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Research Article

Synthesis, analytical characterization, antimicrobial, anti oxidant and anticonvulsant evaluation of some novel 6-fluorobenzothiazole substituted pyrazole analogues

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Abstract

Benzothiazoles and pyrazoles are renowned moieties and they were reported with wide range of pharmacological activities. A Series of 1-(7-chloro-6-fluoro-1,3-benzothiazol-2-yl)-3-methyl-1*H*-pyrazol-5-ol derivatives were synthesized in four phases containing different functional groups. They were synthesized by treating 7- Chloro-6-Fluoro-Aniline with potassium thiocynate and glacial acetic acid resulting in the formation of 7-Chloro-6-fluorobenzo[*d*]thiazol-2-amine.Then it was treated with hydrazine hydrate in the presence of ethylene glycol and concentrated hydrochloric acid. After that it was treated with ethylacetoacetate in the presence of ethanol. In the fourth phase 1-(7-chloro-6-fluoro-1,3-benzothiazole-2-yl)-3-methyl-1*H*-pyrazol-5-ol was treated with equimolar quantities of various substituted anilines like morphline, piperazine aniline presence of 30ml N,N- dimethyl formamide (DMF). The synthesized compounds were characterized by (IR, ^{1}H-NMR and MASS) spectroscopic methods. Further, they were screened for anti-microbial, anti-oxidant and anti convulsant activities. *β*-phenyl ethyl amine and *m,o*-toulidines derivatives showed potent activity when compared to the standard drug.

Key words: *β*-phenyl ethyl amine, *o*-toulidine, anti-microbial, anti-oxidant and anti Convulsant activity

1. Introduction

The chemistry and biological study of heterocyclic compounds has been an interesting field in medicinal chemistry for a long time. 2-amino Benzothiazole, a heterocyclic compound containing N and S atoms serve as a unique and versatile scaffold for experimental drug design1 and have varied biological activities like anti-inflammatory, antitumor[2], anthelmintic[3], anti-tubercular[4], anti-convulsant[5] and antimicrobial[6]. Pyrazoles and Pyrazolones are the key structures in numerous compounds. They represent an important class of compounds not only for their theoretical interest but also for their biological activities such as anti-inflammatory[7], hypoglycemic[8] and antimicrobial[9].A review of the literature revealed that many effective antimicrobial agents have a heterocyclic system in their molecule10. Recent observation suggests that several analogs of pyrazole ring system such as Benzoxazole and Benzothiazole derivatives also indicate potential activity with lower toxicity in the antimicrobial Activity.

Pyrazole ring system is of some practical importance, because many drugs and medicines contain a pyrazole ring system. As early as[11] discovered the antipyretic (temperature reducing) action of a pyrazole derivative in human beings and

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due to its antipyretic property, he named the compound "Antipyrine", belong to pyrazoles[12], the literature survey reveals that pyrazole derivatives exhibit anti diabetic, herbicidal activity, anti-inflammatory, antipyretics, analgesics and muscle relaxants[13].

As a continuation of our efforts on the efficient synthesis and potential bio activities of heterocyclic compounds[14], we report the facile synthesis of 1-(7-chloro-6-fluoro-1,3-benzothiazol-2-yl)-3-methyl-1*H*-pyrazol-5-ol containing different functional groups have been synthesized by condensing 7-Chloro-6-Fluoro-2-amino-Benzothiazole (Scheme-1) and screening of anti microbial , anti oxidant and anti Convulsant activities of synthesized compounds.

2. Materials and Methods

2.1.Chemicals and Instrumentation

^{1}H NMR spectra were recorded on BRUKER 300 MHz spectrometer. Solvent is deuterio chloroform. IR Spectra were recorded on ELICO FTIR-8400 spectrophotometer shows different vibration levels of molecules by using KBr pellet technique. Mass spectra were recorded under electron impact at 70 eV on a 70 eV Micromass70E instrument. Melting point was recorded on Thiel's apparatus. Thin layer chromatography was performed using pre coated aluminium plates coated with silica gel GF_{254} [E.Merck]. N-hexane: ethyl acetate was used as the eluent. The spots were visualized in the ultraviolet light chamber. Mass spectrum was recorded on GCMS QP 5000 shimadzu.

2.2 Synthesis of 7-Chloro-6-fluorobenzo [*d*] thiazol-2-amine (1)

To glacial acetic acid (20ml) cooled below room temperature were added 8gm (0.08mol) of potassium thiocyanate and 1.45g (0.01 mol) of fluoro chloro aniline. The mixture was placed in a water bath and stirred with magnetic stirrer while 1.6ml of bromine in 6ml of glacial acetic acid was added from a dropping funnel at such a rate that the temperature never rises beyond room temperature. After all the bromine was added (105min), the solution was stirred 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 ^{0}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 ^{0}C and filtered hot. The combined filtrate was cooled and neutralized with ammonia solution to the pH range 6.0 A dark yellow precipitate was collected. Recrystalised 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 oven at 80^{0}C, the dry material (1gm 51.02%) melted at 210-212^{0}C.

2.3.Synthesis of7-Chloro-6-fluoro-2-hydrazinylbenzo[*d*] thiazole (2)

Take 10 ml of concentrated hydrochloric acid in round bottom flask add 12 ml of hydrazine hydrate drop wise cool the mixture and add 20.2gm (0.1 mol) of 2-amino Benzothiazole add 40ml of ethylene glycol refluxed for 8hrs, then in hot condition poured into crushed ice. Filter and dry the product and Recrystalised form alcohol.

2.4. Synthesis of 1-(7-Chloro-6-fluorobenzo[*d*]thiazol-2-yl)-4-methyl-1*H*-pyrrol-2-ol (3)

21.7gm (0.1mol) of hydrazine Benzothiazole mix with 13.6 ml (0.1- mol) of ethyl aceto acetate in a round bottom flask into the 40ml ethanol reflux for 2hrs and later excess of ethanol was distilled of and poured onto the crushed ice. The product obtained was filter and Recrystalised form ethanol.

2.5. Synthesis of 1-(7-chloro-6-fluoro-1,3-benzothiazole-2-yl)-3-methyl-1*H*-pyrazol- 5-ol derivatives(4a-l)

To 0.01 mol of 1-(7-chloro-6-fluoro-1,3-benzothiazole-2-yl)-3-methyl-1H-pyrazol-5-ol was treated with equimolar quantities of various substituted aniline, morph line, piperazine and diphenylamine refluxed for 2 hours in oil bath in presence of 30ml N,N- dimethyl formamide (DMF). The mixture was cooled and poured in to crushed ice. The solid separated was filtered off, dried and crystallized from alcohol and benzene.

Scheme: Synthetic Steps for Preparation Some Novel 6-Fluorobenzothiazole Substituted Pyrazole Analogues.

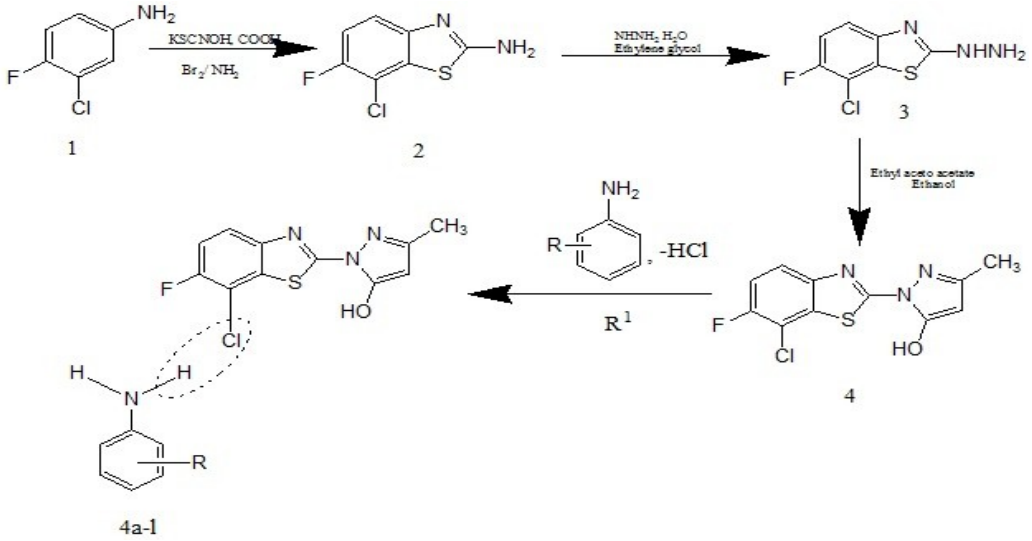
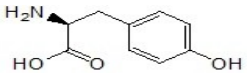
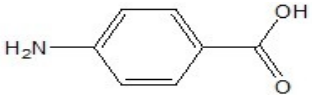
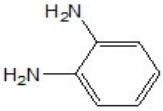
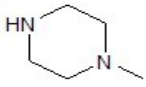
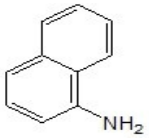


Table -1: Derivatives

Compound	-R	Compound	-R
4a	C_2H_5	4g	
4b	m- CH_3	4h	
4c	o- CH_3	4i	
4d	4-OCH_3	4j	
4e	3-OCH_3	4k	
4f		4l	

3. Statistical Analysis

3.1. Spectral characterization of Synthesis of 7-Chloro-6-fluorobenzo[*d*]thiazol-2-amine(1): Yellow solid; M.P. 210-211°C, ; Molecular formula $C_{19}H_{17}FN_4OS$; Mol. Wt. 368.43; R_f= 0.87 (n-hexane : EtOAc); MS *m/z*: 189 , (100%), 203(M^{+1}); IR (KBr) V_{max}(cm^{-1}) 678.12,848.02,1067, 1336.58, 1541.38, 1642.89; ^{1}HNMR(400MHz DMSO d_6 (δppm); 7.22 (s, 2H, NH_2), 7.53(m, 4H, aromatic).

3.2. Spectral characterization of 7-Chloro-6-fluoro-2-hydrazinylbenzo[*d*] thiazole (2)Off white solid; m.p. 233-234°C; Molecular formula $C_{19}H_{17}FN_4OS$; Mol. Wt. 368.43; R_f =0.89(n--hexane: EtOAc);MS *m/z*:172(100%), 219(M^{+2}).; IR (KBr)712.96,802.80,1072.81,1246.14,1313.91,1547.30,1640.79;^{1}HNMR(400MHz DMSO d_6 (δppm) 4.0(s, 1H, NH),4.59(s,2H, NH_2), 7.53-8.18(M, 4H, aromatic).

3.3. Spectral characterization of 1-(7-Chloro-6-fluorobenzo[*d*]thiazol-2-yl)-4-methyl-1*H*-pyrrol-2-ol(3)Orange white solid; m.p. 233-234°C, Molecular formula $C_{19}H_{17}FN_4OS$; Mol. Wt. 368.43; R_f= 0.89 (n-hexane: EtOAc); MS *m/z*: 246(100%), 282(M^{-1}).; (KBr) V_{max}(cm^{-1}) 678.12, 848.56, 1067.01,1200, 1335.78, 1449.02, 1541.59, 1649.59; ^{1}HNMR (400MHz DMSO d_6 (δppm); 2.04(S, 3H, CH_3), 7.53(M, 6H, aromatic), 11.40(s, 1H, OH).

3.3.1. 1-(7-(4-ethylphenylamino)-6-fluorobenzo[d]thiazol-2-yl)-3-methyl-1H-pyrazol-5-ol (4a): Pale yellow solid; M.P. 140°C; Molecular formula $C_{19}H_{17}FN_4OS$; Mol. Wt. 368.43; R_f= 0.93 ($CHCl_3$:EtOAc: n-Bu 1:2:1); MS *m/z* 254, 289,316(100%), 367(M^{-1}); IR (KBr) V_{max}(cm^{-1}) 860.16, 1677.2, 1542.14, 1076.14, 1449.01, 1200.94; ^{1}HNMR(400MHz DMSO d_6 (δppm) 2.3-2.4(d,6H,2CH_3,*j* =4.0), 2.7 (S,2H,CH_2), 7.2-8.7(m,7H,aromatic), 9.77(s,1H,NH), 11.78 (S, 1H, OH).

3.3.2. 1-(6-fluoro-7-(m-tolylamino)benzo[d]thiazol-2-yl)-3-methyl-1H-pyrazol-5-ol(4b): Brick red solid; M.P. 135°C; Molecular formula $C_{18}H_{15}FN_4OS$; Mol. Wt. 354.40; R_f=0.84($CHCl_3$:EtOAc: n-Bu 1:2:1); MS *m/z* : 286, 301,314(100%), 356(M^{+2}); IR (KBr) V_{max}(cm^{-1}) 843.01, 1642.87, 1541.21, 1068.70, 296.90, -1192.12 ; ^{1}HNMR (400 MHz ,DMSO D_6) (δppm)2.30-2.34(d,6H, 2CH_3, *j* =1.6),7.08-7.30(m,7H,aromatic), 9.77(s,1H,NH) , 11.78 (S, 1H, OH).

3.3.3. 1-(6-fluoro-7-(o-tolylamino)benzo[d]thiazol-2-yl)-3-methyl-1H-pyrazol-5-ol (4c): Brick red solid; M.P. 137°C; Molecular formula $C_{18}H_{15}FN_4OS$; Mol. Wt. 354.40; R_f=0.83($CHCl_3$:EtOAc: n-Bu 1:2:1); MS *m/z* 234,267, 301,323(100%), 355(M^{+1}); IR (KBr) V_{max}(cm^{-1}) 843.01, 1642.87, 1541.21, 1068.70, 296.90, -1192.12 ; ^{1}HNMR (400 MHz, DMSO D_6) (δppm) 2.30-2.34(d,6H, 2CH_3, *j* =1.6),7.08-7.30(m,7H,aromatic), 9.77(s,1H,NH) , 11.78 (S, 1H, OH).

3.3.4. 1-(6-fluoro-7-(4-methoxyphenylamino)benzo[d]thiazol-2-yl)-3-methyl-1H-pyrazol-5-ol(4d): Yash color solid; M.P. 157 °C; Molecular formula $C_{18}H_{15}FN_4O_2S$; Mol. Wt. 370.40; R_f=0.86($CHCl_3$:EtOAc: n-Bu 1:2:1); MS *m/z*256, 284,,319(100%), 370(M^+); IR (KBr) V_{max}(cm^{-1}) 840.151, 1640.56, 1538.45, 1067.02, 1448.58, 1192.01, 2831.90 12 ; ^{1}HNMR (400 MHz ,DMSO D_6) (δppm) ;2.30-3.83(d,6H, 2CH_3, *j* =6.0),7.02-7.55(m,7H,aromatic), 9.77(s,1H,NH) , 11.78 (S, 1H, OH).

3.3.5. 1-(6-fluoro-7-(3-methoxyphenylamino)benzo[d]thiazol-2-yl)-3-methyl-1H-pyrazol-5ol(4e): Yash color solid; M.P. 160 °C; Molecular formula $C_{18}H_{15}FN_4O_2S$; Mol. Wt. 370.40; R_f=0.83($CHCl_3$:EtOAc: n-Bu 1:2:1); MS *m/z*256, 284,319(100%), 370(M^+); IR (KBr) V_{max}(cm^{-1}) 840.151, 1640.56, 1538.45, 1067.02, 1448.58, 1192.01, 2831.90 12 ; ^{1}HNMR (400 MHz ,DMSO D_6) (δppm); 2.30-3.83(d,6H, 2CH_3, *j* =6.12),7.02-7.55(m,7H,aromatic), 9.77(s,1H,NH) , 11.78 (S, 1H, OH).

3.3.6. 1-(6-fluoro-7-(piperizn-1-yl)benzo[d]thiazol-2-yl)-3-methyl-1H-pyrazol-5-ol(4f): Light brown solid; M.P.148 °C; Molecular formula $C_{15}H_{16}FN_5OS$; Mol. Wt. 333.38; R_f=0.62($CHCl_3$:EtOAc: n-Bu 1:2:1); MS *m/z* 268, 298, 312(100%), 333(M^+).; IR (KBr) V_{max}(cm^{-1}) 854.87, 1633.12, 1545.25, 1076.12, 1453.86, 1200.84 ; ^{1}HNMR (400 MHz ,DMSO D_6) (δppm) 2.0(s, 1H, NH), 2.30(S, 3h, CH_3), 2.78-3.46(m, 6H, 3CH_2), 7.08 (m,11H, aromatic), 11.77 (S, 1H, OH).

3.3.7. 1-(6-fluoro-7-amino benzo [d]thiazol-2-yl)-3-methyl-1H-pyrazol-5-ol(4g): Off-white solid; M.P. 182°C; Molecular formula $C_{15}H_{15}FN_4OS$; Mol. Wt. 264.28; R_f=0.81 ($CHCl_3$:EtOAc: n-Bu 1:2:1); MS *m/z* 198, 209, 221(100%), 265(M^{+2}); IR (KBr) V_{max}(cm^{-1}) 848.65, 1646.25, 1545.01, 1069.41, 1449.15, 1193.47 ; ^{1}HNMR (400 MHz ,DMSO D_6) (δppm) 2.30(s, 3H, CH_3),1.92-3.44(d, 8H, 4CH_2, *j*=6.08),7.28(m, 3H, aromatic), 11.77 (S, 1H, OH).

3.3.8.(S)-2-(6-fluoro-2-(5-hydroxy-3-methyl-1H-pyrazol-1-yl) benzo[d]thiazol-7-ylamino)-3- (4-hydroxy phenyl) propanoic acid (4h): Off-white solid; M.P. 176 °C; Molecular formula $C_{20}H_{17}FN_4O_4S$; Mol. Wt. 428.44; R_f=0.55

($CHCl_3$:EtOAc: n-Bu 1:2:1); MS *m/z* 289, 346, 387, 401(100%), 430(M^{+2}); IR (KBr) V_{max}(cm^{-1}) 837.37, 1580.14, 1580.56, 1042.45, 1450.13, 1197.03; ^{1}HNMR (400 MHz ,DMSO D_6) (δppm) 2.30 (s, 3H, CH_3),3.40(s, 2H, CH_2),6.70-7.05(m, 5H, aromatic), 9.13(s, 1H, NH), 11.78-12.58(m, 3H, 3 OH).

3.3.9. 4-(6-fluoro-2-(5-hydroxy-3-methyl-1H-pyrazol-1-yl)benzo[d]thiazol-7-ylamino)-benzoic acid(4i): Brown solid; m.p. =129°C; Molecular formula $C_{18}H_{13}FN_4O_3S$; Mol. Wt. 384.38; R_f=0.69 ($CHCl_3$:EtOAc: n-Bu 1:2:1); MS *m/z* : 291, 325, 346(100%), 383(M^-); IR (KBr) V_{max}(cm^{-1}) 857.72, 1543.78, 1543.66, 1074.89, 1452.10, 1199.1; ^{1}HNMR (400 MHz ,DMSO D_6) (δppm) 2.30 (s, 3H, CH_3), 7.73(m, 7H, aromatic), 9.77(s, 1H, NH), 11.78-12.74(d, 2H, 2 OH, *j*-1.08).

3.3.10. 1-(7-(2-aminophenylamino)-6-fluorobenzo[d]thiazol-2-yl)-3-methyl-1H-pyrazol-5-ol(4j): Brown solid; m.p. =135°C; Molecular formula $C_{17}H_{14}FN_5OS$; Mol. Wt. 355.39; R_f=0.84 ($CHCl_3$:EtOAc: n-Bu 1:2:1); MS *m/z* 267, 319, 349(100%), 357(M^{+2}).; IR (KBr) V_{max}(cm^{-1})1 846.05, 1544.19, 1644.12, 1068.13, 1450.89, 1193.7; ^{1}HNMR (400 MHz ,DMSO D_6) (δppm); 2.30 (s, 3H, CH_3),6.38-7.02(m, 7H, aromatic),5.19(s, 2H, 1NH_2) 9.77(s, 1H, NH), 11.78(d, 1H, 1 OH).

3.3.11. 1-(6-fluoro-7-(4-hydroxyphenylamino)benzo[d]thiazol-2-yl)-3-methyl-1H-pyrazol-5-ol(4k): Black solid; m.p. = 190 °C ; Molecular formula $C_{17}H_{13}FN_4O_2S$; Mol. Wt. 356.37; R_f=0.84 ($CHCl_3$:EtOAc: n-Bu 1:2:1); MS *m/z* 267, 319, 349(100%), 357(M^{+1}); IR (KBr) V_{max}(cm^{-1}) 845.59, 1544.45, 1644.86, 1067, 1448.5, 1191.92; ^{1}HNMR (400 MHz ,DMSO D_6) (δppm) 2.30 (s, 3H, CH_3),6.38-7.02(m, 7H, aromatic), 9.77(s, 1H, NH), 9.43, 11.78(d, 2H, 2 OH *j*=8..48).

3.3.12. 1-(6-fluoro-7-(naphthalene-1-ylamino)benzo[d]thiazol-2-yl)-3-methyl-1H-pyrazol-5-ol(4l): Brown solid; m.p. = 121°C; Molecular formula $C_{21}H_{15}FN_4OS$; Mol. Wt. 368.43; R_f=0.97 ($CHCl_3$:EtOAc: n-Bu 1:2:1); MS *m/z* 247, 299, 318,357(100%), 391(M^{+1}).; IR (KBr) V_{max}(cm^{-1}) 848.19, 1544.85, 1644.86, 1069.58, 1450.1, 1193.4; ^{1}HNMR (400 MHz, DMSO D_6) (δppm) 2.30 (s, 3H, CH_3),6.98-7.38(m, 10H, aromatic), 9.77(s, 1H, NH), 11.78(d, 1H, 1OH,)

4. Elemental Analysis

4.3.1. Tabel-2 Elemental analysis of Synthesised compounds

Compounds	Various Amines used	Mol. Formula	Mol. Weight	M.P°C	%Yield	R_f Value	Elemental analysis Calculated		
							C	H	N
4a	B-phenyl ethyl amine	$C_{19}H_{17}FN_4OS$	368.43	140	13.7%	0.93	58.98	5.22	18.17
4b	m-toulidine	$C_{18}H_{15}FN_4OS$	354.40	135	17%	0.84	58.37	4.08	18.17
4c	o-toulidine	$C_{18}H_{15}FN_4OS$	354.40	157	16%	0.83	58.37	4.08	18.17
4d	m-Anisidine	$C_{18}H_{15}FN_4O_2S$	370.40	160	14%	0.86	58.37	4.52	14.95
4e	p-Anisidine	$C_{18}H_{15}FN_4O_2S$	370.40	137	11%	0.83	58.37	4.27	14.95
4f	Piperazine	$C_{15}H_{16}FN_5OS$	333.38	148	28%	0.62	53.88	4.39	14.95
4g	Pyrrolidine	$C_{15}H_{15}FN_4OS$	264.28	182	16%	0.81	53.88	4.34	16.46
4h	L-Tyrosine	$C_20H_{17}FN_4O4$ S	428.44	176	23%	0.55	5924	4.78	15.13
4i	p-Amino Benzoic Acid	$C_{18}H_{13}FN_4O_3S$	384.38	129	17%	0.69	58.37	4.48	15.13
4j	o-phenylene diamine	$C_{17}H_{14}FN_5O$ S	355.39	135	20%	0.84	52.98	4.48	16.76
4k	4-amino phenol	$C_{17}H_{13}FN_4O_2S$	356.37	190	10%	0.75	52.98	3.23	20.16
4l	Naphthyl amine	$C_{21}H_{15}FN_4OS$	390.43	121	25%	0.97	59.74	4.24	16.23

5. Biological Screening

Chemicals and reagents: Nutrient agar medium, DMSO, Ciprofloxacin, potato dextrose medium, fluconazole, wlister rats, tween 80, Diazepam Sodium.

5.1. Antibacterial Activity

5.1.1. Antibacterial activity against microbial strains[15]: All the compounds were tested for their antibacterial activity against *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumonia* (ATCC 13883), *Escheria coli* (ATCC 25922) using disc diffusion method . DMSO was run as a control and test was performed at different concentrations (25, 50 and 100 µg/ ml) using a solvent DMSO. Ciprofloxacin was used as a standard drug. All the pyrazolo fluoro benzothiazoles derivatives (4a-l) showed antibacterial activity.

5.1.1.1. Determination of the *in vitro* antimicrobial activity by the disc-diffusion method: The antimicrobial activity of the compounds was determined by means of the disc-diffusion method. Cultures of each bacteria were inoculated to nutrient agar broth and incubated at 37 ^{0}C for 16 h, then adjusted to OD625 ¼ 0.08–0.1 (approximately 1 x 10^7 –1x 10^8CFU/mL).The bacterial suspensions (100l L) was placed on to agar in a 60-mm Petri dish and spread homogeneously with a Drigalski tip. Discs (6.0-mm diameter) were impregnated with 25, 50 and 100 µg/ ml concentrations in DMSO solution of the compounds (4a-l) and placed on the surface of the agar containing each bacterium, which was incubated at37C for 24 h. The inhibition zones were measured with a caliper considering the total diameters. Similarly, each plate carried a blank disc containing 25, 50 and 100 µg/ ml concentrations in DMSO and an anti-biotic disc (100 µg/ml for ciprofloxacin).

5.1.2. Anti fungal activity against fungal Strians[15]: All the compounds were tested for their antifungal activity against A*spergillus flavus* and *Aspergillus niger* (NCCS 1196) by disc diffusion method DMSO was run as a control and test was performed at different concentrations (100 and 150 µg/ml) using a solvent DMSO. Fluconazole was used as a standard drug. All the compounds (4a-l) showed antifungal activity.

5.1.2.1. Determination of the *in vitro* antifungal activity by the disc-diffusion method: The fungal activity of the compounds was determined by means of the disc-diffusion method. Cultures of each bacteria were inoculated to potato dextrose broth and incubated at 37 ^{0}C for 16 h, then adjusted to BOD625 ¼ 0.08–0.1 (approximately 1 x 10^7 –1x 10^8CFU/mL).The Fungal suspensions was placed on to dextrose in a 60-mm Petri dish and spread homogeneously with a Drigalski tip. Discs (6.0-mm diameter) were impregnated with 100 and 150 µg/ ml concentrations in DMSO solution of the compounds (4a-l) and placed on the surface of the dextrose broth containing each fungal strain, which was incubated at37C for 24 h. The inhibition zones were measured with a caliper considering the total diameters. Similarly, each plate carried a blank disc containing 100 and 150 µg/ ml concentrations in DMSO and an anti-biotic disc (100 µg/ml Fluconazole).

5.2. Anti-convulsant Activity *(in-vivo)*

5.2.1. Assessment of Anti-Convulsant Activity by Maximum Electroshock induced Seizures (MES)[16]: Anti-Convulsant activity of prepared compounds was evaluated by using Maximum Electroshock induced Seizures (MES). The animals were divided into control, standard and test groups each consisting of 6 animals (n=6, Mice). The first group was treated with Tween80 1% suspension which served as a control. Second group was administered with a dose of 30mg/kg suspension of Diazepam sodium intra-peritoneal which served as a standard and other groups were treated with 30mg/kg of suspension of test compounds in tween 80 30 min before applying of Electroshock (-42mA,0.2sec) Using Carneal electrodes[1]. The duration of tonic hind leg extension was recorded.

5.3. Antioxidant Activity

5.3.1. Antioxidant Activity by *p*-NDA (*p*-Nitroso Dimethyl Aniline) Radical Scavenging Method[16]: To a solution containing ferric chloride (0.1mM, 0.5ml), EDTA(0.1mM, 0.5ml), ascorbic acid (0.1mM, 0.5ml), hydrogen peroxide (2mM, 0.5ml) and p-nitroso dimethyl aniline (0.01mM, 0.5ml) in phosphate buffer (p^H 7.4, 20Mm) were added various concentrations of the test compounds in distilled DMSO or dissolving solvent or alcohol to produce final volume of 3ml. Absorbance was measured at 440nm.

$$p\text{-NDA radical Scavenging activity (\%)} = \frac{[\text{Abs (sample)-Abs (standard)}]}{[\text{Abs (sample)}]} \times 100$$

Where, Abs- Absorbance,

6. Results

We report here the synthesis of some novel 6-fluorobenzothiazole substituted pyarzole analogues. The synthesized compounds were evaluated for anti-microbial (bacterial and fungal) activity , anti Convulsant activity and also for anti-

oxidant activity. The reaction sequence and their derivatives were out lined in scheme-1. 1-(7-chloro-6-fluoro-1,3-benzothiazole-2-yl)-3-methyl-1*H*-pyrazol-5-ol derivatives were prepared by cyclisation of fluoro chloro aniline with potassium thiocyanate in the presence of glacial acetic acid and bromine (1) further reacts with hydrazine hydrate to yield acid hydrate (2) with Ethyl acetoacetate by ring cyclisation to get the derivatives. A series of derivatives were prepared by equimolar quantities of various substituted aniline, morph line, piperazine and diphenylamine refluxed for 2 hours in oil bath in presence of 30ml N, N- dimethyl formamide (DMF). The products were isolated by adding cold water in the reaction mixture and filtering off the precipitated solid. The structure of the parent compound and its derivatives were confirmed by IR, ^{1}H-NMR, and mass - spectral data as described in experimental section.

7. Discussion

7.1. Anti-bacterial Activity

Synthesized compounds(4a-l) were screened for anti bacterial activity using disc plate method at concentrations 25, 50 and 100µg/ml using gram +ve and gram –ve strains. The strains used for the screening are *Bacillus subtilis*, *Staphylococcus aureus* (gram +ve), *Klebsiella pneumonia* (gram –ve strains), *Escheria coli*. Ciprofloxacin as the standard drug. (Table-3). Among the screened compounds, **4a** and **4f** had shown the potent activity against the standard. Compounds **4d, 4e** and **4i** had shown moderate activity Compounds **4j** and **4k** had shown lesser activity**.** These compounds increased the permeability of the microbial cell resulting in higher activity**.**

Table-3: Anti-Microbial Activity of synthesized Compounds (4a-l) against Bacterial Strains

Compounds	**Mean Zone of inhibition**											
	BS			**SA**			**K.p**			***E.Coli***		
	25 µg/disc	**50 µg/disc**	**100 µg/disc**	**25 µg/disc**	**50 µg/disc**	**100 µg/disc**	**25 µg/disc**	**50 µg/disc**	**100 µg/disc**	**25 µg/disc**	**50 µg/disc**	**100 µg/disc**
Control	5.9	6	6.2	5.8	6.6	6.10	6.4	7.7	8.1	6.2	7.4	7.6
4a	8	7	9	14	18	16	9	10	11	6.6	7.1	7.8
4b	6.1	6.9	7	6.4	8	10	7	8	12	6.7	8	9.6
4c	6	8	13	7	10	11	8	8	12	11	10	14
4d	8	9	13	7	10	11	8	8.10	12	9.9	12	13
4e	7	8	11	6	9	13	6.6	7	10	9	16	18
4f	7	11	12	7	7.10	13	6	8	9	6.5	9	11
4g	6	8	11	12	14	19	9	8	14	7	8	10
4h	7	9	10	7	7.10	6	7	8	9	7	9	13
4i	6	6.5	11	6	9	10	9	10	13	8	8.8	9
4j	6.1	7	8	12	13	14	7	7.8	11	7	8.4	11
4k	6	8	11	6	7	9	12	12	10	11	13	10
4l	7	7.6	8	11	15	16	8	8.9	12	6.11	8	9
Standard	18			22			19			24		

7.2. Antifungal Activity:

Synthesized compounds (4a-l) were screened for anti fungal activity using disc plate method at concentrations 150 and 200µg/ml and the strains used for the screening are *Aspergillus niger* and *Aspergillus flavus.* Fluconazole as the standard drug. (Table - 4). Among the screened compounds, **4a** and **4c** had shown the potent activity against the standardCompounds **4e, 4g** and **4k** had shown moderate activity. Compounds **4d** and **4f** had shown lesser activity.

Table-4: Anti-Fungal Activity of synthesized Compounds (4a-l) against Fungal Strains

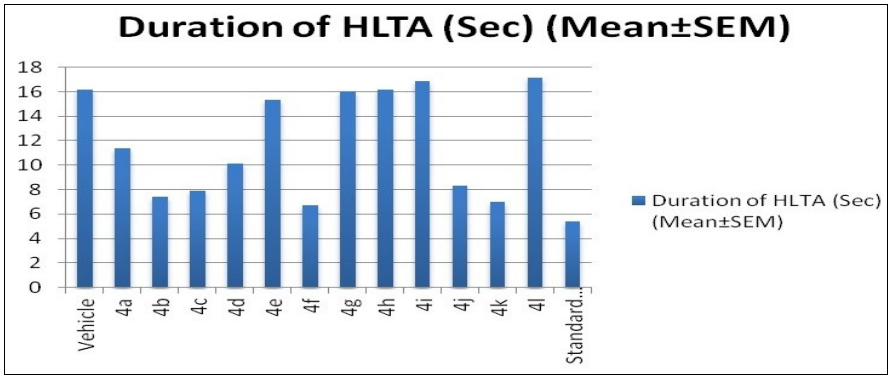
Compounds	Mean Zone of inhibition			
	Aspergillus flavus		*Aspergillus niger*	
	150 µg/disc	200 µg/disc	150 µg/disc	200 µg/disc
Control	-	-	-	-
4a	11	13	6	9
4b	11	13	8	14
4c	8	11	9	12
4d	6	10	8	14
4e	9	12	10	13
4f	6	11	7	9
4g	11	14	9	14
4h	6	10	11	14
4i	7	9	6	8
4j	7	9	6	8
4k	6	14	7	9
4l	8	9	9	14
Standard	22		18	

7.3.Anti-Convulsant Activity: Synthesized compounds (4a-l) were screened for anti-Convulsant Activity by Maximum Electroshock induced Seizures (MES). Compounds of different concentrations are screened for the activity using Diazepam as standard. The duration of seizures is calculated (Table-5). Among the screened compounds, **4l, 4k, 4i** had shown the potent activity against the standard. Compounds **4h, 4fa**and 4i had shown the moderate activity**.** Compound **4f** had shown the lesser activity against the standard.

Tabel-5: Anti-Convulsant Activity of Synthesized Compounds

S. No	Experimental groups	Duration of HLTA (Sec) (Mean±SEM)
1	Vehicle	16.15±0.61
2	4a	11.36±0.21
3	4b	7.41±0.36
4	4c	7.89±0.12
5	4d	10.12±0.41
6	4e	15.34±0.11
7	4f	6.71±0.18
8	4g	16.03±0.92
9	4h	16.18±0.16
10	4i	16.89±0.34
11	4j	8.34±0.21
12	4k	6.98±0.13
13	4l	17.12±0.12
14	Standard (Diazepam)	5.43±0.76

Graph-1: Anti-Convulsant Activity by Maximum Electroshock induced Seizures (MES)

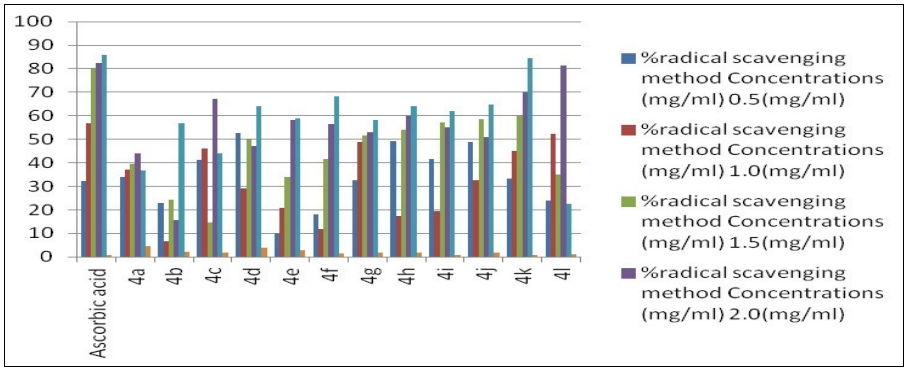


7.4.Anti - Oxidant Activity: Synthesized compounds (4a-l) were screened for anti-oxidant activity. Compounds of different concentrations are screened for the activity using Ascorbic acid as standard. The % inhibition of the compounds at various concentrations is calculated from their absorbance values. (Table-6) Among the screened compounds, **4a** had shown the potent activity against the standard. Compounds **4b, 4f** and 4i had shown the moderate activity**.** Compound **4j** had shown the lesser activity against the standard.

Table 6. Anti-oxidant Activity against Free Radical Scavenging Method

Compound	%radical scavenging method Concentrations (mg/ml)					
	0.5	**1.0**	**1.5**	**2.0**	**2.5**	**IC50**
Standard (Ascorbic acid)	32.3	56.79	80.45	82.34	85.84	0.64
4a	33.82	37.18	39.48	44.12	36.7	4.67
4b	22.74	6.5	24.13	15.74	56.79	2.19
4c	41.2	45.98	14.58	67.14	44.15	1.76
4d	52.56	29.09	49.74	47.25	64.12	3.85
4e	10.09	20.86	34.02	58.34	58.80	2.74
4f	17.9	11.68	41.58	56.34	68.12	1.48
4g	32.69	48.74	51.49	52.86	58.08	1.68
4h	49.09	17.46	54.19	59.93	64.12	1.95
4i	41.45	19.57	57.26	55.22	62.03	0.75
4j	48.78	32.57	58.45	51.06	64.71	1.73
4k	33.15	44.89	59.98	69.81	84.46	0.82
4l	23.89	52.46	35.15	81.25	22.46	0.96

Graph-2: Anti-oxidant Activity against Free Radical Scavenging Method:



8. Conclusion

It can be concluded that the projected structures of the synthesized compounds are well supported by spectroscopic data and biological evaluation. Benzothiazole substituted with anisidine, pyrrolidine, *o*-phenylene diamine on seventh position enhances the anti-microbial, anti-oxidant activities.

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