Full Paper

Synthesis and Comparative Study of Anti-*Mycobacterium* Activity of a Novel Series of Fluoronitrobenzothiazolo-pyrazoline Regioisomers

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In an attempt to find a new and a safer drug for tuberculosis, we have synthesized a series of fluoronitrobenzothiazolopyrazolines for antitubercular activity. The series comprises three subclasses: fluorobenzothiazolopyrazolines (**11a–f**), fluoronitrobenzothiazolopyrazoline, nitro group at 5^{th} position (**12a–f**) and 4^{th} position (**13a–f**). All compounds were screened for their *in-vitro* antitubercular activity against *Mycobacterium tuberculosis* H37Rv strain by using Middlebrook 7H-9 broth. An introduction of $-NO_2$ group at 5^{th} position of benzothiazole ring (**12a–f**) increased the antitubercular activity whereas introduction of $-NO_2$ group at 4^{th} position (**13a–f**) was found to decrease the activity remarkably. Two compounds from each series showing good antitubercular activity were tested for cytotoxicity on THP-1 cell lines and they showed low cytotoxicity.

Keywords: Antitubercular activity / ATP phosphoribosyl transferase / Benzothiazole / Pyrazoline / Regioisomers

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Introduction

There were an estimated 11.1 million (range 9.6–13.3 million) prevalent cases of *Tubercle bacillus* (TB) in 2008, equivalent to 164 cases per 100 000 population [1]. India and China alone account for an estimated 35% of TB cases worldwide. Of the 9.4 million incident cases in 2008, an estimated 1.2–1.6 million (13–16%) were HIV-positive, as their immune systems are compromised by immunosuppressive drugs, substance abuse, or AIDS [2–5].

As a result there is a dire need to develop novel, faster acting chemotherapeutics with a lower toxicity for the treatment of tuberculosis. A new TB treatment should offer at least one of three improvements over the existing regimens: (a) It should shorten the total duration of effective treatment and/or significantly reduce the total number of doses needed to be taken under Directly Observed Therapy Short-course (DOTS) supervision; (b) it should improve the treatment of multidrug-resistant tuberculosis (MDR-TB), which cannot be treated with Isoniazid (INH) and Rifampin (RIF) and/or (c) it should provide more effective treatment of latent/dormant TB infection, which is essential for eliminating the TB.

In the recent years nitrobenzothiazole has been discovered as an inhibitor for *Mycobacterium tuberculosis* ATP phosphoribosyl transferase (HisG) [6]. HisG is an ATP phosphoribosyl transferase (ATP-PRTase) that catalyzes the first step in the biosynthetic pathway for histidine, which also leads to intermediates that play a significant role in purine biosynthesis. HisG condenses ATP with phosphoribosyl pyrophosphate (PRPP) to produce phosphoribosyl ATP (PR-ATP) and inorganic pyrophosphate (PP_i). Histidine biosynthesis is metabolically expensive, requiring 10 enzymatic reactions and consuming an estimated 41 ATP molecules. Therefore the pathway is tightly regulated through negative feedback via allosteric inhibition of HisG by histidine. Partly due to its central role in metabolism and its uniqueness to prokaryotes and lower eukaryotes, HisG represents a potential drug target for combating tuberculosis. HisG in *Mycobacterium tuberculosis* has an α/β-fold containing three domains: two N-terminal catalytic

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domains, with a large, relatively solvent exposed active site formed between them, and a C-terminal regulatory domain [7]. The active site is formed by residues contributed by both domains I and II and contains putative binding sites for both ATP and PRPP, inferred based on co-crystal structures of complexes with AMP, PRPP and PR-ATP (product) [7–9]. Domain II contains a 13-residue signature sequence (residues 149–161) associated with binding PRPP, whereas conserved residues for ATP-binding are primarily found in domain I on the opposing face of the cleft. Reaction follows ordered bi-bi kinetics, with sequential binding of ATP followed by PRPP, condensation, and then release of products PPi and PR-ATP [10]. The C-terminal domain (III) binds histidine at a site approximately 40 Å away from the active site, which causes a rotation between domains and a conformational shift in packing within the hexameric complex [7], which ultimately inhibits catalytic activity by reducing k_{cat} [11].

The pyrazoline derivatives constitute an interesting class of organic compounds, which are associated with diverse chemical and pharmacological properties [12–13].

Fluorine has been incorporated into the molecule [14]. Fluorine, being the second smallest substituent next to hydrogen, closely mimics hydrogen in enzyme receptor interactions. The substitutions of fluorine by hydrogen increases lipid solubility which in turn increases the transport and absorption of drug *in vivo*, and a strong electron withdrawing inductive effect of fluorine can significantly influence reactivity and stability of functional groups and the reactivity of neighboring reaction centers.

Drawing on structural knowledge obtained from our past work we herein synthesized fluorobenzothiazolopyrazolines, and fluoronitrobenzothiazolopyrazolines (**11a–f**, **12a–f** and **13a–f**) for their antitubercular activity study and cytotoxicity study of some of the compounds.

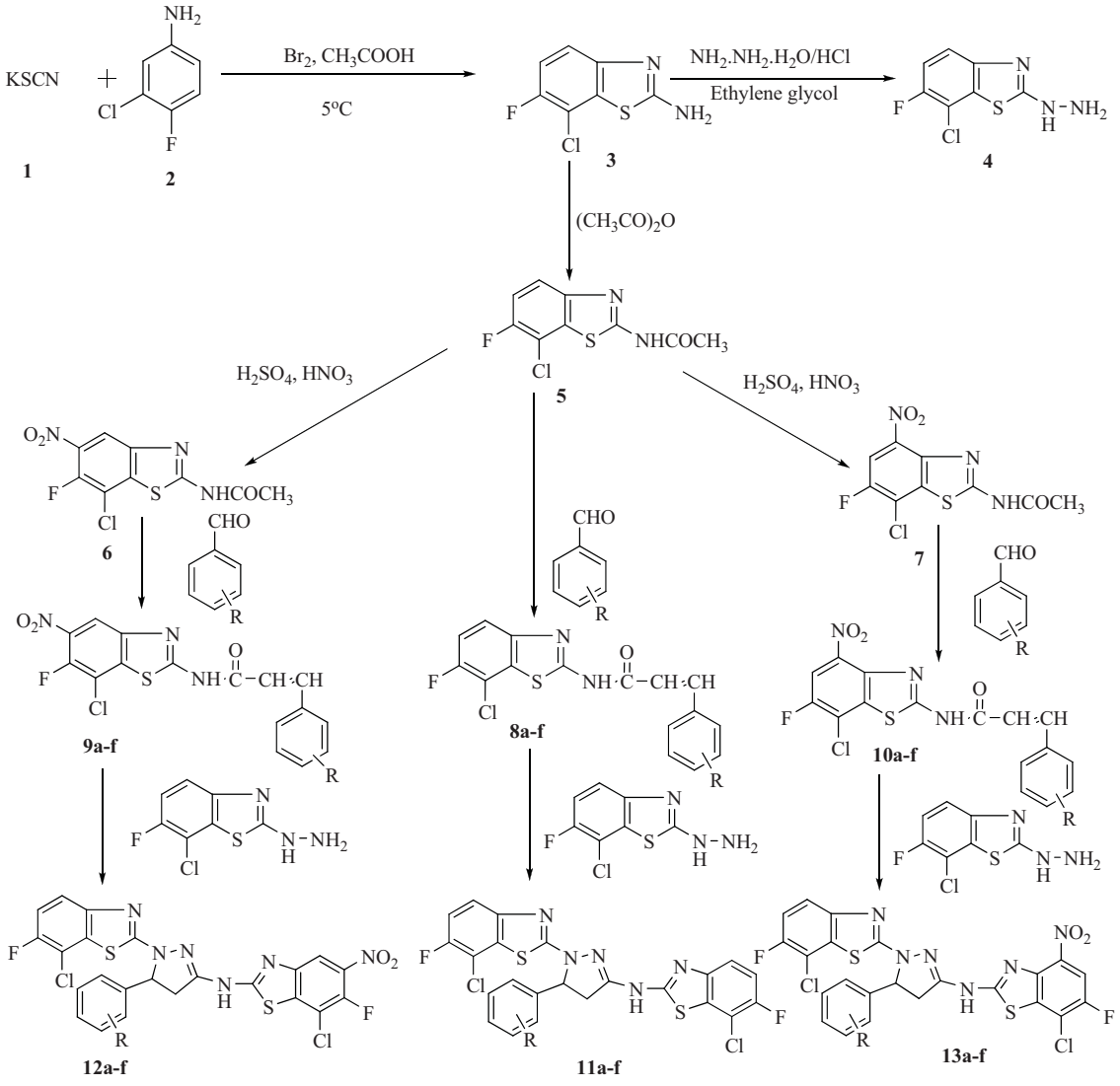
Results and discussion

Chemistry

The preparation of final compounds **11a–f**, **12a–f** and **13a–f** was accomplished by synthetic sequence illustrated in Scheme 1. The compound **3** was synthesized from 4-fluoro-3-chloro aniline and potassium thiocyanate with bromine in acetic acid. The starting material then was treated with hydrazine hydrate and conc. HCl in the presence of ethylene glycol yielding compound **4**. Alternatively the compound **3** was again treated with acetic anhydride to get compound **5**. Upon nitration with conc. HNO_3 & conc. H_2SO_4 afforded **6** & **7** two regioisomers. The NMR spectra (in $CDCl_3$) of compound **6** showed absorption of 4th proton at δ 7.75–7.73 ppm as doublet due to C–F coupling accounting for 1 proton, J = 7.60 Hz, NH at δ 9.16 ppm singlet 1 proton and CH_3 at δ 2.34 ppm singlet 3 protons. Whereas compound **7** showed (in DMSO-d_6) absorption of 5th proton at δ 8.40–8.38 ppm as doublet due to C–F coupling accounting for 1 proton, J = 9.44 Hz, NH at δ 13.21 ppm singlet 1 proton and CH_3 at δ 2.27 ppm singlet 3 protons. Compounds **5**, **6** & **7** were treated with different aromatic aldehydes in basic medium as in Claisen-Schmidt condensation yielding **8a–f**, **9a–f** & **10a–f**, respectively (chalcones). The presence of aromatic peak in the aromatic region, vinylic peak in δ 4.5 to 5.5 ppm and absence of CH_3 peak in between δ 2 to 3 ppm showed the formation of chalcones. The synthesis of target compounds **11a–f**, **12a–f** & **13a–f** was carried out by refluxing **8a–f**, **9a–f** & **10a–f** with hydrazino benzothiazole (**4**) in alcohol. Presence of singlet in between δ 3 to 5.5 ppm for pyrazoline hydrogen showed the formation of target compounds. This was further confirmed by the mass spectra and CHN elemental analysis.

Antitubercular activity

The results of the *in-vitro* evaluation of antituberculosis activity are reported in Table 1. The compounds were evaluated against *M. tuberculosis* H37Rv using Middlebrook 7H-9 broth. The ability of compounds to inhibit the growth of *Mycobacterium* species was determined by Ziehl-Neelsen staining. Compounds **12b**, **12c** and **12d** have shown 100% inhibition at 25 μg/mL, **11b**, **11c** and **11d** at 50 μg/mL and **13b**, **13c** and **13d** at 100 μg/mL, whereas the standard drug pyrazinamide and streptomycin showed 100% inhibition at 7.5 μg/mL. Compounds having a nitro group in 5th position (**12a–f**) of the benzothiazole ring show a better antitubercular activity than they do without any nitro group (**11a–f**) in the benzothiazole ring. The nitro group at the 4th position (**13a–f**) showed a reduced antitubercular activity than 5th position nitro compound as well as without a nitro group in the structure. The antitubercular activity of compounds with presence of nitro group in 5th position (**12a–f**) may be because this series of compounds packs in the part of the active site responsible for binding the PRPP substrate [6]. This may bind with the Tyr116 in the 1NH8 receptor, which is thought to interact with ATP, along with Glu141 and Asp154, which are expected to interact with PRPP. Compounds without any nitro group (**11a–f**) and a nitro group at the 4th position (**13a–f**) in the benzothiazole ring probably missed the binding site. Electron donating substituent in the aromatic ring showed a good antitubercular activity. 4-OH-3-OCH_3, 4-OCH_3, 4-OH showed good antitubercular activity, whereas 2-OH substituent does not show any antitubercular activity, irrespective of nitro substituent. This may conclude that nitro group at 5th position of benzothiazole ring and 4-OH-3-OCH_3, 4-OCH_3 and 4-OH group in benzene ring in fluoronitrobenzothiazolopyrazolines moiety enhanced antitubercular activity.



R = **11-13a** = 4-N$(CH_3)_2$, **11-13b** = 4-OH, **11-13c** = 4-OH, 3-OCH_3, **11-13d** = 4-OCH_3, **11-13e** = 2-OH, **11-13f** = H.

Scheme 1. Synthesis of **11a–f, 12a–f** and **13a–f**.

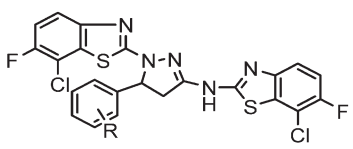
In-vitro cytotoxicity evaluation

The six compounds were tested for cytotoxicity (CTC_{50}) in THP-1 cell lines at concentrations 1000, 500, 250 and 125 μg/mL. Table 2 shows the cytotoxicity to the host cells of compounds **11b**, **11c**, **12b**, **12c**, **13b** and **13c**. Compounds **12b** & **12c** were found to be cytotoxic at the CTC_{50} values 0.324, 0.300 mM, though this concentration is much higher than the concentration used for antitubercular activity. Other compounds have shown comparatively less cytotoxicity. In the compounds **12b** & **12c** carrying nitro group at 5th position, the cytotoxicity may be the position of the nitro group, whereas a nitro group at 4th position and compounds that do not have any nitro group show less cytotoxicity. All compounds have higher antitubercular activity against *M. tuberculosis* as compared to cytotoxic activity in eukaryotic cell.

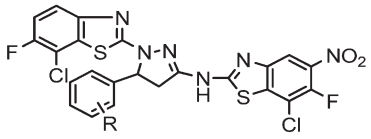
Conclusion

In the present study, we have synthesized regioisomers of fluoronitrobenzothiazolopyrazoline, and their activity on *Mycobacterium tuberculosis* H37Rv strain was compared to the

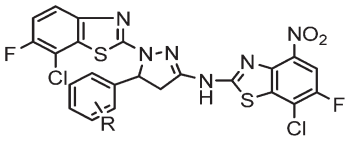
Table 1. *In-vitro* antitubercular evaluation.



11a-f



12a-f



13a-f

SL. No.	Comp. No.	R	Antitubercular activity (MIC)			Min. conc. in mM showed activity
			25 μg/mL	50 μg/mL	100 μg/mL	
1	**11a**	4-N(CH_3)$_2$	R	R	S	0.174
2	**11b**	4-OH	R	S	S	0.091
3	**11c**	4-OH-3-OCH_3	R	S	S	0.086
4	**11d**	4-OCH_3	R	S	S	0.089
5	**11e**	2-OH	R	R	R	—
6	**11f**	-H	R	R	R	—
7	**12a**	4-N(CH_3)$_2$	R	S	S	0.080
8	**12b**	4-OH	S	S	S	0.042
9	**12c**	4-OH-3-OCH_3	S	S	S	0.040
10	**12d**	4-OCH_3	S	S	S	0.041
11	**12e**	2-OH	R	S	S	0.084
12	**12f**	-H	R	R	R	—
13	**13a**	4-N(CH_3)$_2$	R	R	R	—
14	**13b**	4-OH	R	R	S	0.168
15	**13c**	4-OH-3-OCH_3	R	R	S	0.160
16	**13d**	4-OCH_3	R	R	S	0.165
17	**13e**	2-OH	R	R	R	—
18	**13f**	-H	R	R	R	—
19	Pyrazinamide		7.5 μg/mL			0.060
20	Streptomycin		7.5 μg/mL			0.014

R = resistant; S = sensitive

standard drugs streptomycin and pyrazinamide. Two possible regioisomers of 4th and 5th positions of benzothiazole ring were produced during the nitration of *N*-(7-chloro-6-fluorobenzo[*d*]thiazol-2-yl)acetamide (**5**), and both the compounds were separated. The final compounds fluorobenzothiazolopyrazolines (**11a–f**), fluoronitrobenzothiazolopyrazoline in which nitro group at 5th position (**12a–f**) and fluoronitrobenzothiazolopyrazoline, nitro group at 4th position (**13a–f**) were tested for *in-vitro* antitubercular activity against *Mycobacterium tuberculosis* H37Rv strain. Compounds with a nitro substituent have shown better activity than those without a nitro group. Nitro group at position 5th of the benzothiazole ring has shown a better activity compared to 4th position, but nitro group at 5th position is more cytotoxic than 4th position. Electron donating substituent in the aromatic ring shows a better antitubercular activity. Introduction of nitro group at 5th position has shown higher cytotoxicity where as introduction of nitro group at 4th has not shown any significant increase in cytotoxicity.

Table 2. *In-vitro* cytotoxicity evaluation on THP-1 cell line.

Sl. No.	Comp. No.	R	CTC_{50}, μg/mL	CTC_{50}, mM
1	**11b**	4-OH	447.00	0.817
2	**11c**	4-OH-3-OCH_3	423.50	0.733
3	**12b**	4-OH	192.00	0.324
4	**12c**	4-OH-3-OCH_3	187.50	0.300
5	**13b**	4-OH	432.50	0.730
6	**13c**	4-OH-3-OCH_3	412.50	0.663

Experimental

Chemistry

The melting points were determined with an electrothermal melting point apparatus and are uncorrected. Infrared spectra (KBr disc) were performed on FTIR-8400 Shimadzu and the frequencies were expressed in cm^{-1}. ^{1}H-NMR spectra were recorded on Bruker-Avance 400 MHz instrument with TMS (0 ppm) as an internal standard; the chemical shifts (δ) are reported in ppm and coupling constants (*J*) are given in Hertz (Hz). Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), ds (double singlet), dd (double doublet), m (multiplet) and

br s (broad singlet). Mass spectra were recorded on ESI-MS, Thermo, Finnigan LCQ deca xp max. Elemental analyses were performed on Perkin-Elmer 2400 CHN elemental analyzer. Analyses indicated by the symbols of the elements of functions were within ±0.4% of the theoretical values. The purity of the compounds was checked on Merck precoated silica gel 60 F-254. Column chromatography was performed using P.D. fine chem. silica gel (100–200 mesh). Yields were not optimized. All the solvents and reagents were used without further purification.

*Synthesis of 7-chloro-6-fluorobenzo[d]thiazol-2-amine **3***

To glacial acetic acid (40 mL) precooled at 5°C were added 40 g (0.4123 mol) potassium thiocyanate and 7.25 g (0.0498 mol) of 4-fluoro-3-chloro aniline. The mixture was stirred, during which 6 mL of bromine in 24 mL of glacial acetic acid was added at such a rate that the temperature was not allowed to rise beyond 5°C, for a period of 2 h. The stirring was continued for an additional 2 h at the same temperature, and further at room temperature for 10 h. It was allowed to stand overnight during which an orange precipitate was settled at the bottom. 30 mL of water was added and slurry was heated at 85°C on a steam bath and filtered hot. The filtrate was cooled and neutralized with strong ammonia solution to pH 6, a light yellow precipitate obtained was collected. The resulting product was recrystallized by toluene.

Yield 76%; slight yellowish crystalline; mp 180–182°C; IR (ν_{max}, cm^{-1}, KBr): 3480 (N–H), 1199 (C–F), 681 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 4.21 (s, 2H, NH_2), 7.62–7.60 (d, 1H, J = 7.22 Hz, ArH), 7.70–7.68 (d, 1H, ArH, J = 8.59 Hz); ESI-MS, m/z: 201.98 $[M]^+$, 203.86 $[M+2]^+$; anal. calcd. for $C_7H_4ClFN_2S$: C, 41.49; H, 1.99; N, 13.82. Found: C, 41.51; H, 2.03; N, 13.81.

*Synthesis of 7-chloro-6-fluoro-2-hydrazinylbenzo[d]-thiazole **4***

10 mL of conc. HCl was added drop-wise with stirring to 10 mL (0.3 mol) hydrazine hydrate at 5–10°C followed by ethylene glycol 40 mL. To the above solution 2.025 g (0.01 mol) of compound **3** in portion was added and the resulting mixture was refluxed for 2 h. On cooling solid separated out, was filtered and washed with water, dried and recrystallized from ethanol.

Yield 66%, mp 217–219°C, light brown coloured needle shaped crystals; IR (ν_{max}, cm^{-1}, KBr): 3380 (N–H), 1200 (C–F), 683 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 5.09 (s, 2H, NH_2), 7.42–7.41 (d, 1H, ArH, J = 6.64 Hz), 7.81–7.79 (d, 1H, ArH, J = 9.24 Hz), 9.20 (s, 1H, NH); ESI-MS, m/z: 217.02 $[M]^+$, 219.05 $[M+2]^+$; anal. calcd. for $C_7H_5ClFN_3S$: C, 38.63; H, 2.32; N, 19.31. Found: C, 38.61; H, 2.34, N, 19.31.

*Synthesis of N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-acetamide **5***

A mixture of compound **3**, 2.025 g (0.01 mol), and 10 mL of acetic anhydride was refluxed for 1 h. After that the reaction mixture was cooled, the separated out solid was heated with water, filtered and washed with water. The product was then recrystallized in ethanol.

Yield 98%, mp 232–233°C, whitish needle shaped crystal; IR (ν_{max}, cm^{-1}, KBr): 3318 (N–H), 1681 (C=O), 1189 (C–F), 652 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 2.34 (s, 3H, CH_3), 7.26–7.24 (d, 1H, ArH, J = 7.57 Hz), 7.75–7.73 (d, 1H, ArH, J = 7.60 Hz), 9.05 (s, 1H, NH); ESI-MS, m/z: 244.03 $[M]^+$, 246.02 $[M+2]^+$; anal. calcd. for $C_9H_6ClFN_2OS$: C, 44.18; H, 2.47; N, 11.45. Found: C, 44.17; H, 2.49; N, 11.44.

*Synthesis of N-(7-chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)acetamide & N-(7-chloro-6-fluoro-5-nitrobenzo[d]-thiazol-2-yl)acetamide **6 & 7***

A mixture of compound **5**, 100 mg (0.000409 mol), and 0.3 mL of ice-cold conc. H_2SO_4 was stirred under ice-cooled condition. To this 0.1 mL conc. HNO_3 was added drop-wise, continued to be stirred at room temperature for 2 h. Then, 0.1 mL conc. HNO_3 was further added to the reaction mixture and stirred overnight. The reaction mixture was poured into a large amount of water. The solids obtained were filtered and washed with water thoroughly and dried under vacuum. The compound obtained was a mixture of **6** & **7**, which was separated by column chromatography employing *n*-hexane/ethyl acetate (9:1) as an eluent.

*N-(7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-yl)-acetamide **6***

Yield 56%; mp 297–298°C; buff color crystalline solid; IR (ν_{max}, cm^{-1}, KBr): 3301 (N–H), 1535 (NO_2), 1680 (C=O), 1186 (C–F), 661 (C–Cl); ^{1}H-NMR (400 MHz, $CDCl_3$), δ (ppm): 2.34 (s, 3H, CH_3), 7.75–7.73 [d, 1H, ArH, J = 7.60 Hz (C–F)], 9.16 (s, 1H, NH); ESI-MS, m/z: 288.99 $[M]^+$, 290.97 $[M+2]^+$; anal. calcd. for $C_9H_5ClFN_3O_3S$: C, 37.32; H, 1.74; N, 14.51. Found: C, 37.30; H, 1.76; N, 14.50.

*N-(7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-acetamide **7***

Yield 30%; mp 336–337°C; white crystalline solid; IR (ν_{max}, cm^{-1}, KBr): 3311 (N–H), 1530 (NO_2), 1680 (C=O), 1185 (C–F), 665 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 2.27 (s, 3H, CH_3), 8.40–8.38 (d, 1H, ArH, J = 9.44 Hz (C–F)), 13.21 (s, 1H, NH); ESI-MS, m/z: 288.95 $[M]^+$, 290.95 $[M+2]^+$; anal. calcd. for $C_9H_5ClFN_3O_3S$: C, 37.32; H, 1.74; N, 14.51. Found: C, 37.31; H, 1.76; N, 14.52.

*Synthesis of N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-3-substituted phenyl acrylamide **8a–f***

A mixture of compound **5**, 2.445 g (0.01 mol), in ethanol (25 mL) and 8 mL of 10% NaOH was stirred at room temperature. To this mixture equimolar quantity of substituted aromatic aldehyde (0.01 mol) was added in small portions. Stirring was continued for overnight in room temperature. The excess of solvent was distilled off, cool, filter and residue was thoroughly washed with cold water. The product was then recrystallized from acetone/water mixture.

*N-(7-Chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(4-dimethylamino)phenyl)acrylamide **8a***

Yield 89%; mp 140–142°C; yellowish needle shaped crystal; IR (ν_{max}, cm^{-1}, KBr): 3306 (N–H), 1664 (C=O), 1170 (C–F), 667 (C–Cl); ^{1}H-NMR (200 MHz, $CDCl_3$), δ (ppm): 3.09 (s, 6H, CH_3), 4.58–4.35 (dd, 2H, CH, J = 7.34, 7.39 Hz), 6.73–6.68 (d, 2H, ArH, J = 8.88 Hz), 7.39–7.35 (d, 1H, ArH, J = 8.35 Hz), 7.56–7.54 (d, 1H, ArH, J = 7.50 Hz), 7.76–7.72 (d, 2H, ArH, J = 8.88 Hz), 9.74 (s, 1H, NH); ESI-MS, m/z: 375.09 $[M]^+$, 377.09 $[M+2]^+$; anal. calcd. for $C_{18}H_{15}ClFN_3OS$: C, 57.52; H, 4.02; N, 11.18. Found: C, 57.53; H, 4.03; N, 11.17.

*N-(7-Chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(4-hydroxyphenyl) acrylamide **8b***

Yield 83%; mp 227–229°C; buff colored powder; IR (ν_{max}, cm^{-1}, KBr): 3480 (N–H), 3152 (O–H), 1647 (C=O), 1189 (C–F), 664 (C–Cl);

^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 4.15 (s, 1H, OH), 4.54–5.52 (d, 1H, CH, J = 7.32 Hz), 4.68–4.66 (d, 1H, CH, J = 7.30 Hz), 7.19–6.92 (d, 2H, ArH, J = 8.71 Hz), 7.49–7.25 (d, 2H, ArH, J = 8.78 Hz), 7.56–7.52 (d, 1H, ArH, J = 8.50 Hz), 7.78–7.76 (d, 2H, ArH, J = 8.82 Hz), 9.65 (s, 1H, NH); ESI-MS, *m/z*: 348.07 $[M]^+$, 350.05 $[M+2]^+$; anal. calcd. for $C_{16}H_{10}ClFN_2O_2S$: C, 55.10; H, 2.89; N, 8.03. Found: C, 55.12; H, 2.90; N, 8.02.

N-(7-Chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(4-hydroxy-3-methoxyphenyl)acryl-amide ***8c***

Yield 81%; mp 185–187°C; white powder; IR (ν_{max}, cm^{-1}, KBr): 3485 (N–H), 3095 (OH), 1685 (C=O), 1250 C–O), 1200 (C–F), 685 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.02 (s, 3H, OCH_3), 4.22 (s, 1H, OH), 5.03–4.90 (d, 1H, J = 7.44 Hz, CH), 5.16 (d, 1H, CH, J = 7.40Hz), 7.76–7.73 (m, 3H, ArH), 7.96–7.94 (d, 2H, ArH, J = 8.26 Hz), 9.85 (s, 1H, NH); ESI-MS, *m/z*: 378.06 $[M]^+$, 380.07 $[M+2]^+$; anal. calcd. for $C_{17}H_{12}ClFN_2O_3S$: C, 53.90; H, 3.19; N, 7.40. Found: C, 53.92; H, 3.21; N, 7.39.

N-(7-Chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(4-methoxyphenyl)acrylamide ***8d***

Yield 87%; mp 175–178°C; orange powder; IR (ν_{max}, cm^{-1}, KBr): 3485 (N–H), 1680 (C=O), 1270 (C–F), 675 (C–Cl), 1260 C–O); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.20 (s, 3H, OCH_3), 4.62–4.58 (d, 1H, J = 7.93 Hz, CH), 5.28–5.26 (d, 1H, CH, J = 7.49 Hz), 7.78–7.52 (m, 4H, ArH), 7.82–7.80 (d, 2H, ArH, J = 7.08 Hz), 9.87 (s, 1H, NH); ESI-MS, *m/z*: 362.08 $[M]^+$, 364.09 $[M+2]^+$; anal. calcd. for $C_{17}H_{12}ClFN_2O_2S$: C, 56.28; H, 3.33; N, 7.72. Found: C, 56.29; H, 3.35; N, 7.71.

N-(7-Chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(2-hydroxyphenyl) acrylamide ***8e***

Yield 56%; mp 178–181°C; ash colored powder; IR (ν_{max}, cm^{-1}, KBr): 3487 (N–H), 3095 (O–H), 1695 (C=O), 1210 (C–F), 680 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 9.25 (s, 1H, NH), 7.86–7.84 (d, 2H, ArH, J = 7.42 Hz), 7.46–7.42 (m, 4H, ArH), 4.77–4.73 (d, 1H, CH, J = 7.85 Hz), 5.72 (d, 1H, CH, J = 7.48 Hz), 4.09 (s, 1H, OH); ESI-MS, *m/z*: 348.09 $[M]^+$, 350.11 $[M+2]^+$; anal. calcd. for $C_{16}H_{10}ClFN_2O_2S$: C, 55.10; H, 2.89; N, 8.03. Found: C, 55.11; H, 2.91; N, 8.02.

N-(7-Chloro-6-fluorobenzo[d]thiazol-2-yl)-3-phenylacrylamide ***8f***

Yield 58%; mp 212–214°C; orange crystalline solid; IR (ν_{max}, cm^{-1}, KBr): 3489 (N–H), 1705 (C=O), 1203 (C–F), 676 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 4.40–4.36 (d, 1H, CH, J = 7.31 Hz), 4.49–4.43 (d, 1H, CH, J = 7.29 Hz), 7.56–7.55 (m, 5H, ArH), 7.78–7.77 (d, 2H, ArH, J = 8.22 Hz), 9.64 (s, 1H, NH); ESI-MS, *m/z*: 332.08 $[M]^+$, 334.07 $[M+2]^+$; anal. calcd. for $C_{16}H_{10}ClFN_2OS$: C, 57.75; H, 3.03; N, 8.42. Found: C, 57.77; H, 3.06; N, 8.41.

Synthesis of N-(7-chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-yl)-3-(substituted phenyl) acrylamide ***9a–f***

A mixture of compound **6**, 2.895 g (0.01 mol), in ethanol (25 mL) and 8 mL of 10% NaOH was stirred at room temperature. To this mixture equimolar quantity of substituted aromatic aldehydes (0.01 mol) was added in small portions. Stirring was continued for overnight in room temperature. The excess of solvent was distilled off, cooled, filtered and the residue was thoroughly washed with cold water. The product was then recrystallized from acetone-water mixture.

N-(7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-yl)-3-(4-(dimethylamino)phenyl)acrylamide ***9a***

Yield 71%; mp 286–288°C; light orange crystal; IR (ν_{max}, cm^{-1}, KBr): 3429 (N–H), 1537 (NO_2), 1688 (C=O), 1194 (C–F), 667 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.12 (s, 6H, CH_3), 5.01–4.29 (d, 1H, CH, J = 7.65 Hz), 5.28–5.09 (d, 1H, CH, J = 7.68 Hz), 7.72–7.70 (m, 4H, ArH), 7.76–7.75 (d, 1H, ArH, J = 7.62 Hz), 9.50 (s, 1H, NH); ESI-MS, *m/z*: 420.02 $[M]^+$, 422.02 $[M+2]^+$; anal. calcd. for $C_{18}H_{14}ClFN_4O_3S$: C, 51.37; H, 3.35; N, 13.31. Found: C, 51.40; H, 3.37; N, 13.30.

N-(7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-yl)-3-(4-hydroxyphenyl)acrylamide ***9b***

Yield 68%; mp 240–242°C; buff color powder; IR (ν_{max}, cm^{-1}, KBr): 3450 (N–H), 3198 (O–H), 1688 (C=O), 1629 (C=C), 1538 (NO_2), 1196 (C–F), 640 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.95 (s, 1H, OH), 4.85–4.83 (d, 1H, CH, J = 8.65 Hz), 5.02–4.94 (d, 1H, CH, J = 8.32 Hz), 7.62–7.60 (m, 4H, ArH), 7.87–7.85 (d, 1H, ArH, J = 7.61 Hz), 9.61 (s, 1H, NH); ESI-MS, *m/z*: 393.04 $[M]^+$, 395.06 $[M+2]^+$; anal. calcd. for $C_{16}H_9ClFN_3O_4S$: C, 48.80; H, 2.30; N, 10.67. Found: C, 48.85; H, 2.33; N, 10.65.

N-(7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-yl)-3-(4-hydroxy-3-methoxyphenyl) acrylamide ***9c***

Yield 73%; mp 275–278°C; yellowish crystal; IR (ν_{max}, cm^{-1}, KBr): 3452 (N–H), 3191 (O–H), 1695 (C=O), 1537 (NO_2), 1258 & 1050 (C–O), 1202 (C–F), 637 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.52 (s, 3H, OCH_3), 3.93 (br s, 1H, OH), 4.81–4.79 (d, 1H, CH, J = 6.61 Hz), 5.02–4.93 (d, 1H, CH, J = 6.24 Hz), 7.64–7.61 (m, 3H, ArH), 7.80–7.79 (d, 1H, ArH, J = 7.62 Hz), 9.69 (s, 1H, NH), ESI-MS, *m/z*: 423.07 $[M]^+$, 425.07 $[M+2]^+$; anal. calcd. for $C_{17}H_{11}ClFN_3O_5S$: C, 48.18; H, 2.62; N, 9.92. Found: C, 48.21; H, 2.66; N, 9.94.

N-(7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-yl)-3-(4-methoxyphenyl)acrylamide ***9d***

Yield 77%; mp 222–226°C; yellow powder; IR (ν_{max}, cm^{-1}, KBr): 3442 (N–H), 1668 (C=O), 1523 (NO_2), 1252 & 1051 (C–O), 1197 (C–F), 620 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.62 (s, 3H, OCH_3), 4.91–4.86 (d, 1H, CH, J = 7.29 Hz), 5.03–5.01 (d, 1H, CH, J = 7.32 Hz), 7.78–7.75 (m, 4H, ArH), 7.90–7.88 (d, 1H, ArH, J = 7.63 Hz), 10.00 (s, 1H, NH); ESI-MS, *m/z*: 407.02 $[M]^+$, 409.09 $[M+2]^+$; anal. calcd. for $C_{17}H_{11}ClFN_3O_4S$: C, 50.07; H, 2.72; N, 10.30. Found: C, 50.11; H, 2.76; N, 10.33.

N-(7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-yl)-3-(2-hydroxyphenyl)acrylamide ***9e***

Yield 51%; mp 205–208°C; light gray powder; IR (ν_{max}, cm^{-1}, KBr): 3425 (N–H), 3152 (O–H), 1678 (C=O), 1515 (NO_2), 1198 (C–F), 670 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 4.26 (br s, 1H, OH), 5.30 (d, 1H, CH, J = 7.35 Hz), 5.77 (d, 1H, CH, J = 7.35 Hz), 7.63–7.61 (m, 4H, ArH), 7.87–7.85 (d, 1H, ArH, J = 7.62 Hz), 9.65 (s, 1H, NH); ESI-MS, *m/z*: 393.04 $[M]^+$, 395.06 $[M+2]^+$; anal. calcd. for $C_{16}H_9ClFN_3O_4S$: C, 48.80; H, 2.30; N, 10.67. Found: C, 48.87; H, 2.34; N, 10.65.

N-(7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-yl)-3-phenylacrylamide ***9f***

Yield 56%; mp 234–237°C; yellow crystalline solid; IR (ν_{max}, cm^{-1}, KBr): 3434 (N–H), 1697 (C=O), 1523 (NO_2), 1208 (C–F) 637 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 4.86–4.84 (d, 1H, CH, $J = 7.39$ Hz), 5.12–4.96 (d, 1H, CH, $J = 7.40$ Hz), 7.46–7.43 (m, 5H, ArH), 7.72–7.70 (d, 1H, ArH, $J = 7.61$ Hz), 9.55 (s, 1H, NH); ESI-MS, *m/z*: 377.01 $[M]^+$, 379.02 $[M+2]^+$; anal. calcd. for $C_{16}H_9ClFN_3O_3S$: C, 50.87; H, 2.40; N, 11.12. Found: C, 50.92; H, 2.46; N, 11.17.

Synthesis of N-(7-chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-(substituted phenyl) acrylamide ***10a–f***

A mixture of compound **6**, 2.895 g (0.01 mol), in ethanol (25 mL) and 8 mL of 10% NaOH was stirred at room temperature. To this mixture equimolar quantity of substituted aromatic aldehydes (0.01 mol) was added in small portions. Stirring was continued for overnight in room temperature. The excess of solvent was distilled off, cooled, filtered and the residue was thoroughly washed with cold water. The product was then recrystallized from acetone-water mixture.

N-(7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-(4-(dimethylamino)phenyl)acrylamide ***10a***

Yield 75%; mp 266–268°C; orange crystal; IR (ν_{max}, cm^{-1}, KBr): 3402 (N–H), 1698 (C=O), 1525 (NO_2), 1197 (C–F), 630 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.12 (s, 6H, CH_3), 4.43–4.36 (dd, 2H, CH, $J = 7.32$, 7.40 Hz), 7.76–7.72 (m, 4H, ArH, $J = 8.88$ Hz), 8.40–8.38 (d, 1H, ArH, $J = 9.44$ Hz), 9.65 (s, 1H, NH); ESI-MS, *m/z*: 420.05 $[M]^+$, 422.05 $[M+2]^+$; anal. calcd. for $C_{18}H_{14}ClFN_4O_3S$: C, 51.37; H, 3.35; N, 13.31. Found: C, 51.42; H, 3.39; N, 13.29.

N-(7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-(4-hydroxyphenyl)acrylamide ***10b***

Yield 70%; mp 270–272°C; yellow crystal IR (ν_{max}, cm^{-1}, KBr): 3402 (N–H), 3102 (O–H), 1675 (C=O), 1515 (NO_2), 1184 (C–F), 670 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.94 (br s, 1H, OH), 4.46–4.40 (dd, 2H, CH, $J = 7.34$, 7.41 Hz), 7.82–7.79 (m, 4H, ArH), 8.41–8.40 (d, 1H, ArH, $J = 9.43$ Hz), 9.61 (s, 1H, NH); ESI-MS, *m/z*: 393.01 $[M]^+$, 395.02 $[M+2]^+$; anal. calcd. for $C_{16}H_9ClFN_3O_4S$: C, 48.80; H, 2.30; N, 10.67. Found: C, 48.93; H, 2.38; N, 10.82.

N-(7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide ***10c***

Yield 72%; mp 266–269°C; Yellow crystal; IR (ν_{max}, cm^{-1}, KBr): 3400 (N–H), 3090 (O–H), 1672 (C=O), 1508 (NO_2), 1251 & 1052 (C–O), 1198 (C–F), 645 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.22 (s, 3H, OCH_3), 3.93 (br s, 1H, OH), 4.52–4.47 (dd, 2H, CH, $J = 7.27$, 7.33 Hz), 7.34–7.30 (m, 3H, ArH), 8.42–8.40 (d, 1H, ArH, $J = 9.42$ Hz), 9.25 (s, 1H, NH); ESI-MS, *m/z*: 423.03 $[M]^+$, 425.03 $[M+2]^+$; anal. calcd. for $C_{17}H_{11}ClFN_3O_5S$: C, 48.18; H, 2.62; N, 9.92. Found: C, 48.32; H, 2.74; N, 9.90.

N-(7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-(4-methoxyphenyl)acrylamide ***10d***

Yield 66%; mp 272–274°C; yellow crystal; IR (ν_{max}, cm^{-1}, KBr): 3402 (N–H), 1649 (C=O), 1516 (NO_2), 1249 & 1051 (C–O), 1202 (C–F), 680 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.42 (s, 3H, OCH_3), 4.35–4.31 (d, 1H, CH, $J = 7.92$ Hz), 4.46–4.40 (d, 1H, CH, $J = 7.95$ Hz), 7.78–7.75 (m, 4H, ArH), 8.45–8.44 (d, 1H, ArH, $J = 9.40$ Hz), 9.29 (s, 1H, NH); ESI-MS, *m/z*: 407.02 $[M]^+$, 409.03 $[M+2]^+$; anal. calcd. for $C_{17}H_{11}ClFN_3O_4S$: C, 50.07; H, 2.72; N, 10.30. Found: C, 50.16; H, 2.67; N, 10.37.

N-(7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-(2-hydroxyphenyl)acrylamide ***10e***

Yield 54%; mp 280–281°C; greenish powder; IR (ν_{max}, cm^{-1}, KBr): 3422 (N–H), 3156 (O–H), 1681 (C=O), 1523 (NO_2), 1192 (C–F), 676 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.97 (br s, 1H, OH), 4.42–4.37 (dd, 2H, CH, $J = 7.38$, 7.46 Hz), 7.43–7.38 (m, 4H, ArH), 8.31–8.26 (d, 1H, ArH, $J = 9.42$ Hz), 9.64 (s, 1H, NH); ESI-MS, *m/z*: 393.01 $[M]^+$, 395.03 $[M+2]^+$; anal. calcd. for $C_{16}H_9ClFN_3O_4S$: C, 48.80; H, 2.30; N, 10.67. Found: C, 48.74; H, 2.30; N, 10.61.

N-(7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-phenylacrylamide ***10f***

Yield 52%; mp 263–265°C; yellow crystal; IR (ν_{max}, cm^{-1}, KBr): 3431 (N–H), 1691 (C=O), 1533 (NO_2), 1186 (C-F), 645 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 4.86–4.81 (dd, 1H, CH, $J = 7.39$, 7.52 Hz), 7.56–7.51 (m, 5H, ArH), 8.67–8.63 (d, 1H, ArH, $J = 9.42$ Hz), 9.57 (s, 1H, NH); ESI-MS, *m/z*: 377.03 $[M]^+$, 379.04 $[M+2]^+$; anal. calcd. for $C_{16}H_9ClFN_3O_3S$: C, 50.87; H, 2.40; N, 11.12. Found: C, 50.69; H, 2.48; N, 11.06.

Synthesis of 7-chloro-N-(1-(7-chloro-6-fluorobenzo[d]-thiazol-2-yl)-5-substituted phenyl-4,5-dihydro-1H-pyrazol-3-yl)-6-fluorobenzo[d]thiazol-2-amine ***11a–f***

A mixture of compound **8a–f** (0.002 mol) and 7-chloro-6-fluoro-2-hydrazinylbenzo[d]thiazole **4** (0.002 mol) in ethanol (25 mL) containing 3–4 drops of glacial acetic acid was heated under reflux for 8 h. After completion of reaction, the reaction mixture was concentrated under reduced pressure. On cooling, solid separated out, was collected and recrystallized by DMF-water mixture.

7-Chloro-N-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-5-(4-(dimethylamino) phenyl)-4, 5-dihydro-1H-pyrazol-3-yl)-6-fluorobenzo[d]thiazol-2-amine ***11a***

Yield 82%; mp 240–242°C; buff color powder; IR (ν_{max}, cm^{-1}, KBr): 3440 (N-H), 1100 (C–F), 640 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 2.05 (s, 6H, CH_3), 3.27 (1H, dd, $J = 5.8$, 18.20 Hz, H-4 *trans* (pyrazoline)), 4.04 (1H, dd, $J = 12.10$, 18.20 Hz, H-4 *cis* (pyrazoline)), 5.06 (s, 1H, NH), 5.22 (1H, dd, $J = 5.80$, 12.00 Hz, H-5 (pyrazoline)), 7.23–7.19 (m, 4H, ArH), 7.68–7.64 (m, 4H, ArH); ESI-MS, *m/z*: 574.06 $[M]^+$, 576.05 $[M+2]^+$, 578.05 $[M+4]^+$; anal. calcd. for $C_{25}H_{18}Cl_2F_2N_6S_2$: C, 52.18; H, 3.15; N, 14.60. Found: C, 52.23; H, 3.21; N, 14.52.

4-(1-(7-Chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(7-chloro-6-fluorobenzo[d]thiazol-2-yl-amino)-4,5-dihydro-1H-pyrazol-5-yl)phenol ***11b***

Yield 78%; mp 170–171°C; yellow crystal; IR (ν_{max}, cm^{-1}, KBr): 3482 (N–H), 3120 (O–H), 1160 (C–F), 638 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.31 (1H, dd, $J = 5.7$, 18.4 Hz, H-4 *trans* (pyrazoline)), 4.04 (1H, dd, $J = 12.3$, 18.5 Hz, H-4 *cis* (pyrazoline)),

4.49 (bs, 1H, OH), 5.12 (s, 1H, NH), 5.25 (1H, dd, $J = 5.83, 12.07$ Hz, H-5 (pyrazoline)), 7.43–7.40 (m, 4H, ArH), 7.72–7.69 (m, 4H, ArH); ESI-MS, *m/z*: 547.01 $[M]^+$, 549 $[M+2]^+$, 551 $[M+4]^+$; anal. calcd. for $C_{23}H_{13}Cl_2F_2N_5OS_2$: C, 50.37; H, 2.39; N, 12.77. Found: C, 50.37; H, 2.39; N, 12.77.

4-(1-(7-Chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(7-chloro-6-fluorobenzo[d]thiazol-2-ylamino)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol ***11c***

Yield 72%; mp 185–186°C; buff color powder; IR (ν_{max}, cm^{-1}, KBr): 3490 (N–H), 3037 (O–H), 1251 & 1050 (C–O), 1178 (C–F), 660 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.27 (1H, dd, $J = 5.8$, 18.2 Hz, H-4 *trans* (pyrazoline)), 3.63 (s, 3H, OCH_3), 4.03 (1H, dd, $J = 12.30$, 18.50 Hz, H-4 *cis* (pyrazoline)), 4.51(bs, 1H OH), 5.03 (s, 1H, NH), 5.23 (1H, dd, $J = 5.9$, 12.04 Hz, H-5 (pyrazoline)), 7.58–7.55 (m, 3H, ArH), 7.77–7.73 (m, 4H, ArH); ESI-MS, *m/z*: 577.02 $[M]^+$, 579.05 $[M+2]^+$, 581.03 $[M+4]^+$; anal. calcd. for $C_{24}H_{15}Cl_2F_2N_5O_2S_2$: C, 49.83; H, 2.61; N, 12.11. Found: C, 49.92; H, 2.69; N, 12.16.

7-Chloro-N-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-fluorobenzo[d]thiazol-2-amine ***11d***

Yield 80%; mp 180–181°C; light yellow crystal IR (ν_{max}, cm^{-1}, KBr): 3489 (N–H), 1252 & 1055 (C–O), 1178 (C–F), 643 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.29 (1H, dd, $J = 5.80$, 18.2 Hz, H-4 *trans* (pyrazoline)), 3.64 (s, 3H, OCH_3), 4.07 (1H, dd, $J = 11.99$, 18.0 Hz, H-4 *cis* (pyrazoline)), 5.04 (s, 1H, NH), 5.23 (1H, dd, $J = 5.7$, 11.97 Hz, H-5 (pyrazoline)), 7.48–7.45 (m, 4H, ArH), 7.73–7.69 (m, 4H, ArH); ESI-MS, *m/z*: 561.04 $[M]^+$, 563.06 $[M+2]^+$, 565.01 $[M+4]^+$; anal. calcd. for $C_{24}H_{15}Cl_2F_2N_5OS_2$: C, 51.25; H, 2.69; N, 12.45. Found: C, 51.31; H, 2.76; N, 12.41.

2-(1-(7-Chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(7-chloro-6-fluorobenzo[d]thiazol-2-ylamino)-4,5-dihydro-1H-pyrazol-5-yl)phenol ***11e***

Yield 62%; mp 175–177°C; white crystal; IR (ν_{max}, cm^{-1}, KBr): 3475 (N–H), 3119 (O–H), 1176 (C–F), 641 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.32 (1H, dd, $J = 5.86$, 18.19 Hz, H-4 *trans* (pyrazoline)), 4.12 (1H, dd, $J = 12.03$, 18.06 Hz, H-4 *cis* (pyrazoline)), 4.41 (bs, 1H OH), 5.06 (s, 1H, NH), 5.25 (1H, dd, $J = 5.70$, 12.02 Hz, H-5 (pyrazoline)), 7.38–7.35 (m, 4H, ArH), 7.84–7.80 (m, 4H, ArH); ESI-MS, *m/z*: 547 $[M]^+$, 549.02 $[M+2]^+$, 551 $[M+4]^+$; anal. calcd. for $C_{23}H_{13}Cl_2F_2N_5OS_2$: C, 50.37; H, 2.39; N, 12.77. Found: C, 50.49; H, 2.45; N, 12.81.

7-Chloro-N-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-6-fluorobenzo[d]-thiazol-2-amine ***11f***

Yield 69%; mp 190–192°C; gray color powder; IR (ν_{max}, cm^{-1}, KBr): 3465 (N–H), 1188 (C–F), 633 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.27 (1H, dd, $J = 5.8$, 18.2 Hz, H-4 *trans* (pyrazoline)), 4.07 (1H, dd, $J = 11.99$, 18.0 Hz, H-4 *cis* (pyrazoline)), 4.98 (s, 1H, NH), 5.23 (1H, dd, $J = 5.70$, 11.97 Hz, H-5 (pyrazoline)), 7.26–7.23 (m, 5H, ArH), 7.73–7.68 (m, 4H, ArH); ESI-MS, *m/z*: 531 $[M]^+$, 533.01 $[M+2]^+$, 535 $[M+4]^+$; anal. calcd. for $C_{23}H_{13}Cl_2F_2N_5S_2$: C, 51.89; H, 2.46; N, 13.15. Found: C, 51.96; H, 2.52; N, 13.11.

Synthesis of 7-chloro-N-(1-(7-chloro-6-fluorobenzo[d]-thiazol-2-yl)-5-substituted phenyl-4,5-dihydro-1H-pyrazol-3-yl)-6-fluoro-5-nitrobenzo[d]thiazol-2-amine ***12a–f***

A mixture of compound **9a–f** (0.002 mol) and 7-chloro-6-fluoro-2-hydrazinylbenzo[*d*] thiazole **4** (0.002 mol) in ethanol (25 mL) containing 3–4 drops of glacial acetic acid was heated under reflux for 8 h. After completion of reaction, the reaction mixture was concentrated under reduced pressure. On cooling, solid was separated out, collected and recrystallized by DMF/water mixture.

7-Chloro-N-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-5-(4-(dimethylamino) phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-fluoro-5-nitrobenzo[d]thiazol-2-amine ***12a***

Yield 77%; mp 104–105°C; orange color crystal; IR (ν_{max}, cm^{-1}, KBr): 3508 (N–H), 1513 (NO_2), 1190 (C–F), 685 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 2.78 (s, 6H, CH_3), 3.26 (1H, dd, $J = 5.6$, 18.19 Hz, H-4 *trans* (pyrazoline)), 4.08 (1H, dd, $J = 12.06$, 18.07 Hz, H-4 *cis* (pyrazoline)), 5.16 (s, 1H, NH), 5.27 (1H, dd, $J = 5.73$, 11.99 Hz, H-5 (pyrazoline)), 7.39–7.36 (m, 4H, ArH), 7.81–7.78 (m, 3H, ArH); ESI-MS, *m/z*: 619.04 $[M]^+$, 621.04 $[M+2]^+$, 623.02 $[M+4]^+$; anal. calcd. for $C_{25}H_{17}Cl_2F_2N_7O_2S_2$: C, 48.39; H, 2.76; N, 15.80. Found: C, 48.51; H, 2.79; N, 15.87.

4-(3-(7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-ylamino)-1-(7-chloro-6-fluorobenzo[d] thiazol-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)phenol ***12b***

Yield 73%; mp 182–184°C; buff color powder; IR (ν_{max}, cm^{-1}, KBr): 3392 (N–H), 3180 (O–H), 1536 (NO_2), 1186 (C–F), 682 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.27 (1H, dd, $J = 5.5$, 18.17 Hz, H-4 *trans* (pyrazoline)), 4.06 (1H, dd, $J = 12.02$, 18.01 Hz, H-4 *cis* (pyrazoline)), 4.46 (bs, 1H, OH), 5.12 (s, 1H, NH), 5.22 (1H, dd, $J = 5.74$, 11.98 Hz, H-5 (pyrazoline)), 7.33–7.30 (m, 4H, ArH), 7.75–7.71 (m, 3H, ArH); ESI-MS, *m/z*: 591.99 $[M]^+$, 594 $[M+2]^+$, 595.97 $[M+4]^+$; anal. calcd. for $C_{23}H_{12}Cl_2F_2N_6O_3S_2$: C, 46.55; H, 2.04; N, 14.16. Found: C, 46.67; H, 2.11; N, 14.12.

4-(3-(7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-ylamino)-1-(7-chloro-6-fluorobenzo[d] thiazol-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol ***12c***

Yield 70%; mp 210–213°C; buff color powder; IR (ν_{max}, cm^{-1}, KBr): 3431 (N–H), 3128 (O–H) 1521 (NO_2), 1255 & 1052 (C–O), 1196 (C–F), 630 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.30 (1H, dd, $J = 5.81$, 18.20 Hz, H-4 *trans* (pyrazoline)), 3.73 (s, 3H, OCH_3), 4.12 (1H, dd, $J = 12.02$, 18.01 Hz, H-4 *cis* (pyrazoline)), 4.42 (bs, 1H, OH), 5.14 (s, 1H, NH), 5.22 (1H, dd, $J = 5.74$, 11.98 Hz, H-5 (pyrazoline)), 7.43–7.39 (m, 3H, ArH), 7.79–7.77 (m, 3H, ArH); ESI-MS, *m/z*: 621.98 $[M]^+$, 623.99 $[M+2]^+$, 626 $[M+4]^+$; anal. calcd. for $C_{24}H_{14}Cl_2F_2N_6O_4S_2$: C, 46.24; H, 2.26; N, 13.48. Found: C, 46.32; H, 2.21; N, 13.44.

7-Chloro-N-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-fluoro-5-nitrobenzo[d]thiazol-2-amine ***12d***

Yield 75%; mp 180–182°C; light yellow crystal; IR (ν_{max}, cm^{-1}, KBr): 3508 (N–H), 1520 (NO_2), 1190 (C–F), 656 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.28 (1H, dd, $J = 5.79$, 18.17 Hz,

H-4 *trans* (pyrazoline)), 3.66 (s, 3H, OCH_3), 4.07 (1H, dd, $J = 12.00$, 17.98 Hz, H-4 *cis* (pyrazoline)), 5.12 (s, 1H, NH), 5.19 (1H, dd, $J = 5.72$, 11.99 Hz, H-5 (pyrazoline)), 7.37–7.34 (m, 3H, ArH), 7.76–7.73 (m, 3H, ArH); ESI-MS, *m/z*: 606.03 $[M]^+$, 608.03 $[M+2]^+$, 610.01 $[M+4]^+$; anal. calcd. for $C_{24}H_{14}Cl_2F_2N_6O_3S_2$: C, 47.45; H, 2.32; N, 13.84. Found: C, 47.52; H, 2.41; N, 13.78.

2-(3-(7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-ylamino)-1-(7-chloro-6-fluorobenzo[d] thiazol-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)phenol ***12e***

Yield 65%; mp 166–168°C; light gray; IR (ν_{max}, cm^{-1}, KBr): 3432 (N–H), 3126 (O–H), 1535 (NO_2), 1192 (C–F), 642 (C-Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.32 (1H, dd, $J = 5.92$, 18.24 Hz, H-4 *trans* (pyrazoline)), 4.05 (1H, dd, $J = 12.14$, 18.25 Hz, H-4 *cis* (pyrazoline)), 4.34 (br s, 1H, OH), 5.12 (s, 1H, NH), 5.25 (1H, dd, $J = 5.86$, 12.04 Hz, H-5 (pyrazoline)) 7.35–7.32 (m, 4H, ArH), 7.92–7.88 (m, 3H, ArH); ESI-MS, *m/z*: 592.02 $[M]^+$, 594.01 $[M+2]^+$, 596 $[M+4]^+$; anal. calcd. for $C_{23}H_{12}Cl_2F_2N_6O_3S_2$: C, 46.55; H, 2.04; N, 14.16. Found: C, 46.62; H, 2.12; N, 14.19.

7-Chloro-N-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-6-fluoro-5-nitrobenzo[d]thiazol-2-amine ***12f***

Yield 69%; mp 177–178°C; light yellow powder; IR (ν_{max}, cm^{-1}, KBr): 3498 (N–H), 1515 (NO_2), 1191 (C–F), 655 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.30 (1H, dd, $J = 5.80$, 18.21 Hz, H-4 *trans* (pyrazoline)), 4.02 (1H, dd, $J = 12.12$, 18.24 Hz, H-4 *cis* (pyrazoline)), 5.13 (s, 1H, NH), 5.23 (1H, dd, $J = 5.81$, 12.03 Hz, H-5 (pyrazoline)), 7.35–7.32 (m, 5H, ArH), 7.87–7.84 (m, 3H, ArH); ESI-MS, *m/z*: 575.99 $[M]^+$, 578 $[M+2]^+$, 580 $[M+4]^+$; anal. calcd. for $C_{23}H_{12}Cl_2F_2N_6O_2S_2$: C, 47.84; H, 2.09; N, 14.55. Found: C, 47.96; H, 2.18; N, 14.59.

Synthesis of 7-chloro-N-(1-(7-chloro-6-fluorobenzo[d]-thiazol-2-yl)-5-substituted phenyl-4,5-dihydro-1H-pyrazol-3-yl)-6-fluoro-4-nitrobenzo[d]thiazol-2-amine ***13a–f***

A mixture of compound **10a–f** (0.002 mol) and 7-chloro-6-fluoro-2-hydrazinylbenzo[d] thiazole **4** (0.002 mol) in ethanol (25 mL) containing 3–4 drops of glacial acetic acid was heated under reflux for 8 h. After completion of reaction, the reaction mixture was concentrated under reduced pressure. On cooling, solid was separated out, collected and recrystallized by DMF-water mixture.

7-Chloro-N-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-5-(4-(dimethylamino) phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-fluoro-4-nitrobenzo[d]thiazol-2-amine ***13a***

Yield 70%; mp 258–260°C; light green crystalline; IR (ν_{max}, cm^{-1}, KBr): 3422 (N–H), 1516 (NO_2), 1197 (C–F), 662 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 2.76 (s, 6H, CH_3), 3.27 (1H, dd, $J = 5.78$, 18.21 Hz, H-4 *trans* (pyrazoline)), 4.04 (1H, dd, $J = 12.11$, 18.21 Hz, H-4 *cis* (pyrazoline)), 5.06 (s, 1H, NH), 5.24 (1H, dd, $J = 5.79$, 12.07 Hz, H-5 (pyrazoline)), 7.50–7.47 (m, 4H, ArH), 7.84–7.81 (d, 1H, $J = 8.28$ Hz, ArH), 7.92–7.90 (d, 1H, ArH, $J = 8.31$ Hz), 8.47–8.45 (d, 1H, ArH, $J = 9.44$ Hz); ESI-MS, *m/z*: 619.04 $[M]^+$, 621.02 $[M+2]^+$, 623 $[M+4]^+$; anal. calcd. for $C_{25}H_{17}Cl_2F_2N_7O_2S_2$: C, 48.39; H, 2.76; N, 15.80. Found: C, 48.53; H, 2.82; N, 15.84.

4-(3-(7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-ylamino)-1-(7-chloro-6-fluorobenzo[d] thiazol-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)phenol ***13b***

Yield 66%; mp 210–212°C; light green crystalline; IR (ν_{max}, cm^{-1}, KBr): 3401 (N–H), 3098 (O–H), 1525 (NO_2), 1190 (C–F), 688 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.26 (1H, dd, $J = 5.79$, 18.18 Hz, H-4 *trans* (pyrazoline)), 4.06 (1H, dd, $J = 12.11$, 18.12 Hz, H-4 *cis* (pyrazoline)), 4.21 (br s, 1H, OH), 5.13 (s, 1H, NH), 5.26 (1H, dd, $J = 5.78$, 12.11 Hz, H-5 (pyrazoline)), 7.54–7.51 (m, 4H, ArH), 7.92–7.87 (dd, 2H, ArH, $J = 7.02$, 8.36 Hz), 8.52–8.49 (d, 1H, ArH, $J = 9.42$ Hz); ESI-MS, *m/z*: 592.03 $[M]^+$, 594 $[M+2]^+$, 596 $[M+4]^+$; anal. calcd. for $C_{23}H_{12}Cl_2F_2N_6O_3S_2$: C, 46.55; H, 2.04; N, 14.16. Found: C, 46.52; H, 2.12; N, 14.13.

4-(3-(7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-ylamino)-1-(7-chloro-6-fluorobenzo[d] thiazol-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol ***13c***

Yield 68%; mp 218–221°C; green powder; IR (ν_{max}, cm^{-1}, KBr): 3402 (N–H), 3102 (O–H), 1518 (NO_2), 1192 (C–F), 666 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.27 (1H, dd, $J = 5.81$, 18.22 Hz, H-4 *trans* (pyrazoline)), 3.78 (s, 3H, OCH_3), 4.05 (1H, dd, $J = 12.14$, 18.27 Hz, H-4 *cis* (pyrazoline)), 4.41 (br s, 1H, OH), 5.13 (s, 1H, NH), 5.24 (1H, dd, $J = 5.86$, 12.03 Hz, H-5 (pyrazoline)), 7.32–7.29 (s, 1H, ArH), 7.36–7.34 (d, 1H, ArH, $J = 8.23$ Hz), 7.89 (s, 1H, CH), 7.99–7.96 (d, 1H, ArH, $J = 8.01$ Hz), 8.10–8.07 (d, 1H, ArH, $J = 8.73$ Hz), 8.59–8.57 (d, 1H, ArH, $J = 9.44$ Hz); ESI-MS, *m/z*: 622.02 $[M]^+$, 624.01 $[M+2]^+$, 626 $[M+4]^+$; anal. calcd. for $C_{24}H_{14}Cl_2F_2N_6O_4S_2$: C, 46.24; H, 2.26; N, 13.48. Found: C, 46.31; H, 2.33; N, 13.51.

7-Chloro-N-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-6-fluoro-4-nitrobenzo[d]thiazol-2-amine ***13d***

Yield 66%; mp 228–231°C; light off color powder; IR (ν_{max}, cm^{-1}, KBr): 3422 (N–H), 3123 (O–H), 1515 (NO_2), 1189 (C–F), 654 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.27 (1H, dd, $J = 5.79$, 18.16 Hz, H-4 *trans* (pyrazoline)), 3.70 (s, 1H, OCH_3), 4.03 (1H, dd, $J = 12.13$, 18.26 Hz, H-4 *cis* (pyrazoline)), 5.11 (s, 1H, NH), 5.25 (1H, dd, $J = 5.83$, 12.07 Hz, H-5 (pyrazoline)), 7.47–7.44 (m, 4H, ArH), 7.95–7.92 (dd, 2H, ArH, $J = 7.13$, 9.66 Hz), 8.48–8.45 (d, 1H, ArH, $J = 9.44$ Hz); ESI-MS, *m/z*: 606.02 $[M]^+$, 608.03 $[M+2]^+$, 610 $[M+4]^+$; anal. calcd for $C_{24}H_{14}Cl_2F_2N_6O_3S_2$: C, 47.45; H, 2.32; N, 13.84. Found: C, 47.57; H, 2.39; N, 13.88.

2-(3-(7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-ylamino)-1-(7-chloro-6-fluorobenzo[d] thiazol-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)phenol ***13e***

Yield 59%; mp 193–194°C; light green crystalline; IR (ν_{max}, cm^{-1}, KBr): 3405 (N–H), 3108 (O–H), 1521 (NO_2), 1183 (C–F), 674 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.30 (1H, dd, $J = 5.85$, 18.19 Hz, H-4 *trans* (pyrazoline)), 4.09 (1H, dd, $J = 12.14$, 18.26 Hz, H-4 *cis* (pyrazoline)), 4.37 (br s, 1H, OH), 5.10 (s, 1H, NH), 5.24 (1H, dd, $J = 5.87$, 12.03 Hz, H-5 (pyrazoline)), 7.42–7.39 (m, 4H, ArH), 7.98–7.95 (dd, 2H, ArH, $J = 7.11$, 9.02 Hz), 8.53–8.51 (d, 1H, ArH, $J = 9.44$ Hz); ESI-MS, *m/z*: 591.98 $[M]^+$, 593.99 $[M+2]^+$, 596 $[M+4]^+$; anal. calcd. for $C_{23}H_{12}Cl_2F_2N_6O_3S_2$: C, 46.55; H, 2.04; N, 14.16. Found: C, 46.52; H, 2.14; N, 14.13.

7-Chloro-N-(1-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-6-fluoro-4-nitrobenzo[d]thiazol-2-amine ***13f***

Yield 64%; mp 226–228°C; gray color powder; IR (ν_{max}, cm^{-1}, KBr): 3423 (N-H), 3118 (O–H), 1517 (NO_2), 1199 (C–F), 685 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 3.29 (1H, dd, J = 5.80, 18.20 Hz, H-4 *trans* (pyrazoline)), 4.32 (br s, 1H, OH), 4.00 (1H, dd, J = 12.1, 18.2 Hz, H-4 *cis* (pyrazoline)), 5.04 (s, 1H, NH), 5.22 (1H, dd, J = 5.8, 12.0 Hz, H-5 (pyrazoline)), 7.40–7.37 (m, 4H, ArH), 7.90–7.87 (dd, 2H, ArH, J = 7.27, 8.94 Hz), 8.51–8.49 (d, 1H, ArH, J = 9.44 Hz); ESI-MS, *m/z*: 576.01 $[M]^+$, 578 $[M+2]^+$, 589.98 $[M+4]^+$; anal. calcd. for $C_{23}H_{12}Cl_2F_2N_6O_2S_2$: C, 47.84; H, 2.09; N, 14.55. Found: C, 47.88; H, 2.18; N, 14.57.

Antitubercular activity

The ability of compounds to inhibit the growth of *Mycobacterium* species was determined by Ziehl-Neelsen stain and they were grown in Middlebrook 7H-9 broth. The standard strain was used for *Mycobacterium tuberculosis* H37Rv (ATCC 27294). The basal medium was prepared according to manufacturer's instructions (Hi-Media) and sterilized by autoclaving. 4.5 mL of broth was taken in each sterile bottle and to this 0.5 mL of ADC supplement was added which containing catalase, dextrose and bovine serum albumin fraction. Then, the compound solution was transferred to media bottles to achieve the final concentrations 25, 50, 100 μg/mL. Finally a 10-μL suspension of *Mycobacterium tuberculosis* strain (100 000 organisms/mL, adjusted by McFarland's turbidity standard) was transferred to each of the tubes which were incubated at 37°C for three weeks. A control without compound as well as drug was also set up and was inspected for growth twice a week. The appearance of turbidity was considered as growth and the indicator of the resistance to the compound. The growth was confirmed by making a smear from each bottle and performing a Ziehl-Neelsen stain.

In-vitro cytotoxicity evaluation

The compounds were tested for cytotoxicity (CTC_{50}) against THP-1 cell lines [15].

Procedure with THP-1 culture:

The cell suspension from the confluent culture flask was transferred to sterile tubes, centrifuged at 2000 rpm for 10 min and cell pellet was separated. Known volume of media was added to the pellet and cells were resuspended and cell count was adjusted to 1.0×10^5 cells/mL using RPMI-1640 medium containing 10% fetal bovine serum. To each well of the 96 well microtitre plate, 0.1 mL of the diluted cell suspension (approximately 10 000 cells) was added. After 2 h, 100 μL of different test concentrations of test drugs were added onto the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, 10 μL of 3-(4,5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT) (5 mg/mL) in phosphate buffered saline was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO_2 atmosphere. Microtitre plates were centrifuged at 2000 rpm for 15 min and supernatant was removed. 100 μL of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

$$\%\,Growth\ Inhibition = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

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