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Synthesis and Biological Screening of Benzothiazole Derivatives with Pyrazole Moiety

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Abstract: Benzothiazole is one of the most important heterocyclic compound, weak base, having varied biological activities and still of great scientific interest now a days. They are widely found in bioorganic and medicinal chemistry with application in drug discovery. In the present study some novel benzothiazole derivatives were synthesized according to the scheme. All the synthesized benzothiazole derivatives have been characterized by using elemental analysis, FT-IR, ^{1}HNMR, ^{13}C NMR spectroscopy and further supported by mass spectroscopy. Purity of all the compounds has been checked on thin layer chromatographic plate and HPLC technique. All the synthesized compounds were tested for their antibacterial and antifungal activity (MIC) in vitro by broth dilution method with two Gram-positive bacteria, two Gram-negative bacteria and two fungal strains. The biological activities of the synthesized compounds have been compared with standard drugs Ciprofloxacin and Flucanazole. Analgesic activities were tested by Tail-flick method and Writhing method. The anti-pyretic activity was evaluated using Brewer's yeast induced pyrexia in rats. The compounds exhibited significant and moderate antibacterial, antifungal, analgesic and anti-pyretic activities. These compounds can be further exploited to get the potent lead compounds. The detailed synthesis and the antimicrobial screening of the new compounds are reported.

INTRODUCTION:

Heterocyclic compound is one which possesses a cyclic structure with at least two different kinds of heteroatoms in the ring. Heterocyclic compounds are very widely distributed in nature and are essential to life in various ways. Benzothiazole is a heterocyclic compound, weak base, is made from thiazole ring fused with benzene ring, having various biological activities and still of great scientific interest now a days. Benzothiazole possess interesting biological activities like anti-tumor (Yin GL et al, 2014), anti-microbial (Vikas S. Padalkar, 2011), anti-tubercular (Telvekar VN, 2011), anti-convulsant (Nadeem Siddiqui et al, 2012), anthelmintic (Balaji.P.N, 2014), anti-oxidant (Nagaraju G, 2015), analgesic (Govinda *et al, 2015*), anti-inflammatory (Achaiah, Garlapati et al, 2014), antifungal (Herrera Cano N, 2015), anti-leishmanial (Delmas F, 2004), antipsychotic (Gollapalli Naga Raju et al, 2015), anti-ulcer ((Gollapalli Naga Raju et al, 2015)), local anesthetic (Geronikaki A, 2009) and diuretic (Husain A, 2016) activities. In the 1950s, a number of benzothiazole derivatives were intensively studied, as the benzothiazole scaffold is one of privileged structure in medicinal chemistry. Based on these findings, synthesis of some compounds featuring benzothiazole derivatives fused with pyrazole moiety with the aim of obtaining more potent pharmacologically active compounds.

EXPERIMENTAL:

Material and Methods:

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV light. All compounds were purified by recrystallization with suitable organic solvents. IR spectra were recorded on Brooker-ALPHA FT-IR instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. ^{1}H NMR and ^{13}C NMR was determined in $CDCl_3$ solution on a Bruker Ac 400 MHz spectrometer. Purity of the synthesized compounds was checked by HPLC Agilent. The results are in agreements with the structures assigned.

Synthesis of 7-chloro-6-fluorobenzo[d]thiazol-2-amine: 3-Chloro-4-fluoro aniline (3.7gm 0.025mol) mixed with potassium thiocyanate (20 gm 1.2mol) and glacial acetic acid (20 ml) in a three necked 250ml RBF with magnetic stirrer. The reactants are precooled to 0-5^{0}C by using freezing mixture. 30 ml of bromine water was added slowly by the help if funnel and maintain the temperature 0-5^{0}C. The mixture was further stirred 2hrs at 0-5^{0}C and 10 hrs at room temperature.

Synthesis of 1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)hydrazine: Hydrazine hydrate 5ml was placed in 100ml three necked RBF fitted with a mechanical stirrer and cool this to 5^{0}C using an ice bath. To the add 5ml concentrated HCl followed by 20ml ethylene glycol with continuous stirring and maintain the temperature 5^{0} – 10^{0} C. 7-chloro-6-

fluorobenzo[d]thiazol-2-amine (2 gm, 9.90mol) was added in four portions with a gap of 2 minutes between each addition with continuous stirring. The reaction mixture is refluxed 4 hrs, cooled and filtered by vacuum pump and washed with10 ml of ethyl acetate to remove unreacted 7-chloro-6-fluorobenzo[d]thiazol-2-amine.

Synthesis of7-chloro-6-fluoro-2-(3,5-dimethyl-1H-pyrazol-1-yl)benzo[d]thiazole: In a 200ml two necked RBF fitted with a condenser and placed 1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)hydrazine (2.17gm, 0.01mol), pentane-2,4-dione (1gm, 0.01 mol) and 50 ml of ethanol and refluxed 10 hrs. The reaction mixture was poured into 100 gm of crushed ice and separate the solid which was filtered and recrystallized in alcohol.

Removal of chlorine by different groups from 7-chloro-6-fluoro-2-(3,5-dimethyl-1H-pyrazol-1-yl)benzo[d]thiazole: In a dry 25 ml two necked RBFwith a condenser and nitrogen inlet assembly was placed 7-chloro-6-fluoro-2-(3,5-dimethyl-1H-pyrazol-1-yl)benzo[*d*]thiazole (3.55 mol) and added 10 ml of dry solvent, anhydrous K_2CO_3, 1.2 – 1.5 equivalents of phenol / amine / alcohol (aniline, morpholine, piperdine and phenols), 1 – 2.5 mol % cuprous salt catalyst. The entire assembly was flushed two times with dry nitrogen and refluxed 16 – 24 hrs under nitrogen atmosphere. The reaction mixture was poured into 100ml of cold water, extracted with 3×20 ml of ethyl acetate. The organic layer was dried by anhydrous sodium sulphate and evaporated in vaccuo to obtain the desired product and recrystallized by using 2:1 mixture of hexane: ethyl acetate.

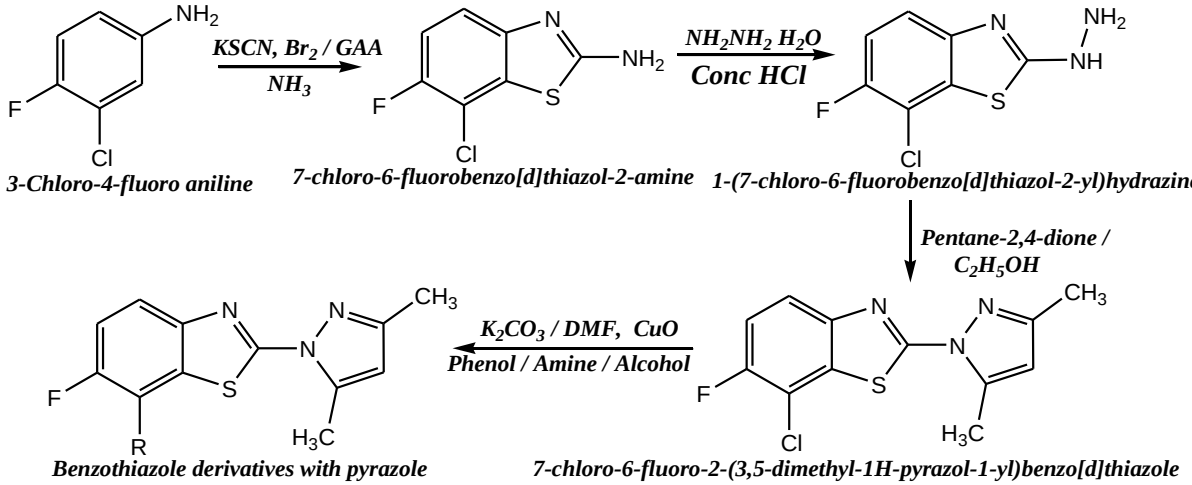
***N*-Cyclohexyl-6-fluoro-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)benzo[*d*]thiazol-7-amine:** Yield: 81%; M.P. 206 ºC; IR (cm^{-1}): 1114 (C – F stretching), 1470 (C=C), 2924 (C – H stretching), 3446 (N – H stretching); Anal. Calcd. for$C_{18}H_{21}FN_4S$: C, 62.53; H, 6.25; N, 16.37, F, 5.55; S, 9.35. Found: C, 62.76; H, 6.15; N, 16.27; F, 5.53; S, 9.31%.

***N*-Butyl-6-fluoro-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)benzo[*d*]thiazol-7-amine:** Yield: 65 %; M.P. 178 ºC; IR (cm^{-1}): 1212 (C – F stretching), 1450 (C=C), 3090 (C – H stretching), 1649 (N – H def); ^{1}H NMR ($CDCl_3$) δ ppm: 0.82 – 0.88 (m, 2H, aliphatic), 0.92 – 1.06 (t, 3H, aliphatic), 1.42 – 1.47 (m, 4H, aliphatic),2.06 (s, 3H, CH_3 at 3), 2.74 (s, 3H, CH_3 at 5), 4.60 (s, 1H, NH), 6.05 (s, 1H, ArH), 7.33 – 7.37 (d, 1H, ArH, J=8.9 Hz); 7.51 – 7.56 (d, 1H, ArH, J= 6.43 Hz); Anal. Calcd. for$C_{16}H_{19}FN_4S$: C, 60.37; H, 6.03; N, 17.65; F, 5.98; S, 10.07. Found: C, 60.35; H, 6.02; N, 17.65; F, 5.96; S, 10.09 %.

Table-1: Physical constants ofbenzothiazole derivatives with pyrazole moiety

Comp no	R	M.F	M.W	Reaction time	Yield (%)	R_f	M.P
PB-1	Cyclohexylamine	$C_{18}H_{21}FN_4S$	344.54	18hr	81	0.34	206
PB-2	Butylamine	$C_{16}H_{19}FN_4S$	318.41	25hr	65	0.39	178
PB-3	Morpholine	$C_{16}H_{17}FN_4OS$	332.39	16hr	52	0.48	177
PB-4	*N*-Methyl piperazine	$C_{17}H_{20}FNOS$	345.43	23hr	58	0.31	220
PB-5	Aniline	$C_{18}H_{15}FN_4S$	338.40	20hr	95	0.44	235
PB-6	Phenol	$C_{18}H_{14}FN_3OS$	339.38	24hr	76	0.56	158
PB-7	*p*-Cresol	$C_{19}H_{16}FN_3OS$	353.41	20hr	55	0.54	192
PB-8	*p*-Nitro aniline	$C_{18}H_{14}FN_5O_2S$	383.39	22hr	92	0.41	170
PB-9	*p*-Nitro phenol	$C_{18}H_{13}FN_4O_3S$	384.38	24hr	52	0.54	181
PB-10	Benzyl alcohol	$C_{19}H_{16}FN_3OS$	353.41	23hr	61	0.58	194

Scheme 1: Synthesis of benzothiazole derivatives with pyrazole



3-Chloro-4-fluoro aniline — *7-chloro-6-fluorobenzo[d]thiazol-2-amine* — *1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)hydrazine*

Benzothiazole derivatives with pyrazole — *7-chloro-6-fluoro-2-(3,5-dimethyl-1H-pyrazol-1-yl)benzo[d]thiazole*

6-Fluoro-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-7-morpholinobenzo[*d*]thiazole: Yield: 52 %; M.P. 177 ºC; IR (cm^{-1}): 1232 (C – F stretching), 1454 & 1526 (C=C), 2849 (C – H stretching), 1649 (N – H def); ^{1}H NMR ($CDCl_3$) δ ppm: 1.56 – 1.71 (m, 8H, aliphatic), 2.30 (s, 3H, CH_3 at 3), 2.76 (s, 3H, CH_3 at 5), 4.1 (br-s, 1H, NH), 6.05 (s, 1H, ArH), 7.55 – 7.59 (d, 1H, ArH, J=8.9 Hz); 7.87 – 7.90 (d, 1H, ArH, J= 6.43 Hz); MS: m/z 319; Anal. Calcd. for$C_{16}H_{17}FN_4OS$: C, 57.91; H, 5.10; N, 16.76; F, 5.65; S, 9.71. Found: C, 57.81; H, 5.15; N, 16.86; F, 5.72; S, 9.65%.

6-Fluoro-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-7-(4-methylpiperazin-1-yl)benzo[*d*]thiazole:Yield: 58 %; M.P. 220 ºC; IR (cm^{-1}): 1212 (C – F stretching), 1450 & 1546 (C=C), 3088 (C – H stretching), 1648 (N – H def); Anal. Calcd. for$C_{16}H_{17}FN_4OS$: C, 59.20; H, 5.88; N, 20.37; F, 5.55; S, 9.31. Found: C, 59.11; H, 5.84; N, 20.27; F, 5.50; S, 9.28 %.

6-Fluoro-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-N-phenylbenzo[*d*]thiazol-7-amine:Yield: 95 %; M.P. 235 ºC; IR (cm^{-1}): 1251 (C – F stretching), 1452 (C=C), 2980 (C – H stretching), 1620 (N – H def); Anal. Calcd. for$C_{18}H_{15}FN_4S$: C, 63.82; H, 4.45; N, 16.50; F, 5.65; S, 9.30. Found: C, 63.89; H, 4.46; N, 16.56; F, 5.61; S, 9.28 %..

6-Fluoro-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-7-phenoxybenzo[*d*]thiazole:Yield: 76 %; M.P. 158 ºC; IR (cm^{-1}): 1193 (C – O), 1259 (C – F stretching), 1546 (C=C), 3088 (C – H stretching), 1649 (N – H def); A
nal. Calcd. for$C_{18}H_{14}FN_3OS$: C, 36.78; H, 4.19; N, 12.35; F, 5.65; S, 9.40. Found: C, 36.70; H, 4.16; N, 12.38; F, 5.60; S, 9.45 %.

7-(*p*-Tolyloxy)-6-fluoro-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)benzo[*d*]thiazole:Yield: 55 %; M.P. 192 ºC; IR (cm^{-1}): 1193 (C – O), 1250 (C – F stretching), 1452 (C=C), 3090 (C – H stretching), 1649 (N – H def); Anal. Calcd. for$C_{19}H_{16}FN_3OS$: C, 64.62; H, 3.60; N, 11.80; F, 5.30; S, 9.10. Found: C, 64.57; H, 3.68; N, 11.84; F, 5.38; S, 9.07 %.

6-Fluoro-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-N-(4-nitrophenyl)benzo[*d*]thiazol-7-amine:Yield: 92 %; M.P. 170 ºC; IR (cm^{-1}): 1127 (C – F stretching), 1458 & 1526 (C=C), 1604 (N=O), 3091 (C – H stretching), 1649 (N – H def); Anal. Calcd. for$C_{18}H_{14}FN_5O_2S$: C, 59.42; H, 3.62; N, 18.37; F, 4.98; S, 8.34. Found: C, 59.39; H, 3.68; N, 18.27; F, 4.96; S, 8.36 %

7-(4-Nitrophenoxy)-6-fluoro-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)benzo[*d*]thiazole:Yield: 52 %; M.P. 181 ºC; IR (cm^{-1}): 1193 (C – O), 1217 (C – F stretching), 1458 & 1526 (C=C), 1612 (N=O), 3081 (C – H stretching), 1644 (N – H def); Anal. Calcd. for$C_{18}H_{13}FN_4O_3S$: C, 56.30; H, 3.45; N, 14.55; F, 4.92; S, 8.36. Found: C, 56.24; H, 3.41; N, 14.58; F, 4.94; S, 8.34 %.

7-(Benzyloxy)-6-fluoro-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)benzo[*d*]thiazole:Yield: 61 %; M.P. 194 ºC; IR (cm^{-1}): 1193 (C – O), 1251 (C – F stretching), 1452 & 1526 (C=C), 3068 (C–H stretching), 1649 (N – H def); Anal. Calcd. for$C_{16}H_{17}FN_4OS$: C, 64.60; H, 5.88; N, 11.90; F, 5.40; S, 9.10. Found: C, 64.57; H, 4.56; N, 11.84; F, 5.38; S, 9.07 %.

BILOGICAL SCREENING:

ANTIMICROBIAL STUDIES:

All the synthesized compounds 2–10 were screened for their antibacterial and antifungal activity at 50 µg/disc by the disc diffusion method. Test was carried out on four bacterial strains, namely *Staphylococcus aureus*(MTCC 96), *Staphylococcus pyogenus, Pseudomonas aeruginosa*(MTCC 1688),*Escherichia coli* (MTCC 443). Ciprofloxacin was used as standard. Antifungal study was carried out in two fungal strains, namely *Candida albicans*(MTCC 227) and *Aspergilla niger* (MTCC 282). Fluconazole was used as standard.The results obtained from antimicrobial susceptibility testing are depicted in Table 2.

ANALGESIC ACTIVITY:

Swiss Albino mice (weighing 20-25 gm) and Wistar rats (weighing 150-200 gm) were used for studying *in vivo* analgesic and anti-pyretic activities. Animals were maintained under standard laboratory conditions (24±2 ^{0}C; relative humidity 60-70%). Study protocol was approved by the Institutional Animal Ethics Committee before conducting the experiments (CIPS/IAEC/08/2015-2016). The mice and rats were used in the study. The animals were kept in polypropylene cages and maintained on balanced diet with free access to drinking water. All the experimental procedures were conducted in accordance with the guide for Care and Use of Laboratory Animals and in accordance with the Local Animal Care and Use Committee.

The analgesic activity was carried out by Tail-flick and writhing methods.

Table-2: Antimicrobial activity ofbenzothiazole derivatives with pyrazole moiety

Compound	Diameter of the zone of inhibition in mm (Relative inhibition %)					
	Antibacterial Activity				Antifungal activity	
	S. aureus	*S. pyogenus*	*E.coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
PB-1	08 (40)	10 (50)	10 (42)	32 (35)	12 (50)	11 (55)
PB-2	09 (47)	11 (55)	08 (44)	12 (48)	13 (54)	12 (40)
PB-3	10 (52)	12 (60)	10 (55)	13 (52)	14 (58)	13 (43)
PB-4	15 (78)	16 (80)	12 (66)	17 (68)	19 (79)	26 (86)
PB-5	17 (89)	19 (95)	15 (83)	20 (80)	20 (83)	28 (93)
PB-6	11 (57)	12 (60)	11 (61)	14 (56)	15 (62)	14 (46)
PB-7	16 (84)	18 (90)	14 (77)	18 (72)	22 (91)	30 (100)
PB-8	14 (73)	13 (65)	13 (72)	16 (64)	18 (75)	21 (70)
PB-9	13 (68)	14 (70)	13 (72)	15 (60)	16 (66)	20 (66.7)
PB-10	12 (63)	15 (75)	12 (66)	14 (56)	15 (62)	19 (63.3)
Ciprofloxacin	19 (100)	20 (100)	18 (100)	25 (100)	NA	NA
Fluconazole	NA	NA	NA	NA	24 (100)	30 (100)

Tail-flick method:

After over-night fasting, the rats were divided into different groups (n=6) as shown in Table 3.The reaction time was measured at the end of 0, 30, 60 and 90 min after the administration of the compounds. The drugs were dispersed in 0.5% w/v of sodium carboxy methyl cellulose (sodium CMC) and administered orally. The control group (no drug) was administered with 0.5 ml of 0.5% w/v of sodium CMC. The tail-flick latency was assessed by considering the time taken by the rat to withdraw its tail from the hot water bath (55 ± 0.5^0C) . The tail-flick latency of treated animals was compared with control animals.

Writhing method:

After activity an over-night fast, the mice were distributed into different groups and treated as shown in Table 4**.** The drugs were dispersed in 0.5% w/v of sodium CMC and administered orally. The control group (no drug) was administered with 0.5 ml of 0.5% w/v of sodium CMC. The standard drug used was nimesulide (12.5 mg/ kg). One hour after the treatment, the mice were given an intraperitoneal injection of 0.7% v/v acetic acid solution (volume of injection was 0.1 ml/ 10 gm body weight). The number of writhes produced in these animals was counted for 30 min. The analgesic was evaluated in terms of the percentage of writhe inhibitions.

Table 3: Analgesic activity ofbenzothiazole derivatives with pyrazoleby tail flick method on rats

Compound	Average reaction time (sec) at different time intervals[b,c]			
	0 min	**30 min**	**60 min**	**90 min**
PB-1	3.94±0.34	4.18±0.35	4.20±0.28	4.35±0.030
PB-2	3.86±0.32	4.08±0.29	4.15±0.21	4.25±0.21
PB-3	3.92±0.42	4.28±0.48	4.11±0.20	4.38±0.36
PB-4	3.85±0.24	4.15±0.28	4.69±0.37*	5.01±0.33*
PB-5	3.77±0.26	4.45±0.52*	5.05±0.46*	5.05±0.46*
PB-6	3.91±0.38	4.18±0.31	4.57±0.35	4.55±0.35
PB-7	3.95±0.36	4.55±0.50*	5.12±0.35*	5.46±0.35*
PB-8	3.82±0.22	4.11±0.29	4.15±0.21	4.42±0.30
PB-9	3.88±0.37	4.27±0.58	4.46±0.47	4.65±0.25
PB-10	3.67±0.29	4.28±0.59	4.62±0.45	4.98±0.45*
[a]Control	3.70±0.36	3.76±0.28	4.07±0.36	4.01±0.36

[a]Control group was administered (p.o.) with 0.5 ml of 0.5% w/v CMC;
[b]Test drugs were administered (p.o.) at a dose level of 100 mg/ kg;
[c]All the values are expressed as Mean±SD, n=6;
* Significant ($p<0.05$) compared to control.

ANTI-PYRETIC ACTIVITY:

Prior to the experiment, the rats were maintained in separate cages for 7 days and the animals with approximately constant rectal temperature were selected for the study. The anti-pyretic activity was evaluated using Brewer's yeast induced pyrexia in rats. Fever was induced by injecting 20 ml/kg (s.c.) of 20% w/v aqueous suspension of Brewer's yeast in normal saline intramuscularly. After 18 hrs, the animals developed 0.5 °C or more rise in the rectal temperature (about 60% of the total animals injected). The rats were divided into different groups (n=6) and treated orally as shown inTable 5.The compounds were dispersed in 0.5% w/v of sodium CMC for administration. The control group was fed with 0.5 ml of 0.5% w/v of sodium CMC orally. Standard drug group was administered orally with nimesulide (9 mg/ kg; p.o.). At different time intervals the rectal temperature was recorded using clinical thermometer. Percentage reduction in rectal temperature was calculated by considering the total fall in temperature to normal level as 100%.

Table 4: Analgesic activity of benzothiazole derivatives with pyrazole by Writhe method on mice

[c]Compound	No. of Writhes	Inhibition (%)
PB-1	68±3*	9.23
PB-2	67±4*	6.84
PB-3	68±4*	5.59
PB-4	46±4*#	36.36
PB-5	43±4*#	39.41
PB-6	53±4*#	26.54
PB-7	42±4*#	42.92
PB-8	66±3#	8.42
PB-9	42±4*#	40.67
PB-10	49±3*#	32.15
[a]Control	72±3	-----
[b]Standard drug	32±3*	54.92

[a]Control group was administered (p.o.) with 0.5 mL of 0.5% w/v CMC;
[b]Standard drug group was administered (p.o.) with 12.5 mg/ kg of Nimesulide;
[c]Test drugs were administered (p.o.) at a dose level of 100 mg/ kg; All the values are expressed as Mean±SD, n=6;
**Significant ($p<0.05$) compared to control; #Significant ($p<0.05$) compared to standard drug*

RESULTS AND DISCUSSION:

In the present work we are reporting the synthesis of 7-(substituted)-6-fluoro-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)benzo[*d*]thiazole derivatives. To prepare 7-chloro-6-fluorobenzo[d]thiazol-2-amine, 3-Chloro-4-fluoro aniline was mixed with potassium thiocyanate (20 gm 1.2mol) and glacial acetic acidand maintain the temperature 0-5^{0}C. Then hydrazine hydrate, concentrated HCl followed by 20ml ethylene glycol with continuous stirring was added to the prepare 7-chloro-6-fluorobenzo[d]thiazol-2-amine to synthesize 1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)hydrazine. To this pentane-2,4-dioneand 50 ml of ethanol and refluxed 10 hrs to get 7-chloro-6-fluoro-2-(3,5-dimethyl-1H-pyrazol-1-yl)benzo[*d*]thiazole. Finally chlorine atom was substituted by different substituents by adding anhydrous K_2CO_3 in presence of a dry solvent.The structures of all the synthesized compounds in the present investigation were confirmed by the support of analytical data and spectral data given in the experimental section.

ANTIMICROBIAL STUDIES:

The investigation of antibacterial screening data (Table 2) revealed that all the tested compounds showed moderate to good microbial inhibition. In the series, the compounds PB-6 and PB-8 exhibited potent activities compared to others. The compound PB-6 is potent antibacterial than PB-8, whereas vice-versa is true for antifungal activity. All the synthesized compounds were subjected to antimicrobial screening bythe disc diffusion method.

ANALGESIC STUDIES:

The synthesized compounds PB-1 to PB-10 were screened for analgesic activity by tail-flick method (in rats) and acetic acid induced writhing method (in mice). Tail-flick test was employed to assess centrally mediated analgesia by synthesized compounds. Tail flick responses to thermal stimuli are mediated via supra-spinal centres. The results of analgesic activity by tail flick method are shown in Table 3. The results indicated that there was a little increase in the reaction time to flick the tail in all the treated groups. However the increase in reaction and hence analgesic activity was not appreciable, although the time required to flick the tail at 90 min was significantly different ($p<0.05$) than that of control. The compoundsPB-6 and PB-8 showed comparatively better analgesic activity than others.

The results of analgesic activity by writhing method are shown in Table 4. The acetic acid-induced writhing method is generally used for the evaluation of peripheral antinociceptive activity because of its sensitivity in detecting antinociceptive potential of the compounds which may appear inactive in other models. Writhing is demonstrated as acute pain due to tissue damage and sensitization of nociceptors by inflammatory mediators. Synthesized compounds inhibited painful writhes suggesting its inhibitory action on these mediators of inflammation and pain. Control group showed 72±3 writhes. The standard drug (nimesulide) and all the compounds showed significantly ($p<0.05$) less number of writhes in comparison with control. Among the series, the compounds PB-6 and PB-8 showed highest analgesic activity (Compound PB-6 - No. of writhes: 43±4 and % Reduction: 39.41%; Compound PB-8 - No. of writhes: 42±4 and % Reduction: 42.92). Although, the reduction in number of writhes by compounds were

significantly less compared to control, the values were not significantly different ($p>0.05$) than that of nimesulide, which exhibited highest reduction in number of writhes. The extent of analgesia produced by compounds in writhe method was considerably better than that in tail flick method. This indicates that the compounds may act as analgesic agents by acting peripherally instead of centrally. However more experiments with different models are necessary to precisely know the mechanism by which these metal complexes exhibit their activity.

ANTI-PYRETIC STUDIES:

All the synthesized compounds were screened for anti-pyretic activity by using the Brewer's yeast-induced pyrexia method in rats. The results of anti-pyretic activity of synthesized compounds are shown in Table 5. All the synthesized compounds showed significant ($p<0.05$) reduction in the rectal temperature at all the time intervalscompared to control. The compound PB-8 showed better percentage of reduction in pyrexia (44.11±5.12, 51.47±5.42 and 57.35±6.12% at 1, 2 and 3 h, respectively) among all the synthesized compounds. Nimesulide (standard drug) showed highest antipyretic and all the % reduction values from all the tested compounds were significantly ($p<0.05$) lower than those of nimesulide.

The results of biological screening studies demonstrate the significant analgesic and anti-pyretic effect of synthesized compounds in comparison with respective control groups. Although the compounds PB-6 and PB-8 showed appreciable pharmacological activities, the effect was significantly lower than that of standard drug. The results indicate the need to carry out the similar studies at different dose levels of synthesized compounds in different preclinical experimental models to precisely check the extent and mechanism of pharmacological effects.

CONCLUSION

A series of novel 7-(substituted)-6-fluoro-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)benzo[*d*]thiazole derivativesPB-1 to PB-10 have been synthesized in good yield and screened for their antimicrobial, analgesic and antipyreticactivities. In the series, the compounds PB-6 and PB-8exhibited potent activities compared remaining. However these in vivo evaluations in different experimental models and detailed toxicological studies are necessary to further support these results.

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Table 5: Anti-pyretic activity of benzothiazole derivatives with pyrazoleon Brewer's yeast induced pyrexia in rats

Compound	Rectal temperature (^{0} C)		[d] Rectal temperature after administration of drugs (^{0} C)		
	Normal (A)	18 hrs after administration of yeast (B)	1 h (C_1)	2 h (C_2)	3 h (C_3)
PB-1	37.75±0.51	38.42±0.35	39.30±0.42 (2.85±0.61) #	38.50±0.25 (4.20±0.52) #	38.42±0.35 (5.54±0.655 #
PB-2	37.65±0.41	38.36±0.28	38.34±0.31 (2.81±0.51) #	38.33±0.29 (4.22±0.62) #	38.32±0.25 (5.63±0.68) #
PB-3	37.65±0.50	38.35±0.29	38.32±0.31 (4.28±0.52) #	38.32±0.31 (4.28±0.52) #	38.30±0.29 (7.14±0.69) #
PB-4	37.62±0.48	38.21±0.32	38.06±0.35 (25.42±3.01) #*	37.98±0.29 (39.98±3.88)* #	37.96±0.32 (42.37±4.55)* #
PB-5	37.82±0.52	38.47±0.28	38.20±0.31 (41.53±4.26)* #	38.17±0.32 (46.15±5.15)* #	38.15±0.41 (49.23±5.21)* #
PB-6	37.32±0.51	38.00±0.36	37.83±0.38 (25.00±3.21)* #	37.75±0.40 (36.76±4.52)* #	37.72±0.32 (41.17±3.68)* #
PB-7	37.61±0.49	38.29±0.31	37.99±0.39 (44.11±5.12)* #	37.94±0.37 (51.47±5.42)* #	37.90±0.42 (57.35±6.12)* #
PB-8	37.62±0.61	38.21±0.21	38.16±0.25 (8.47±0.92) #	38.15±0.29 (10.16±1.26) #	38.14±0.35 (11.86±1.51) #
PB-9	37.65±0.42	38.30±0.29	38.15±0.31 (23.07±3.12)* #	38.08±0.32 (33.84±4.01)* #	38.05±0.40 (38.46±4.55)* #
PB-10	37.55±0.55	38.25±0.32	38.06±0.40 (27.14±3.12)* #	37.98±0.42 (38.57±4.82)* #	37.45±4.12 (42.85±5.32)* #
[a]Control	37.85±0.41	38.50±0.32	38.48±0.15 (3.07±0.61)	38.48±0.15 (3.07±0.61)	38.47±0.20 (4.61±0.81)
[b]Standard drug	37.31±0.48	38.00±0.31	37.60±0.21 (57.97±4.11)*	37.50±0.25 (72.46±4.56)*	37.47±0.28 (76.81±5.12)*

[a]Control group was administered (p.o.) with 0.5 mL of 0.5% w/v CMC;
[b]Standard drug group was administered (p.o.) with 9 mg/ kg of Nimesulide;
[c]Test drugs were administered (p.o.) at a dose level of 100 mg/ kg; All the values are expressed as Mean±SD, n=6;
[d]Percentage reduction in rectal temperature after administration of drugs is given in parenthesis; Statistics was applied to percentage reduction values;
[*]Significant ($p<0.05$) compared to control;
 # Significant ($p<0.05$) compared to standard drug. Percentage reduction = (B-Cn)/(B-A) × 100, where n = 1, 2 or 3.

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