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Design and Synthesis of Biologically active Azetidinones nucleus containing 1, 3, 4-thiadiazole derivatives and evaluate their Tuberculosis activity

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Abstract: In present study, a series of 1,3,4-thiadiazol derivatives have been synthesized from Schiff base. All these newly synthesized compounds for biological activity compaired against antibacterial, antifungal and antituberculosis standards. Most of the these newly synthesized azetidinone derivatives have shown significant antibacterial activity against Gram-positive bacteria and Gram- negative bacteria .The synthesized compounds were showed better results for antibacterial evaluation against gram-positive (Staphylococcus aureus, Bacillus subtilis), gram-negative (Escherichia coli, Salmonella, Pseudomonas aeruginosa) and showed significant antifungal activity against fungal strains (Candida albicans, A. niger) and showed better antitubaeculosis activity against Mycobacterium tuberculosis CIP and M. tuberculosis H37Rv. The newly synthesized compounds found to be good antibacterial, antifungal and also antituberculosis agents.

Keywords: 1,3,4-thiadiazol, Azetidinones, Antibacterial, Antifungal, Antitubercular.

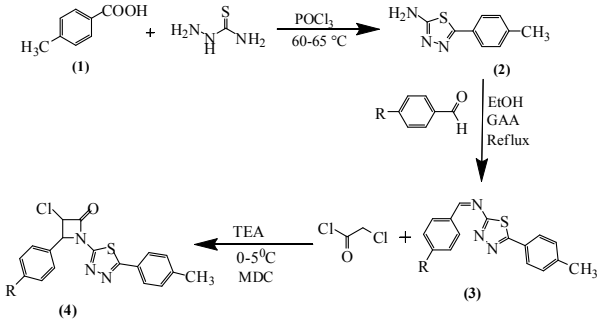
Introduction: The 1,3,4-thiadiazol nucleus is important and famous heterocyclic nuclei, which shows common and essential features of a variety of natural products and medicinal agents [1]. 1,3, 4-thiadiazol, and azetidinones these moiety is present into synthesis of a new class of heterocyclic molecules and attempt to develop possible biological active molecules. Natural and synthetic azetidinone derivatives occupy a central place among medicinally important compounds due to their different and interesting biological activities [2]. For such kind of activity, we want to synthesize new derivatives having 1, 3, 4- thiadiazole and azetidinone moiety into it. It is also well established that various derivatives of azetidinone show wide range of pharmacological properties such as anti-inflammatory [2], antiviral [3], anticonvulsant [4,5], antifungal [6], antibacterial [7], antimicrobial [8], antituberculosis [9]. Natural and synthetic 1,3,4-thiadiazol derivatives also show medicinally important compounds due to

their different and interesting antibiotic activities [10-13]. Many natural occurring biologically active compounds possesses heterocyclic moiety including organic resources also which is essential to the life of modern society and is dependent on synthetic heterocycles compound for new discovery of drugs. The structures of these compounds were confirmed by means of IR, MS, ^{1}H NMR.

Materials and method

The entire chemicals and solvents are used in the present investigation were purchased from commercial suppliers and used without further purification. The reactions and purity of the products were monitored by thin layer chromatography (TLC) using silica gel coated aluminum sheets. Melting points are determined on open capillary tubes and are uncorrected. Mass spectra were recorded on Shimadzu mass-spectrometer. NMR spectra were recorded on a Bruker advance II 400 NMR Spectrometer.

Scheme:



Scheme-1. Synthetic route of target compounds

Where R=H, CH_3, OCH_3, OC_2H_5, Cl, Br & EtOH=C_2H_5OH, GAA=Glacial Acetic Acid, TEA=Triethylamine,MDC=Dichloromethane.

Results and Discussion:

Desired products (4) were synthesized using an identical effective and conventional synthetic route outlined in Scheme. Compound (4) was synthesized by reacting with 4-methyl benzoic acid and thiosemicarbazide in presence of phosphorus oxychloride at 60-65^{0}C for one hour. The reaction mass was cooled and diluted with water and again refluxed for 3 h. The reaction was monitored by thin layer chromatography and filtered after completion of reaction. The filtrate was basified with Potasium hydroxide and the precipitate was filtered off and then recrystallized from ethanol to give the desired compound (2) and then compound (2) was reacted with substituted aromatic aldehyde to form Schiff's base (3) and further compound (3) was reacted with chloroacetyl chloride in presence of triethyl amine and MDC (dichloromethane) as a solvent at 0-5^{0}C to form substituted compound (4) as a product in good yield.

General procedure for the synthesis of the derivative 5-p-tolyl-1,2,4-thiadiazol-2-yl) benzamide:

5-p-tolyl-1,3,4-thiadiazol-2-amine: (2)

In a clean four Neck RBF, take a solution of 4-methyl benzoic acid (0.05 mol) was refluxed with thiosemicarbazide (0.05 mol) in the presence of phosphorus oxychloride (15 ml) for 1 h. The reaction mixture was cooled and diluted with water and again refluxed for 4 h. The reaction was monitored by thin layer chromatography and filtered after completion. The filtrate was basified with potassium hydroxide and the precipitate was filtered off and then recrystallized from ethanol to give the desired compound 2. mp:204-206^{0}C ^{1}H-NMR d (ppm) δ: 2.35 (3H, s, CH_3); 7.29-7.67 (4H, m, Ar-H); 7.22 (2H, s, -NH_2) MS: m/z 192 (M + 1).

(4-methylbenzylidene)-5-p-tolyl-1,3,4-thiadiazol-2-amine: (3)

A mixture of (2) (0.01 mol) and aromatic

aldehyde (0.01 mol) were dissolved in 30ml of ethanol containing few drops of glacial acetic acid. The reaction was refluxed for 5h. Cooled and then poured into crushed ice and the resultant solid was recrystallized from ethanol.

3-chloro-4-p-tolyl-1-(5-p-tolyl-1,3,4-thiadiazol-2-yl)azetidin-2-one: (4)

Compound (3) (0.01 mol) was dissolved in ethanol (40ml) and triethylamine (0.02 mol) was added to it. Chloroacetyl chloride (0.02 mol) was introduced dropwise at 0-5^{0}C over a period of 1h with constant stirring. After addition of chloroacetyl chloride the reaction mixture was refluxed for 2h. After cooling, the reaction mixture was washed with water and recrystallized from ethanol.

1) 3-chloro-4-phenyl-1-(5-p-tolyl-1,3,4-thiadiazol-2-yl)azetidin-2-one: $C_{18}H_{14}ClN_3OS$ mp:207-210^{0}C.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.36 (3H, s, $-CH_3$), 7.31-7.69 (4H, m, Ar-H), 7.27-7.41 (5H, m, Ar-H), 5.02 (1H, s, N-CH), 5.44 (1H, s, CH-Cl), M/Z: m/z 356 [M+1].

2) 3-chloro-4-p-tolyl-1-(5-p-tolyl-1,3,4-thiadiazol-2-yl)azetidin-2-one: $C_{19}H_{16}ClN_3OS$ mp:226-227^{0}C.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.32 (3H, s, $-CH_3$), 2.35 (3H, s, $-CH_3$), 7.27-7.67 (4H, m, Ar-H), 7.28-7.43 (4H, m, Ar-H), 5.04 (1H, s, N-CH), 5.45 (1H, s, CH-Cl), M/Z: m/z 371 [M+1].

3) 3-chloro-4-(4-hydroxyphenyl)-1-(5-p-tolyl-1,3,4-thiadiazol-2-yl)azetidin-2-one: C18H14ClN3O2S mp:218-2210C.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.33 (3H, s, $-CH_3$), 7.30-7.69 (4H, m, Ar-H), 6.70-7.12 (4H, m, Ar-H), 5.03 (1H, s, N-CH), 5.47 (1H, s, CH-Cl), 9.43 (1H, s, Ar-OH), M/Z: m/z 373 [M+1].

4) 3-chloro-4-(3-hydroxyphenyl)-1-(5-p-tolyl-1,3,4-thiadiazol-2-yl)azetidin-2-one: $C_{18}H_{14}ClN_3O_2S$ mp:200-202^{0}C.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.31 (3H, s, $-CH_3$), 7.25-7.66 (4H, m, Ar-H), 6.73-7.15 (4H, m, Ar-H), 5.01 (1H, s, N-CH), 5.43 (1H, s, CH-Cl), 9.45 (1H, s, Ar-OH), M/Z: m/z 373 [M+1].

5) 3-chloro-4-m-tolyl-1-(5-p-tolyl-1,3,4-thiadiazol-2-yl)azetidin-2-one: $C_{19}H_{16}ClN_3OS$ mp:208-210^{0}C.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.33 (3H, s, $-CH_3$), 2.36 (3H, s, $-CH_3$), 7.26-7.70 (4H, Ar-H), 7.27-7.41 (4H, m, Ar-H), 5.02 (1H, s, N-CH), 5.44 (1H, s, CH-Cl), M/Z: m/z 371 [M+1].

6) 3-chloro-4-(4-methoxyphenyl)-1-(5-p-tolyl-1,3,4-thiadiazol-2-yl)azetidin-2-one: $C_{19}H_{16}ClN_3O_2S$ mp:198-201^{0}C.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.38 (3H, s, $-CH_3$), 3.83 (3H, s, $-OCH_3$), 7.23-7.71 (4H, m, Ar-H), 6.94-7.18 (4H, m, Ar-H), 5.03 (1H, s, N-CH), 5.47 (1H, s, CH-Cl), M/Z: m/z 389 [M+1].

7) 3-chloro-4-(4-ethoxyphenyl)-1-(5-p-tolyl-1,3,4-thiadiazol-2-yl)azetidin-2-one: $C_{20}H_{18}ClN_3O_2S$ mp:203-205^{0}C.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.32 (3H, s, $-CH_3$), 1.32- 4.10 (5H, m, $-OC_2H_5$), 7.22-7.68 (4H, m, Ar-H), 6.93-7.20 (4H, m, Ar-H), 5.01 (1H, s, N-CH), 5.45 (1H, s, CH-Cl), M/Z: m/z 404 [M+1].

8) 3-chloro-4-(3-methoxyphenyl)-1-(5-p-tolyl-1,3,4-thiadiazol-2-yl)azetidin-2-one: $C_{19}H_{16}ClN_3O_2S$ mp:190-192^{0}C.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.36 (3H, s, $-CH_3$), 3.85 (3H, s, $-OCH_3$), 7.25-7.69 (4H, m, Ar-H), 6.91-7.18 (4H, m, Ar-H), 5.04 (1H, s, N-CH), 5.49 (1H, s, CH-Cl), M/Z: m/z 389 [M+1].

9) 4-(4-bromophenyl)-3-chloro-1-(5-p-tolyl-1,3,4-thiadiazol-2-yl)azetidin-2-one: $C_{18}H_{13}BrClN_3OS$ mp:221-223^{0}C.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.31 (3H, s,

-CH_3), 7.22-7.671 (4H, m, Ar-H), 7.21-7.95 (4H, m, Ar-H), 5.07 (1H, s, N-CH), 5.42 (1H, s, CH-Cl), M/Z: m/z 439 [M+1].

10) 3-chloro-4-(4-chlorophenyl)-1-(5-p-tolyl-1,3,4-thiadiazol-2-yl)azetidin-2-one: $C_{18}H_{13}Cl_2N_3OS$ mp:212-214^{0}C.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.30 (3H, s, -CH_3), 7.30-7.67 (4H, s, Ar-H), 7.41-7.50 (4H, m, Ar-H), 5.02 (1H, s, N-CH), 5.44 (1H, s, CH-Cl), M/Z: m/z 394 [M+1].

11) 4-(3-bromophenyl)-3-chloro-1-(5-p-tolyl-1,3,4-thiadiazol-2-yl)azetidin-2-one: $C_{18}H_{13}BrClN_3OS$ mp:205-208^{0}C.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.28 (3H, s, -CH_3), 7.32-7.66 (4H, m, Ar-H), 7.18-7.92 (4H, m, Ar-H), 5.07 (1H, s, N-CH), 5.46 (1H, s, CH-Cl), M/Z: m/z 439 [M+1].

12) 3-chloro-4-(3-chlorophenyl)-1-(5-p-tolyl-1,3,4-thiadiazol-2-yl)azetidin-2-one: $C_{18}H_{13}Cl_2N_3OS$ mp:200-201^{0}C.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.33 (3H, s, -CH_3), 7.29-7.68 (4H, m, Ar-H), 7.44-7.48 (4H, m, Ar-H), 5.02 (1H, s, N-CH), 5.45 (1H, s, CH-Cl), M/Z: m/z 394 [M+1].

Table:1

Sr. No	-R-	Product	Yield (%)
1	-C_6H_5	$C_{18}H_{14}ClN_3OS$	80
2	p-CH_3-C_6H_4	$C_{19}H_{16}ClN_3OS$	81
3	p-OH-C_6H_4	$C_{18}H_{14}ClN_3O_2S$	84
4	m-OH-C_6H_4	$C_{18}H_{14}ClN_3O_2S$	78
5	m-CH_3-C_6H_4	$C_{19}H_{16}ClN_3OS$	82
6	p-OCH_3-C_6H_4	$C_{19}H_{16}ClN_3O_2S$	84
7	p-OC_2H_5-C_6H_4	$C_{20}H_{18}ClN_3O_2S$	76
8	m-OCH_3-C_6H_4	$C_{19}H_{16}ClN_3O_2S$	79
9	p-Br-C_6H_4	$C_{18}H_{13}BrClN_3OS$	76
10	p-Cl-C_6H_4	$C_{18}H_{13}Cl_2N_3OS$	78
11	m- Br-C_6H_4	$C_{18}H_{13}BrClN_3OS$	75
12	m- Cl-C_6H_4	$C_{18}H_{13}Cl_2N_3OS$	71

Antibacterial Activity:

In vitro antibacterial activity was determined by standardized disk diffusion methods. The newly synthesized compounds (1-12) were tested for their antimicrobial activity. In this work Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa bacterial strains were used. The test bacteria were subcultured using nutrient agar medium. Freshly prepared sterilized nutrient agar media were poured 20 ml into each petri plate and allowed to solidify. with the respective strains of bacteria was transferred aseptically. The plates were kept undisturbed for at least 20 min in refrigerator to allow diffusion of the solution properly in the nutrient agar medium. The plates were incubated at 37 ± 1^{0}C for 24 h. Then a well 0.5cm was made in the medium by using sterile cork borer, 50µl of the every compound were transferred into separate wells. Then these plates were incubated at 37^{o}C for 24 hours. After incubation period the results were observed and measured the diameter of inhibitor zone around the each well. The zones of growth inhibition around the well were measure after 24 hours at 37^{0}C. Streptomycin was used as standard drug for antibacterial activity.

Table 2 : Synthesized Antibacterial Derivatives:

Sr. No	C Compound	Product	S. aureus ZOI (mm)	B. subtilis ZOI (mm)	E. coli ZOI (mm)	P. aeruginosa ZOI (mm)
1	-C_6H_5	$C_{16}H_{13}N_3OS$	16	14	15	16
2	p-CH_3-C_6H_4	$C_{17}H_{15}N_3OS$	13	11	12	10
3	p-OH-C_6H_4	$C_{17}H_{15}N_3O_2S$	10	13	12	10
4	m-OH-C_6H_4	$C_{17}H_{15}N_3OS$	15	16	15	14
5	m-CH_3-C_6H_4	$C_{17}H_{15}N_3O_2S$	16	17	18	16
6	p-OCH_3-C_6H_4	$C_{18}H_{17}N_3OS$	11	10	10	12
7	p-OC_2H_5-C_6H_4	$C_{18}H_{17}N_3O_2S$	13	12	13	11

8	m - O C H $_3$ - C_6H_4	$C_{17}H_{14}ClN_3OS$	**12**	**11**	**12**	**11**
9	p-Br-C_6H_4	$C_{16}H_{12}ClN_3OS$	**10**	**13**	**13**	**11**
10	p-Cl-C_6H_4	$C_{16}H_{12}BrN_3OS$	**11**	**13**	**14**	**10**
11	m- Br-C_6H_4	$C_{17}H_{14}ClN_3OS$	**14**	**15**	**16**	**14**
12	m- Cl-C_6H_4	$C_{17}H_{14}BrN_3OS$	**14**	**16**	**16**	**13**
13	--- -	**Steptomycin(Std)**	**17**	**18**	**20**	**18**

Antifungal Activity:

In vitro antifungal activity was determined by agar well diffusion method. The newly synthesized compounds (1-12) were screened for their antifungal activity. In this work and Candida albicans and A.niger fungal strains were used. The test fungi were subcultured using potato dextrose agar medium. Freshly prepared sterilized potato dextrose agar media were poured 20 ml into each petri plate and allowed to solidify. The test fungal cultures were evenly spread over using sterile cotton swab. Then a well 0.5cm was made in the medium by using sterile cork borer, 50µl of the every compound were transferred into separate wells. Then these plates were incubated at 27^{o}C for 48-96 hours. After incubation period the results were observed and measured the diameter of inhibitor zone around the each well. The zones of growth inhibition around the well were measure after 48 to 96 hours at 28°C. Nystatin was used as standard drug for antifungal activity.

Table 3 : Synthesized Antifungal Derivatives:

Sr. No	Compound	Product	C. albicans ZOI (mm)	*A. niger* ZOI (mm)
1	-C_6H_5	$C_{16}H_{13}N_3OS$	**12**	**14**
2	p-CH_3-C_6H_4	$C_{17}H_{15}N_3OS$	**15**	**12**
3	p-OH-C_6H_4	$C_{17}H_{15}N_3O_2S$	**17**	**16**
4	m-OH-C_6H_4	$C_{17}H_{15}N_3OS$	**20**	**19**
5	m-CH_3-C_6H_4	$C_{17}H_{15}N_3O_2S$	**19**	**17**
6	p-OCH_3-C_6H_4	$C_{18}H_{17}N_3OS$	**15**	**18**
7	p-OC_2H_5-C_6H_4	$C_{18}H_{17}N_3O_2S$	**16**	**17**
8	m-OCH_3-C_6H_4	$C_{17}H_{14}ClN_3OS$	**18**	**19**
9	p-Br-C_6H_4	$C_{16}H_{12}ClN_3OS$	**12**	**14**
10	p-Cl-C_6H_4	$C_{16}H_{12}BrN_3OS$	**11**	**18**
11	m- Br-C_6H_4	$C_{17}H_{14}ClN_3OS$	**14**	**13**
12	m- Cl-C_6H_4	$C_{17}H_{14}BrN_3OS$	**13**	**15**
13	- -	**Nystatin (Std)**	**24**	**25**

Antitubercular activity

The antimycobacterial activity of compounds (**1-12**) was assessed against *M. tuberculosis* using the microplate Alamar blue assay (MABA). This methodology is nontoxic, uses thermally stable reagent and showed good correlation with proportional and BACTEC radiometric methods 25 and the activity is expressed as the minimum inhibitory concentration (MIC) in µg/mL. The final drug concentration tested was 0.01-100 µg/mL. A blue colour in the well was interpreted as no bacterial growth and pink colour was scored as growth. The MIC was defined as the lowest drug concentration, which prevented a colour change from blue to pink. The MICs of the compounds were given in Table-4. Streptomycin, pyrazinamide and Ciprofloxacin were used as standards. All the tested compounds showed satisfactory result *in vitro* activity against streptomycin, pyrazinamide and Ciprofloxacin as standard drugs.

Table 4 : Synthesized Antifungal Derivatives:

Sr. No	Compound	Product	MIC(µg/mL)
1	-C_6H_5	$C_{16}H_{13}N_3OS$	**13.6**
2	p-CH_3-C_6H_4	$C_{17}H_{15}N_3OS$	**6.21**
3	p-OH-C_6H_4	$C_{17}H_{15}N_3O_2S$	**6.13**
4	m-OH-C_6H_4	$C_{17}H_{15}N_3OS$	**24.41**

5	m-CH_3-C_6H_4	$C_{17}H_{15}N_3O_2S$	**22.87**
6	p-OCH_3-C_6H_4	$C_{18}H_{17}N_3OS$	**7.21**
7	p-OC_2H_5-C_6H_4	$C_{18}H_{17}N_3O_2S$	**6.32**
8	m-OCH_3-C_6H_4	$C_{17}H_{14}ClN_3OS$	**21.34**
9	p-Br-C_6H_4	$C_{16}H_{12}ClN_3OS$	**6.43**
10	p-Cl-C_6H_4	$C_{16}H_{12}BrN_3OS$	**6.11**
11	m- Br-C_6H_4	$C_{17}H_{14}ClN_3OS$	**24.82**
12	m- Cl-C_6H_4	$C_{17}H_{14}BrN_3OS$	**25**
13	-	**Streptomycin (Std)**	**6.25**
14	-	**Pyrazinamide (Std)**	**3.25**
15	-	**Ciprofloxacine (Std)**	**3.25**

Conclusion:

In conclusion, we have designed and synthesized biologically active Azetidinones nucleus containing 1, 3, 4-thiadiazole derivatives by suitable methods. The satisfactory results obtained by the synthesized compounds, and the attracting significance of thiadiazole can be better explored in future as an effective candidate for antitubercular activity. It is also note that the toxicity studies have been carried out for these compounds and least toxicity is being found in all these compounds.The most compounds shows high valuable character for further evaluation.

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