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## Synthesis of benzothiazolopyrazoloisonicotinohydrazide derivatives and their nitro regioisomers for antitubercular activity

Kuntal Hazra<sup>\*a</sup> & B Nandha<sup>b</sup>

<sup>a</sup>Department of Pharmaceutical Chemistry, Bharat Technology, Uluberia, Howrah 711 316, India

<sup>b</sup>Department of Pharmaceutical Chemistry, Vivekananda College of Pharmacy, Bangalore 560 055, India

E-mail: [kuntalhazra@gmail.com](mailto:kuntalhazra@gmail.com)

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The history of incessant struggle and the current global burden associated with emerging infectious disease especially tuberculosis, guided us to define the scope of this research project, and to identify research gaps in synthesizing some heterocyclic compounds for anti-tubercular activity. In this research project, we have synthesized a series of novel heterocyclic (benzothiazoles) compounds *viz* *N'*-((1-(7-chloro-6-fluorobenzo[*d*]thiazol-2-yl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)isonicotinohydrazide **12a-c** their 5-nitro **15a-c** and 4-nitro **18a-c** derivatives, by a series of reactions of their respective synthons. The completion of reaction and the purity of the synthesized compounds have been established by chromatographic analysis. All the newly synthesized compounds satisfactorily show acceptable analysis for their anticipated structures, which have been confirmed based on physicochemical and spectral data. These newly synthesized compounds have been primarily evaluated for their *in vitro* anti-tubercular activity by Ziehl-Neelsen stain method. Compounds **15a**, **15b**, **15c**, **18b** and **18c** have shown 100% inhibition at 25 mg/mL and MIC values of around 45 nM against *M. tuberculosis* H<sub>37</sub>Rv (ATCC 27294). Cytotoxicity on THP-1 cell line shows that all the tested compounds are safe.

**Keywords:** Nitrobenzothiazoles, antitubercular activity, pyrazole, regioisomers

Bacterial resistance to antibacterial agents or antibiotics is of serious concern in the medical community, as many species of bacteria have evolved resistance to certain well known antibiotics and synthetic agents. Therefore, there could be a rapidly growing global crisis in the clinical management of life-threatening infectious diseases caused by multidrug-resistant strains. To meet this catastrophe successfully, many researchers across the globe are working to discover new compounds which can selectively attack novel targets in microorganisms. Hence, the development of novel, potent, and unique antibacterial agents is the supreme way to overcome bacterial resistance and develop effective therapies. Agents that reduce the duration and complexity of the current therapy would have a major impact on the overall cure rate, meaning, there is an urgent need for new antimycobacterials<sup>1</sup>. In India, each year, approx. 220,000 deaths are reported due to tuberculosis. India is the highest TB burden country with World Health Organisation (WHO) statistics for 2011 giving an estimated incidence figure of 2.2 million cases of TB for India out of a global incidence of 9.6 million cases<sup>2,3</sup>. Tuberculosis is India's biggest health issue,

but what makes this issue worse is the recently discovered phenomenon of Totally Drug-Resistant Tuberculosis (TDR-TB). This issue of drug-resistant TB began with Multi Drug-Resistant Tuberculosis (MDR-TB), and moved on to Extremely Drug Resistant Tuberculosis (XDR-TB). Gradually, the most dangerous form has situated itself in India as TDR-TB. We have previously reported that certain benzothiazoles especially nitro benzothiazoles have good anti-tubercular activity<sup>4</sup>. These compounds exhibit several properties which make them highly interesting as potential drugs against tuberculosis. It has been estimated that approximately 30 million people will die from tuberculosis in about ten years. Benzothiazole derivatives are found in commercial products in nature. A derivative of benzothiazole is the light-emitting component of luciferin, found in fireflies. Pyrazole is extensively used as useful synthons in organic syntheses. INH, when it is attached to the benzothiazole derivatives the anti tubercular activity of the compounds will altered. Compounds containing nitro groups (nitrophenols, nitrobenzenes and nitrofurans) possess high physiological activity. Chloramphenicol, nitrofurans

are the very good antibiotics available in the market contains nitro aromatic in the structure<sup>5</sup>. In the recent years nitrobenzothiazole has been discovered as an inhibitor for *Mycobacterium tuberculosis* ATP phosphoribosyl transferase (HisG)<sup>6</sup>.

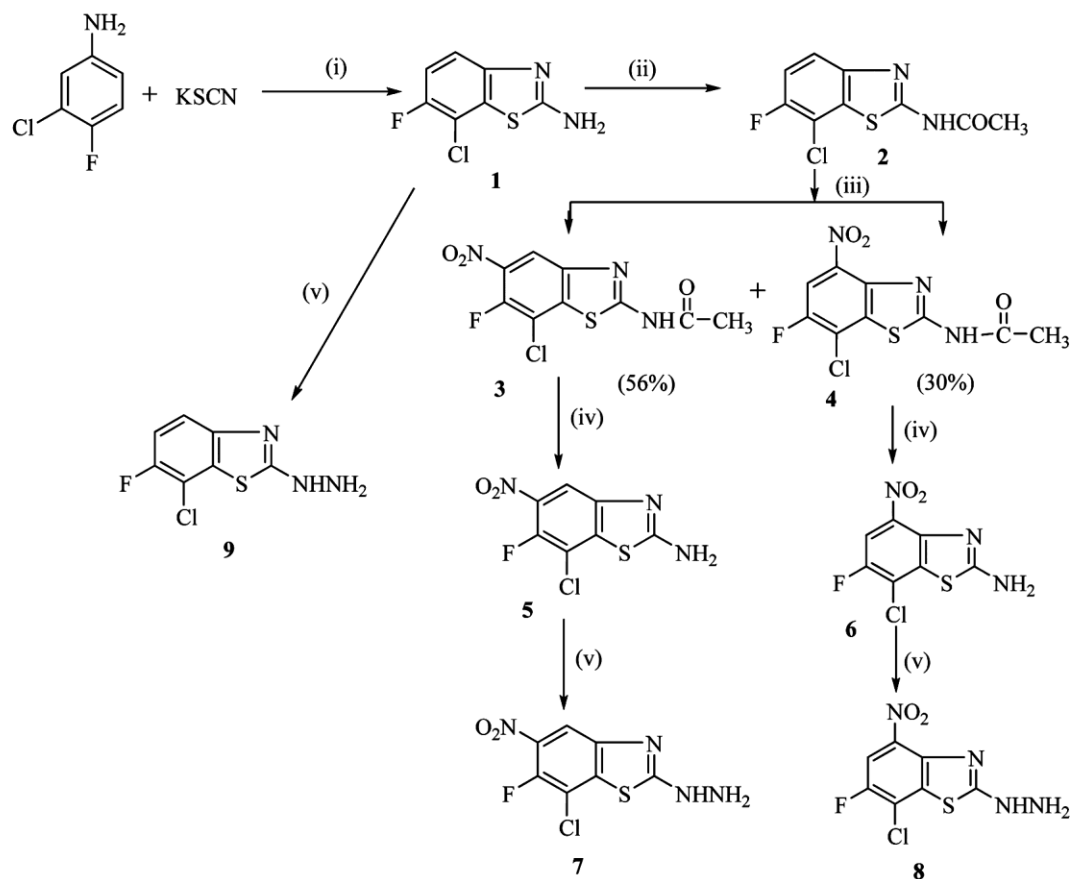
Fluorine has been incorporated into the benzothiazole molecule. Because, being the second smallest substituent next to hydrogen, intimately mimics hydrogen in enzyme receptor interactions. The substitutions of fluorine by hydrogen increases lipophilicity which in turn increases the transport and absorption 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 adjacent reaction centres<sup>7</sup>.

## Results and Discussion

### Chemistry

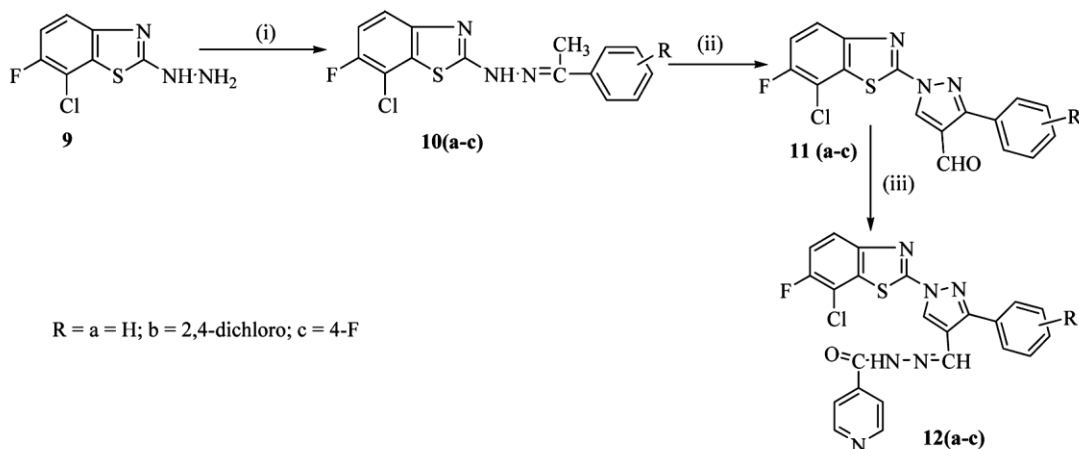
In the course of our ongoing program devoted to the synthesis of some nitro benzothiazoles as anti-

tubercular activity we describe the synthesis of *N'*-(1-(7-chloro-6-fluorobenzo[*d*]thiazol-2-yl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)isonicotinohydrazide (**12a-c**) their 5-nitro (**15a-c**) and 4-nitro (**18a-c**) derivatives through the versatile and efficient synthetic route outlined in Scheme I, Scheme II, Scheme III and Scheme IV. In Scheme I, we describe the synthesis of 7-chloro-6-fluoro-2-hydrazinylbenzo[*d*]thiazole (**9**) and their 5 nitro (**7**) and 4 nitro (**8**) derivatives. The compound **1** was synthesized from 4-fluoro-3-chloro aniline and potassium thiocyanate with bromine in acetic acid at 5°C. The starting material then was treated with hydrazine hydrate and conc. HCl in the presence of ethylene glycol yielding compound **9**. Alternatively the compound **1** was treated with acetic anhydride to protect the primary amino group and generate compound **2**, which upon nitration with conc. HNO<sub>3</sub> and conc. H<sub>2</sub>SO<sub>4</sub> produce **3** and **4** two regioisomers. Compounds **3** and **4** separately deprotected by using 70% H<sub>2</sub>SO<sub>4</sub> to obtain compounds



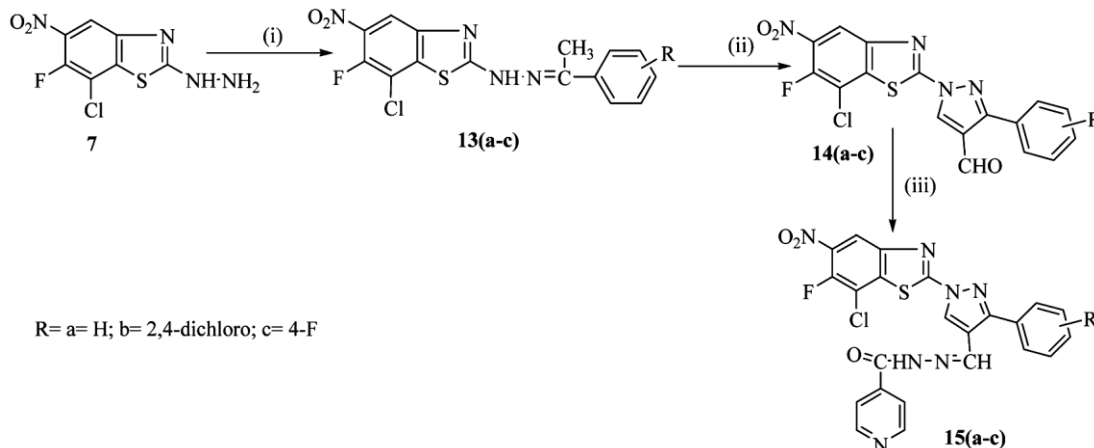
**Reagents and conditions:** (i) Br<sub>2</sub>, CH<sub>3</sub>COOH, 5°C, stir, NH<sub>3</sub>; (ii) (CH<sub>3</sub>CO)<sub>2</sub>O, reflux 1 h; (iii) conc. HNO<sub>3</sub> and conc. H<sub>2</sub>SO<sub>4</sub>, stir, RT (iv) 70% H<sub>2</sub>SO<sub>4</sub>, reflux, 30 min; (v) NH<sub>2</sub>.NH<sub>2</sub>.H<sub>2</sub>O/ HCl, ethylene glycol, reflux 2 h

Scheme I — Synthetic route of 7-chloro-6-fluoro-2-hydrazinylbenzo[*d*]thiazole **9** and 5 and 4 nitro derivatives **7** and **8**.



**Reagents and conditions:** (i) substituted acetophenone, 2-3 drops glacial acetic acid, methanol, reflux, 0.5 h; (ii)  $\text{POCl}_3$ -DMF, stir, 60-65°C, 3 h; (iii) isoniazid, toluene, 2-3 drops glacial acetic acid, reflux, 24 h

Scheme II — Synthetic route of novel series of *N'*-((1-(7-chloro-6-fluorobenzo[*d*]thiazol-2-yl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)isonicotinohydrazide **12a-c**



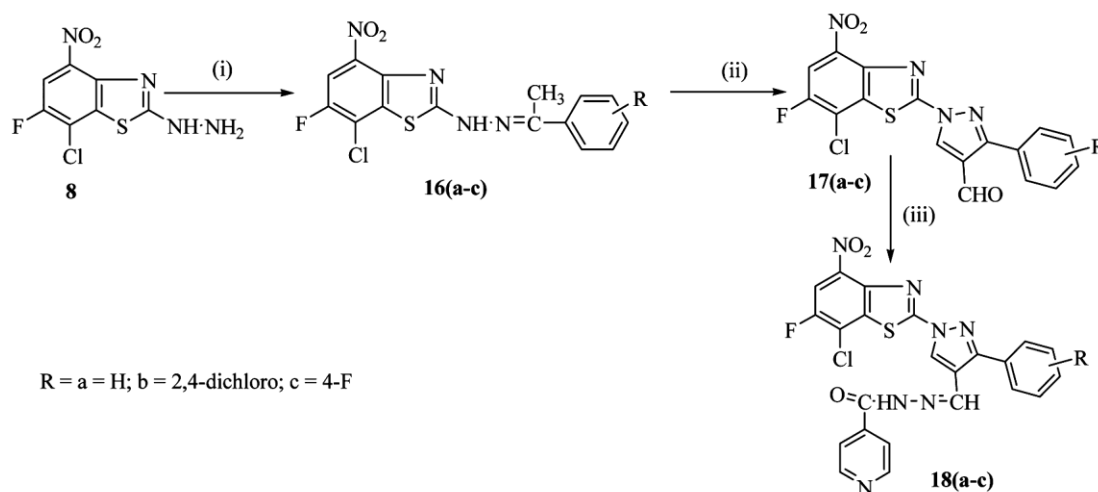
**Reagents and conditions:** (i) substituted acetophenone, 2-3 drops glacial acetic acid, methanol, reflux, 0.5 h; (ii)  $\text{POCl}_3$ -DMF, stir, 60-65°C, 3 h; (iii) isoniazid, toluene, 2-3 drops glacial acetic acid, reflux, 24 h

Scheme III — Synthetic route of a novel series of *N'*-((1-(7-chloro-6-fluoro-5-nitrobenzo[*d*]thiazol-2-yl)-3-substituted phenyl-1*H*-pyrazol-4-yl)methylene)isonicotinohydrazide **15a-c**

5 and 6. These were further treated with hydrazine hydrate and conc. HCl in the presence of ethylene glycol to obtain compounds 7 and 8 respectively. The NMR spectra (in  $\text{DMSO-}d_6$ ) of compound 7 showed absorption of 4<sup>th</sup> proton at  $\delta$  7.89 - 7.91 ppm as doublet due to C-F coupling accounting for 1 proton,  $J = 7.62$  Hz, NH at  $\delta$  9.18 - 9.20 ppm triplet accounting for 1 proton and  $\text{NH}_2$  at  $\delta$  5.05 ppm as doublet, accounting for 1 proton. Whereas compound 8 showed (in  $\text{DMSO-}d_6$ ) absorption of 5<sup>th</sup> proton at  $\delta$  8.53–8.55 ppm as doublet due to C-F coupling accounting for 1 proton,  $J = 9.42$  Hz, NH at  $\delta$  12.16 - 12.18 ppm

as singlet accounting for 1 proton and  $\text{NH}_2$  at  $\delta$  5.10 ppm as doublet accounting for 1 proton.

In Scheme II, we describe the synthesis of *N'*-((1-(7-chloro-6-fluorobenzo[*d*]thiazol-2-yl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)isonicotinohydrazide (**12a-c**) derivative. The compound 10 (a-c) was synthesized from compound 9 was treated with substituted acetophenone. Syntheses of *N'*-((1-(7-chloro-6-fluorobenzo[*d*]thiazol-2-yl)-3-phenyl-1*H*-pyrazol-4-yl)ethylene)isonicotinohydrazide (**12a-c**), their 5-nitro (**15a-c**) and 4-nitro (**18a-c**) derivatives were achieved through the versatile and efficient



**Reagents and conditions:** (a) substituted acetophenone, 2-3 drops glacial acetic acid, methanol, reflux, 0.5 h; (b)  $\text{POCl}_3$ -DMF, stir, 60-65°C, 3 h; (c) isoniazid, toluene, 2-3 drops glacial acetic acid, reflux, 24 h

Scheme IV — Synthetic route of a novel series of  $N'$ -((1-(7-chloro-6-fluoro-4-nitrobenzo [d]thiazol-2-yl)-3-substituted phenyl-1H-pyrazol-4-yl)methylene)isonicotinohydrazide **18a-c**

synthetic route outlined in Scheme II, Scheme I and Scheme IV respectively. Compound 7-chloro-6-fluoro-2-(2-(1-substituted phenylethylidene)hydrazinyl)benzo[d]thiazole (**10a-c**), their 5-nitro (**13a-c**) and 4-nitro (**16a-c**) derivatives were synthesized by reacting 7-chloro-6-fluoro-2-hydrazinylbenzo[d]thiazole (**9**), its 5-nitro (**7**) and 4-nitro (**8**) derivatives with substituted acetophenone in methanol with 2-3 drops of glacial acetic acid. Absence of primary amine peak and the presence on  $\text{N}=\text{C}$  peak at around 1550 in IR confirmed the formation of the product. In  $^1\text{H}$  NMR a singlet peak for methyl proton at  $\delta$  2.3 accounting three protons, benzothiazole and aromatic protons at aromatic region and the absent of  $\text{NH}_2$  peak confirmed the product. These compounds [**10(a-c)**, **13(a-c)** and **16(a-c)**] were then subjected to Vilsmeier-Haack reagent prepared from DMF and  $\text{POCl}_3$  at 60-65°C to form 1-(7-chloro-6-fluorobenzo [d] thiazol-2-yl)-3-substituted phenyl-1H-pyrazole-4-carbaldehyde (**11a-c**) their 5-nitro (**13a-c**) and 4-nitro (**17a-c**) derivatives. Preliminary conformation was done by TLC, melting point and overlapping IR. A strong  $\text{C}=\text{O}$  peak for aldehyde and the lacking of secondary amine peak in IR spectra confirmed the desired product. Two singlet peak for aldehydic and pyrazole proton at around  $\delta$  10.0 and  $\delta$  9.5, and the nonappearance of secondary amine and methyl protons confirmed the formation of the desired product. Latter these compounds were treated with isoniazid in toluene with 2-3 drops of glacial acetic acid in Dean-Stark apparatus. Isoniazid

tablet was dissolve in water, filter and filtrate was recrystallized by adding few drops of ethanol. In IR spectra presence of  $\text{C}=\text{O}$ ,  $\text{N}=\text{CH}$  and secondary amine peak confirmed the product. Which was further confirmed by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and mass spectra. In  $^1\text{H}$  NMR, a singlet peak for NH at around  $\delta$  12.0,  $\text{N}=\text{CH}$  at around  $\delta$  8.6 accounting one proton each, pyridine protons at around  $\delta$  8.7 and 7.7 as doublet and benzothiazole and aromatic protons at aromatic region confirmed the desired product. In  $^{13}\text{C}$  NMR peaks appeared at around  $\delta$  161 for  $\text{C}=\text{O}$ , pyridine carbons at around  $\delta$  156, 157, 140, 132 and 130,  $\text{N}=\text{CH}$  carbon at around  $\delta$  150 and other carbons appeared in the peak, confirmed the formation of the product.

#### Antitubercular activity

The *in vitro* anti tubercular activity of compounds **12a-c**, **15a-c** and **18a-c** were screened against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) using Middlebrook 7H-9 broth. The ability of compounds to inhibit the growth of *Mycobacterium* species was determined by Ziehl-Neelsen staining. The minimum inhibitory concentration (MIC) was defined as the minimum concentration of compound required to completely inhibit bacterial growth. The MICs of the synthesized compounds along with the standard pyrazinamide and streptomycin drugs are mentioned in Table I. Nine novel synthesized compounds along with two standard drugs were

Table I — *In vitro* anti-tubercular and cytotoxicity evaluation

S.No	Compd	R	R <sub>1</sub>	Anti-tubercular (MIC in nM)	Cytotoxicity (IC <sub>50</sub> nM)
1	<b>12a</b>	H	H	104.84	645.879
2	<b>12b</b>	2,4-dichloro	H	91.60	641.260
3	<b>12c</b>	4-fluoro	H	101.03	653.733
4	<b>15a</b>	H	5-NO <sub>2</sub>	47.90	162.863
5	<b>15b</b>	2,4-dichloro	5-NO <sub>2</sub>	42.31	160.798
6	<b>15c</b>	4-fluoro	5-NO <sub>2</sub>	46.30	168.317
7	<b>18a</b>	H	4-NO <sub>2</sub>	95.80	469.429
8	<b>18b</b>	2,4-dichloro	4-NO <sub>2</sub>	42.31	495.650
9	<b>18c</b>	4-fluoro	4-NO <sub>2</sub>	46.30	555.658
10	Pyrazinamide	–	–	60.095	NT
11	Streptomycin	–	–	14.387	NT

NT: Not tested, MIC: Minimum inhibitory concentration. IC<sub>50</sub>: Concentration of test compound needed to inhibit cell growth by 50%.

screened for antimycobacterial activity. Three different final concentrations 25, 50, 100 µg/ mL of compounds were prepared and used for activity. The appearance of turbidity was considered as growth and indicates resistance to the compound. The growth was confirmed by making a smear from each bottle and performing a Ziehl-Neelsen stain. From the results it has been observed that compounds 15a, 15b, 15c, 18b and 18c were shown 100% inhibition at 25 mg/ mL and MIC values of around 40 nM against *M. tuberculosis* H<sub>37</sub>Rv. Whereas the standard drug pyrazinamide and streptomycin have been showed 100% inhibition at 60 nM and 14 nM respectively.

Presence nitro group increases the antitubercular activity irrespective of the position in benzothiazole ring. Only electron withdrawing substituent in this series were synthesized and screened, so activity towards the electron donating substituent yet to be carried out.

The IC<sub>50</sub> values of all the tested nine compounds were ranging from 160 to 653 nM. Presence of a nitro group in to the compound increases the toxicity of the compound. Nitro group at position 5<sup>th</sup> exhibited more toxic than when it is in 4<sup>th</sup> position, though this concentration is far more than the concentration tested for the antitubercular activity. Cytotoxicity on THP-1 cell line showed that all the tested compounds are safe.

#### Experimental Section

The research chemicals and reagents were purchased from Himedia, Rankem, Loba chem., Merck, Spectrochem, SD Fine (India), Sigma-Aldrich (St. Louis, Missouri, USA), Lancaster Co. (Ward Hill, MA, USA) used as such for the reactions. Solvents, except laboratory reagent (LR) grade were dried and purified according to the literature when necessary. Reactions were monitored and purity of compounds

was examined by thin layer chromatography (TLC) on pre-coated silica gel plates from E. Merck and Co. (Darmstadt, Germany). Compounds visualized on UV cabinet at 365 nm/ 254 nm, exposure to iodine vapours, different visualising reagent depending on the requirement.

The melting points were determined with an electrothermal melting point apparatus and were uncorrected. Infrared spectra (KBr disc) were performed on FTIR-8400 Shimadzu and the frequencies were expressed in cm<sup>-1</sup>. <sup>1</sup>H NMR and <sup>13</sup>C 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), dd (double doublet), m (multiplet) and br s (broad singlet). Mass spectra were recorded on ESI-MS, Thermo, Finnigan LCQ deca xp max. 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 compound 1

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) 4-fluoro-3-chloro aniline. The mixture was stirred while 6 mL of bromine in 24 mL of glacial acetic acid was added at such a rate that the temperature did not rise beyond 5°C, for a period of 2 h. The stirring was continued for an additional 2 h at the same temperature, and at room temperature for 10 h. It was allowed to stand overnight during

which an orange precipitate settled at the bottom. 30 mL of water was added and slurry was heated at 85°C on a water bath and filtered hot. The filtrate was cooled and neutralized with strong ammonia solution to pH 6, a light yellow precipitate was collected. The resulting product was recrystallized by toluene.

**7-Chloro-6-fluorobenzo[d]thiazol-2-amine, 1:** Yield: 87%; m.p.: 180-182°C; slight yellowish crystalline solid; IR ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ , KBr): 3480 (N-H), 1247 (C-F), 1094 (C-Cl);  $^1\text{H NMR}$  (400MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 4.21 (s, 2H,  $\text{NH}_2$ ), 7.60-7.62 (d, 1H,  $J = 7.22$ , ArH), 7.64-7.66 (d, 1H, ArH,  $J = 8.59$  Hz); ESI-MS,  $m/z$ : 201.98  $[\text{M}]^+$ , 203.86  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_7\text{H}_4\text{ClFN}_2\text{S}$ : C, 41.49; H, 1.99; N, 13.82. Found: C, 41.51; H, 2.03; N, 13.81.

#### Synthesis of compound 2

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

**N-(7-Chloro-6-fluorobenzo[d]thiazol-2-yl)acetamide, 2:** Yield: 92%, m.p.: 232-233°C, white star shaped crystalline solid; IR ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ , KBr): 3318 (N-H), 1681 (C=O), 1239 (C-F), 1082 (C-Cl);  $^1\text{H NMR}$  (400MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 2.34 (s, 3H,  $\text{CH}_3$ ), 7.61-7.63 (d, 1H, ArH,  $J = 8.67$  Hz), 7.73-7.75 (d, 1H, ArH,  $J = 8.85$  Hz), 9.05 (s, 1H, NH); ESI-MS,  $m/z$ : 244.03  $[\text{M}]^+$ , 246.02  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_9\text{H}_6\text{ClFN}_2\text{OS}$ : C, 44.18; H, 2.47; N, 11.45. Found: C, 44.17; H, 2.49; N, 11.44.

#### Synthesis of compound 3 and 4

A mixture of compound (**2**) (100 mg, 0.000409 mol) and 0.3 mL of ice cold conc.  $\text{H}_2\text{SO}_4$  was stirred under ice cold condition. To this 0.1 mL conc.  $\text{HNO}_3$  was added drop wise, continued stirring at room temperature for 2 h. Then 0.1 mL conc.  $\text{HNO}_3$  was further added to the reaction mixture and stirred overnight at room temperature. 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 **3** and **4**, 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, 3:** Yield: 56%; m.p.: 297-298°C; buff colour needle shaped crystalline solid; IR ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ , KBr): 3301 (N-H), 1535, 1337 ( $\text{NO}_2$ ), 1680 (C=O),

1186 (C-F), 661 (C-Cl);  $^1\text{H NMR}$  (400MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 2.34 (s, 3H,  $\text{CH}_3$ ), 7.93-7.95 [d, 1H, ArH,  $J = 7.60$  Hz (C-F)], 9.16 (s, 1H, NH); ESI-MS,  $m/z$ : 288.99  $[\text{M}]^+$ , 290.97  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_9\text{H}_5\text{ClFN}_3\text{O}_3\text{S}$ : 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, 4:** Yield: 30%; m.p.: 336-337°C; white crystalline solid; IR ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ , KBr): 3311 (N-H), 1530, 1341 ( $\text{NO}_2$ ), 1680 (C=O), 1245 (C-F), 1092 (C-Cl);  $^1\text{H NMR}$  (400MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 2.27 (s, 3H,  $\text{CH}_3$ ), 8.40-8.38 [d, 1H, ArH,  $J = 9.44$  Hz (C-F)], 13.21 (s, 1H, NH); ESI-MS,  $m/z$ : 288.95  $[\text{M}]^+$ , 290.95  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_9\text{H}_5\text{ClFN}_3\text{O}_3\text{S}$ : C, 37.32; H, 1.74; N, 14.51. Found: C, 37.31; H, 1.76; N, 14.52.

#### Synthesis of compound 5 and 6

Compounds **3** and **4** (1 g, 0.004040 mole), separately taken in two different round bottom flask, add 70%  $\text{H}_2\text{SO}_4$  in both flask and was refluxed for 30 min. Then poured the clear solution into 50 mL of cold water and neutralized it with dil. sodium hydroxide solution. Filter the solid product and recrystallized both product with DMF- water mixture.

**7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-amine, 5:** Yield: 76%; m.p.: 187-188°C; light yellowish needle shaped crystalline solid; IR ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ , KBr): 3450 (N-H), 1543, 1348 ( $\text{NO}_2$ ), 1243 (C-F), 1089 (C-Cl);  $^1\text{H NMR}$  (400MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 4.26 (s, 2H,  $\text{NH}_2$ ), 7.90-7.92 (d, 1H,  $J = 7.62$ , ArH); ESI-MS,  $m/z$ : 247.63  $[\text{M}]^+$ , 249.35  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_7\text{H}_3\text{ClFN}_3\text{O}_2\text{S}$ : C, 33.95; H, 1.22; N, 16.97. Found: C, 34.07; H, 1.21; N, 16.95.

**7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-amine, 6:** Yield: 94%; m.p.: 193-194°C; yellowish needle shaped crystalline solid; IR ( $\nu_{\max}$ ,  $\text{cm}^{-1}$ , KBr): 3452 (N-H), 1550, 1333 ( $\text{NO}_2$ ), 1247 (C-F), 1087 (C-Cl);  $^1\text{H NMR}$  (400MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 4.29 (s, 2H,  $\text{NH}_2$ ), 8.40-8.42 (d, 1H,  $J = 9.42$ , ArH); ESI-MS,  $m/z$ : 247.28  $[\text{M}]^+$ , 249.14  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_7\text{H}_3\text{ClFN}_3\text{O}_2\text{S}$ : C, 33.95; H, 1.22; N, 16.97. Found: C, 34.01; H, 1.23; N, 16.91.

#### Synthesis of compound 7, 8 and 9

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 0.01 mol of compound (**1**, **5** and **6**), in portion was added (in separate RBF) 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.

**7-Chloro-6-fluoro-2-hydrazinyl-5-nitrobenzo[d]thiazole, 7:** Yield 66%, m.p.: 257-258°C, gray coloured crystals,  $\nu$ : 3458 (N-H), 1542, 1349 (NO<sub>2</sub>) 1241 (C-F), 1085 (C-Cl); <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>),  $\delta$  (ppm): 5.05 (s, 2H, NH<sub>2</sub>), 7.89-7.91 (d, 1H, J = 7.62 Hz, ArH), 9.18-9.20 (t, 1H, NH); ESI-MS, m/z: 261.91 [M]<sup>+</sup>, 263.53 [M+2]<sup>+</sup>; Anal. calcd. for C<sub>7</sub>H<sub>4</sub>ClFN<sub>4</sub>O<sub>2</sub>S: C, 32.01; H, 1.54; N, 21.33. Found: C, 32.03; H, 1.55; N, 21.35.

**7-Chloro-6-fluoro-2-hydrazinyl-4-nitrobenzo[d]thiazole, 8:** Yield 66%, m.p.: 292-294°C, gray coloured crystals; IR ( $\nu$ , cm<sup>-1</sup>, KBr): 3412 (N-H), 1532, 1342 (NO<sub>2</sub>), 1232 (C-F), 1074 (C-Cl); <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>),  $\delta$  (ppm): 5.10 (s, 2H, NH<sub>2</sub>), 8.53-8.55 (d, 1H, J = 9.42 Hz, ArH), 12.16-12.18 (t, 1H, NH); ESI-MS, m/z: 261.93 [M]<sup>+</sup>, 263.66 [M+2]<sup>+</sup>; Anal. calcd. for C<sub>7</sub>H<sub>4</sub>ClFN<sub>4</sub>O<sub>2</sub>S: C, 32.01; H, 1.54; N, 21.33. Found: C, 32.04; H, 1.55; N, 21.34.

**Synthesis of 7-chloro-6-fluoro-2-hydrazinyl benzo[d]thiazole, 9:** Yield 66%, mp 217-219 °C, light brown coloured needle shaped crystals; IR ( $\nu_{\max}$ , cm<sup>-1</sup>, KBr): 3380 (N-H), 1200 (C-F), 683 (C-Cl); <sup>1</sup>H NMR (400MHz, DMSO-d<sub>6</sub>),  $\delta$  (ppm): 5.09 (s, 2H, NH<sub>2</sub>), 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]<sup>+</sup>, 219.05 [M+2]<sup>+</sup>; Anal. calcd. for C<sub>7</sub>H<sub>5</sub>ClFN<sub>3</sub>S: C, 38.63; H, 2.32; N, 19.31. Found: C, 38.61; H, 2.34, N, 19.31.

#### Synthesis of compound 10a-c

A mixture of compound 9 (0.01 mol) and appropriate acetophenone (0.01 mol) in methanol (30 mL) containing 2-3 drop of glacial acetic acid was refluxed for 0.5 h. The reaction was monitored by TLC. After completion of the reaction, solid that separated out on cooling, was filtered and recrystallized by methanol.

**7-Chloro-6-fluoro-2-(2-(1-phenylethylidene)hydrazinyl)benzo[d]thiazole, 10a:** Yield 92%; m.p.: 120-122°C; colourless needle shaped crystals; IR ( $\nu$ , cm<sup>-1</sup>, KBr): 3432 (NH), 1610 (N=C), 1195 (C-F), 1028 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>),  $\delta$  (ppm): 2.34 (s, 3H, CH<sub>3</sub>), 7.39-7.47 (m, 5H, ArH), 7.61-7.63 (d, 1H, benzothiazole, J = 7.24 Hz), 7.65-7.68 (d, 1H, benzothiazole, J = 8.61 Hz) 10.75 (s, 1H, NH); ESI-MS, m/z: 319.72 [M]<sup>+</sup>, 317.65 [M+2]<sup>+</sup>; Anal. calcd. for C<sub>15</sub>H<sub>11</sub>ClFN<sub>3</sub>S: C, 56.34; H, 3.47; N, 5.94. Found: C, 56.33; H, 3.49, N, 5.91.

**7-Chloro-2-(2-(1-(2,4-dichlorophenyl)ethylidene)hydrazinyl)-6-fluorobenzo [d]thiazole, 10b:** Yield 95%; mp 192-194°C; colourless needle shaped crystals; IR ( $\nu$ , cm<sup>-1</sup>, KBr): 3142 (NH), 1612 (N=C), 1190 (C-F), 1030 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>),  $\delta$  (ppm): 2.37 (s, 3H, CH<sub>3</sub>), 7.42-7.44 (m, 3H, ArH), 7.78-7.79 (d, 2H, benzothiazole), 10.76 (s, 1H, NH); ESI-MS, m/z: 388.67 [M]<sup>+</sup>, 390.85 [M+2]<sup>+</sup>; Anal. calcd. for C<sub>15</sub>H<sub>9</sub>Cl<sub>3</sub>FN<sub>3</sub>S: C, 46.35; H, 2.33; N, 10.81. Found: C, 46.33; H, 2.34, N, 10.79.

**7-Chloro-6-fluoro-2-(2-(1-(4-fluorophenyl)ethylidene)hydrazinyl)benzo[d] thiazole, 10c:** Yield 95%; m.p.: 224-226°C; colourless crystalline solid; IR ( $\nu$ , cm<sup>-1</sup>, KBr): 3180 (N-H), 1610 (N=C), 1212 (C-F), 1080 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>),  $\delta$  (ppm): 2.37 (s, 3H, CH<sub>3</sub>), 7.41-7.43 (m, 4H, ArH), 7.78-7.79 (d, 2H, benzothiazole), 10.75 (s, 1H, NH); ESI-MS, m/z: 337.77 [M]<sup>+</sup>, 339.45 [M+2]<sup>+</sup>; Anal. calcd. for C<sub>15</sub>H<sub>10</sub>ClF<sub>2</sub>N<sub>3</sub>S: C, 53.34; H, 2.98; N, 12.44. Found: C, 53.33; H, 2.99, N, 12.40.

#### Synthesis of compound 11a-c

To the Vilsmeier-Haack reagent prepared from DMF (10 mL) and POCl<sub>3</sub> (1.1 mL, 0.012 mol), hydrazone 10(a-c) (0.01 mol) was added and the reaction mixture was stirred at 60-65°C for 3 h. The reaction was monitored by TLC. After completion of the reaction, the mixture was then poured in to ice cold water. The solid that separated on neutralization with NaHCO<sub>3</sub> was filtered, washed with water and recrystallized from DMF-water.

**1-(7-Chloro-6-fluorobenzo[d]thiazol-2-yl)-3-phenyl-1H-pyrazole-4-carbaldehyde, 11a:** Yield 84%; m.p.: 185-186°C; gray coloured solid; IR ( $\nu$ , cm<sup>-1</sup>, KBr): 1691(C=O), 1203 (C-F), 1078 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>),  $\delta$  (ppm): 7.28-7.32 (m, 5H, ArH), 7.63-7.64 (d, 1H, benzothiazole, J = 7.23 Hz), 7.69-7.71(d, 1H, benzothiazole, J = 8.55 Hz) 9.45 (s, 1H, pyrazole), 9.95 (s, 1H, CHO); ESI-MS, m/z: 357.79 [M]<sup>+</sup>, 359.45 [M+2]<sup>+</sup>; Anal. calcd. for C<sub>17</sub>H<sub>9</sub>ClFN<sub>3</sub>OS: C, 57.07; H, 2.54; N, 11.74. Found: C, 57.09; H, 2.57, N, 11.71.

**1-(7-Chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(2,4-dichlorophenyl)-1H-pyrazole-4-carbaldehyde, 11b:** Yield 84%; m.p.: 120-123°C; gray coloured solid; IR ( $\nu$ , cm<sup>-1</sup>, KBr): 1692 (C=O), 1145 (C-F), 1027 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>),  $\delta$  (ppm) 7.28-7.31 (m, 3H, ArH), 7.61-7.64 (d, 1H, benzothiazole, J = 7.24 Hz), 7.66-7.68 (d, 1H, benzothiazole, J = 8.60 Hz) 9.51 (s, 1H, pyrazole),

9.93 (s, 1H, CHO); ESI-MS,  $m/z$ : 426.68  $[M]^+$ , 428.57  $[M+2]^+$ ; Anal. calcd. for  $C_{17}H_7Cl_3FN_3OS$ : C, 47.85; H, 1.65; N, 9.85. Found: C, 47.88; H, 1.66, N, 9.82.

**1-(7-Chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(4-fluorophenyl)-1H-pyrazole-4-carbaldehyde, 11c:** Yield 85%; m.p.: 250-251°C; buff coloured solid; IR (v,  $cm^{-1}$ , KBr): 1691(C=O), 1262 (C-F), 1078 (C-Cl);  $^1H$  NMR (400 MHz, DMSO- $d_6$ ),  $\delta$  (ppm) 7.24-7.28 (m, 4H, Ar-H), 7.62-7.64 (d, 1H, benzothiazole,  $J = 7.22$  Hz), 7.68-7.70 (d, 1H, benzothiazole,  $J = 8.59$  Hz), 9.49 (s, 1H, pyrazole), 9.91 (s, 1H, CHO); ESI-MS,  $m/z$ : 375.78  $[M]^+$ , 377.51  $[M+2]^+$ ; Anal. calcd. for  $C_{17}H_8ClF_2N_3OS$ : C, 54.34; H, 2.15; N, 11.18. Found: C, 54.36; H, 2.17, N, 11.16.

#### Synthesis of compound 12a-c

To a mixture of **11(a-c)** (0.01 mol), isoniazid (0.01 mol) and 2-3 drops of glacial acetic acid in dry toluene to a 100 mL round bottom flask, reflux it for 24 h in Dean-Stark apparatus. The reaction was monitored by TLC. After completion of the reaction, solid separated out on cooling, was filtered and recrystallized from toluene.

**N'-((1-(7-Chloro-6-fluorobenzo[d]thiazol-2-yl)-3-phenyl-1H-pyrazol-4-yl)methylene)isonicotine hydrazide, 12a:** Yield 82%; m.p.: 228-230°C; off white solid; IR (v,  $cm^{-1}$ , KBr): 3454 (NH), 1652 (C=O), 1560 (N=CH), 1205 (C-F), 1037 (C-Cl);  $^1H$  NMR (400 MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 7.81-7.82 (d, 2H, pyridine,  $J = 8.82$ Hz), 7.31-7.36 (m, 5H, ArH), 8.21(s, 1H, NH), 7.68-7.70 (d, 1H, benzothiazole,  $J = 7.23$  Hz), 7.75-7.77 (d, 1H, benzothiazole,  $J = 7.69$  Hz), 8.57 (s, 1H, N=CH), 8.77-8.78 (m, 2H, pyridine), 8.97 (s, 1H, pyrazole), 12.08 (s, 1H, NH);  $^{13}C$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  ppm; 161.23 (C=O), 160.54 (C-2 of benzothiazole), 154.07 (C-F of benzothiazole), 150.47 (C-2 of pyridine), 150.29 (C-6 of pyridine), 147.38 (C-4 of benzothiazole), 140.65 (CH=N), 140.24 (C-3 of pyrazole), 132.85 (C-4 of pyridine), 130.46 (C-9 of benzothiazole), 129.46 (C-5 of pyrazole), 128.84 (C-3 of pyridine), 128.52 (C-5 of pyridine), 128.26 (C-4 of pyrazole), 127.26 (C-Cl of benzothiazole), 123.32 (C-1 of Ar), 121.41 (C-2 of Ar), 121.26 (C-6 of Ar), 119.53 (C-3 of Ar), 119.34 (C-5 of Ar), 119.05 (C-4 of Ar), 110.70 (C-6 of benzothiazole), 110.43 (C-5 of benzothiazole); ESI-MS,  $m/z$ : 475.73  $[M]^+$ , 477.43  $[M+2]^+$ ; Anal. calcd. for  $C_{23}H_{14}ClFN_6OS$ : C, 57.92; H, 2.96; N, 17.62. Found: C, 57.90; H, 2.98, N, 17.60.

**N'-((1-(7-Chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(2,4-dichlorophenyl)-1H-pyrazol-4-yl) methylene)isonicotinohydrazide, 12b:** Yield 82%; m.p.: 260-262°C; light brown coloured solid; IR (v,  $cm^{-1}$ , KBr): 3450 (NH), 1692 (C=O), 1565 (N=CH), 1167 (C-F), 977 (C-Cl);  $^1H$  NMR (400 MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 7.85-7.87 (d, 2H, pyridine,  $J = 8.56$ Hz), 7.31-7.36 (d, 2H, ArH,  $J = 9.53$  Hz), 7.58 (s, 1H, ArH), 8.25(s, 1H, NH), 7.66-7.68 (d, 1H, benzothiazole,  $J = 7.26$  Hz), 7.75-7.76 (d, 1H, benzothiazole,  $J = 7.65$  Hz), 8.51 (s, 1H, N=CH), 8.70-8.72 (m, 2H, pyridine), 8.99 (s, 1H, pyrazole), 12.01 (s, 1H, NH); ESI-MS,  $m/z$ : 545.8  $[M]^+$ , 547.3  $[M+2]^+$ ; Anal. calcd. for  $C_{23}H_{12}Cl_3FN_6OS$ : C, 50.61; H, 2.22; N, 19.49. Found: C, 50.62; H, 2.25, N, 19.47.

**N'-((1-(7-Chloro-6-fluorobenzo[d]thiazol-2-yl)-3-(4-fluorophenyl)-1H-pyrazol-4-yl) methylene)isonicotinohydrazide, 12c:** Yield 89%; m.p.: 325-326°C; off white solid; IR (v,  $cm^{-1}$ , KBr): 3397 (NH), 1696 (C=O), 1600 (N=CH), 1224 (C-F), 1076 (C-Cl);  $^1H$  NMR (400 MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 7.37-7.41 (t, 2H, ArH,  $J = 8.84$  Hz and 8.88 Hz), 7.80-7.81 (d, 2H, pyridine), 7.87-7.91 (q, 2H, ArH), 7.61-7.63 (d, 1H, benzothiazole,  $J = 7.24$  Hz), 7.69-7.70 (d, 1H, benzothiazole,  $J = 8.68$  Hz), 8.54 (s, 1H, N=CH), 8.77-8.78 [d, 2H, (pyridine)], 9.00 (s, 1H, pyrazole) 12.03 (s, 1H, NH); ESI-MS,  $m/z$ : 494.9  $[M]^+$ , 496.5  $[M+2]^+$ ; Anal. calcd. for  $C_{23}H_{13}ClF_2N_6OS$ : C, 55.82; H, 2.65; N, 16.98. Found: C, 55.76; H, 2.68, N, 16.97.

#### Synthesis of compound 13a-c

A mixture of compound **7**, (2.626 g, 0.01 mol) and substituted acetophenone (0.01 mol) in methanol (30 mL) containing 2-3 drop of glacial acetic acid was refluxed for 0.5 h. After completion of the reaction, the solid that separated out on cooling, was filtered and recrystallized by methanol.

**7-Chloro-6-fluoro-5-nitro-2-(2-(1-phenylethylidene)hydrazinyl)benzo[d]thiazole, 13a:** Yield 94%; m.p.: 212-214°C; colourless crystal; IR (v,  $cm^{-1}$ , KBr): 3432 (NH), 1615 (N=C), 1194 (C-F), 1011 (C-Cl);  $^1H$  NMR (400 MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 2.30 (s, 3H,  $CH_3$ ), 7.50-7.46 (m, 5H, ArH), 7.93-7.96 (d, 1H, benzothiazole,  $J = 7.60$  Hz), 10.76 (s, 1H, NH); ESI-MS,  $m/z$ : 364.74  $[M]^+$ , 366.25  $[M+2]^+$ ; Anal. calcd. for  $C_{15}H_{10}ClFN_4O_2S$ : C, 49.39; H, 2.76; N, 15.36. Found: C, 49.36; H, 2.78, N, 15.34.

**7-Chloro-2-(2-(1-(2,4-dichlorophenyl)ethylidene)hydrazinyl)-6-fluoro-5-nitrobenzo[d]thiazole, 13b:**



Yield 85%; m.p.: 205-206°C; off white crystal; IR (v, cm<sup>-1</sup>, KBr): 3321 (N-H), 1537 (NO<sub>2</sub>), 1612 (N=C), 1222 (C-F), 1091 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>), δ (ppm): 2.33 (s, 3H, CH<sub>3</sub>), 7.42 (s, 1H, ArH), 7.32-7.35 (d, 2H, ArH), 7.94-7.97 (d, 1H, benzothiazole, J = 7.61 Hz), 10.45 (s, 1H, NH); ESI-MS, m/z: 433.66 [M]<sup>+</sup>, 435.14 [M+2]<sup>+</sup>; Anal. calcd. for C<sub>15</sub>H<sub>8</sub>Cl<sub>3</sub>FN<sub>4</sub>O<sub>2</sub>S: C, 41.54; H, 1.86; N, 12.92. Found: C, 41.38; H, 1.88, N, 12.91.

**7-Chloro-6-fluoro-2-(2-(1-(4-fluorophenyl) ethylidene)hydrazinyl)-5-nitrobenzo[d]thiazole,**

**13c:** Yield 84%; m.p.: 230-232°C; off white crystal; IR (v, cm<sup>-1</sup>, KBr): 3469 (N-H), 1606 (N=C), 1546 (NO<sub>2</sub>), 1193 (C-F), 1012 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>), δ (ppm): 2.36 (s, 3H, CH<sub>3</sub>), 7.41-7.44 (m, 4H, ArH), 7.93-7.95 (d, 1H, benzothiazole, J = 7.65 Hz), 10.72 (s, 1H, NH); ESI-MS, m/z: 382.68 [M]<sup>+</sup>, 384.25 [M+2]<sup>+</sup>; Anal. calcd. for C<sub>15</sub>H<sub>9</sub>ClF<sub>2</sub>N<sub>4</sub>O<sub>2</sub>S: C, 47.07; H, 2.37; N, 14.64. Found: C, 47.02; H, 2.34, N, 14.62.

**Synthesis of compound 14a-c**

To the Vilsmeier-Haack reagent prepared from DMF (10 mL) and POCl<sub>3</sub> (1.1 mL, 0.012 mol), 7-chloro-6-fluoro-5-nitro-2-(2-(1-substitutedphenyl ethylidene)hydrazinyl)benzo [d] thiazole **13(a-c)** (0.01 mol) was added and the reaction mixture was stirred at 60-65°C for 3 h. The reaction was monitored by TLC. After completion of the reaction, the mixture was poured in to ice cold water. The solid that separated on neutralization with NaHCO<sub>3</sub> was filtered, washed with water and recrystallized by DMF-water.

**1-(7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-yl)-3-phenyl-1H-pyrazole-4-carbaldehyde, 14a:** Yield 72%; m.p.: 230-232°C; off white crystal; IR (v, cm<sup>-1</sup>, KBr): 1691 (C=O), 1546 (NO<sub>2</sub>), 1205 (C-F), 1076 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>), δ (ppm): 7.29-7.32 (m, 5H, ArH), 7.92-7.94 (d, 1H, benzothiazole, J = 7.67 Hz), 9.43 (s, 1H, pyrazole), 9.96 (s, 1H, CHO); ESI-MS, m/z: 402 [M]<sup>+</sup>, 404.2 [M+2]<sup>+</sup>; Anal. calcd. for C<sub>17</sub>H<sub>8</sub>ClFN<sub>4</sub>O<sub>3</sub>S: C, 50.69; H, 2.00; N, 13.91. Found: C, 50.66; H, 2.00, N, 13.90.

**1-(7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-yl)-3-(2,4-dichlorophenyl)-1H-pyrazole-4-carbaldehyde, 14b:** Yield 67%; m.p.: 215-217°C; garish granular; IR (v, cm<sup>-1</sup>, KBr): 1695 (C=O), 1542 (NO<sub>2</sub>), 1201 (C-F), 1072 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>), δ (ppm): 7.32-7.35 (m, 3H, ArH), 7.91-7.94 (d, 1H, benzothiazole, J = 7.70 Hz), 9.42 (s, 1H, pyrazole),

9.97 (s, 1H, CHO); ESI-MS, m/z: 470 [M]<sup>+</sup>, 472.15 [M+2]<sup>+</sup>; Anal. calcd. for C<sub>17</sub>H<sub>6</sub>Cl<sub>3</sub>FN<sub>4</sub>O<sub>3</sub>S: C, 43.29; H, 1.28; N, 11.88. Found: C, 43.23; H, 1.32, N, 11.84.

**1-(7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-yl)-3-(4-fluorophenyl)-1H-pyrazole-4-carbaldehyde, 14c:**

Yield 69%; mp 262-264°C; off white crystal; IR (v, cm<sup>-1</sup>, KBr): 1691 (C=O), 1543 (NO<sub>2</sub>), 1224 (C-F), 1045 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>), δ (ppm): 7.23-7.27 (m, 4H, ArH), 7.91-7.93 (d, 1H, benzothiazole, J = 7.65 Hz), 9.44 (s, 1H, pyrazole), 9.95 (s, 1H, CHO); ESI-MS, m/z: 420 [M]<sup>+</sup>, 422.34 [M+2]<sup>+</sup>; Anal. calcd. for C<sub>17</sub>H<sub>7</sub>ClF<sub>2</sub>N<sub>4</sub>O<sub>3</sub>S: C, 48.52; H, 1.68; N, 13.32. Found: C, 48.50; H, 1.69, N, 13.30.

**Synthesis of compound 15a-c**

To a mixture of compound **14(a-c)** (0.01 mol), isoniazid 1.371 g (0.01 mol) and 2-3 drops of glacial acetic acid in dry toluene to a 100 mL round bottom flask, reflux it for 24 h in Dean-Stark apparatus. The reaction was monitored by TLC. After completion of the reaction, solid that separated out on cooling, was filtered and recrystallized by toluene.

**N'-((1-(7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-yl)-3-phenyl-1H-pyrazol-4-yl)methylene)isonicotinohydrazide, 15a:**

Yield 77%; m.p.: 302-304°C; light yellow granular; IR (v, cm<sup>-1</sup>, KBr): 3472 (NH), 1654 (C=O), 1535 (NO<sub>2</sub>), 1606 (N=CH), 1222 (C-F), 1070 (C-Cl); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>), δ (ppm): 7.35-7.38 (m, 5H, ArH), 7.93-7.95 (d, 1H, benzothiazole, J = 7.67 Hz), 7.98-8.00 (d, 2H, pyridine, J = 8.83 Hz), 8.57 (s, 1H, -N=CH), 8.75-8.78 [m, 2H, (pyridine, J = 8.88 Hz)], 8.98 (s, 1H, pyrazole), 12.07 (s, 1H, NH); <sup>13</sup>C NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm: 161.26 (C=O), 160.54 (C-2 of benzothiazole), 158.29 (C-F of benzothiazole), 156.29 (C-2 of pyridine), 156.07 (C-6 of pyridine), 150.70 (C-4 of benzothiazole), 150.29 (CH=N), 147.38 (C-3 of pyrazole), 140.90 (C-4 of pyridine), 140.24 (C-9 of benzothiazole), 132.85 (C-5 of pyrazole), 132.43 (C-3 of pyridine), 130.46 (C-5 of pyridine), 129.46 (C-NO<sub>2</sub> of benzothiazole), 128.85 (C-4 of pyrazole), 128.52 (C-1 of Ar), 128.26 (C-2 of Ar), 127.26 (C-6 of Ar), 123.32 (C-3 of Ar), 121.41 (C-5 of Ar), 121.26 (C-4 of Ar), 119.53 (C-5 of benzothiazole), 110.43 (C-Cl of benzothiazole); ESI-MS, m/z: 521.00 [M]<sup>+</sup>, 523.40 [M+2]<sup>+</sup>; Anal. calcd. for C<sub>23</sub>H<sub>13</sub>ClFN<sub>7</sub>O<sub>3</sub>S: C, 52.93; H, 2.51; N, 18.79. Found: C, 52.95; H, 2.54, N, 18.76.

**N'-((1-(7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-yl)-3-(2,4-dichlorophenyl)-1H-pyrazol-4-yl)methylene)isonicotinohydrazide, 15b:** Yield 71%;

m.p.: 292-294°C; whitish amorphous powder; IR (v,  $\text{cm}^{-1}$ , KBr): 3385 (N-H), 1697 (C=O), 1610 (N=CH), 1537 ( $\text{NO}_2$ ), 1222 (C-F), 971 (C-Cl);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 7.36-7.39 (m, 3H, ArH), 7.92-7.93 (d, 1H, benzothiazole,  $J = 7.66$  Hz), 7.97-7.99 (d, 2H, pyridine,  $J = 8.83$  Hz), 8.48 (s, 1H, N=CH), 8.77-8.78 [m, 2H, (pyridine,  $J = 8.80$  Hz)], 8.98 (s, 1H, pyrazole), 12.09 (s, 1H, NH); ESI-MS,  $m/z$ : 589  $[\text{M}]^+$ , 591  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_{23}\text{H}_{11}\text{Cl}_3\text{FN}_7\text{O}_3\text{S}$ : C, 46.76; H, 1.88; N, 16.60. Found: C, 46.71; H, 1.84, N, 16.62.

**N'-((1-(7-Chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-yl)-3-(4-fluorophenyl)-1H-pyrazol-4-yl)methylene)isonicotinohydrazide, 15c:** Yield 70%; m.p.: 325-327°C; light yellow amorphous powder; IR (v,  $\text{cm}^{-1}$ , KBr): 3476 (N-H), 1641 (C=O), 1610 (N=CH), 1537 ( $\text{NO}_2$ ), 1222 (C-F), 1017 (C-Cl);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 7.37-7.41 (m, 4H, ArH), 7.92-7.94 (d, 1H, benzothiazole,  $J = 7.62$  Hz), 7.98-7.80 (d, 2H, pyridine,  $J = 8.83$  Hz), 8.54 (s, 1H, N=CH), 8.77-8.78 [d, 2H, (pyridine,  $J = 8.89$  Hz)], 9.01 (s, 1H, pyrazole), 12.09 (s, 1H, NH); ESI-MS,  $m/z$ : 539  $[\text{M}]^+$ , 541  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_{23}\text{H}_{12}\text{ClF}_2\text{N}_7\text{O}_3\text{S}$ : C, 51.17; H, 2.24; N, 18.16. Found: C, 51.19; H, 2.28, N, 18.13.

#### Synthesis of compound 16a-c

A mixture of compound **8**, (2.626 g, 0.01 mol) and substituted acetophenone (0.01 mol) in methanol (30 mL) containing 2-3 drop of glacial acetic acid was refluxed for 0.5 h. The reaction was monitored by TLC. After completion of the reaction, solid separated out on cooling, was filtered and recrystallized from methanol.

**7-Chloro-6-fluoro-4-nitro-2-(2-(1-phenylethylidene)hydrazinyl)benzo[d]thiazole, 16a:** Yield 87%; m.p.: 244-246°C; white crystal; IR (v,  $\text{cm}^{-1}$ , KBr): 3330 (N-H), 1615 (N=C), 1540 ( $\text{NO}_2$ ), 1191 (C-F), 1045 (C-Cl);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 2.31 (s, 3H,  $\text{CH}_3$ ), 7.45-7.48 (m, 5H, ArH), 8.38-8.40 (d, 1H, benzothiazole,  $J = 9.41$  Hz), 10.86 (s, 1H, NH); ESI-MS,  $m/z$ : 364  $[\text{M}]^+$ , 366  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_{15}\text{H}_{10}\text{ClFN}_4\text{O}_2\text{S}$ : C, 49.39; H, 2.76; N, 15.36. Found: C, 49.41; H, 2.75, N, 15.35.

**7-Chloro-2-(2-(1-(2,4-dichlorophenyl)ethylidene)hydrazinyl)-6-fluoro-4-nitrobenzo[d]thiazole, 16b:** Yield 81%; mp 236-238°C; off white crystal; IR (v,  $\text{cm}^{-1}$ , KBr): 3321 (N-H), 1612 (N=C), 1537 ( $\text{NO}_2$ ), 1193 (C-F), 1041 (C-Cl);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 2.30 (s, 3H,  $\text{CH}_3$ ), 7.44-7.49 (m,

3H, ArH), 8.39-8.41 (d, 1H, benzothiazole,  $J = 9.42$  Hz), 10.87 (s, 1H, NH); ESI-MS,  $m/z$ : 432  $[\text{M}]^+$ , 434  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_{15}\text{H}_8\text{Cl}_3\text{FN}_4\text{O}_2\text{S}$ : C, 41.54; H, 1.86; N, 12.92. Found: C, 41.53; H, 1.85, N, 12.90.

**7-Chloro-6-fluoro-2-(2-(1-(4-fluorophenyl)ethylidene)hydrazinyl)-4-nitrobenzo[d]thiazole, 16c:** Yield 85%; m.p.: 274-276°C; white crystal; IR (v,  $\text{cm}^{-1}$ , KBr): 3462 (N-H), 1608 (N=C), 1546 ( $\text{NO}_2$ ), 1193 (C-F), 1078 (C-Cl);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 2.29 (s, 3H,  $\text{CH}_3$ ), 7.43-7.47 (m, 4H, ArH), 8.38-8.40 (d, 1H, benzothiazole,  $J = 9.42$  Hz), 10.84 (s, 1H, NH); ESI-MS,  $m/z$ : 382.02  $[\text{M}]^+$ , 384.21  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_{15}\text{H}_9\text{ClF}_2\text{N}_4\text{O}_2\text{S}$ : C, 47.07; H, 2.37; N, 14.64. Found: C, 47.05; H, 2.36, N, 14.65.

#### Synthesis of compound 17a-c

To the Vilsmeier-Haack reagent prepared from DMF (10 mL) and  $\text{POCl}_3$  (1.1 mL, 0.012 mol), 7-chloro-6-fluoro-5-nitro-2-(2-(1-substituted phenylethylidene)hydrazinyl)benzo [d] thiazole (**16a-c**) (0.01 mol) was added and the reaction mixture was stirred at 60-65°C for 3 h. The reaction was monitored by TLC. After completion of the reaction, the mixture was poured in to ice cold water. The solid that separated on neutralization with  $\text{NaHCO}_3$  was filtered, washed with water and recrystallized by DMF-water.

**1-(7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-phenyl-1H-pyrazole-4-carbaldehyde, 17a:** Yield 72%; m.p.: 270-272°C; gray coloured crystal; IR (v,  $\text{cm}^{-1}$ , KBr): 1691 (C=O), 1537 ( $\text{NO}_2$ ), 1205 (C-F), 1035 (C-Cl);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 7.30-7.34 (m, 5H, ArH), 8.41-8.43 (d, 1H, benzothiazole,  $J = 9.26$  Hz), 9.60 (s, 1H, pyrazoline), 9.97 (s, 1H, CHO); ESI-MS,  $m/z$ : 402.01  $[\text{M}]^+$ , 404.11  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_{17}\text{H}_8\text{ClFN}_4\text{O}_3\text{S}$ : C, 50.69; H, 2.00; N, 13.91. Found: C, 50.68; H, 2.04, N, 13.88.

**1-(7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-(2,4-dichlorophenyl)-1H-pyrazole-4-carbaldehyde, 17b:** Yield 64%; m.p.: 290-292°C; off white crystal;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 7.35-7.39 (m, 3H, ArH), 8.42-8.44 (d, 1H, benzothiazole,  $J = 9.46$  Hz), 9.59 (s, 1H, pyrazoline), 9.98 (s, 1H, CHO); ESI-MS,  $m/z$ : 469.98  $[\text{M}]^+$ , 472.05  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_{17}\text{H}_6\text{Cl}_3\text{FN}_4\text{O}_3\text{S}$ : C, 43.29; H, 1.28; N, 11.88. Found: C, 43.28; H, 1.30, N, 11.87.

**1-(7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-(4-fluorophenyl)-1H-pyrazole-4-carbaldehyde, 17c:** Yield 61%; m.p.: 302-304°C; gray coloured crystal;

IR (v,  $\text{cm}^{-1}$ , KBr): 1691 (C=O), 1543 ( $\text{NO}_2$ ), 1207 (C-F), 1083 (C-Cl);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 7.33-7.37 (m, 4H, ArH), 8.42-8.44 (d, 1H, benzothiazole,  $J = 9.46$  Hz), 9.58 (s, 1H, pyrazoline), 9.97 (s, 1H, CHO); ESI-MS,  $m/z$ : 420.00  $[\text{M}]^+$ , 421.94  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_{17}\text{H}_7\text{ClF}_2\text{N}_4\text{O}_3\text{S}$ : C, 48.52; H, 1.68; N, 13.32. Found: C, 48.51; H, 1.65, N, 13.29.

#### Synthesis of compound 17a-c

To a mixture of compound 17(a-c) (0.01 mol), isoniazid 1.371 g (0.01 mol) and 2-3 drops of glacial acetic acid in dry toluene to a 100 mL round bottom flask, reflux it for 24 h in Dean-Stark apparatus. The reaction was monitored by TLC. After completion of the reaction, solid separated out on cooling, was filter and recrystallized by toluene.

**N'-((1-(7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-phenyl-1H-pyrazol-4-yl) methylene)isonicotinohydrazide, 18a:** Yield 77%; m.p.: 300-302°C; light yellow crystal; IR (v,  $\text{cm}^{-1}$ , KBr): 3471 (N-H), 1696 (C=O), 1602 (N=CH), 1537 ( $\text{NO}_2$ ), 1211 (C-F), 1031 (C-Cl);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 7.34-7.38 (m, 5H, ArH), 7.68-7.69 (d, 2H, pyridine,  $J = 9.01$  Hz), 8.49-8.52 (d, 1H, benzothiazole,  $J = 8.71$  Hz), 8.74 (s, 1H, N=CH), 9.13-9.15 (d, 2H, pyridine,  $J = 9.01$  Hz), 9.38 (s, 1H, pyrazole), 12.12 (s, 1H, NH); ESI-MS,  $m/z$ : 521.05  $[\text{M}]^+$ , 523.00  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_{23}\text{H}_{13}\text{ClFN}_7\text{O}_3\text{S}$ : C, 52.93; H, 2.51; N, 18.79. Found: C, 52.91; H, 2.50, N, 18.78.

**N'-((1-(7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-(2,4-dichlorophenyl)-1H-pyrazol-4-yl) methylene)isonicotinohydrazide, 18b:** Yield 68%; m.p.: 308-310°C; light yellow crystal; IR (v,  $\text{cm}^{-1}$ , KBr): 3446 (N-H), 1696 (C=O), 1610 (N=CH), 1537 ( $\text{NO}_2$ ), 1224 (C-F), 1074 (C-Cl);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 7.32-7.37 (m, 3H, ArH), 7.72-7.74 (d, 2H, pyridine,  $J = 9.05$  Hz), 8.52-8.54 (d, 1H, benzothiazole,  $J = 8.75$  Hz), 8.75 (s, 1H, N=CH), 9.15-9.16 (d, 2H, pyridine,  $J = 9.05$  Hz), 9.39 (s, 1H, pyrazole), 12.08 (s, 1H, NH); ESI-MS,  $m/z$ : 588.95  $[\text{M}]^+$ , 590.89  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_{23}\text{H}_{11}\text{Cl}_3\text{FN}_7\text{O}_3\text{S}$ : C, 46.76; H, 1.88; N, 16.60. Found: C, 46.77; H, 1.89, N, 16.57.

**N'-((1-(7-Chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-yl)-3-(4-fluorophenyl)-1H-pyrazol-4-yl) methylene)isonicotinohydrazide, 18c:** Yield 77%; m.p.: 325-327°C; off white crystal; IR (v,  $\text{cm}^{-1}$ , KBr): 3169 (N-H), 1656 (C=O), 1606 (N=CH), 1537 ( $\text{NO}_2$ ),

1224 (C-F), 1027 (C-Cl);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ),  $\delta$  (ppm): 7.37-7.41 (m, 4H, ArH), 7.62-7.65 (d, 2H, pyridine,  $J = 9.24$  Hz), 8.54-8.57 (d, 1H, benzothiazole,  $J = 8.18$  Hz), 8.74 (s, 1H, N=CH), 9.07-9.09 (d, 2H, pyridine,  $J = 9.24$  Hz), 9.41 (s, 1H, pyrazole), 12.07 (s, 1H, NH); ESI-MS,  $m/z$ : 539.60  $[\text{M}]^+$ , 541.00  $[\text{M}+2]^+$ ; Anal. calcd. for  $\text{C}_{23}\text{H}_{12}\text{ClF}_2\text{N}_7\text{O}_3\text{S}$ : C, 51.17; H, 2.24; N, 18.16. Found: C, 51.19; H, 2.23, N, 18.17.

#### Antitubercular activity

The ability of compounds to inhibit the growth of *Mycobacterium* species was determined by Ziehl-Neelsen stain which was grown in Middlebrook 7H-9 broth. The standard strain *Mycobacterium tuberculosis* H37Rv (ATCC 27294) was used. The basal medium was prepared according to manufacturer's instructions (Hi-Media) and sterilized by autoclaving. 4.5 mL of broth were taken into each sterile bottles to that 0.5 mL of ADC supplement was added which contains catalase, dextrose and bovine serum albumin fraction. Then compound solution was then transferred to media bottles to achieve final concentrations 25, 50, 100  $\mu\text{g}/\text{mL}$ . Finally 10  $\mu\text{L}$  suspension of *Mycobacterium tuberculosis* strain (100000 organisms/ mL, adjusted by McFarland's turbidity standard) was transferred to each of the tubes and were incubated at 37°C for three weeks. One 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 indicates 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

##### Chemicals

3-(4, 5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), RPMI-1640 medium (RPMI) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai.

##### Cell lines and culture medium

THP-1 (Human, Monocytes) cell cultures were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells of THP-1 was cultured in RPMI, supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ mL),

streptomycin (100  $\mu\text{g}/\text{mL}$ ) and amphotericin B (5  $\mu\text{g}/\text{mL}$ ) in a humidified atmosphere of 5%  $\text{CO}_2$  at 37  $^\circ\text{C}$  until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). For THP-1 cells, the confluent cell suspension was centrifuged at 2000 rpm for 10 min and the cell pellet was resuspended in fresh medium. The stock cultures were grown in 25  $\text{cm}^2$  culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

#### Preparation of test solutions

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with RPMI supplemented with 2% inactivated FBS to obtain a stock solution of 1  $\text{mg}/\text{mL}$  concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

#### Cytotoxicity ( $\text{CTC}_{50}$ ) THP-1 cell lines

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  $1.0 \times 10^5$  cells/ $\text{mL}$  using RPMI-1640 medium containing 10% fetal bovine serum. To each well of the 96 well microtitre plate, 0.1  $\text{mL}$  of the diluted cell suspension (approximately 10,000 cells) was added. After 2 h, 100  $\mu\text{L}$  of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37 $^\circ\text{C}$  for 3 days in 5%  $\text{CO}_2$  atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, 10  $\mu\text{L}$  of 3-(4, 5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT) (5  $\text{mg}/\text{mL}$ ) in phosphate buffered saline was added to each well. The plates were gently shaken and incubated for 3 h at 37 $^\circ\text{C}$  in 5%  $\text{CO}_2$  atmosphere. Microtitre plates were centrifuged at 2000 rpm for 15 min and supernatant was removed. 100  $\mu\text{L}$  of propanol was added and the plates were gently shaken to solubilise 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% ( $\text{CTC}_{50}$ ) values is generated from the dose-response curves for each cell line<sup>8</sup>.

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

#### Conclusion

Benzothiazole derivatives and there nitro regioisomers combined with pyrazoles attached with isoniazid for better antitubercular activity. Nine final compounds has been synthesised and there *in vitro* antimycobacterial activity on *Mycobacterium tuberculosis* H<sub>37</sub>Rv (ATCC 27294) has shown the compounds 15a, 15b, 15c, 18b and 18c are potent anti-tubercular agents (MIC = 40.19 to 64.96 nM). *In vitro* cytotoxicity studies on mammalian THP-1 (Human, Monocytes) cell line and  $\text{CTC}_{50}$  value was calculated. Presence of a nitro group in the compound is shown to increase the toxicity, though this concentration is far more than the concentration tested for the antitubercular activity.

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#### References

- 1 Ian O, *Antimicrob Agents Chemother*, 45 (2001) 1943.
- 2 "TB India 2016 Revised National TB Control Programme Annual Status Report", New Delhi (2016) <https://www.tbfacts.org/tb-india/>
- 3 Global Tuberculosis Report, World Health Organisation (2015).
- 4 Kuntal H, Nargund L V G, Rashmi P, Narendra Sharath Chandra J N, Nandha B & Harish M S, *Arch Pharm Chem Life Sci*, 345(2) (2012) 137.
- 5 Kawamoto T, Ikeuchi Y, Hiraki J, Eikyu Y, Shimizu K & Tomishima M, *Bioorg Med Chem Lett*, 5(18) (1995) 2109.
- 6 Cho Y, Ioerger T R & Sacchettini J C, *J Med Chem*, 51 (2008) 5984.
- 7 Lion C J, Matthews C S, Wells G, Bradshaw T D, Stevens M G F & Westwell A D, *Bioorg Med Chem Lett*, 16 (2006) 5005.
- 8 Francis D & Rita L, *J Immunol Methods*, 89 (1986) 271.