars and polyamino ethers (Alcaide and Almendros, 2001) the cyclic 2-azetidinone skeleton has been extensively used as a template to build the heterocyclic structure fused to the four-membered ring. This provides an access to diverse structural type of synthetic target molecules lacking ß-lactam ring structure (Brickner et al., 1992).

Azetidinones are of great biological interest, especially as anti-tubercular (Kagthara et al., 2000), antibacterial (Singh et al., 2005, Bhanvesh and Desai 2004; Patel and Patel, 2004; Pratibha et al., 2004; Ashok et al., 2003; Devendra and Sharma, 2002; More et al., 2002; Choudhari and Mulwad, 2003; Oza et al, 2003; Padam and Saksena., 2003; Freddy and Sushil, 2004), antifungal

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(Pandey et al., 2005; Mehta et al., 2006) and as anti-inflammatory agents (Srivastava et al. 1999, 2002; Gdupi et al, 1996; Shalabh et al., 2006). Patel and Mehta (2006) carried out the synthesis of azetidinone and thiazolidinone derivatives from 2-amino-6-(2-naphthalenyl)thiazolo[3,2-d]thiadiazole. Singh (2004) has also reviewed beta latcams in the new millennium, that is, monobatcams and carbapenems. Wang et al. (2009) synthesized fourteen derivatives of 2-azetidinones and reported for cholesterol absorption inhibitory action. Singh et al. (2007) have prepared some new 2-azetidones from N-(salicylidene) amine and 2-diazo-1,2-diarylethanones.

The chemical structure of phenothiazine provides a most valuable molecular template for the development of agents able to interact with a wide variety of biological processes. Phenothiazine derivatives possess potential biological activities as antinociceptive (Gildasio et al., 2004) anticonvulsant (Mia et al., 1998), anti-tumour (Andreani et al., 1991), antimalarial (Martha et al., 2002), anti-tubercular (Aaron et al., 2007), anti-emetic (Manish et al., 2003), antihistaminic (Oliver et al., 2006) and antipsychotic (Bateman, 2003) agents. The synthesis of phenothiazines fused with azetidinones are not reported so for. Hence, it was thought worthwhile to synthesize new congeners by incorporating phenothiazine and azetidinone moieties in a single molecular frame work and to evaluate their antimicrobial and anti-inflammatory activity.

MATERIALS AND METHODS

All melting points were taken by open capillary tubes and were uncorrected. Thin layer chromatography was performed on precoated Silica Gel 60 F_{254} plates from E.Merck using chloroform and benzene as mobile phase (75:25) and visualized by exposure to iodine vapors. IR spectra were recorded on a Perkin Elmer IR spectrophotometer, using KBr pellets. ^{1}HNMR spectra were recorded on Bruker DRX 300 (300 MHz) NMR spectrophotometer in DMSO-d_6 using TMS as internal standard and Mass spectra on Jeol SX 102 (FAB) Mass spectrophotometer.

Synthesis of 2-chloro-1-(10H-phenothiazin-10-yl) ethanone (1)

Phenothiazine (0.1 mol) and chloroacetyl chloride (0.15 mol) in dry benzene were refluxed for 2.5 h on a water bath. The solid product was filtered off and recrystallized from benzene. The IR spectrum of compound 1 revealed a sharp strong absorption band around 1680 cm^{-1} due to the presence of the carbonyl function in the structure. The methylene protons resonate as singlet at δ 4.3 and the aromatic protons resonate as multiplets at δ 6.70-6.92 ppm, its mass spectra revealed a molecular ion peak at m/z 275 (M^+) corresponding to the molecular formula $C_{14}H_{10}ClNOS$.

Synthesis of 2-hydrazinyl-1-(10H-phenothiazin-10-yl) ethanone (2)

Compound 1 (0.1 mol) and hydrazine hydrate (0.1 mol) in benzene was refluxed for 3 h on a water bath. Solid mass obtained was filtered and recrystallized from benzene. The IR spectrum of 2 showed the absence of acid chloride stretching frequency, instead it gave a band at 1657 cm^{-1} for carbonyl group and showing two broad bands in the region of 3300-3400 and at 3100-3400 cm^{-1} for NH_2 and NH frequencies, respectively. ^{1}H NMR spectrum of compound 2 exhibited signals at δ 10.12 and δ 4.62 ppm for -NH and -NH_2 (D_2O exchangeable) of hydrazide respectively. The structure was further confirmed by recording its mass spectra. It gave the molecular ion peak at m/z 271 (M^+) corresponds to molecular formula $C_{14}H_{13}N_3OS$.

Synthesis of Schiff's bases (3a-g)

A mixture of equimolar quantities of compound 2 (0.01 mol) and appropriate aryl aldehyde (benzaldehyde, 2, 4-dimethoxybenzaldehyde, anisaldehyde, 4-nitrobenzaldehyde, 4-chlorobenzaldehyde, 4-hydroxybenzaldehyde and 3, 4-dichlorobenzaldehyde) (0.01 mol) were dissolved in ethanol (95%). The contents were refluxed for a period of 3 h on a steam bath. The solid obtained was separated out and recrystallized from ethanol. The yield and melting point were reported in Table 1. The IR spectra of compounds 3a-g showed strong absorption bands for carbonyl group (1657 cm^{-1}), aromatic C-H Stretching (3100 cm^{-1}) and aromatic C=C Stretching (1600 and 1500 cm^{-1}). Compound 3d showed absorption bands for nitro group (1315 & 1515 cm^{-1}). Compound 3f showed absorption bands for hydroxyl group (3280 - 3450 cm^{-1}). ^{1}H NMR spectrum of compounds 3a-g showed a quartet for methine protons at δ 7.8 (1H, N=CH-R), multiplets at 6.9-8.1 (Ar-H). Compounds 3b and 3c showed a singlet at δ 3.82 due to the signals of methoxyl protons. Compound 3f showed a singlet for hydroxyl protons at δ 5. Compounds 3a-g gave molecular ion peak at m/z 359, 419, 389, 404, 393, 375 and 428 (M+) respectively for their corresponding molecular formulae (Table 2).

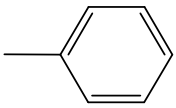
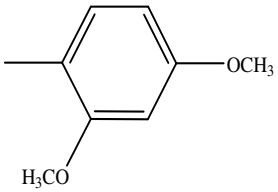
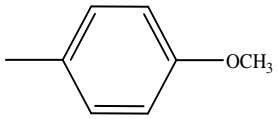
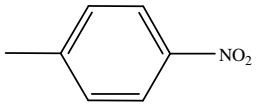
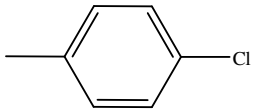
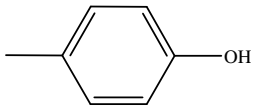
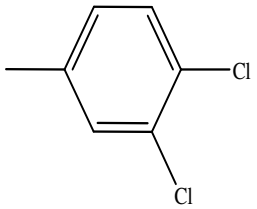
Synthesis of azetidinones (4a-g)

Triethylamine (0.01 mol) in 1,4-dioxane, chloroacetyl chloride (0.01 mol) was added drop wise to a solution of the compounds 3a-g (0.005 mol) and at room temperature. The reaction mixture was stirred for 30 min. The mixture was then refluxed for 3 h on a water bath. The solid obtained after removal of 1,4-dioxane was recrystallized from ethanol. The yield, melting point and spectral characterization of the compounds were reported in Tables 3, 4 and Figure 1.

Anti-tubercular activity

Synthesized compounds 4a-g were screened for antitubercular activity against Mycobacterium tuberculosis $H_{37}Rv$ strain using Lowenstein-Jensen medium method (Cambau et al. 2000). Ten milligram of each synthesized compound was dissolved in 10 ml of dimethyl sulfoxide to get a concentration of 1000 µg/L. Further dilutions were made with dimethyl sulphoxide to get different concentrations such as 100, 10 and 1 µg/mL. 0.8 ml of each concentration was used for the study. To this, 7.2 ml of Lowenstein-Jensen medium was added. Pyrazinamide (M/s Sigma Chemical Co.) was used as the standard drug. The dilution of pyrazinamide was made with dimethyl sulphoxide to get different concentrations of 100, 10 and 1 µg/mL. 0.8 ml of each concentration was used for the study. A sweep from M. tuberculosis $H_{37}R_V$ strain culture was discharged with the help of nichrome wire loop with a 3 mm external

Table 1. Physical and analytical data of newly synthesized compounds.

Compound	R	M.P. (°C)	Yield (%)	Rf value*	Molecular formula
3a		228-230	72	0.78	$C_{21}H_{17}N_3OS$
3b		168-170	62	0.67	$C_{23}H_{21}N_3O_3S$
3c		198-200	68	0.67	$C_{22}H_{19}N_3O_2S$
3d		278-280	70	0.50	$C_{21}H_{16}N_4O_3S$
3e		240-242	60	0.70	$C_{21}H_{16}ClN_3OS$
3f		290-292	59	0.57	$C_{21}H_{17}N_3O_2S$
3g		205-207	64	0.73	$C_{21}H_{15}Cl_2N_3OS$

*methanol and benzene as mobile phase, spot detection-Iodine vapour.

diameter, into a sterile distilled bijou bottle containing six 3 mm glass beads and 4 ml of sterile distilled water.

The bottle was shaken with the help of a mechanical shaker for 2 min. Then using nichrome wire loop, 3 mm external diameter, a loopful of suspension was inoculated on the surface of each of Lowenstein-Jensen medium containing the test compounds. Lowenstein-Jensen medium containing pyrazinamide as well as control were inoculated with Mycobacterium tuberculosis $H_{37}R_V$ strain. The inoculated medium was incubated at 37°C for 4 weeks. At the end of 4 weeks readings were taken and recorded in Table 5.

Anti-bacterial activity

Antibacterial activity was evaluated by agar cup plate method (Shanmugakumar et al., 2006). Nutrient agar medium was used for the study. After sterilization the nutrient agar medium was melted, cooled and inoculated with two Gram-positive organisms *S. aureus*, *B. subtilis* and two Gram-negative organisms *E. coli*, *P. aeruginosa* and poured into sterile Petri dish to get a uniform thickness of 6 mm. Cups were made out in the other plate using sterile cork borer (6 mm dia). The standard antibacterial agent streptomycin (100 µg L^{-1}), solvent control (0.5% v/v Tween 80) and the synthesized compounds in a concentration of 100 µg L^{-1} were added with the sterile micro pipette into each cup. The plates were then incubated at 37°C for 24 h and the diameter of zone of inhibition were measured and recorded in Table 5.

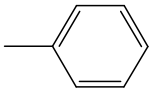
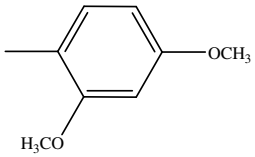
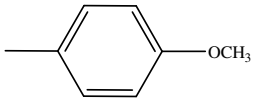
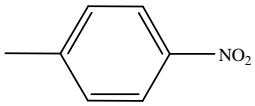
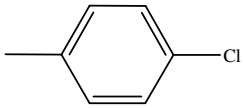
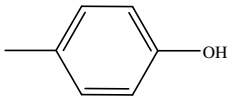
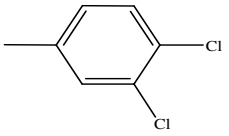
Anti-fungal activity

Anti-fungal susceptibility test was done by disc diffusion method (Mahoto et al., 2002) using Sabouraud's dextrose agar medium. After sterilization the medium was inoculated with Candida albicans and Aspergillus fumigatus. The standard antifungal agent clotrimazole (100 µg L^{-1}), solvent control (0.5% v/v Tween 80) and the synthesized compounds in a concentration of 100 µg L^{-1} were then added by sterile micro pipette. The plates were the incubated

Table 2. Spectral data of newly synthesized compounds.

Compound	IR (ν, cm^{-1})		^{1}H-NMR in DMSO (δ, ppm)	Mass spectra m/z value
3a	1690	C=O	1.8 (d, 1H, N-CH-C)	359
	3454	N-H	6.6-7.1 (m, 13H, Ar-H)	
	3080, 1600,790	Aromatic	10.1 (s, 1H, N=CH)	
	1569	N=CH-		
3b	1690 cm^{-1}	C=O	1.8 (d, 1H, N-CH-C)	419
	3454	N-H	3.82 (s, 6H, OCH_3)	
	3080, 1600,790	Aromatic	6.9-8.0 (m, 11H, Ar-H)	
	1360	C-N	10.1 (s, 1H, N=CH)	
	1569	N=CH-		
3c	1690 cm^{-1}	C=O	1.8 (d, 1H, N-CH-C)	387
	3454	N-H	3.82 (s, 3H, OCH_3)	
	3080, 1600,790	Aromatic	6.9-8.1 (m, 12H, Ar-H)	
	1360	C-N	10.1 (s, 1H, N=CH)	
	1569	N=CH-		
3d	1315,1515	NO_2	1.8 (d, 1H, N-CH-C)	404
	1690 cm^{-1}	C=O	6.9-8.1 (m, 12H, Ar-H)	
	3454	N-H	10.1 (s, 1H, N=CH)	
	3080, 1600,790	Aromatic		
	1569	N=CH-		
	1360	C-N		
3e	1690 cm^{-1}	C=O	1.8 (d, 1H, N-CH-C)	393
	3454	N-H	6.9-7.4 (m, 8H, Ar-H)	
	3080, 1600,790	Aromatic	7.7 – 7.8 (m, 2H +2H, Ar-H) p-chloro phenyl ring	
	1569	N=CH-	10.1 (s, 1H, N=CH)	
	1360	C-N		
3f	3280 - 3450	OH	1.8 (d, 1H, N-CH-C)	375
	1690 cm^{-1}	C=O	5 (s, 1H, OH)	
	3454	N-H	6.9-8.5 (m, 12H, Ar-H)	
	3080, 1600,790	Aromatic	10.1 (s, 1H, N=CH)	
	1569	N=CH-		
	1360	C-N		
3g	1690 cm^{-1}	C=O	1.8 (d, 1H, N-CH-C)	428
	3454	N-H	6.9-8.4 (m, 11H, Ar-H)	
	3080, 1600,790	Aromatic	10.1 (s, 1H, N=CH)	
	1569	N=CH-1360 C-N		

Table 3. Physical and analytical data of newly synthesized compounds.

Compound	R	M.P. (°C)	Yield (%)	Rf value*	Molecular formula
4a		211-215	71	0.66	$C_{23}H_{18}ClN_3O_2S$
4b		160-163	64	0.59	$C_{25}H_{22}ClN_3O_4S$
4c		180-187	67	0.63	$C_{24}H_{20}ClN_3O_3S$
4d		195-197	65	0.56	$C_{23}H_{17}ClN_4O_4S$
4e		117-120	60	0.73	$C_{23}H_{17}Cl_2\ N_3O_2S$
4f		209 -213	64	0.76	$C_{23}H_{18}ClN_3O_3S$
4g		173-175	55	0.72	$C_{23}H_{16}Cl_3N_3O_2S$

*methanol and benzene as mobile phase, spot detection-Iodine vapour.

at 37°C for 24 h and the diameter of zone of inhibition were measured and recorded in Table 5.

Acute toxicity study

This involves the estimation of the median lethal dose (LD_{50}), which is the dose that will kill 50% of the animal population within 24 h post treatment of the test substance. The method of Miller and Tainter (1944) was adopted. The animals used for the studies were in accordance with principles of laboratory animal care and were approved by Institutional animal ethical committee. Swiss Albino mice were starved of feed but allowed access to water 24 h prior to the study and were then grouped (five mice per group). They were treated intraperitoneally with different doses of the test compounds (200, 400, 600, 800 and 1000 mg kg^{-1}). The animals were then observed for 24 h for any behavioral effects such as nervousness, excitement, dullness, in-coordination or even death. The LD_{50} was estimated from the geometric mean of the dose that caused 100% mortality and the dose which caused no lethality at all.

Anti-inflammatory activity

The anti-inflammation activity was evaluated by carrageenan induced paw edema method (Winter et al., 1962). Albino rats of Wistar strain weighing 100-200 g of either sex were divided into nine groups each of six animals. The animals were maintained under normal environmental conditions. They were fed ad libitum with standard feed and water. Tween 80 suspension (0.5% v/v) of the test compounds were administered intraperitoneally in a dose of 100 mg kg^{-1}. The control group was given only 0.5% v/v Tween 80 (0.5 ml) suspension. One group was administered with phenylbutazone as standard, intraperitoneally in a dose of 100 mg kg^{-1}. After 30 min of the administration of test compounds and phenylbutazone, paw edema was induced in albino rats by injecting 0.1 ml of carrageenan (0.9% v/v in normal saline) suspension, into subplantar region of the left hind paw of each rat. After 3 h of carrageenan injection, the increase in paw volume was measured by a plethysmometer. The anti-inflammatory activity was measured in terms of percentage inhibition of edema and is analyzed statistically by students “t” test and recorded in Table 6.

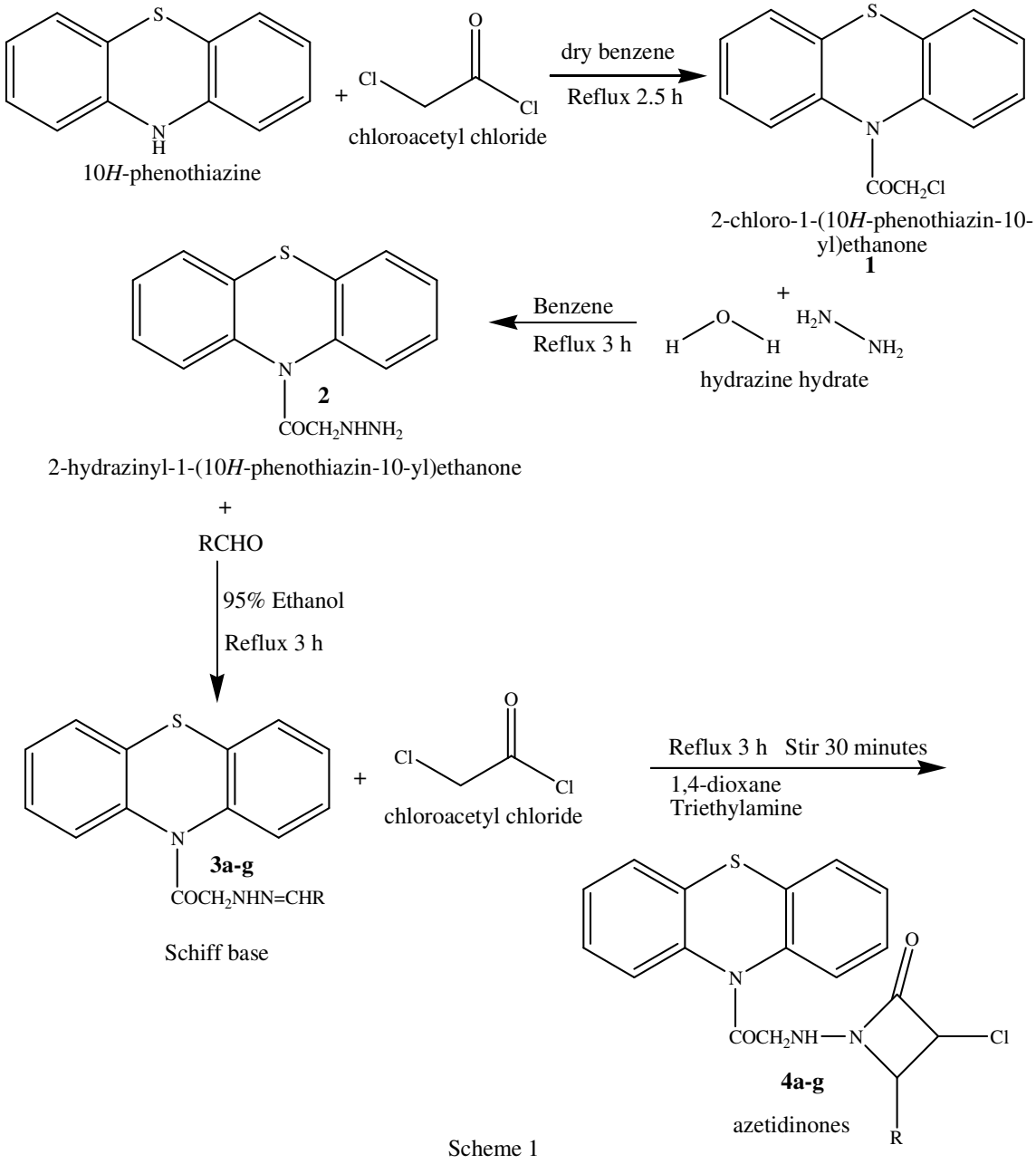
RESULTS AND DISCUSSION

The present protocol describes a simple and efficient method for the synthesis of azetidinones by different Schiff bases of phenothiazines. It has been demonstrated

Table 4. Spectral data of newly synthesized compounds.

Compound	IR (ν, cm^{-1})		^{1}H-NMR in DMSO (δ, ppm)	Mass spectra m/z value
4a	1657	C=O azetidinone	1.8 (d, 1H, N-CH-C)	436
	3420	N-H	2.5 (d, 1H, C-CH-Cl)	
	3100, 1500,770	Aromatic	6.9-7.8 (m, 13H, Ar-H)	
	727	C-Cl	10.45 (s, 1H, CONH)	
4b	1657	C=O azetidinone	1.8 (d, 1H, N-CH-C)	496
	3420	N-H	2.5 (d, 1H, C-CH-Cl)	
	1360	C-N	3.82 (s, 6H, OCH_3)	
	3100, 1500,770	Aromatic	6.9-8.0 (m, 11H, Ar-H)	
	727	C-Cl	10.45 (s, 1H, CONH)	
4c	1657	C=O azetidinone	1.8 (d, 1H, N-CH-C)	466
	3420	N-H	2.5 (d, 1H, C-CH-Cl)	
	1360	C-N	3.82 (s, 3H, OCH_3)	
	3100, 1500,770	Aromatic	6.9-8.1 (m, 12H, Ar-H)	
	727	C-Cl	10.45 (s, 1H, CONH)	
4d	1315,1515	NO_2	1.8 (d, 1H, N-CH-C)	481
	1657	C=O azetidinone	2.5 (d, 1H, C-CH-Cl)	
	3420	N-H	6.9-8.1 (m, 12H, Ar-H)	
	1360	C-N	10.45 (s, 1H, CONH)	
	3100, 1500,770	Aromatic		
	727	C-Cl		
4e	1657	C=O azetidinone	1.8 (d, 1H, N-CH-C)	470
	3420	N-H	2.5 (d, 1H, C-CH-Cl)	
	1360	C-N	6.9-7.4 (m, 8H, Ar-H)	
	3100, 1500,770	Aromatic	7.7 – 7.8 (m, 2H +2H, Ar-H) p-chloro phenyl ring	
	727	C-Cl	10.45 (s, 1H, CONH)	
4f	3280 - 3450	OH	1.8 (d, 1H, N-CH-C)	452
	1657	C=O azetidinone	2.5 (d, 1H, C-CH-Cl)	
	3420	N-H	5 (s, 1H, OH)	
	1360	C-N	6.9-8.5 (m, 12H, Ar-H)	
	3100, 1500,770	Aromatic	10.45 (s, 1H, CONH)	
	727	C-Cl		
4g	1657	C=O azetidinone	1.8 (d, 1H, N-CH-C)	505
	3420	N-H	2.5 (d, 1H, C-CH-Cl)	
	1360	C-N	6.9-8.4 (m, 11H, Ar-H)	
	3100, 1500,770	Aromatic	10.45 (s, 1H, CONH)	
	727	C-Cl		

that cyclocondensation of Schiff bases with chloroacetyl chloride in triethylamine revealed with fairly high yields in a relatively short reaction time and easy work-up procedures. These conditions enable this method to be applicable for the synthesis of 2-azetidinone based heterocyclic. The purity of the synthesized compounds were confirmed by performing TLC, which gave single spot (Tables 1 and 2). IR absorption band at 1569 cm^{-1}



Scheme 1

Figure 1. Synthesis of azetidinones (4a-g).

for stretching vibration of -CH=N- and ^{1}H NMR signals for the presence of one imine proton (CH=N-) at 10.0357 ppm (1H, s), confirms the condensation of reactants to form Schiff-base. Similarly IR, ^{1}HNMR and mass spectraldata obtained were in correlation with synthesized azetidinones.

The synthesized compounds 4a-g was evaluated for their *in vitro* anti-tuberculosis activity against M. tuberculosis strain $H_{37}Rv$ at 1, 10 and 100 µg mL^{-1} concentration. Pyrazinamide was used as the standard drug for comparison. The antitubercular data revealed that the all synthesized azetidinones proved to be active against the test organism M. tuberculosis, $H_{37}R_V$ strain, at 100, 10 and 1 µg mL^{-1} levels.

The compounds tested for anti-bacterial activity proved to be effective particularly against the Gram-positive organisms like *S. aureus* and *B. subtilis*. None of the compounds produced inhibition zone against Gram-

Table 5. Antimicrobial activity data of the titled compounds.

Compound	Zone of inhibition in mm						Concentration in µg mL^{-1}		
	Bacteria				Fungi		Mycobacterium tuberculosis		
	S.a	B.s	E.c	P.a	C.a	A.f	100	10	1
Control	No zone inhibition						++	++	++
Streptomycin	19	18	19	20	--	--	--	--	--
Clotrimazole	---	--	-	--	20	21	--	--	--
Pyrazinamide	--	--	--	--	--	--	-ve	-ve	-ve
4a	09	08	00	00	00	12	-ve	-ve	-ve
4b	10	08	00	00	00	12	-ve	-ve	-ve
4c	08	10	00	0	00	11	-ve	-ve	-ve
4d	08	09	00	00	00	09	-ve	-ve	-ve
4e	15	18	00	00	00	10	-ve	-ve	-ve
4f	09	07	00	00	00	11	-ve	-ve	-ve
4g	09	08	00	00	00	12	-ve	-ve	-ve

S.a-*Staphylococcus aureus*, B.s-*Bacillussubtilis*, E.c-*Escherichia coli*, P.a-*Pseudomonas aeruginosa*, C.a- *Candida albicans*, A.f-*Aspergillus fumigatus*, -ve indicates complete inhibition of H_{37} RV, ++ indicates intensive growth of *M. tuberculosis.*

Table 6. Evaluation of anti-inflammatory activity of the synthesized compounds by carrageenan induced paw edema method.

Compound (100 mg/kg^{-1})	Paw volume (mean ± SEM)	Percentage Inhibition after 3 h
Control	0.44 ± 0.190	---
Phenylbutazone	0.17 ± 0.020	61.07*
4a	0.42 ± 0.020	13.26*
4b	0.49 ± 0.030	14.92*
4c	0.38 ± 0.038	12.98*
4d	0.47 ± 0.030	14.32*
4e	0.44 ± 0.020	13.88*
4f	0.36 ± 0.030	12.44*
4g	0.52 ± 0.220	15.12*

*$p < 0.05$ represent significant difference when compared with control groups.

negative organisms like *E. coli* and *P. aeruginosa*. Compound 4e having 4-chloro phenyl substitution showed potent anti-bacterial activity, which is equipotent activity with the reference standard streptomycin against *B. subtilis*. It also exhibited a good anti-bacterial activity against *S. aureus*. The anti-fungal activity was tested against the fungal species *A. fumigatus* and *C. albicans* at 100 µg mL^{-1} concentration. All the compounds 4a-g showed almost equal anti-fungal activity against *A. fumigatus*, but none of the compounds produced inhibition zone against *C. albicans*.

All the doses employed for acute toxicity studies found to be non toxic, since there is no mortality observed up to the dose of 1000 mg kg^{-1}. The compounds 4a-g afforded 12-15% protection against carrageenan induced paw edema, where as the standard drug phenylbutazone under similar conditions showed 61% inhibition after 3 h of carrageenan injection. All the tested compounds have demonstrated poor anti-inflammation properties.

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