Synthesis and Antimicrobial Activity of Some New [1,2,4]Triazolo[3,4-B][1,3,4]Thiadiazole Derivatives

Mukesh M. Mudgal, Rajeev K. Singla, Varadaraj Bhat G.*, Gautham Shenoy G.

Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences,

Manipal University, Manipal-576104, Karnataka(India)

(E-mail: varad.g@manipal.edu)

Summary

Recent observations of literature suggests that molecular manipulation, combination of two or more active moieties into one molecule and synthesis of totally newer moieties have been the method of research. In view of this we have undertaken synthesis of various 6-(pyrazin-2-yl)-3-substituted [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives and their antibacterial and antifungal activity. The Structures of the synthesized compounds were established with spectral characterizations using UV, IR, ^{1}H-NMR and mass spectra. The Synthesized molecules were tested against bacterial strains (*Micrococcus luteus*, *Bacillus subtilis*, *Bacillus cereus*, *Enterobacter aerogenes*, *E. Coli, Klebsiella pneumonia*, *Proteus microbilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, *Salmonella typhii*, *Streptococcus pneumoniae*), fungal strains (*Candida albicans*, *Cryptococcus albidus*, *Trychophyton rubrum*, *Apergillus niger*, *A. flavus*, *A. spinulosus*, *A. terrcues* and *A. nidulans*) by agar diffusion method. The results of preliminary screening showed that all synthesized 6-(pyrazin-2-yl)-3-substituted [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole derivatives has good antibacterial activity against all strains but only few of them showing good activity against *Streptococcus pneumoniae* . Similarly some of these molecules showed good antifungal activity against all strains except against trychophyton species.

Keywords : Triazole, Thiadiazole, Antibacterial, Antifungal.

Introduction

The increase in clinical resistant for microbial pathogens has lent additional urgency for microbiological and antifungal research. Several five membered aromatic systems having three hetero atoms at symmetrical position have been studied because of their interesting physiological properties[1-3]. Well-known azole derivatives, having a gem-phenyl-(1H-imidazol-1-ylmethyl) moiety which is thought to be largely responsible for imparting, antifungal activity, such as clotrimazole, miconazole , econazole, and ketoconazole, have been developed for clinical uses. SAR studies revealed that imidazole and phenyl rings which are also pharmacophoric portion of all these molecules can be replaced by the triazole[4-6].

It is also a well established fact that various derivatives of 1,2,4-triazole, 1,3,4-thiadiazole (which incorporates N-C-S linkage) exhibit broad spectrum of pharmacological properties such as antibacterial, antifungal, antitubercular activities. So synthesis of new molecules to explore their potential antimicrobial activity is still draws continued interest. Molecular manipulation, combination of two or more biologically active moieties into one molecule and synthesis of totally newer moieties have been the method of research and this can possibly result in augmenting the activity, removal of unwanted effects and particularly prevent the development of resistance by the infectious organisms.

So a triazolo- thiadiazole system may be viewed as a cyclic analogue of two very important components. In this paper we describe the synthesis of some new 1,2,4-triazole derivatives and a screen for their *in vitro* activity against some species of aerobic bacteria and fungi.

EXPERIMENTAL

The melting points were taken in open capillaries in liquid paraffin and are uncorrected. IR spectra were recorded in KBr on Shimadzu FTIR-8310 spectrometer. PMR spectra on AMX 400 (400 MHz) instrument (chemical shift in ∂ ppm) using TMS as internal standard. Mass spectra were recorded in Shimadzu GC-MS QP 5050. The structure of the synthesized compounds were confirmed by spectral analysis.

Synthesis of acid ester and hydrazide (1): To a solution of 2-Chlorobenzoic acid (10mM) in Methanol (20mM), three drops of sulphuric acid was added and the solution was heated under reflux for a period of 24 hours. Hydrazine hydrate 99-100% (30mM) was added to it and was heated under refluxed for a period of 8 hours. The progress of the reaction was monitored using TLC (solvent system: Methanol-Benzene (15-85). After completion of the reaction the product was concentrated under reduced pressure and cooled. The solid obtained was washed with cold water and recrystallised from absolute ethanol.

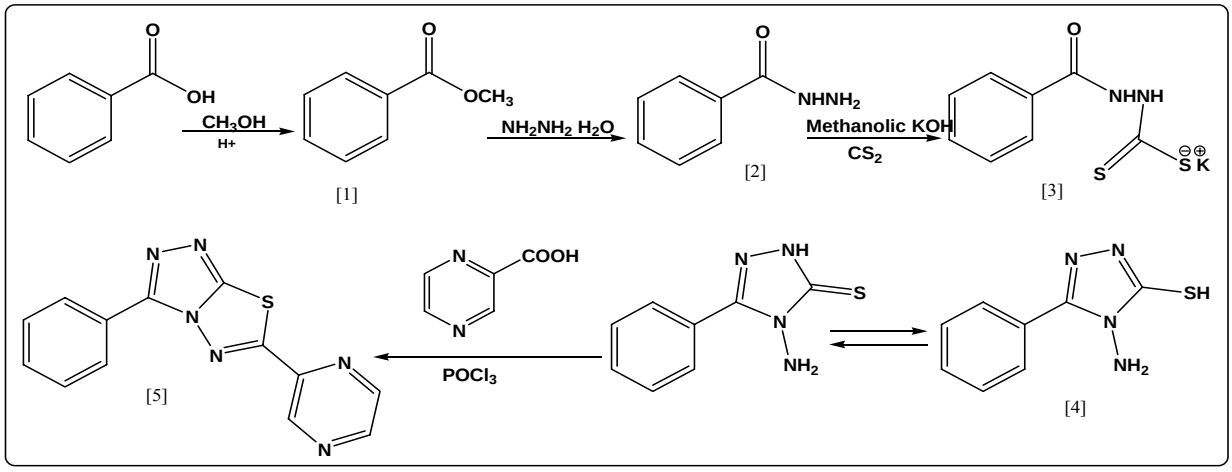
Synthesis of Potassium Dithiocarbazinate salt (2): 2-Chlorobenzohydrazide (10mM) was added with carbon disulphide (12mM) followed by alcoholic KOH (15mM) drop wise. The reaction mixture was then kept for stirring for about 4 hours to get potassium 2-[(2-chlorophenyl) carbonyl] hydrazinecarbodithioate. The progress of the reaction was monitored using TLC (solvent system: Methanol-Benzene (15-85).

Synthesis of 4-amino-5- (2-chlorophenyl)-4H-1, 2, 4- triazole-3-thiol (3): To 2-[(2-chlorophenyl)carbonyl] hydrazinecarbodithioate (10mM), added Hydrazine hydrate 99-100% (30mM) and was heated under reflux for a period of 6-8 Hrs. The reaction mixture was poured to minimum quantity of ice cold water and was acidified to get the title compounds. It was filtered and recrytallised from absolute ethanol. The progress of the reaction was monitored using TLC (solvent system: Methanol-Benzene (15-85).

Synthesis of 3-(2`- Chlorophenyl)-6-(2`` pyrazine)- [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (**M2**): A mixture of triazole (M2) (10mM) and pyrazinoic acid (10mM) and phosphorous oxychloride (100mM) were heated to reflux for 10hrs and excess of phosphorous oxychloride was distilled off under reduced pressure. The concentrated residual mass was poured onto a crushed ice and neutralized with sodium bicarbonate. The solid separated was filtered, washed with cold water, dried and recrystallised from absolute ethanol. The progress of the reaction was monitored using TLC (solvent system: Methanol-Benzene (15-85).

M.P: 214-216 ^{0}C. Yield 70%.GC-MS: 314 m/z value. IR (KBr): 655(C-Cl str.), 727(C-S str.), 1539(C=N str.), 761.8(C-H def.), 2920(C-H str.), 1604,1571& 1463(C=C str.). ^{1}H-NMR(DMSO): 7.2-8.1(Ar-H, Pyrazine-H) broad peak confirms the compound **M2**.

Figure 1 Reaction scheme



All the compounds were synthesized by the same procedure and their formulas, melting points, yields and analytical data are shown in **Table No. 1.**

Table 1: Physicochemical properties of the synthesized compounds

CODE	R	Mol. Formula	% Yield	M.P. (^oC)	R_f	λmax (nm)	IR(cm^{-1})
M1	-Cl(p)	$C_{13}H_7N_6SCl$	67	194-196	0.52	259	1604.7,1473.5,2922,763.8, 1425.3,833.2,721
M2	-Cl(o)	$C_{13}H_7N_6SCl$	70	214-216	0.54	252	1604,1571,1463,2920, 761.8,1539,727,655
M3	-NO_2(p)	$C_{13}H_7N_7O_2S$	56	222-224	0.48	261	1612,1479,2918,721, 1303.8,3213
M4	-NO_2(o)	$C_{13}H_7N_7O_2S$	60	239-241	0.47	231	1570,1618.2,2850.6,754.1, 1479.3,690.5,1267.1, 1569.9
M5	-NH_2(p)	$C_{13}H_7N_6S$	64	236-238	0.51	289.20	1606.6,1463.9,1510.2, 3055,1423.4,3305.8,3398.3,1 311.5
M6	-NO_2(m), -NO_2(m)	$C_{13}H_6N_8O_2S$	50	258-260	0.44	254.20	1463.9,1604.7,1548.7, 3058.9,761.8,1425.3,715.5 ,3313.5,1548.7,1496.7
M7	- NO_2(m), -NO_2(m), -OH(o)	$C_{14}H_6N_8O_7S$	47	197-199	0.42	259	1625.9,2848.7,790.8,742.5, 709.8,1236.3,1325.0, 1481.2
M8	-H	$C_{13}H_8N_6S$	72	185-187	0.56	257	1604.7,1564.2,1463.9, 1496.7,3058.9,1425.3, 769.5,1294.1
M9	-OH(o), -NO_2(m)	$C_{13}H_7N_7O_3S$	48	257-259	0.49	262.20	1607,1503,28501203, 837,746.4,1344.3
M10	Phenoxy in place of R-aryl ring	$C_{15}H_{10}N_6O_3S$	62	259-261	0.50	278.20	1500,1625.9,711.7,750.3, 1299.9,1080.1
M11	-CH_3(o)	$C_{14}H_{10}N_6S$	48	199-201	0.47	258.60	1544.9,1460,2908,765.7, 723,1245.9,875.6
M12	Pyridine ring in place of R-aryl ring	$C_{12}H_6N_7S$	71	223-225	0.42	255.40	1606.6,1471.6,2725.2, 1419.5,719.4,1315.4, 1282.6
M13	-OH(o)	$C_{14}H_8N_6O_3S$	65	159-161	0.53	243.20	1600,1510,1465,2931, 1228.6,3205,1423,761
M14	-Cl(m), -Cl(m), -OH(o)	$C_{14}H_6N_6O_3SCl_2$	60	197-199	0.47	261.20	1438.8,1579.6,2852.5, 1409.9,761.8,619.1,700.1
M15	-OH(p)	$C_{13}H_8N_6OS$	67	195-197	0.55	258.40	1463.9,1608.5,2927.7, 1220.9,3201,1425.3,763.8

Microbiology

The assessment of the antimicrobial action of the synthesized compounds was performed using disc diffusion method. Microorganisms used in this study were; Gram-positive bacteria: *Micrococcus luteus, Bacillus subtilis, Bacillus cereus, Klebsiella pneumonia, Staphylococcus aureus, Staphylococcus pneumonia* and Gram-negative bacteria: *Enterobacter aerogens, Escherichia coli, Proteus microbilis, Pseudomonas aeruginosa* and *Salmonella typhii.* For testing antifungal activities of the compounds following strains used were; *Candida albicans, Cryptococcus albidus, Trychophyton rubrum, Aspergillus niger, A. flavus, A. spinulosus, A. terrcues, A.nidulans.* Antimicrobial activity was tested under standard conditions using Mueller Hinton Agar medium (Himedia) for bacteria and Potato dextrose agar medium with 2% glucose for yeasts, according to CLSI guide lines[7].

The compounds were dissolved in alcohol. Alcohol was applied to paper disc as negative control. Sterile filter paper disc (Himedia, Mumbai) with a diameter of 6mm were dripped with solutions of tested compounds to load 2mg/disc. Dried discs were placed on the surface of prepared agar medium. The diameter of Growth Inhibition Zone was read after 18 hrs of incubation at 35^0C. The final inoculum of all bacteria was 10^8CFU/ml and all fungi was10^4 spore/ml. Gentamycin and Ketoconazole were used as standard.

Results and Discussion

All the tattle molecules were synthesized starting from different benzoic acid derivatives. The structure of molecules M1-M15 was confirmed on the basis of the spectral analysis. All the synthesized molecules were screened for their antibacterial activity against *Micrococcus luteus (106), Bacillus subtilis (121), Bacillus cereus (430), Klebsiella pneumonia (109), Staphylococcus aureus (96), Staphylococcus pneumonia (2672), Enterobacter aerogens (111), Escherichia coli (443), Proteus microbilis (1429), Pseudomonas aeruginosa (429)* and *Salmonella typhii (98);* using disc diffusion method . All the molecules were screened using 50µg/ml and Gentamycin was used as standard drug. The Growth inhibition zone(giz) for the molecules M1, M2, M6, M7, M13, M15 for *micrococcus luteus* (106) were 19,24.4,21.5,22,21.8,23mm respectively and giz for *proteus microbilis*(1429) were 22.8, 29.5, 22, 23, 22 for M1, M2, M6, M7, M15 respectively of which M2 appeared to be more effective than standard. The detailed results are tabulated in **Table no. 2.** As seen through results, electronegative groups are more effective if o/m substituted as compared to p-substitution. Electron donating groups are seems to be less significant as substituting groups.

All the synthesized molecules were also screened for their antifungal activity against *Candida albicans (183), Cryptococcus albidus (2661), Trychophyton rubrum (296), Aspergillus niger (16406), A. flavus (1973), A. spinulosus (16919), A. terrcues (1782), A.nidulans (11267)* using disc diffusion method. All molecules were screened using a concentration 50µg/ml and Ketoconazole was used as standard drug. The GIZ for molecules M1, M3 and M5 for strain *A. spinulosus* (16919) were 21.5, 20.5, 21.5 mm respectively. Growth inhibition zone for molecule M1 was 20mm for *candida albicans* (183). The detailed results are tabulated in **Chart 1.** The results reveals that the substitution of the aromatic ring(by the electronegative groups) at C3 of **[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole** are somehow crucial for the antifungal activity. The hydroxyl and methyl substitutions do not contribute to activity.

Table 1: Antibacterial Activity of Synthesized Compounds expressed as the Growth Inhibition Zone (GIZ, in mm)

Compound Code	GIZ (mm) / Bacterial Strain										
	106	**121**	**430**	**111**	**443**	**109**	**1429**	**424**	**96**	**98**	**2672**
M1	19	15	14.5	17	15.5	19.5	22.8	24.5	17	11.5	19.5
M2	24.5	22.5	21	18.5	20.5	20	29.5	22	19	25	15.5
M3	14	14	10.5	11.5	11.5	12	14.5	11.5	12.5	12.5	11.5
M4	18.5	20.5	18.5	16	15	19.5	18.5	17.5	20.5	16	18
M5	17.5	11.5	8.5	8.5	R	9.5	12	8.5	10.5	7.5	R
M6	21.5	18.5	10.5	12.5	R	14.5	22	10.5	15.5	10	R
M7	22	22	25	16	24	18	23	28	18	25	38
M8	12	R	14.5	8	9	10.5	8	7	R	R	R
M9	14	8	R	9	8	R	11	8	R	R	R
M10	15.5	9	8	11.5	10.5	9.5	12	7.5	R	7	R
M11	16	12.5	12	12	10	10	12.5	8	10	7	R
M12	16.5	11.5	9.5	14.5	12	9.5	12	10	12	8	R
M13	21.5	18.5	11.5	12	14.5	11.5	12.5	10.5	15.5	10	R
M14	18	16.5	15.5	17.5	19.5	15.5	18	14	R	R	R
M15	23	24.5	25	14	22.5	18	22	28.5	18	25	35
Gentamycin	26	28	31	19.5	30.5	25	24	34	26	32	44

Micrococcus luteus (106); B. subtilis (121); B. Cereus (430); Enterobacter aerogens (111); E. coli (443); Klebisiella pneumoniae (109); Proteus microbilis (1429); Pseudomonas aeruginosa (429); S. aureus (96); Salmonella Typhii (98); S.pneumoniae (2672) and Resistance (R)

Chart 1: Antifungal Activity of Synthesized Compounds expressed as the Growth Inhibition Zone(GIZ,mm)

Compound Code	GIZ(mm)/ Fungal strains							
	183	**2661**	**16404**	**1973**	**16919**	**1782**	**12267**	**296**
M1	20	11.5	12.5	17.5	21.5	17.5	7.5	9
M2	12.5	22.5	12.5	11	18.5	16	8.5	R
M3	9.5	17.5	8.5	7.5	20.5	R	5.5	R
M4	9.5	5.5	R	9	17.5	9.5	6	R
M5	17	12	R	14	21.5	14.5	7.5	R
M6	12.5	9.5	R	16.5	9.5	15.5	8	R
M7	11.5	10.5	5.5	8.5	9.5	12.5	9.5	R
M8	11	R	R	R	R	R	R	R
M9	R	R	R	R	R	R	R	R
M10	R	R	R	R	R	R	R	R
M11	R	R	R	R	R	R	R	R
M12	11	R	R	R	16	R	R	R
M13	R	R	R	7	R	R	R	R
M14	R	R	R	R	14	R	R	R
M15	R	R	R	7	14	R	R	R
Ketoconazole	24	30	21	28	22	28	23	32

Candida albicans (183); Cryptococcus albidus (2661); Trycophyton rubrum (296); Aspergillus niger (16406); A.flavus (1973); A.spinulosus (16919); A.terrcues (1782); A.nidulans (11267) & Resistance (R)

Conclusion

A Total of 15 molecules were synthesized and passed preliminary screening for their antibacterial and antifungal activity. The present work has demonstrated that the triazole derivatives are potential heterocycles which can be explored further in depth for their antibacterial, antifungal and antitubercular activities. The antifungal activity can be attributed to triazole ring as it contains the toxophoric group (N-C-N). Hence it can be concluded that 3-substitution of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole can lead to potential bioactivity.

Acknowledgements

This work was funded by Manipal College of Pharmaceutical Sciences, Manipal University. Indian Institute of Science, Bangalore provided support for the acquisition of H1-NMR Spectra. NBRI, Lucknow rendered help for the evaluation of antimicrobial activity.

Refernces

1. Hetzhein A & Mockel K. Adv. Heterocycle Chem.,1996;7: 183.
2. Sandstrom J. Adv. Heterocycle chem..,1968;9:165.
3. Sabir Hussain et al., Synthesis and Antimicrobial Activities of 1,2,4-triazole and 1,3,4-thiadiazole derivatives of 5-Amino-2-hydroxybenzoic acid.E-Journal of Chemistry.2008;5(4): 963-968.
4. Rossello A. et al., J. Med. Chem..2002;45:4903.
5. Wahbi Y. et al.,Eur. J. Med. Chem.1995;30:955.
6. Ozden Ozel Guven et al., Synthesis and antimicrobial activity of some novel phenyl and benzimidazole substituted benzyl ethers. Bioorganic and medicinal chemistry letters.2007;17:2233-2236.
7. Clinical and Laboratory standard institute. Performance standards for antimicrobial disc Susceptibility tests; approved standard M2-A8. Clinical Laboratory Standards Institute, Wayne,Pa.2003.
8. Turan-Zitouni. G et al. Synthesis and antimicrobial activity of 4-phenyl/cyclohexyl-5-(1-phenoxyethyl)-3-[N-(2-thiazolyl)acetamido]thio-4H-1,2,4-triazole derivatives. Eur. J. Med Chem. 2005;40(6):607-613.
9. Almajan. G.L. et al. Carbonic anhydrase inhibitors. Inhibition of cytosolic and tumor-associated carbonic anhydrase isozymes I,II and IX with a series of 1,3,4-thiadiazole- and 1,2,4-triazole-thiols. Biorg. Med Chem. Let. 2005;15(9):2347-2352.
10. R.R.Somani et al. Synthesis of some antifungal and antitubercular 1,2,4-triazole analogues. International journal of chemtech research.2009;1(2):168-173.
11. Monika Wujec et al. Synthesis, lipophillicity and anticrobial activity of new derivatives of thiosemicarbazides and 1,2,4-triazoline-5-thione. Acta Poloniae pharmaceutica- Drug research. 2009;66(1):73-82
12. Nurhan G. et al. Synthesis and antimicrobial activities of some new 1,2,4-triazole derivatives. Turk J Chem. 2007;31:335-348.
13. Shipra Baluja et al. A facile synthesis and antimicrobial acivity of some 4-aryltriazoles. J.Serb.Chem.Soc.2007;72(6):539-544.
14. Gabriela Laura almajan et al. Synthesis , Characterization and antibacterial activity of some triazole Mannich bases carrying diphenylsulfone moieties. Eur. J. Med. Chem. 2009;44:3083-3089.
15. Charansingh Harnamsingh gill et al. SAR study of clubbed [1,2,4]-triazolyl with fluorobenzimidazoles as antimicrobial and antitubercular agents. Eur. J. Med. Chem. 2009;44:2930-2935.