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ORIGINAL ARTICLE

Synthesis and Characterization of 1,2,4-Triazolo [3,4-B] [1,3,4]-Thiadiazole Derivatives as Potent Antimicrobial Agent

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Abstract:

The chemistry of heterocyclic compounds has been an interesting field of study for a long time. The heterocyclic nucleus 1,2,4-triazolo [3,4-b] [1,3,4]-thiadiazole derivatives constitutes an important class of compounds development. New drug synthesis of novel 1,2,4-triazolo [3,4-b][1,3,4]-thiadiazol derivatives and investigation of their chemical and biological behavior have gained more importance in recent decades. Different 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles were prepared by condensation of 4-amino-5-(2-(4-isobutyl phenyl) propyl)-4H-1,2,4-triazole-3-thiol (4) with acetic acid and substituted aromatic acids through a one-pot reaction. These compounds were investigated for their antimicrobial activity. Some of the tested compounds also showed moderate antimicrobial activity against tested bacterial and fungal strains.

Introduction

Due to a number of causes, such as newly emerging infectious diseases and an increase in multi-drug resistance microbial pathogens, treating infectious diseases remains a big and challenging problem. Despite the abundance of antibiotics and chemotherapeutics that are now used in medicine, the development of both new and old forms of antibiotic resistance over the past few decades has highlighted the urgent need for new classes of antimicrobial drugs. There is a genuine need for the development of novel molecules endowed with antimicrobial activity, possibly working through mechanisms distinct from those of well-established classes of antibacterial drugs, to which many clinically relevant infections are currently resistant. It was discovered that 1,2,4-triazole and their derivatives may be regarded as antibacterial agents through the numerous compounds that were designed and synthesized.[1]

A variety of 1,2,4 triazole derivatives and their N-bridged heterocyclic analogues' biological activity. Triazole has a variety of uses in the realm of medicine when fused with other five- or six-membered ring structures. [2]

Triazoles are fused with thiadiazole, which exhibits antifungal, antiviral, antibacterial, analgesic, anti-inflammatory, anticancer, and anthelmintic activity. The activity occurs because N-C-S linkage is incorporated, as in the frame of 1,2,4 triazolo[3,4-b], 1,3,4-thiadiazole, which exhibits a broad spectrum of antimicrobial activity.[2-6]

The main objective is to synthesize, characterize, and assess 1,2,4-triazolo [3,4-b]-1,3,4-thiadiazoles substituted compounds that exhibit improved and broad range antibacterial activity.

Experimental Work

The present work comprises of the preparation 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles derivatives 3-(2-(4-isobutyl phenyl) propyl)-6-(alkyl/aryl substituted) 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles derivatives**.**

2-(4-isobutyl phenyl) propanoate. (**2**)

A mixture of Ibuprofen (2.068gm, 10mmol), absolute ethanol 50ml and Conc. H_2SO_4 0.5ml was taken in a round bottom flask and refluxed the reaction mixture for 8 – 10 hrs. The reaction mixture was cooled to 5^oC and solvent should be distilled under vaccum. Added water to the reaction mixture and treated with 1% sodium bicarbonate solution. The reaction mixture was extracted with ethyl acetate (3x5). Extracted ethyl acetate layer should be separated and dried over magnesium sulfate. Distilled the excess solvent and collected the oily ester (**2**).[15]

2-(4-isobutyl phenyl) propane hydrazide. (**3**)

Dissolved 2-(4-isobutyl phenyl) propanoate (**2**) (2.34gm, 9.98mmol) in 30ml absolute ethanol and hydrazine hydrate (99%) (1.005gm, 14.98mmol) was added drop wise to the reaction mixture with stirring. The resulting mixture was allowed to reflux for 6hr. Excess ethanol was distilled off and content was allowed to cool. The crystal formed was filtered, washed with water and dried. The completion of reaction monitored by TLC using petroleum ether and ethyl acetate (1:1) as eluent. Recrystallised the product with ethanol to obtained 2-(4-isobutyl phenyl) propane hydrazide (**3**).[16]

4-amino-5-(2-(4-isobutyl phenyl) propyl)-4H-1,2,4-triazole-3-thiol.(**4**)

Potassium hydroxide (0.84gm, 14.97mmol) was dissolved in absolute ethanol 200ml. To this solution 2-(4-isobutyl phenyl) propane hydrazide(**3**) (2.203gm;9.99mmol) was added and cooled the solution in ice. To this carbon disulfide (1.2ml, 15mmol) was added in small portion with constant stirring the reaction mixture was stirred continuously at room temperature for period of 10 -12 hrs. It was then diluted with anhydrous diethyl ether. The precipitated potassium thiocarbazide salt was collected by filtration the precipitate was further washed with anhydrous ether and dried under vaccum. The potassium salt obtained was in quantitative yield and used in next step without further purification. A suspension of Potassium thiocarbazide salt in water(50ml) and hydrazine hydrate (99%) (2.015ml, 30mmol) was refluxed for 18-20hrs with occasional shaking. The color of reaction mixture was change to deep green color with evolution of H_2S gas. A homogenous reaction mixture was obtained during the reaction process. The reaction mixture was cooled to room temperature and diluted with water(100ml) on acidification with Conc. HCl, the required triazole was precipitated out, filtered and washed thoroughly with cold water. Recrystallized the crude product with absolute ethanol to obtain 4-amino-5-(2-(4-isobutyl phenyl) propyl)-4H-1,2,4-triazole-3-thiol.(**4**).[16]

Procedure for synthesis of 3-(2-(4-isobutyl phenyl) propyl)-6-(alkyl/aryl substituted) 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles derivatives 5(a - g))

An equimolar mixture of 4-amino-5-(2-(4-isobutyl phenyl) propyl)-4H-1,2,4-triazole-3-thiol.(**4**) (2.904gm, 9.98mmol) and substituted acetic acid (2.4ml; 39,98mmol) in phosphorus oxy chloride (10ml) was refluxed for 5 – 7 hrs. The reaction mixture was cooled to room temperature and then gradually poured onto crushed ice with stirring. Finely powdered Potassium Carbonate and required amount of Potassium Hydroxide were added till the pH of mixture raised to 8, to remove excess of $POCl_3$. The reaction mixture was allowed to stand overnight and solid separated out. It was filtered, washed thoroughly with cold water, dried and recrystallised from hot ethanol to obtained 3-(2-(4-isobutyl phenyl) propyl)-6-alkyl/aryll-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazole (5(a-g))**.**

All physicochemical data and Spectral analysis were summarized in Table 1 and 2.

Result and discussion

The present work comprises of synthesis of new antimicrobial agent, in which iso butyl propane was used as a starting material to which the heterocyclic rings i.e. thiadiazole and triazole are substituted (it is reported that the presence of heterocyclic ring in lead structure increases the probability of that compound to be a good antimicrobial activity), so due to the presence of above features in new compounds we can say that

the drug should exhibit good antimicrobial activity. 1,2,4 triazole, 1,3,4 thiadiazole and their condensed heterocyclic derivatives i.e. 1,2,4 – triazolo[3,4-b] thiadiazole have been reported to possess anti-microbial activity.

Table No. 1 Physicochemical data

Compound Code	COMPOUND	M.P. (^oC)	% YIELD	MOL. WT.	MOL. FORMULA
2	2-(4-isobutyl phenyl) propanoate.	132	68.38	234.3340	$C_{15}H_{22}O_2$
3	2-(4-isobutyl phenyl) propane hydrazide.	210	57.07	220.3107	$C_{13}H_{20}N_2O$
4	4-amino-5-(2-(4-isobutyl phenyl) propyl)-4H 1,2,4 triazole-3-thiol.	155	66.50	290.4270	$C_{15}H_{22}N_4S$
5 (a)	3-(2-(4-isobutyl phenyl) propyl)-6-methyl-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazole.	130	33.26	314.4484	$C_{17}H_{22}N_4S$
5 (b)	3-(2-(4-isobutyl phenyl) propyl)-6-phenyl-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazole.	122	25.07	376.5178	$C_{22}H_{24}N_4S$
5 (c)	3-(2-(4-isobutyl phenyl) propyl)-6- (4-chloro phenyl)-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazole.	168	32.82	410.9628	$C_{22}H_{23}ClN_4S$
5 (d)	3-(2-(4-isobutyl phenyl) propyl)-6-(4-methoxy phenyl)-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazole.	160	35.56	406.5437	$C_{23}H_{36}N_4OS$
5 (e)	3-(2-(4-isobutyl phenyl) propyl)-6-(4-nitro phenyl)-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazole.	174	38.76	421.5153	$C_{22}H_{23}N_5O_2S$
5 (f)	3-(2-(4-isobutyl phenyl) propyl)-6-(4-amino phenyl)-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazole	142	31.45	391.5324	$C_{22}H_{25}N_5S$
5 (g)	3-(2-(4-isobutyl phenyl) propyl)-6-(4-hydroxy phenyl)-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazole	168	34.92	392.5172	$C_{22}H_{24}N_4OS$

Table 2 Spectral data of 1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazoles derivatives: -

S.No.	Compound	I.R. (KBr) cm^{-1}	^1H NMR ($CDCl_3$) (δ ppm)
4	4-amino-5-(2-(4-isobutyl phenyl) propyl)-4H 1,2,4triazole-3-thiol.	3280.1 N-H st. (Het.Aro.), 3001.9 C-H st. (aro), 2868.8 C-H st. (ali) 2601.7 S-H st. (Thiol.), 1682.6 N-N st. (Het.Aro.), 1475.2 C=N st. (Het.Aro.) 1414.6 C=C st. (aro) 1359.0 Ring st. (Het.Aro.) 822.3 C-C st. (ali) 659.5 C-S st. (Het.Aro.)	7.18(d, 2H, J=7.5Hz, Ar-***H***). 7.08(d, 2H, J=7.5Hz, Ar-***H***). 3.37(q, 1H, J=6.0Hz, C***H***). 3.12(s, 1H, S***H***). 2.54(d, 2H, J=6.0Hz, C$\boldsymbol{H}_2$). 2.18(m, 1H, J=3.6, C***H***). 1.77(s, 2H, N$\boldsymbol{H}_2$), 1.42(d, 3H, J=6.0, C$\boldsymbol{H}_3$). 1.06(d, 6H, J=6.0Hz,C$\boldsymbol{H}_3)_2$)

5 (a)	3-(2-(4-isobutyl phenyl) propyl)-6-methyl-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazole.	3026.5 C-H st. (aro) 2921.4 C-H st. (ali) 1643.5 N-N st. (Het.Aro.) 1511.0 C=N st. (Het.Aro.) 1474.9 C=C st. (aro) 1332.6 Ring st. (Het.Aro.) 749.6 C-C st. (ali) 645.0 C-S st. (Het.Aro.)	7.20(d, 2H, J=7.5Hz, Ar-***H***) 7.09(d, 2H, J=7.5Hz, Ar-***H***) 3.48(q, 1H, J=2.4Hz, C***H***); 2.51(s, 3H, C$\boldsymbol{H}_3$) 2.53(d, 2H, J=7.3Hz, C$\boldsymbol{H}_2$) 2.12(m, 1H, J=6.0, C***H***) 1.43(d, 3H, J=6.0, C$\boldsymbol{H}_3$) 1.07(d,6H,J=6.0Hz, (C$\boldsymbol{H}_3$)$_2$)
5 (b)	3-(2-(4-isobutyl phenyl) propyl)-6-phenyl-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazole.	3056.9 C-H st. (aro) 2893.7 C-H st. (ali) 1643.6 N-N st. (Het.Aro.) 1483.1 C=N st. (Het.Aro.) 1419.5 C=C st. (aro) 1344.0 Ring st. (Het.Aro.) 842.3 C-C st. (ali) 657.6 C-S st. (Het.Aro.)	7.48(d, 2H, J=3.6Hz, Ar-***H***) 7.34(t, 3H, J=4.0Hz, Ar-***H***) 7.01(d, 4H, J=4.0Hz, Ar-***H***) 3.46(q, 1H, J=4.0Hz, C***H***) 2.84(d, 3H,J=2.4 C$\boldsymbol{H}_3$) 2.54(d, 2H, J=0.8Hz, C$\boldsymbol{H}_2$) 2.28(m, 1H, J=3.2, C***H***) 1.61(d, 6H, J=4.0Hz, (C$\boldsymbol{H}_3$)$_2$)
5 (c)	3-(2-(4-isobutyl phenyl) propyl)-6- (4-chloro phenyl)-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazole.	3005.5 C-H st. (aro) 2868.8 C-H st. (ali) 1645.1 N-N st. (Het.Aro.) 1531.3 C=N st. (Het.Aro.) 1455.2 Ring st. (Het.Aro.) 1421.7 C=C st. (aro) 1092.6C-Cl st (Aro.Halide) 849.3 C-C st. (ali) 632.2 C-S st. (Het.Aro.)	7.48(d, 2H, J=1.6Hz, Ar-***H***) 7.33(d, 2H, J=1.6Hz, Ar-***H***) 7.14(d, 4H, J=3.6Hz, Ar-***H***) 3.34(q, 1H, J=3.2Hz, C***H***) 2.84(d, 3H, J=2.4, C$\boldsymbol{H}_3$) 2.52(d, 2H, J=1.6Hz, C$\boldsymbol{H}_2$) 2.24(m, 1H, J=4.0, C***H***) 1.42(d, 6H, J=4.0Hz, (C$\boldsymbol{H}_3$)$_2$)
5 (d)	3-(2-(4-isobutyl phenyl) propyl)-6-(4-methoxy phenyl)-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazole.	3084.7 C-H st. (aro) 2965.6 C-H st. (ali) 1647.0 N-N st. (Het.Aro.) 1475.4 C=N st. (Het.Aro.) 1443.7 Ring st. (Het.Aro.) 1421.3 C=C st. (aro) 1260.9 C-O-C st. 845.5 C-C st. (ali) 626.1 C-S st. (Het.Aro.)	7.43(d, 2H, J=3.2Hz, Ar-***H***) 7.42(d, 2H, J=2.4Hz, Ar-***H***) 6.82(d, 4H, J=1.6Hz, Ar-***H***) 3.84(s, 3H, OCH_3) 3.42(q, 1H, J=3.6Hz, C***H***) 2.94(d, 3H, J=1.6, C$\boldsymbol{H}_3$) 2.54(d, 2H, J=0.8Hz, C$\boldsymbol{H}_2$) 2.20(m, 1H, J=4.0, C***H***) 1.42(d, 6H, J=2.8Hz, (C$\boldsymbol{H}_3$)$_2$)
5 (e)	3-(2-(4-isobutyl phenyl) propyl)-6-(4-nitro phenyl)-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazole.	3005.1 C-H st. (aro) 2885.3 C-H st. (ali) 1616.2 N-N st. (Het.Aro.) 1502.8 C=N st. (Het.Aro.) 1458.0 C=C st. (aro) 1368.5 Ring st. (Het.Aro.) 1264.5 N=Ost. (Nitro. Aro) 790.2 C-C st. (ali) 609.4 C-S st. (Het.Aro.)	8.24(d, 2H, J=7.5Hz, Ar-***H***) 8.02(d, 2H, J=7.5Hz, Ar-***H***) 7.19(d, 2H, J=7.5Hz, Ar-***H***) 7.05(d, 2H, J=7.5, Ar-***H***) 3.42(q, 1H, J=2.7Hz, C***H***) 2.53(d, 2H, J=3.2Hz, C$\boldsymbol{H}_2$) 2.10(m, 1H, J=6.0, C***H***) 1.46(d. 3H, J=5.2Hz, C$\boldsymbol{H}_3$) 0.99(d, 6H, J=6.0Hz, (C$\boldsymbol{H}_3$)$_2$)
5 (f)	3-(2-(4-isobutyl phenyl) propyl)-6-(4-amino phenyl)-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazole	3367.4 N-H st. (Amine) 3067.8 C-H st. (aro) 2880.2 C-H st. (ali) 1663.3 N-N st. (Het.Aro.) 1593.0 C=N st. (Het.Aro.) 1434.9 C=C st. (aro) 1325.0 Ring st. (Het.Aro.) 1261.3 C-N st. (Amine)	7.65(d, 2H, J=7.5Hz, Ar-***H***) 7.19(d, 2H, J=7.5Hz, Ar-***H***) 7.07(d, 2H, J=7.5Hz, Ar-***H***) 6.53(d, 2H, J=7.5, Ar-***H***) 4.12(s, 2H, N$\boldsymbol{H}_2$) 3.40(q, 1H, J=6.0Hz, C***H***) 2.45(d, 2H, J=7.5Hz, C$\boldsymbol{H}_2$) 2.15(m, 1H, J=7.3, C***H***) 1.46(d, 3H, J=6.0, C$\boldsymbol{H}_3$)

		734.8 C-C st. (ali) 600.6 C-S st. (Het.Aro.)	1.06(d, 6H, J=6.0Hz, (C***H***$_3$)$_2$)
5 (g)	3-(2-(4-isobutyl phenyl) propyl)-6-(4-hydroxy phenyl)-[1,2,4] triazolo [3,4-*b*] [1,3,4] thiadiazole	3596.7 O-H st. (Hydroxy) 3011.4 C-H st. (aro) 2805.1 C-H st. (ali) 1643.5 N-N st. (Het.Aro.) 1479.3 C=N st. (Het.Aro.) 1421.4 C=C st. (aro) 1301.9 Ring st. (Het.Aro.) 1236.3 C-O st. (Alcohol) 752.1 C-C st. (ali) 626.8 C-S st. (Het.Aro.)	7.61(d, 2H, J=7.5Hz, Ar-***H***) 7.19(d, 2H, J=7.5Hz, Ar-***H***) 7.05(d, 2H, J=7.5Hz, Ar-***H***) 6.88(d, 2H, J=7.5, Ar-***H***) 4.87(s, 1H, O***H***) 3.42(q, 1H, J=3.4Hz, C***H***) 2.48(d, 2H, J=7.3Hz, C***H***$_2$) 2.11(m, 1H, J=6.0, C***H***) 1.46(d, 3H, J=6.0, C***H***$_3$) 1.05(d, 6H, J=6.0Hz, (C***H***$_3$)$_2$)

Antimicrobial Activity.[16]

Escherichia coli, *Bacillus subtilis*, and *Staphylococcus aureus*, which represent Gram-negative bacteria, Gram-positive bacteria, and fungi, respectively, were used to test the antibacterial activity of the compounds 5(a-g). The results of each compound's antibacterial activity were recorded as a zone of inhibition in millimeters and are displayed in Tables 3. The outcome showed that the majority of recently synthesized compounds had positive antibacterial and antifungal properties. In comparison to ciprofloxacin, the test compounds 5(e - g) often shown good effectiveness against Gram-positive bacteria. Other substances displayed moderate anti-gram-positive bacterial action. In comparison to ciprofloxacin, compounds 5(b-d and e) shown good action against Gram-negative bacteria. When compared to ciprofloxacin, compound 5(a), 5(b), and 5(d) have superior antifungal activity against Saccharomyces cerevisiae. Other substances, such 5(c) and 5(e-g), shown strong antifungal action.

Table 3 Antimicrobial activity data at 100µg/ml (after 24 hr)

S.NO.	**COMPOUNDS**	**ZONE OF INHIBITION IN mm (after 24 hr) [**100 µg/ml]			
		Antibacterial			Antifungal
		S. aureus	*B.subtilis*	*E.coli*	*S. Cerevisiae*
1.	5 (a)	13	12	13	13
2.	5 (b)	14	12	13	13
3.	5 (c)	13	15	12	16
4.	5 (d)	12	14	13	16
5.	5 (e)	16	17	15	15
6.	5 (f)	17	16	16	14
7.	5 (g)	13	12	15	16
8.	Ciprofloxacin	30	29	30	27

Minimum Inhibitory Concentration

Using the broth microdilution method, the minimum inhibitory concentration (MIC) values for the synthesized compounds were determined. From **Table 4.** it has been observed that Compound BT4 was most active in minimum concentrations of approximately 3 and 4 µg/mL

Table 10. Minimum Inhibitory Concentration of selected compounds against *B. subtilis*, *E. coli* and *S. Cerevisiae*.

S. No.	**Compound Code**	**MIC µg/mL**		
		B. subtilis	*E. coli*	*S. Cerevisiae*
1.	5a	7	8	9

2.	5b	7	7	8
3.	5c	6	5	5
4.	5d	3	3	2
5.	5e	3	4	3
6.	5f	2	2	3
7.	5g	4	2	4
8.	Ciprofloxacin	>0.5	>0.5	>0.5

Conclusion

The outcome showed that the majority of recently synthesised compounds had positive antibacterial and antifungal properties. Comparing the synthesised compounds to ciprofloxacin, compound 5(e-g) shown good action against Gram-positive bacteria. Other substances displayed moderate anti-gram-positive bacterial action. In comparison to ciprofloxacin, compounds 5(b-d and e) shown good action against Gram-negative bacteria. When compared to ciprofloxacin, compound **5(a), 5(b),** and **5(d)** had superior antifungal activity against Saccharomyces Cerevisiae. In general, triazolo-thiadiazole rings with methyl, phenyl, and 4-amino phenyl groups at the C-6 position exhibited good antibacterial action.

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