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Synthesis, Characterization and Anti-microbial activity of Fluoro benzothiazole incorporated with 1,3,4-Thiadiazole

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Abstract:
Fluorobenzothiazole incorporated with 1,3,4-thiadiazole derivatives have been synthesized and evaluated for their anti-microbial activity. Structures of these compounds have been established by IR, [1]HNMR data. Significant anti-microbial activities were observed for members of this series.
Key Words: Fluorobenzothiazole, Thiadiazoles, Anit-microbial.

INTRODUCTION

We report herein the new and unreported yet the synthesis of fluoro benzothiazoles[1-4] comprising thiadiazole[5-10] derivatives. The chemistry and pharmacology of thiadiazole have been of great interest because of its various biological activities, so that the biological and pharmacological activity of thiadiazole with fluoro benzothiazoles 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 benzothiaole moiety.

In search for new bioactive potent molecule, it was thought worth while to incorporate some additional heterocyclic moieties in the thiadiazole nucleus and study their biological and pharmacological activity, the review of literature reveal prompted us to synthesis of substituted Fluoro benzothiazolyl thiadiazole compounds and those will be screened for antimicrobial[11-13], anti-inflammatory and anthelmintic activity to get potent bioactive molecule.

The thiadiazole drugs were the first effective chemotherapeutic agents to be employed systematically for the prevention and cure of bacterial infection in human beings. They are also choice of drug as diuretic (eg. Acetazolamide). Benzothiazole with thiadiazole group etc. were reported to posses various pharmacological activity of clinical importance.

Thiadiazole derivatives are well known to have number of biological and pharmacological activities.

MATERIALS AND METHODS:

Chemical and Reagents

4-fluoro-3-chloro aniline, Potassium thiocyanate, Glacial acetic acid, Bromine, carbon disulphide, ammonia, alcohol, hydrazine hydrate, 2-phenyl quinolin-4-carboxylic acid, pyridine, dimethyl formamide, N-phenyl antranilic acid, aromatic primary & secondary amines.

Experimental section:

Preparation of 6-fluoro-7-chloro (1, 3) benzothiazole 2-thiosemicarbazide :

2-amino benzothiozole (0.1mol) 20.25gm was dissolved in ethanol (95%) 50ml and ammonia solution was added to it. The reaction mixture was cooled below 30˚c and carbon disulphide (8ml) was added slowly within 15 minutes with continuous shaking. After complete addition of disulphide the solution was cooled to stand for 1 hour. then sodium chloro acetate (0.1mol) 9.4 gm was added to it. The reaction was exothermic. To it 50% hydrazine hydrate (20ml) 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 recrystalised from ethanol.

Preparation of 6-fluoro-7-chloro-2-[5'-(2 phenyl quinoline-4-yl) 1',3',4'-thiadiazol-2'-yl] amino (1, 3)- benzothiazoles :

An intimate mixture of 13.9 gm (0.05 mol) of 6-fluoro-7-chloro (1,3) benzothiazoles-2-thiosemicarbazides 2-phenyl quinolin-4-carboxylic acid (0.05 mol) 6.8gm and pyridine (100ml) heated at 170˚c-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 and recrystalised from ethanol.

Preparation of 2[5'-(2-phenyl quinolin-4-yl)-1',3',4'-thiadiazol-2'-yl)amino]-6-fluoro-7-substituted (1, 3) benzothiazoles:

To 6-fluoro-7-chloro-2[5'-(2-henylquinoline -4-yl)–1',3',4'-thiadiazol-2'yl] amino (1,3) benzothiazoles was treated with equimolar quantity (0.01mol) of various substituted aromatic primary and secondary amines, and refluxed for 2 hours in oil bath 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 recrystalised from benzene and absolute alcohol (1:1).

6-fluoro-7-chloro-2[5' (o anilino phenyl) 1',3',4'-thiadiazol-2'-yl amino (1,3) benzothiazoles :

An intimate mixture of 13.9 gm (0.05 mol) of (1,3) benzothiazoles 6-fluoro-7-chloro-2-thiosemicarbazides and N-phenyl Anthranilic acid (0.05 mol) 7.8 gm and pyridine (100ml) heated at 170˚c-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 recrystalised from ethanol.

2[5'-(o-anilino phenyl) -1',3',4'-thidiazol-2',-yl) amino 6-fluoro-7-substituted-(1,3) -benzothiazoles:

The 0.01 mol 6-Fluoro-7-chloro-2[5'-(o-anilino phenyl) -1',3',4'-thiadiazol-2'-yl amino (1,3) benzothiazoles was treated with equimolar quantity (0.01mol) of various substituted aromatic primary and secondary amines and refluxed for 2 hrs in the presence of DMF (dimethyl formamide) then the mixture was cooled and poured in to crushed ice. The solid was filtered off, dried and recrystallised from benzene and absolute alcohol (1:1).

General Procedure

Melting point was determined by open capillary tube method and are uncorrected. T.L.C was run on silica gel G plates using ethyl acetate and chloroform (2:1) as developing solvent for the purity of the compounds. I.R. Spectra were recorded on Shimadzu FTIR Spectrophotometer by using Nujol mull technique.

Anti-bacterial activity: Cup plate method (Diffusion method) & Minimal inhibitory concentration (MIC) method.

All the compounds synthesized were screened for antibacterial and antifungal activities at two different concentrations (50μg/ml, 100μg/ml) against *Staphylococcus aureus*, *Escherchia coli, streptocci, pseudomonas aureus* and *Candida albicans*, *Aspergillus Niger* by cup plate method using Procaine Penicillin, Streptomycin and Griseoflavin respectively as standards.

The antibacterial activities of synthesized compounds were tested in vitro on strains of four microorganisms-*Escherichia coli, Bacillus subtilis*, *Pseudomonas typhii* and *Staphylococcus aureus.*

The antibacterial activity was evaluated by tube dilution method (turbidometric method). The turbidometric method depends upon the inhibition of growth of microbial culture in a uniform solution of antibacterial in a fluid medium that is favourable to its rapid growth in the absence of the antibacterial agent. In this method minimal inhibitory concentration (MIC) of the lowest concentration of an antibacterial agent that

Table No. 1: Analytical Data

Sl. No.	Compound code	M.P/ B.P ˚C	% Yield	MOL FORM	Calculated %			
					M. Wt	C%	H%	N%
1	V_1	214-215	65%	$C_{30}H_{18}FN_7O_2S_2$	591.63	60.90	3.07	16.57
2	V_2	218-220	67%	$C_{30}H_{18}FN_7O_2S_2$	591.63	60.90	3.07	16.57
3	V_3	218-220	68%	$C_{30}H_{18}FN_7O_2S_2$	591.63	60.90	3.07	16.57
4	V_4	214-215	65%	$C_{30}H_{18}ClFN_6S_2$	581.08	62.01	3.12	14.46
5	V_5	190-192	68%	$C_{30}H_{18}ClFN_6S_2$	581.08	62.01	3.12	14.46
6	V_6	218-220	70%	$C_{30}H_{18}ClFN_6S_2$	581.08	62.01	3.12	14.46
7	V_7	212-215	66%	$C_{30}H_{19}FN_6S_2$	546.64	65.92	3.50	15.57
8	V_8	213-215	69%	$C_{28}H_{21}FN_6OS_2$	540.63	62.20	3.92	15.54
9	V_9	210-212	67%	$C_{28}H_{22}FN_7S_2$	539.64	62.32	4.11	18.17
10	P_1	202-204	68%	$C_{28}H_{21}FN_6OS_2$	540.63	62.20	3.92	15.54
11	P2	205-208	70%	$C_{28}H_{21}FN_6OS_2$	540.63	62.20	3.92	15.54
12	P_3	205-206	69%	$C_{28}H_{21}FN_6OS_2$	540.63	62.20	3.92	15.54

inhibits the growth of test organism can be detected.

The synthesized compounds were dissolved in DMF (dimethyl formamide) to prepare a stock solution of 1 mg/ml concentration. With this stock solution different dilutions 800µg - 5µg/ml were prepared. The ciprofloxacin was also prepared in DMF to obtain a concentration of 800 µg – 5µg/ml.

The solid ingredients were dissolved in water and pH was adjusted to 7.4 $\pm$ 0.2 and media was sterilized by autoclaving at 15 lb/psi for 15 minutes.

Preparation of suspension of microorganisms:

Transfer the microorganisms from culture to 5 ml of sterile normal saline (0.09%) solution made of each microorganism.

Determination of minimal inhibitory concentration:

The sterile test tube containing 1 ml of sterile media were added with 1 ml of

Table No. 2: Characteristics IR absorption bands of similar compounds

Sl.No	Compound code	Ar-NH_2 cm^{-1}	Ar C=C cm^{-1}	Ar.C=O (COOH)	Cyclic C=N cm^{-1}	C-F cm^{-1}	C-Cl cm^{-1}	NO_2 cm^{-1}	C-S-C cm^{-1}
1	CFA	3433	1494	-	1646	1259	762	-	-
2	2AB	3479	1460	-	1640	1193	685	-	-
3	2TH	3300	1380	-	1680	1200	685	-	700
4	Acid 1	-	1560	1720	1640	-	-	-	-
5	Acid 2	3280	1540	1720	-	-	-	-	-
6	V_1	3300	1370	-	1640	1200	-	720	680
7	V_2	3350	1370	-	1640	1200	-	720	680
8	V_3	3300	1370	-	1640	1200	-	720	680
9	V_4	3320	1365	-	1640	1200	660	-	680
10	V_5	3320	1370	-	1640	1200	660	-	680
11	V_6	3320	1380	-	1640	1200	660	-	700
12	V_7	3320	1380	-	1640	1200	-	-	700
13	V_8	3310	1380	-	1640	1200	-	-	700
14	V_9	3320	1380	-	1640	1200	-	-	700
15	P_1	3320	1380	-	1640	1200	-	-	700
16	P_2	3320	1380	-	1640	1200	-	-	700
17	P_3	3320	1380	-	1640	1200	-	-	700

Table No. 3: NMR Spectral Data

Sl. No.	Compound Code	Hydrogen	δ (ppm)	Multiplicity	Solvent
1	V_3	ArH, 16H, NH, 1H	7.15-7.28 6.40	m s	DMF
2	V_6	ArH, 16H, NH, 1H	7.06-7.26 6.57	m s	DMF
3	V_9	ArH, 11H NH, 1H CH_2, 8H	7.25-7.44 6.7 2.9	m s s	DMF
4	P_3	ArH, 15H NH, 1H CH_3, 3H	7.25-7.50 6.8 2.9	m s s	DMF

Table No. 4: Antibacterial activity

Sl. No.	Name of the compounds	*Mean zone of inhibition (in mm)*			
		Staphylococcus aureus		***Escherichia coli***	
		50µg	100µg	50µg	100µg
01	Procaine penicillin	18	14	-	-
02	Streptomycin	-	-	14	18
03	V_1	10(0.5)	10(0.7)	10(0.7)	10(0.5)
04	V_2	10(0.5)	10(0.7)	10(0.7)	10(0.5)
05	V_3	10(0.5)	13(0.9)	11(0.7)	10(0.5)
06	V_4	19(1.0)	10(0.7)	10(0.7)	15(0.8)
07	V_5	13(0.7)	15(1.07)	16(1.14)	13(0.7)
08	V_6	20(1.1)	10(0.7)	10(0.7)	14(0.7)
09	V_7	13(0.7)	12(0.8)	10(0.7)	13(0.7)
10	V_8	18(1.0)	10(0.7)	20(1.4)	18(1.0)
11	V_9	10(0.5)	14(1.0)	10(0.7)	10(0.5)
12	P_1	22(1.2)	10(0.7)	14(1.0)	11(0.6)
13	P_2	10(0.5)	10(0.7)	13(0.9)	12(0.6)
14	P_3	10(0.5)	10(0.7)	13(0.8)	14(0.7)

M.I.C

Sl. No	Compound code	***Staphylococcus aurous (G+ve)***	***Bacillus subtilis (G+ve)***	***Escherichia coli (G-ve)***	***Pseudomonas (G-ve)***
1	V_4	25µg/ml	25 µg/ml	25 µg/ml	50 µg/ml
2	V_6	12.5 µg/ml	25 µg/ml	50 µg/ml	25 µg/ml
3	V_8	50 µg/ml	50 µg/ml	50 µg/ml	25 µg/ml
4	P_1	25 µg/ml	12.5 µg/ml	6.5 µg/ml	12.5 µg/ml

Table No. 5: Antibacterial activity

Sl. No.	Name of the compounds	*Mean zone of inhibition (in mm)*			
		Streptococci		***Pseudomonas aureus***	
		50µg	100µg	50µg	100µg
01	Cefalotoxin	20	20	-	-
02	Sporaflaxin	-	-	22	20
03	V_1	10(0.5)	10(0.5)	10(0.4)	10(0.5)
04	V_2	10(0.5)	10(0.5)	14(0.6)	10(0.5)
05	V_3	10(0.5)	10(0.5)	10(0.4)	10(0.5)
06	V_4	10(0.5)	10(0.5)	10(0.4)	10(0.5)
07	V_5	10(0.5)	10(0.5)	10(0.4)	10(0.5)
08	V_6	10(0.5)	10(0.5)	14(0.6)	10(0.5)
09	V_7	10(0.5)	10(0.5)	10(0.4)	10(0.5)
10	V_8	10(0.5)	10(0.5)	10(0.4)	10(0.5)
11	V_9	10(0.5)	10(0.5)	10(0.4)	10(0.5)
12	P_1	10(0.5)	10(0.5)	10(0.4)	10(0.5)
13	P_2	10(0.5)	10(0.5)	10(0.4)	17(0.8)
14	P_3	12(0.6)	10(0.5)	10(0.4)	10(0.5)

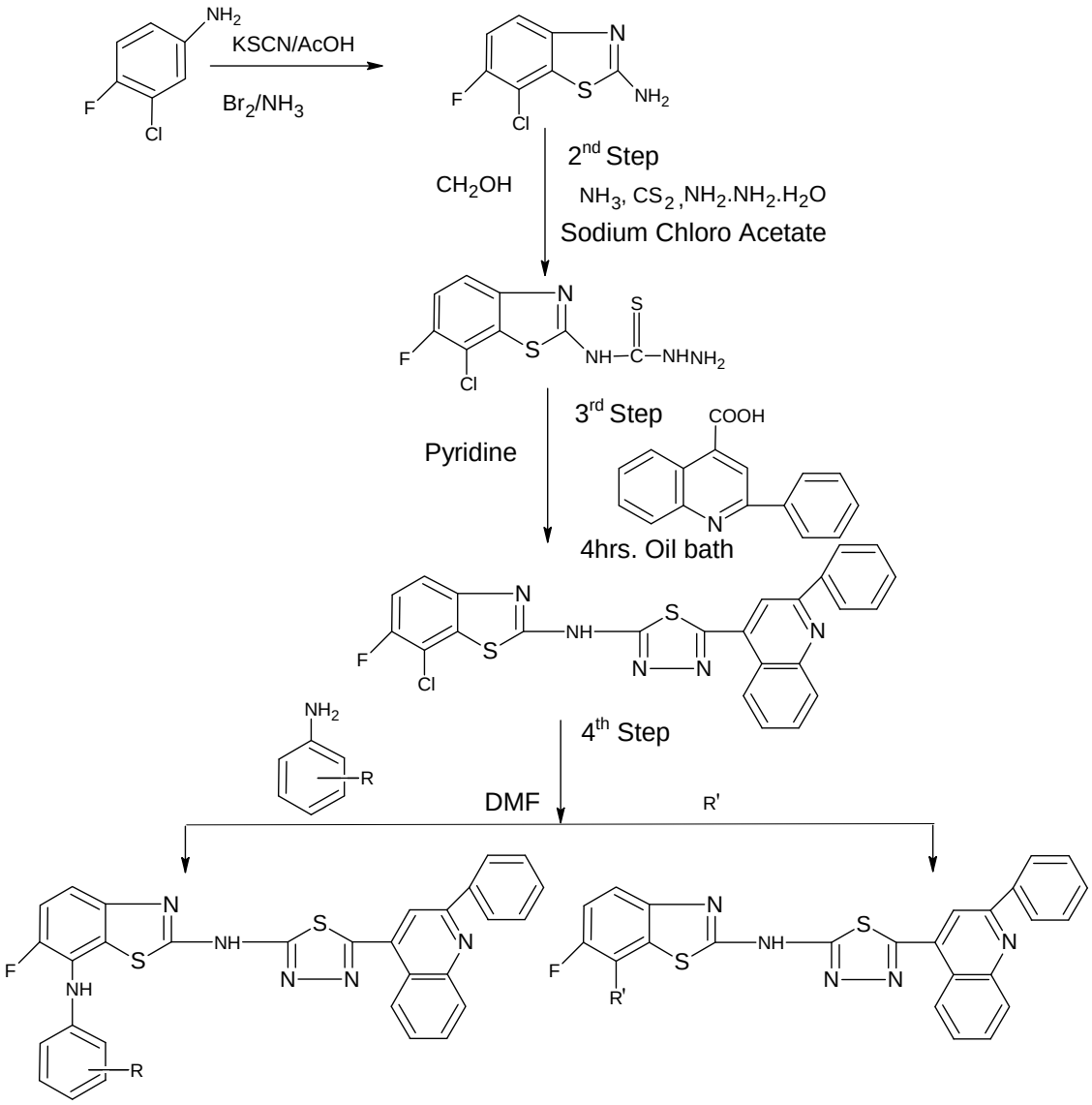
different serially diluted test samples. To these tubes 0.1 ml of normal saline solution suspended with respective microorganisms were inoculated and incubated at $37^{\circ} \pm 2$ C for 15 to 24 hours.

The growth in the tubes was observed visually for turbidity and inhibition was determined by lowest concentration of sample that prevented the development of turbidity. The procedure was repeated to confirm the MIC.

Table No. 6: Antifungal activity

Sl. No.	Name of the compounds	*Mean zone of inhibition (in mm)*			
		Candida albicans		*Aspergillus Niger*	
		50µg	100µg	50µg	100µg
01	Griseofulvin	15	16	15	14
02	V_1	10(0.6)	10(0.6)	10(0.6)	10(0.7)
03	V_2	18(1.2)	20(1.25)	10(0.6)	10(0.7)
04	V_3	15(1.0)	12(0.75)	16(1.0)	14(1.0)
05	V_4	13(0.8)	18(1.12)	10(0.6)	10(0.7)
06	V_5	20(1.3)	15(0.9)	11(0.7)	12(0.8)
07	V_6	18(1.2)	15(0.9)	10(0.6)	10(0.7)
08	V_7	16(1.0)	12(0.75)	15(1.0)	10(0.7)
09	V_8	13(0.8)	15(0.9)	10(0.6)	10(0.7)
10	V_9	10(0.6)	12(0.75)	13(0.8)	15(1.1)
11	P_1	15(1.0)	15(0.9)	13(0.8)	13(0.9)
12	P_2	15(1.0)	18(1.12)	12(0.8)	10(0.7)
13	P_3	15(1.0)	16(1.0)	15(1.0)	20(1.4)

SCHEME-I



R = o, m, p nitro aniline (V_1-V_3)

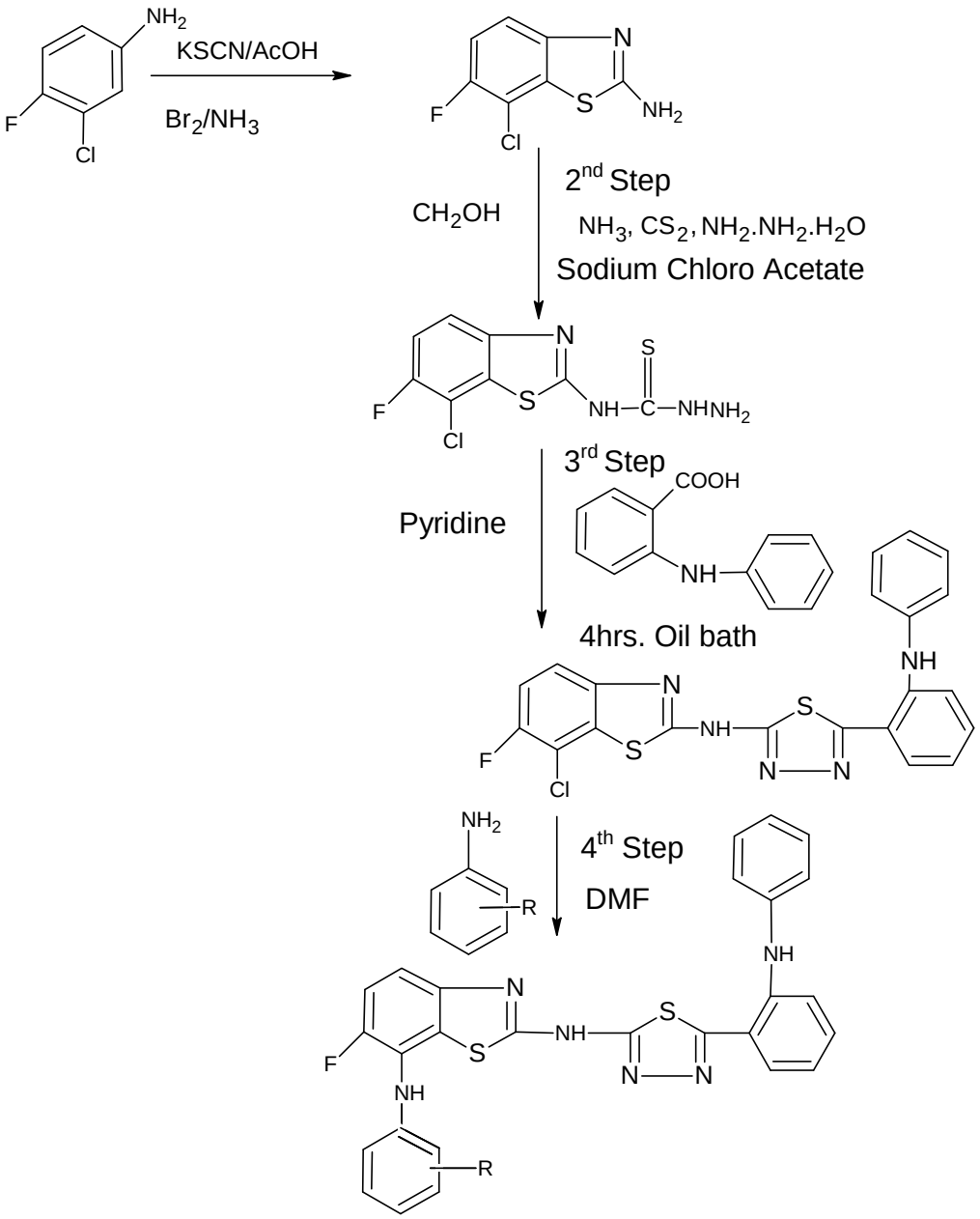
= o, m, p chloro aniline (V_4-V_6)

= aniline (V_7)

R` = Morpholine (V_8)

= Piperazine (V_9)

SCHEME-II



R = o-Anisidine
= m-Anisidine
= p Anisidine

RESULTS AND DISCUSSION

Synthesis and screening of 2-[5'-(-phenyl quinolin-4-yl) 1',3',4'-thiadiazol-2-yl amino] 6-fluoro-7-substituted (1,3) benzothiazoles and 2[5'-(o-anilino phenyl)-1',3',4'-thiadiazol-2-yl amino]-6-fluoro-7-substituted (1,3) benzothiazoles were tested for the antibacterial activity against following bacteria.

a) i) *S. aureus,* ii) *Streptococci* (gram +ve) ; b) iii) *E. coli,* iv) *pseudomonas* (gram –ve)

The test compounds V_3, V_4, V_6, V_7, V_8, V_9, P_1 showed better antibacterial activity against *Staphylococci* (gram +ve) at lower and higher concentrations and compounds V_4, V_5, V_6, V_8, P_1, P_2, P_3 showed promising antibacterial activity against *E. Coli* (gram –ve) at higher and lower concentrations.

Only compound P_3 has shown moderate antibacterial activity against *Streptococci* (gram +ve) where as the test compounds V_2, V_6 and P_2 showed moderate antibacterial activity compared to cefalotoxin (gram +ve) and Sporoflaxin against *pseudomonas* (gram –ve).

2) Antifungal activity :

The above compounds were screened for antifungal activity against *Candida albicans* and *Aspergillus niger*.

Among the compounds tested V_2, V_3, V_4, V_5, V_6, V_7, V_8, V_9, P_1, P_2, P_3 showed comparatively better antifungal activity against *Candida albicans* at both concentrations compared to standard *Griseofulvin*, where as V_3, V_5, V_7,V_9, P_1, P_2,and P_3 showed better antifungal activity against *Aspergillus niger*.

SUMMARY AND CONCLUSION

Scheme –I

In present work fluoro chloro aniline was treated with KSCN in the presence of bromine in glacial acetic acid and ammonia to get 2-amino-6-fluoro-7-chloro(1,3)-benzothiazole, which was condensed with hydrazine hydrate in presence of ammonia, carbon disulphide with ethanol and sodium chloroacetate to get 6-fluoro-7-chloro-(1,3)-benzothiazole-2-thiosemicarbazide, which is further condensed with 2-phenyl quinolin-4-carboxylic acid in the presence of pyridine to get 6-fluoro-7-chloro-2-yl)amino(1,3) benzothiazoles. To the above product different aromatic primary and secondary amines, in the presence of DMF (dimethyl formamide were treated to get newly synthesized compounds through replacing at 7^{th} position of chlorine.

Scheme - II

In scheme-II to get 6-Fluoro-7-chloro-2[5'-(o-anilino phenyl) -1',3',4'-thiadiazol-2'-yl amino (1,3) benzothiazoles, the said compound, 6-fluoro-7-chloro-(1,3)-benzothiazole-2-thiosemicarbazide was treated with N-phenyl anthranilic acid in the presence of pyridine for cyclization. The above said product was treated with different aromatic primary and secondary amines in the presence of DMF to get newly synthesized compound derivatives by replacing chlorine at 7^{th} position.

The lead compounds of scheme-I & II were characterized by melting point TLC elemental analysis, UV, IR, and ^{1}H NMR spectral study. The compound were tested for antibacterial& antifungal activity.

The compounds tested for antibacterial studies, V_2, V_3, V_4, V_5, V_6, V_7, V_8, V_9, P_1, P_2, and P_3 showed promising antibacterial activity.

The compounds tested for antifungal studies, V_2, V_3, V_4, V_5, V_6, V_7, V_8, V_9, P_1, P_2, and P_3 showed significant activity at low and high concentration compound to standard, still further studies are requested.

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References:

[1] Filler.R J,. Fluorine Chem 1986; 33: 361-375.
[2] Conte. L, et al. J. Fluorine Chem. 1995; 70: 175-179.
[3] Costakes E, Tsatsas G. 2-(alkyl amino acyl imino) 3 – methyl benzothiazolines exhibited local

anaesthetic activity Chem. Abstr 1979;90:203935q
[4] Mehra S C, Zamam SJ. Ind Chem. Soc 1980; 57(8):829-32(eng).
[5] Imtiaz Husain. M, Vinay Kumar Synthesis of 3-[2-benzotiazolyl methyl-1,2,4 triazolo [3,4b] [1,3,4] thiadiazole-6-yl-subsituted as possible anthelmintics Ind J Chem. 1992; 31B: 673-676.
[6] Bano. Q., N. Tiwari, Nizamudin Synthesis and fungicidal activities of 3-aryl-loxymethyl-6-substituted-1,2,4-triazole [3,4-b]-1,3,4-thiadiazole Ind J Chem. 1992; 31B: 714-718
[7] Srinivasa. M., G. V. S.Rama Sharma. Synthesis of some new 2-[2, 6, 8-trisubstituted-4(3H)-oxaquinazolin]-5-aryl-1, 3, 4-thiadiazole as potent anti- inflammatory agent Ind J Heterocyclic Chem. 1998; 7:281-284.
[8] Rama Sharma G.V.S, John Thomas, V. Murugan K. Elango Nicotinyl incorporated quinazolinyl thiadiazole as possible NSAID. J Heterocyclic Chem. 1999; 9: 151-152.
[9] Jag Mohan, Anupama Synthesis and antimicrobial activity of s-triazolo [3,4-b][1,3,4] thiadiazole, s-triazolo [3,4-b] [1,3,4]- thiadiazine and s-triazolo [3,4:2,3] [1,3,4] thiadiazino [5,6-b] quinoxaline Ind J Chem. 2001; 40B: 368-371.
[10] Yadav L.D.S, Smita Singh Synthesis and antiviral activity of acylic C-nucleoside incorporating thiazolo-1,3,4-thiadiazole Ind J Chem. 2001;40B: 440-442.
[11] Oseighmah peter, Shesba, Samuel F. Synthesis of (4-isothiazolin-3-one-5-thio) Benzothiazole as microbicides Chem. Abstr 1992; 116:235629.
[12] Trivedi, Bhavani, Shah VH. Synthesis of 2(substituted benzal hydrazine carbomyl Methyl thio) benzothiazoles for antimicrobial activity Chem. Abstr 1992; 116:151637c.
[13] Moharram H. H. Synthesis of some benzothiazole derivatives with potential antibacterial activity Chem. Abstr 1991; 114: 23852 r.