

Synthesis of Fluoro Substituted Benzothiazoles Comprised with 1,3,4-thiadiazoles for Biological and Pharmacological Screening

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ABSTRACT: In the present work an attempt is carried out for the synthesis of fluoro substituted benzothiazoles comprised with 1,3,4-thiadiazoles. 4-fluoro-3-chloro aniline was treated with potassium thiocyanate in presence of glacial acetic acid and bromine and which is neutralized with ammonia to get 2-amino-6-fluoro-7-chloro (1,3) benzothiazole resulting into 2-amino benzothiazole. 2-amino benzothiazole on reaction with carbon disulphide, ammonia with ethanol (95%) followed by addition of hydrazine hydrate yield 2-thiosemicarbazide-6-fluoro-7-chloro (1,3) benzothiazole. Thus synthesized compound was treated with phenyl alanine in the presence of pyridine after the cyclisation it yields N-[5-(1-amino-2-phenylethyl)-1,3,4-thiadiazol-2-yl]-7-chloro-6-fluoro-1,3-benzothiazol-2-amine which was further treated with various aromatic amines o,m,p- nitroanilines, o,m p-methoxyanilines and o-chloroaniline in presence of DMF to obtain various novel derivatives of N-[(1-amino-2-phenylethyl)-1,3,4-thiadiazol-2-yl]-6-fluoro-7-substituted-1,3-benzothiazol-2-amines. The identities of compounds were confirmed on the basis of their spectral (UV-Vis, IR, ¹HNMR and MASS) data. Further they have been screened for their antimicrobial and anthelmintic activities.

Keywords: Fluorobenzothiazole, Thiadiazole, Anti-microbial, Anthelmintic.

INTRODUCTION

The biological activity of the compounds is mainly dependent on their molecular structures. The chemistry and pharmacology of thiadiazoles have been of great interest because of its various biological activities, so that the biological and pharmacological activity of thiadiazole with fluoro benzothiazole may be taken into account for synergism. It is well known that the introduction of fluorine atom into an organic molecule causes dramatic changes in its biological profile, mainly due to high electro negativity of fluorine, the strong carbon-fluorine bond and increased solubility in lipids. Therefore it was thought worthwhile to synthesize better kinds of drugs by incorporating thiadiazole and fluorine atom in benzothiazole moiety. Benzothiazole with thiadiazole group were reported to possess various pharmacological activity of clinical importance. Thiadiazole derivatives are well known to have number of biological and antimicrobial¹⁻⁴, anti-inflammatory⁵⁻⁶, anthelmintic⁷⁻⁹, anti-cancer¹⁰ activities.

Benzothiazole incorporated fluorine gives a promising molecule for biological use. Fluorobenzothiazoles are versatile and possess large range of biological activities. Hence in present work we made an attempt to link Fluorobenzothiazole with thiadiazole in hope of getting novel biodynamic compounds of pharmacological interest. The novel compounds were screened for anti-microbial and anthelmintic activities.

EXPERIMENTAL

Synthesis of 2-amino-6-fluoro-7-chloro (1,3) benzothiazole¹¹⁻¹³

To glacial acetic acid (20ml) cooled below room temperature were added 8gm (0.08mol) of potassium thiocyanate and 1.45gm (0.01mol) of fluoro chloro aniline. The mixture was cooled in a water bath and stirred in magnetic stirrer, while stirring 1.6ml of bromine in 6ml of glacial acetic acid was added from a dropping funnel at such a rate that the temperature never rise beyond room temperature. After all the bromine was added (105min), the solution was stirred in a magnetic

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stirrer for 2 hours below room temperature and at room temperature for 10 hours. It was then allowed to stand overnight, during which period an orange precipitate settle at the bottom. Water (6ml) was added quickly and slurry was heated at 85 °C and filtered hot. The orange residue was placed in a reaction flask and treated with 10ml of glacial acetic acid heated again to 85°C and filtered hot. The combined filtrate was cooled and neutralized with concentrated ammonia solution to pH 6. A dark yellow precipitate was collected. Recrystallized from benzene, ethanol of (1:1) after treatment with animal charcoal gave yellow crystals of 2-amino-6-fluoro-7-chloro-(1,3)-benzothiazole. After drying in an oven at 80 °C, the dry material (1gm 51.02%) melted at 210-212 °C.

Preparation of 6-fluoro-7-chloro (1,3) benzothiazole 2-thiosemicarbazide¹⁴⁻¹⁵

2-amino benzothiazole (0.1mol) 20.25gm was dissolved in ethanol (95%) 50 ml and ammonia solution was added to it. The reaction mixture was cooled below 30 °C and carbon disulphide (8 ml) was added slowly within 15 minutes with continuous shaking. After complete addition of carbon disulphide the solution was cooled to stand for 1 hour. After that sodium chloroacetate (0.1mol) 9.4gm was added to it. The reaction was exothermic. To it 50% hydrazine hydrate (20 ml) was added. The mixture was warmed gently, filtered and boiled to half of its volume and kept overnight. Next day the product thiosemicarbazide was filtered and recrystallized from ethanol.

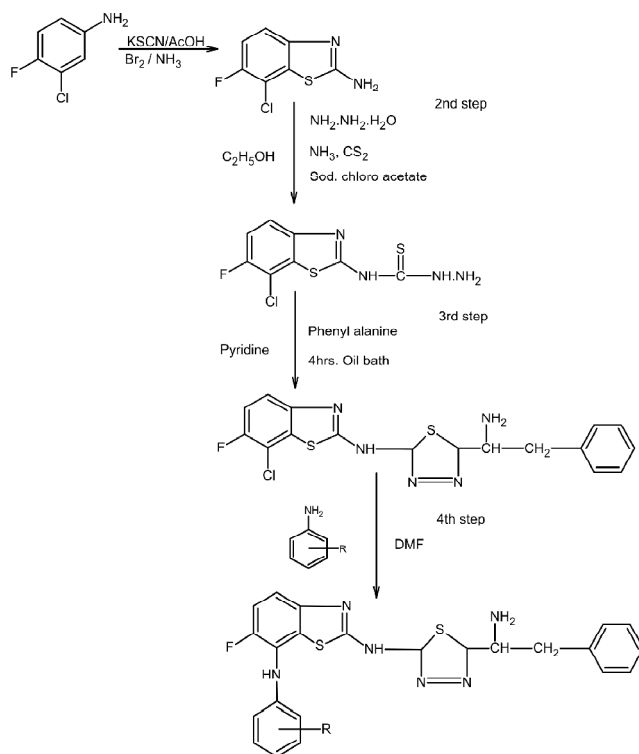
Preparation of N-[5-(1-amino-2-phenylethyl)-1,3,4-thiadiazol-2-yl]-7-chloro-6-fluoro-1,3-benzothiazol-2-amine¹⁶

An intimate mixture of 13.9gm (0.05mol) of (1,3) benzothiazoles 6-fluoro-7-chloro-2-thiosemicarbazide and phenyl alanine (0.05mol) 8.26gm and pyridine (100 ml) heated at 170-210 °C for 4 hours in an oil bath under moisture free condition. The fused material after cooling was treated with cold sodium bicarbonate solution (10%). The resulting solution was filtered, washed and recrystallized from methanol.

Preparation of N-[5-(1-amino-2-phenylethyl)-1,3,4-thiadiazol-2-yl]-6-fluoro-7-substituted 1,3-benzothiazol-2-amine¹⁷

To 0.0025mol of N-[5-(1-amino-2-phenylethyl)-1,3,4-thiadiazol-2-yl]-7-chloro-6-fluoro-1,3-

benzothiazol-2-amine was treated with equimolar quantity (0.0025mol) of various substituted aromatic amines and refluxed for 2 hrs. in the presence of DMF (dimethyl formamide) then the mixture was cooled and poured in the crushed ice. The solid separated was filtered off, dried and recrystallized from benzene and absolute alcohol (1:1).



R = o, m, p - nitro (P1- P3)

R = o, m, p - methoxy (P4 - P6)

R = o - chloro (P7)

Melting points were determined in open capillary method and are uncorrected. Purity of the compounds was checked on Silica gel TLC plates. IR spectra were recorded using KBr disc method. ¹H NMR spectra were recorded on Bruker, DMSO d6 as internal standard.

Anti-bacterial Activity

The antibacterial activities are performed by cup plate method (diffusion technique). The fresh culture of bacteria are obtained by inoculating bacteria into peptone water liquid media and incubated at 37 ± 2 °C for 18 - 24 hours. This culture mixed with nutrient agar media (20%) and poured into petridishes by following aseptic techniques. After solidification of the media, the

Table 1
Thin Layer Chromatography

Sl. No	Compound Code	Solvent system for developing	Proportion of Components	Rf Value
1	P1	n-Butanol: Ethyl acetate: Benzene	1:4:1	0.79
2	P2	n-Butanol: Ethyl acetate: Benzene	1:4:1	0.85
3	P3	n-Butanol: Ethyl acetate: Benzene	1:4:1	0.86
4	P4	n-Butanol: Ethyl acetate: Benzene	1:4:1	0.78
5	P5	n-Butanol: Ethyl acetate: Benzene	1:4:1	0.77
6	P6	n-Butanol: Ethyl acetate: Benzene	1:4:1	0.90
7	P7	n-Butanol: Ethyl acetate: Benzene	1:4:1	0.84

Table 2
Analytical data of the synthesized compounds

Sl. No	Compound Code	M.P / B.PC	% Yield	MOL. FORMULA	M. Wt.	C%	H%	N%
1	P1	235-236	80%	C23H18FN7O2S2	507.56	54.43	3.57	19.32
2	P2	231-233	82%	C23H18FN7O2S2	507.56	54.43	3.57	19.32
3	P3	223-225	65%	C23H18FN7O2S2	507.56	54.43	3.57	19.32
4	P4	239-241	72%	C24H21FN6OS2	492.59	58.52	4.30	17.06
5	P5	225-227	74%	C24H21FN6OS2	492.59	58.52	4.30	17.06
6	P6	222-224	73%	C24H21FN6OS2	492.59	58.52	4.30	17.06
7	P7	224-226	76%	C23H18ClFN6S2	497.01	55.58	3.65	16.91

Table 3
Characteristics of IR absorption bands¹⁸⁻¹⁹

Sl.No.	Compd code	Ar-NH2 cm-1	ArC=C cm-1	Cyclic C=N cm-1	C-F cm-1	C-Cl cm-1	ArNO2 cm 1	C-S-C cm-1
1	P1	3450	1475	1650	1200	-	1540	1070
2	P2	3450	1475	1650	1200	-	1540	1070
3	P3	3450	1475	1650	1200	-	1540	1070
4	P4	3450	1475	1650	1200	-	-	1070
5	P5	3450	1475	1650	1200	-	-	1070
6	P6	3450	1475	1650	1200	-	-	1070
7	P7	3450	1475	1650	1200	690	-	1070

plates were placed in a refrigerator for 2 hours. After two hours of cold incubation, four bores are made at equal distance by using sterile steel cork borer (8mm diameter). Into these cups different concentrations of standard drugs and synthesized compounds are introduced. After introduction of standard drugs and synthesized compounds, the plates were placed in an incubator and maintained at 37 ± 2 °C for 18-24 hours. After the incubation period, the petriplates were observed for zone of inhibition by using vernier scale. The results are evaluated by comparing the zone of inhibition shown by the synthesized compounds with

standard drugs. The results are the mean value of zone of inhibition measured in millimeter of two sets. The results are tabulated in the Table No. 4 and 5.

The standard drugs were dissolved in minimum quantity of distilled water and synthesized compounds were dissolved in minimum quantity of dimethyl formamide (DMF). Procaine penicillin used against *Staphylococcus aureus*, *Streptococcus fecalis* and Streptomycin used against *Escherichia coli*, *Klebsiella* as standard drugs.

Table 4
Antibacterial Activity

Sl. No	Name of the compounds	Mean zone of inhibition (in mm)			
		<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>	
		0.5mg/ml	1mg/ml	0.5mg/ml	1mg/ml
01	Procaine penicillin	18	20	-	-
02	Streptomycin	-	-	19	21
03	P1	17 (0.94)	19 (0.95)	17 (0.89)	19 (0.90)
04	P2	16 (0.88)	18 (0.90)	18 (0.94)	20 (0.95)
05	P3	17 (0.94)	18 (0.90)	17 (0.89)	19 (0.90)
06	P4	17 (0.94)	19 (0.95)	16 (0.84)	18 (0.85)
07	P5	16 (0.88)	18 (0.90)	18 (0.94)	19 (0.90)
08	P6	11 (0.61)	13 (0.65)	17 (0.89)	18 (0.85)
09	P7	15 (0.83)	17 (0.85)	15 (0.78)	17 (0.80)

Table 5
Antibacterial Activity

Sl. No	Name of the compounds	Mean zone of inhibition (in mm)			
		<i>Streptococcus fecalis</i>		<i>Klebsiella</i>	
		0.5mg/ml	1mg/ml	0.5mg/ml	1mg/ml
01	Procaine penicillin	17	19	-	-
02	Streptomycin	-	-	20	21
03	P1	12 (0.70)	16 (0.84)	15 (0.75)	17 (0.80)
04	P2	16 (0.94)	18 (0.94)	13 (0.65)	14 (0.66)
05	P3	15 (0.88)	18 (0.94)	11 (0.55)	18 (0.85)
06	P4	15 (0.88)	18 (0.94)	13 (0.65)	16 (0.76)
07	P5	12 (0.70)	16 (0.84)	14 (0.70)	17 (0.80)
08	P6	16 (0.94)	18 (0.94)	13 (0.65)	15 (0.71)
09	P7	14 (0.82)	16 (0.84)	14 (0.70)	17 (0.80)

Antifungal Activity

The synthesized compounds are screened against two selected fungal strains *Candida albicans* and *Aspergillus fumigatus* by using diffusion method. The 48 hours old fungal culture inoculated into nutrient broth by following aseptic techniques and incubated for 48 hours at 37 ° ± 2 °C in an incubator. This culture mixed with Potato-dextrose agar media (20%) and poured into petriplates. After solidification these plates are placed in a refrigerator at 8°-10 °C for 2 hours. After 2 hours of cold incubation four bores are made at equal distance by using sterile steel cork borer (8mm in diameter). Into these cups different concentrations of standard drug and synthesized

compounds are introduced. After introduction of standard drug and compounds, the petriplates are transferred to incubator and maintained at 37°± 2 °C for 24-36 hours. After the incubation period, the plates were observed for zone of inhibition by using vernier scale. Results are evaluated by comparing the zone of inhibition shown by the synthesized compounds with standard drug. The results are the mean value of zone of inhibition measured in millimeter of two sets. The results are tabulated in the Table 6.

The standard drugs were dissolved in minimum quantity of distilled water and synthesized compounds were dissolved in minimum quantity of dimethyl formamide (DMF). Ciclopirox olamine USP used as a standard drug.

Table 6
Antifungal Activity

Sl. No	Name of the compounds	Mean zone of inhibition (in mm)			
		<i>Candida albicans</i>		<i>Aspergillus fumigatus</i>	
		0.5mg/ml	1mg/ml	0.5mg/ml	1mg/ml
01	Ciclopirox calamine	21	23	19	21
02	P1	13 (0.61)	17 (0.73)	16 (0.84)	20 (0.95)
03	P2	16 (0.76)	18 (0.78)	18 (0.94)	19 (0.90)
04	P3	14 (0.66)	16 (0.69)	17 (0.89)	19 (0.90)
05	P4	14 (0.66)	17 (0.73)	16 (0.84)	18 (0.85)
06	P5	13 (0.61)	16 (0.69)	18 (0.94)	18 (0.85)
07	P6	12 (0.57)	16 (0.69)	16 (0.84)	17 (0.80)
08	P7	12 (0.57)	15 (0.65)	17 (0.89)	19 (0.90)

Anthelmintic Activity [Perituma Posthuma]

The synthesized compounds were screened for anthelmintic activity by using earthworms. Six earthworms of nearly equal size were placed in standard drug solution and test compounds solutions at room temperature. Normal saline was used as control. The standard drug and test compounds were dissolved in minimum quantity of dimethyl formamide (DMF) and adjusted the volume up to 10ml with normal saline solution to get the concentration of 0.1%w/v, 0.2%w/v, and 0.5%w/v. Albendazole was used as a standard drug. The compounds were evaluated by the time taken for complete paralysis and death of earthworms. The mean lethal time for each test compound was recorded and compared with standard drug. The time taken by worms to

become motionless was noted as paralysis time. To ascertain the death of the motionless worms were frequently applied with external stimuli, which stimulate and induce movement in the worms, if active.

The mean lethal time and paralysis time of the earthworms for different test compounds and standard drug are tabulated in Table No.7

Table 7
Anthelmintic Activity

Sl No.	Name of the compound	Time in minutes					
		For Paralysis % of			For Death % of		
		Concentration		Concentration	Concentration		Concentration
		0.1	0.2	0.5	0.1	0.2	0.5
1	Normal saline (Control)	-	-	-	-	-	-
2	Albendazole	20	14	09	14	09	05
3	P1	15	18	13	14	13	09
4	P2	19	17	14	20	15	10
5	P3	21	19	17	19	14	11
6	P4	18	15	13	09	08	05
7	P5	20	18	15	16	14	11
8	P6	22	18	13	15	12	10
9	P7	16	13	10	12	10	08

RESULTS AND DISCUSSION

The synthesized compounds were subjected to various anti-bacterial, anti-fungal and anthelmintic activities by the standard methods.

All the compounds were screened for anti-bacterial activity. However the compounds P1, P3 and P4 showed better antibacterial activity against *Staphylococcus aureus* (gram +ve) at lower and higher concentration when compared with the standard drug Procaine penicillin.

Compounds P2 and P5 showed promoting antibacterial activity against *E. coli* (gram -ve) at lower and higher concentration when compared with the standard drug Streptomycin.

Compounds P2 and P6 showed promising antibacterial activity against *Streptococcus fecalis* (gram +ve) at lower and higher concentration when compared with the standard drug Procaine penicillin.

Compounds P1 and P5 showed good antibacterial activity against *Klebsiella* (gram -

ve) at lower and higher concentration when compared with the standard drug Streptomycin

All the compounds were also screened for anti-fungal activity. Among the compounds tested P2, P3 and P4 showed comparatively better antifungal activity against *Candida albicans* at both concentrations compared to standard Ciclopirox olamine USP.

Compounds P2, P3, P5 and P7 showed promising antifungal activity against *Aspergillus fumigatus* compared to standard Ciclopirox olamine USP.

All the compounds were tested for anthelmintic activity by using earth worms. Among the compounds tested P1, P4 and P7 showed significant anthelmintic activity compared to standard Albendazole.

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References

- [1] Pattan S. R, Kekare P, Dighe N.S, Nirmal S.A, Musmade D.S, Parjane S.K, Daithankar A.V Synthesis and biological evaluation of some 1, 3, 4- thiadiazoles; *J Chem & Pharm. Res.* **2009**; 1(1): 191-198.
- [2] Vedavathi M, Somashekar B, Sreenivasa G. M, Jayachandran E. Synthesis, Characterization and Antimicrobial activity of Fluoro benzothiazole incorporated with 1,3,4-Thiadiazole; *J Pharm. Sci.& Res.* **2010**; 2(1): 53-63.
- [3] Mohd Amir, Arun Kumar, Israr Ali & Khan S. A. Synthesis of pharmaceutically important 1,3,4-thiadiazole and imidazolinone derivatives as antimicrobials; *Ind J Chem.* **2009**; 48B: 1288-1293.
- [4] Jumat S, Nadia S, Ayad H, Hiba I, Emad Y. Synthesis and Antibacterial Activity of Some New 1,3,4-Oxadiazole and 1,3,4-Thiadiazole Derivatives; *J Applied Sci. Res.* **2010**; 6(7): 866-870.
- [5] Sreenivasa Rao D, Jayachandran E, Sreenivasa GM and Shivakumar B. Inhibition of albumin denaturation and anti-inflammatory activity of 2-[N-p-Tolyl sulphon hydrazino]-6-fluoro-7-substituted (1,3) benzothiazoles; *Oriental J Chem.* **2005**; 21(1): 113-116.
- [6] Srinivasa G.M, Jayachandran E, Shivakumar B, Sreenivasa Rao D. Synthesis and pharmacological screening of 2-[3-amino,5-s-met carboxamido pyrazol-1-yl]6-fluoro,7-substituted (1,3)benzothiazole; *Oriental J Chem.* **2004**; 20(1): 103-110.
- [7] Bushan Kumar S Sathe, Sreenivasa GM, Jayachandran E, Sreenivasa Rao D and Nargund LVG. Synthesis and anthelmintic activity for 6-fluoro, 7-substituted (1,3)benzothiazole; *Int J Chem Sci.* **2006**; 4(3): 545-552.

- [8] Sreenivasa GM, Shivkumar B, and Jayachandran E. Anthelmintic activity of 8-fluoro-9-substituted (1,3)-benzothiazolo (5,1-b)-1,2,4-triazole on *perituma posthuma*; *Indian Drugs* **2006**; 43 (4).
- [9] Jayachandran E, Bhatia K, Nargund LVG, and Roy A. Anthelmintic activity 2-[3-amino, 5-s-methyl, 4-carboxamido Pyrozol-1-yl] 6-fluoro-7-substituted (1,3) benzothiazoles on *Perituma-Posthuma*; *Indian Drugs* **2003**; (7) 40.
- [10] Schuunus, Rodney C, Gallaschun, Randall J. *Chem Abstr*, 191; 115: 49484 t.
- [11] Gopikumar P, Jayachandran E, Nagappa AN, Shivkumar B, Nargund LVG, Gurupadiah BM Synthesis and biological activity of 6-fluoro-7-(substituted)-(2-N-p-anilino sulphonamido) benzothiazole; *Ind J Heterocyclic Chem.* **2001**; 11: 39-42.
- [12] Jayachandra E, Nargund LVG, Shivakumar B, Kamal Bhatia Synthesis and pharmacological screening of 2-[3-amino, 5-s-methyl, 4-carboxamido, pyrazol-1-yl]6-fluoro,7-substituted (1,3) benzothiazole; *Oriental J Chem.* **2003**; 19(1): 139-142.
- [13] Shantharam U, L.V.G Nargund, Vasudev Nayak Synthesis of 7-substituted-2-(arylidine)6-fluoro-(1,3) benzothiazole for anti-tumor activity; *Oriental J Chem.* **2007**; 23(3): 1053-1056.
- [14] Pattan S. R, Kekare P, Dighe N.S, Nirmal S.A, Musmade D.S, Parjane S.K, Daithankar A.V Synthesis and biological evaluation of some 1, 3, 4- thiadiazoles; *J Chem & Pharm. Res.* **2009**; 1(1): 191-198.
- [15] Sunil D, Arun M Isloor and Prakash S. Synthesis, characterization and anticancer activity of 1,2,4-Triazol [3,4-b]-1,3,4-thiadiazoles on Hep G2 cell lines; *Der Pharma chemical*, 1(2), **2009**: 19-26.
- [16] Mohamed Al-Omar, Omar A, Al-Deeb, Hamad A, Al-Khamees and Ali A. El-Emam 1,3,4-thiadiazoles. Regioselective o-demethylation on dehydrative cyclisation of 1-(3,4,5-trimethoxybenzoyl)-4-substituted thiosemicarbazides with sulphuric acid, phosphorus, sulfur and silicon. 179,m 2004, 2509-2517.
- [17] Sreenivasa Rao D., Shrinivasa GM., Shivkumar B., Synthesis and antimicrobial activity of 2[NP-tolyi sulphon-hydrazine]-6-fluoro-7-substituted(1,3) benzothiazole. *Ind. J. Het. Chem.*, 14, **2004**, 65-66.
- [18] William Kemp. Infrared spectroscopy, organic spectroscopy ELBS with Mc Millain. IIIrd ed; **1991**, 19-96.
- [19] Robert M, Silverstein, Clayron Bassler G, Terence C, Murill. Proton Magnetic Resonance, Vth ed. John Willey and Sons New York; **1991**:181-212.

