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# SYNTHESIS OF 2*H*-[1,3,5]THIADIAZINO[4,3-*B*][1,3]BENZOTHIAZOLE-3,3-DIOXIDE BY [4+2] DIELS–ALDER CYCLOADDITION REACTION AND COMPARATIVE EVALUATION OF ANTI-*MYCOBACTERIUM* ACTIVITY

**Kuntal Hazra*[1], L.V.G. Nargund[2]**
[1]*Department of Pharmaceutical Chemistry, Bharat Technology, Uluberia, Howrah, INDIA.*
[2]*Department of Pharmaceutical Chemistry, Nargund College of Pharmacy, Bangalore 560085, INDIA.*

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## *ABSTRACT*

*In an attempt to find a new and a safer drug for tuberculosis, series of some novel 9-chloro-8-fluoro-2-substitutedphenyl-2H-[1,3,5]thiadiazino[4,3-b][1,3]benzothiazole-3,3-dioxide (**3a-f**) and their 7 nitro (**9a-f**) and 6 nitro (**12a-f**) derivatives by [4+2] Diels–Alder cycloaddition reaction have been synthesised. All the compounds were screened for their in vitro antimycobacterial activity against Mycobacterium tuberculosis H37Rv strain by using Middlebrook 7H-9 broth. It has been observed that the introduction of -$NO_2$ group on thiadiazinobenzothiazole ring at 7$^{th}$ as well as 6$^{th}$ position irrespective of the phenyl ring substituent decrease the antitubercular activity. Three compounds from each series were tested for cytotoxicity on THP-1 cell lines. The compounds **3b, 3f, 9b, 9f, 12b** and **12f** exhibited antitubercular activity at non-cytotoxic concentration to the mammalian cells.*

***Key Words:*** *Benzothiazole / Diels–Alder cycloaddition reaction / Antitubercular.*

## INTRODUCTION

There were an estimated 11.1 million (range 9.6–13.3 million) prevalent cases of tuberculosis (TB) in 2008, equivalent to 164 cases per 100000 population [1]. Most of the estimated number of cases in 2008 occurred in Asia (55%) and Africa (30%), with small proportions of cases in the Eastern Mediterranean region (7%), the European region (5%) and the region of the Americas (3%). The five countries that rank first to fifth in terms of total numbers of incident cases in 2008 are India (1.6–2.4 million), China (1.0–1.6 million), South Africa (0.38–0.57 million), Nigeria (0.37–0.55 million) and Indonesia (0.34–0.52 million). 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. Of these HIV positive cases, 78% were in the African Region and 13% were in the South-East Asia Region, as their immune systems are compromised by immunosuppressive drugs, substance abuse, or AIDS [2-5]. This is not only because of the lack of proper therapeutic agents for its treatment but also due to the development of drug resistant strains.

There is a dire need to develop a 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.

The heterodiene Diels-Alder reaction is one of the most versatile routes for the synthesis of heterocyclic compounds [6]. Appropriate selection of heterodiene with dienes and heterodienophiles allows for a wide range of structural and functional variations in the adducts and it allows the creation of two bonds and a cycle in one step, with a high level of regio-, stereo- and even enantioselectivity. Although there are numerous reports pertaining to [4+2] cycloaddition reactions of 1,2- and 1,4-diazabutadines, not many are reported for the Diels-Alder cycloaddition reactions of simple 1,3-diazabutadines. Similarly it was observed that highly reactive heterodienophile 'sulfene' either do not add to C=N double bond or had evaded the attention of synthetic chemists. The 1,3,5-thiadiazine and its derivatives are an important class of compounds which posses widespread pharmacological properties such as insecticidal, accaricidal [7], antifungal [8] and antiatherosclerotic activity [9].

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

In the present work an attempt has been made to develop new anti-tubercular agents based on nitrobenzothiazoles, which are reported to be inhibitors for *Mycobacterium tuberculosis* ATP phosphoribosyl transferase (HisG) [10]. 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 role in purine biosynthesis. HisG condenses ATP with phosphoribosyl pyrophosphate (PRPP) to produce phosphoribosyl ATP (PRATP) and inorganic pyrophosphate (PPi). 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.

Based on structural knowledge obtained from our past work, we herein report the synthesis of some new eighteen derivatives of fluorochlorothiadiazinobenzothiazole-3,3-dioxide (**3a-f**) and their corresponding 7-nitro (**9a-f**) and 6-nitro (**12a-f**) derivatives, which possess interesting antimycobacterial and cytotoxic activity profile.

## RESULTS AND DISCUSSION

**Chemistry:**

The preparation of key compounds **3a-f**, **9a-f** and **12a-f** were carried out by using 4-fluoro-3-chloro aniline and potassium thiocyanate with bromine in acetic acid, yielded the starting compound **1,** followed by Schiff base preparation by using different

***Corresponding author:**
***Dr. Kuntal Hazra***
*Associate Professor*
*Department of Pharmaceutical Chemistry*
*Bharat Technology, Uluberia, Howrah*
*Phone: +91-8017946855,*
*E. mail: kuntalhazra@gmail.com, kuntalhazra@ymail.com*

aromatic aldehydes in benzene with catalytic amount of glacial acetic acid. This upon treatment with methane sulfonylchloride in dry 1,4-dioxane and few drops of dry triethylamine yielded the target compounds **3a-f** (Scheme 1). NMR spectra (in DMSO-$d_6$) of these compounds showed absorption of methylene proton at around δ 3.22-3.30 methine proton at δ 3.56-3.71 aromatic protons δ 7.41-7.59 and for benzothiazole ring aromatic protons are δ 7.61-7.78. Acetylation was carried out of the compounds **1** by refluxing with acetic anhydride to protect the primary amine group at the 2nd position, which upon nitration with conc. $HNO_3$ and conc. $H_2SO_4$ afforded compound **5** and **6** two regioisomers (Scheme 2). The NMR spectra (in DMSO-$d_6$) of compound **5** showed absorption of $CH_3$ proton at δ 2.34 ppm singlet 3 protons, aromatic 4th proton at δ 7.95-7.93 ppm as doublet due to C-F coupling accounting 1 proton, J = 7.60Hz and NH proton at δ 9.16 ppm singlet 1 proton. Whereas compound **6** showed (in DMSO-$d_6$) absorption of $CH_3$ at δ 2.27 ppm singlet 3 protons, aromatic 5th proton at δ 8.38-8.40 ppm as doublet due to C-F coupling accounting 1 proton, J = 9.44Hz and NH at δ 13.21 ppm singlet 1 proton. Compound **5** was deacetylated with 70% $H_2SO_4$ yielded compound **7** followed by Schiff base preparation by using different aromatic aldehydes in benzene with catalytic amount of glacial acetic acid. This upon treatment with methane sulfonyl chloride in dry 1,4-dioxane and few drops of dry triethylamine yielded the target compounds **9a-f** (Scheme 3). NMR spectra (in DMSO-$d_6$) of these compounds showed absorption of methylene proton at around δ 3.26-3.31, methine proton at δ 3.58-3.78, aromatic protons δ 7.46-7.65 and for benzothiazole ring aromatic protons are δ 7.88-7.99. Again compound **6** was deacetylated with 70% $H_2SO_4$ yielded compound **10** followed by Schiff base preparation by using different aromatic aldehydes in benzene with catalytic amount of glacial acetic acid. Which upon treatment with methane sulfonylchloride in dry 1,4-dioxane and few drops of dry triethylamine yielded the target compounds **12a-f** (Scheme 4). NMR spectra (in DMSO-$d_6$) of these compounds showed absorption of methylene proton at around δ 3.28-3.34, methine proton at δ 3.59-3.73 and for aromatic protons δ 7.37-7.62 and for benzothiazole ring aromatic protons are δ 8.41-8.54.

**Antitubercular activity:**

The results of the *in vitro* evaluation of antitubercular 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 **3b, 3c, 3f, 9f** have shown 100% inhibition at 25 µg/ mL whereas compounds **3a, 3e, 9b, 9c, 12b, 12f** shown at 50 µg/ mL and **3d, 9a, 9e, 12a, 12c, 12e** at 100 µg/ mL. The standard drug pyrazinamide and streptomycin have shown 100% inhibition at 7.5 µg/ mL. Attachment of the nitro group in both 7th (**9a-f**) and 6th (**12a-f**) position of thiadiazinobenzothiazole ring decreases the antitubercular activity remarkably. Nitro group at the 6th position (**12a-f**) of the thiadiazinobenzothiazole ring have shown least antitubercular activity than the 7th position (**9a-f**). Electron withdrawing substituent (4-Cl) as well as electron donating substituent (4-OH-3-$OCH_3$) in the aromatic ring have shown good antitubercular activity, but 2-OH (**3d, 9d, 12d**) showed least antitubercular activity irrespective of the nitro substituent.

***In vitro* cytotoxicity evaluation:**

Nine compounds three from each series 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 of compounds **3a, 3b, 3f, 9a, 9b, 9f, 12a, 12b** and **12f** to the host cells. Compound **3a, 9a** and **12a** have shown $CTC_{50}$ values at 241, 149.50 and 129.50 µg/ mL. Other six compounds have shown moderate to good $CTC_{50}$ values at around 435-542 µg/ mL. The results indicate that compounds **3b, 3f, 9b, 9f, 12b** and **12f** exhibited antitubercular activity at non-cytotoxic concentration to the mammalian cells. Compounds with nitro group at 7th position have shown slightly less cytotoxic than the compound with nitro group at 6th position. However, compounds with a nitro substituent are slightly more cytotoxic than without a nitro substituent.

In the present study, we have synthesised thiadiazinobenzothiazole (**3a-f**), and the regioisomers of 6 nitro (**9a-f**) and 7 nitro (**12a-f**) thiadiazinobenzothiazole by [4+2] Diels–Alder cycloaddition reaction and tested for antitubercular activity. Their activity on *Mycobacterium tuberculosis* H37Rv strain ware compared to the standard drugs streptomycin and pyrazinamide. Our work has shown the presence of nitro substituent does not increases anti-tubercular activity in these compounds as mentioned in the literature [10]. Probably these nitrobenzothiazoles are not following the same mechanism as mentioned in the literature. The cytotoxicity of the nitro compounds were also high as compared to without a nitro group on the same compound. Electron donating as well as electron withdrawing groups at 4th position of benzene ring is equally active, but an ortho substituent like OH group (**3e, 9e** and **12e**) decreases the antitubercular activity may be because of steric hindrance. Dimethyl amino group at the para position of the benzene ring shows less antitubercular activity and high cytotoxicity. Among the nitro substituent the 7th position of the thiadiazinobenzothiazole (9a-f) is still shows better antitubercular activity as well as less cytotoxic than its isomeric 6th position (**12a-f**).

**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 $cm^{-1}$. $^1H$ 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. Elemental analyses were performed on Perkin-Elmer 2400 CHN Elemental Analyser. 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.

*7-chloro-6-fluorobenzo[d]thiazol-2-amine* **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.

*Yield:* 87%; m.p.: 180-182˚C; slight yellowish crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3480 (N-H), 1247 (C-F), 1094 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 4.21 (s, 2H, $NH_2$), 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 $[M]^+$, 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.

*General procedure for the preparation of N-substitutedbenzylidene-7-chloro-6-fluorobenzo[d]thiazol-2-amine* **2a-f:**

A mixture of **1** (0.01 mole), substituted aromatic aldehydes (0.01 mole) and 2-3 drops of glacial acetic acid in dry benzene was refluxed for 24 h in Dean-Stark apparatus. The reaction was monitored by TLC. After completion of the reaction, mixture was kept in the refrigerator overnight, the solid product obtained was filtered and recrystallized from benzene.

*7-chloro-N-(4-(dimethylamino)benzylidene)-6-fluorobenzo[d]thiazol-2-amine* **2a:**

*Yield:* 68%; m.p.: 160-162˚C; reddish crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1652 (N=CH), 1243 (C-F), 1085 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 2.99 (s, 6H, $N(CH_3)_2$), 7.41-7.44 (m, 4H, ArH), 7.63-7.65 (d, 1H, J = 8.36 Hz, ArH), 7.70-7.72 (d, 1H, J = 8.92 Hz, ArH), 8.71 (s, 1H, N=CH); ESI-MS, m/z: 333.85 $[M]^+$, 335.24 $[M+2]^+$; Anal. calcd. for $C_{16}H_{13}ClFN_3S$: C, 57.57; H, 3.93; N, 12.59. Found: C, 57.61; H, 3.76; N, 12.52.

*4-((7-chloro-6-fluorobenzo[d]thiazol-2-ylimino)methyl)-2-methoxy phenol* **2b:**

*Yield:* 79%; m.p.: 170-172˚C; yellow crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3384 (OH), 1666 (N=CH), 1257, 1042 (C-O-C), 1242 (C-F), 1089 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.89 (s, 3H, $OCH_3$), 7.54-7.57 (m, 3H, ArH), 7.74-7.69 (dd, 2H, ArH), 8.75 (s,

1H, N=CH), 11.38 (br s, 1H, OH); ESI-MS, m/z: 336 $[M]^+$, 337.98 $[M+2]^+$; Anal. calcd. for $C_{15}H_{10}ClFN_2O_2S$: C, 53.50; H, 2.99; N, 8.32. Found: C, 53.61; H, 3.13; N, 8.30.

*4-((7-chloro-6-fluorobenzo[d]thiazol-2-ylimino)methyl)phenol* **2c:**

*Yield:* 74%; m.p.: 215-217°C; yellowish crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3373 (OH), 1653 (N=CH), 1244 (C-F), 1093 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 7.39-7.43 (m, 4H, ArH), 7.68- 7.74 (dd, 2H, ArH), 8.73 (s, 1H, N=CH), 10.09 (br s, 1H, OH); ESI-MS, m/z: 305.95 $[M]^+$, 308.01 $[M+2]^+$; Anal. calcd. for $C_{14}H_8ClFN_2OS$: C, 54.82; H, 2.63; N, 9.13. Found: C, 55.07; H, 3.04; N, 8.97.

*2-((7-chloro-6-fluorobenzo[d]thiazol-2-ylimino)methyl)phenol* **2d:**

*Yield:* 52%; m.p.: 218-222°C; light yellow power; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3384 (OH), 1665 (N=CH), 1242 (C-F), 1075 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 7.39-7.42 (m, 4H, ArH), 7.64-7.66 (d, 1H, J = 8.56 Hz, ArH), 7.71-7.73 (d, 1H, J = 8.76 Hz, ArH), 8.72 (s, 1H, N=CH), 11.36 (br s, 1H, OH); ESI-MS, m/z: 305.95 $[M]^+$, 308.01 $[M+2]^+$; Anal. calcd. for $C_{14}H_8ClFN_2OS$: C, 54.82; H, 2.63; N, 9.13. Found: C, 55.07; H, 3.04; N, 8.97.

*7-chloro-6-fluoro-N-(4-methoxybenzylidene)benzo[d]thiazol-2-amine* **2e:**

*Yield:* 74%; m.p.: 255-257°C; yellowish crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1653 (N=CH), 1243, 1034 (C-O-C), 1241 (C-F), 1092 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm):, 3.84 (s, 3H, $OCH_3$), 7.44-7.47 (m, 4H, ArH), 7.69-7.75 (dd, 2H, ArH), 8.74 (s, 1H, N=CH); ESI-MS, m/z: 320.13 $[M]^+$, 322.42 $[M+2]^+$; Anal. calcd. for $C_{15}H_{10}ClFN_2OS$: C, 56.17; H, 3.14; N, 8.73. Found: C, 56.07; H, 3.24; N, 8.65.

*7-chloro-N-(4-chlorobenzylidene)-6-fluorobenzo[d]thiazol-2-amine* **2f:**

*Yield:* 63%; m.p.: 205-208°C; light yellow crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1676 (N=CH), 1246 (C-F), 1084 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 7.40-7.44 (m, 4H, ArH), 7.65-7.67 (d, 1H, J = 8.64 Hz, ArH), 7.70-7.72 (d, 1H, J = 8.73 Hz, ArH), 8.76 (s, 1H, N=CH); ESI-MS, m/z: 323.95 $[M]^+$, 326.01 $[M+2]^+$; Anal. calcd. for $C_{14}H_7Cl_2FN_2S$: C, 51.71; H, 2.17; N, 8.61. Found: C, 51.75; H, 2.14; N, 8.47.

*General procedure for the preparation of 9-chloro-8-fluoro-2-substitutedphenyl-2H-[1,3,5]thiadiazino[4,3-b][1,3]benzothiazole 3,3-dioxide* **3a-f:**

To a solution of **2a-f** (0.002 mol) in dry 1,4-dioxane (15 mL) was added dry triethylamine (0.555 mL, 0.004 mol) and to this solution was added methanesulfonyl chloride (0.232 mL, 0.003 mol) in 1,4-dioxane (10 mL) was added dropwise at 0-5°C under stirring for 30 min. The stirring was continued for a period of 3-4 h at the same temperature. The reaction mixture was then poured in to crashed ice (100 g) and extracted with chloroform (3×20 mL) three times, then dried over anhydrous sodium sulphate. Removal of the solvent under reduced pressure gave the product. The product was washed with cold dichloromethane.

*N-[4-(9-chloro-8-fluoro-3,3-dioxido-2H-[1,3,5]thiadiazino[4,3-b][1,3]benzothiazol-2-yl)phenyl]-N,N-dimethylamine* **3a:**

*Yield:* 47%; m.p.: 220-221°C; colourless solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1368, 1164 ($SO_2$), 1241 (C-F), 1086 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.00 (s, 6H, $N(CH_3)_2$), 3.22 (s, 2H, $CH_2$), 3.56 (s, 1H, CH), 7.50-7.54 (m, 4H, ArH), 7.67-7.69 (d, 1H, J = 8.38 Hz, ArH), 7.75-7.77 (d, 1H, J = 8.96 Hz, ArH); ESI-MS, m/z: 411.0 $[M]^+$, 413.08 $[M+2]^+$; Anal. calcd. for $C_{17}H_{15}ClFN_3O_2S_2$: C, 49.57; H, 3.67; N, 10.20. Found: C, 49.63; H, 3.70; N, 10.17.

*4-(9-chloro-8-fluoro-3,3-dioxido-2H-[1,3,5]thiadiazino[4,3-b][1,3]benzothiazol-2-yl)-2-methoxyphenol* **3b:**

*Yield:* 49%; m.p.: 266-268°C; greyish powder; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3371 (OH), 1368, 1168 ($SO_2$), 1250, 1038 (C-O-C), 1242 (C-F), 1098 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.30 (s, 2H, $CH_2$), 3.71 (s, 1H, CH), 3.89 (s, 3H, $OCH_3$), 7.55-7.59 (m, 3H, ArH), 7.72-7.78 (dd, 2H, ArH), 8.98 (br s, 1H, OH); ESI-MS, m/z: 414.15 $[M]^+$, 416.00 $[M+2]^+$; Anal. calcd. for $C_{16}H_{12}ClFN_2O_4S_2$: C, 46.32; H, 2.92; N, 6.75. Found: C, 46.45; H, 3.14; N, 6.68.

*4-(9-chloro-8-fluoro-3,3-dioxido-2H-[1,3,5]thiadiazino[4,3-b][1,3]benzothiazol-2-yl)phenol* **3c:**

*Yield:* 54%; m.p.: 287-289°C; buff white powder; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3367 (OH), 1362, 1170 ($SO_2$), 1247 (C-F), 1091 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.25 (s, 2H, $CH_2$), 3.66 (s, 1H, CH), 7.43-7.46 (m, 4H, ArH), 7.69-7.71 (d, 1H, J = 8.74 Hz, ArH), 7.76-7.78 (d, 1H, J = 8.82 Hz, ArH), 8.89 (br s, 1H, OH); ESI-MS, m/z: 383.73 $[M]^+$, 385.64 $[M+2]^+$; Anal. calcd. for $C_{15}H_{10}ClFN_2O_3S_2$: C, 46.82; H, 2.62; N, 7.28. Found: C, 46.89; H, 2.84; N, 7.26.

*2-(9-chloro-8-fluoro-3,3-dioxido-2H-[1,3,5]thiadiazino[4,3-b][1,3]benzothiazol-2-yl)phenol* **3d:**

*Yield:* 43%; m.p.: 276-278°C; light yellow crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3343 (OH), 1372, 1168 ($SO_2$), 1235 (C-F), 1089 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.29 (s, 2H, $CH_2$), 3.57 (s, 1H, CH), 7.42-7.45 (m, 4H, ArH),7.66-7.68 (d, 1H, J = 8.77 Hz, ArH), 7.72-7.74 (d, 1H, J = 8.83 Hz, ArH), 9.00 (br s, 1H, OH); ESI-MS, m/z: 383.79 $[M]^+$, 385.84 $[M+2]^+$; Anal. calcd. for $C_{15}H_{10}ClFN_2O_3S_2$: C, 46.82; H, 2.62; N, 7.28. Found: C, 46.85; H, 2.85; N, 7.26.

*9-chloro-8-fluoro-2-(4-methoxyphenyl)-2H-[1,3,5]thiadiazino[4,3-b][1,3]benzothiazole 3,3-dioxide* **3e:**

*Yield:* 57%; m.p.: 290-292°C; white crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1370, 1165 ($SO_2$), 1265, 1042 (C-O-C), 1243 (C-F), 1083 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.25 (s, 2H, $CH_2$), 3.59 (s, 1H, CH), 3.83 (s, 3H, $OCH_3$), 7.41-7.45 (m, 4H, ArH), 7.72-7.77 (dd, 2H, ArH); ESI-MS, m/z: 398.13 $[M]^+$, 399.93 $[M+2]^+$; Anal. calcd. for $C_{16}H_{12}ClFN_2O_3S_2$: C, 48.18; H, 3.03; N, 7.02. Found: C, 48.27; H, 3.35; N, 7.01.

*9-chloro-2-(4-chlorophenyl)-8-fluoro-2H-[1,3,5]thiadiazino[4,3-b][1,3]benzothiazole 3,3-dioxide* **3f:**

*Yield:* 51%; m.p.: 277-278°C; light yellow crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1362, 1154 ($SO_2$), 1245 (C-F), 1097 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.31 (s, 2H, $CH_2$), 3.68 (s, 1H, CH), 7.41-7.46 (m, 4H, ArH), 7.61-7.63 (d, 1H, J = 8.68 Hz, ArH), 7.74-7.76 (d, 1H, J = 8.83 Hz, ArH); ESI-MS, m/z: 401.86 $[M]^+$, 403.75 $[M+2]^+$; Anal. calcd. for $C_{15}H_9Cl_2FN_2O_2S_2$: C, 44.67; H, 2.25; N, 6.95. Found: C, 44.27; H, 2.35; N, 6.92.

*N-(7-chloro-6-fluorobenzo[d]thiazol-2-yl)acetamide* **4:**

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.

*Yield:* 92%, m.p.: 232-233°C, white star shaped crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3318 (N-H), 1681 (C=O), 1239 (C-F), 1082 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 2.34 (s, 3H, $CH_3$), 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 $[M]^+$, 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.

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

A mixture of compound **4** (100 mg, 0.000409 mol) and 0.3 mL of ice cold conc. $H_2SO_4$ was stirred under ice cold condition. To this 0.1 mL conc. $HNO_3$ was added drop wise, continued stirring at room temperature for 2 h. Then 0.1 mL conc. $HNO_3$ 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 **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* **5:**

*Yield:* 56%; m.p.: 297-298°C; buff colour needle shaped crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3301 (N-H), 1535, 1337 ($NO_2$), 1680 (C=O), 1186 (C-F), 661 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 2.34 (s, 3H, $CH_3$), 7.93-7.95 [d, 1H, ArH, J = 7.60 Hz (C-F)], 9.16 (s, 1H, NH); ESI-MS, m/z: 288.99 $[M]^+$, 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* **6:**

*Yield:* 30%; m.p.: 336-337°C; white crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3311 (N-H), 1530, 1341 ($NO_2$), 1680 (C=O), 1245 (C-F), 1092 (C-Cl); $^1H$ NMR (400MHz, 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.

*7-chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-amine* **7:**

Compound **5** (1 g, 0.004040 mole), in 70% $H_2SO_4$ 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 with DMF- water mixture.

*Yield:* 76%; m.p.: 187-188°C; light yellowish needle shaped crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3450 (N-H), 1543, 1348 ($NO_2$) 1243 (C-F), 1089 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 4.26 (s, 2H, $NH_2$), 7.90-7.92 (d, 1H, J = 7.62, ArH); ESI-MS, m/z: 247.63 $[M]^+$, 249.35 $[M+2]^+$; Anal. calcd. for $C_7H_3ClFN_3O_2S$: C, 33.95; H, 1.22; N, 16.97. Found: C, 34.07; H, 1.21; N, 16.95.

*General procedure for the preparation of N-substitutedbenzylidene-7-chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-amine* **8a-f:**

A mixture of compound **7** (0.01 mole), substituted aromatic aldehydes (0.01 mole) and 2-3 drops of glacial acetic acid in dry benzene was refluxed for 36 h in Dean-Stark apparatus. The reaction was monitored by TLC. After completion of the reaction, mixture was kept in the refrigerator overnight, the solid product obtained was filtered and recrystallized from benzene.

*7-chloro-N-(4-(dimethylamino)benzylidene)-6-fluoro-5-nitrobenzo[d] thiazol-2-amine* **8a:**

*Yield:* 73%; m.p.: 202-204°C; yellowish crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1646 (N=CH), 1538, 1344 ($NO_2$), 1239 (C-F), 1081 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.02 (s, 6H, $N(CH_3)_2$), 7.53-7.57 (m, 4H, ArH), 7.94-7.97 (d, 1H, ArH J = 7.64 Hz), 8.73 (s, 1H, N=CH); ESI-MS, m/z: 378.37 $[M]^+$, 380.18 $[M+2]^+$; Anal. calcd. for $C_{16}H_{12}ClFN_4O_2S$: C, 50.73; H, 3.19; N, 14.79; Found: C, 50.82; H, 3.22; N, 14.81.

*4-((7-chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-ylimino)methyl)-2-methoxyphenol* **8b:**

*Yield:* 73%; m.p.: 217-219°C; yellowish crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3376 (OH), 1659 (N=CH), 1546, 1335 ($NO_2$), 1258, 1043 (C-O-C), 1238 (C-F), 1092 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.88 (s, 3H, $OCH_3$), 7.60-7.64 (m, 3H, ArH), 7.96-7.98 (d, 1H, ArH, J = 7.71Hz), 8.84 (s, 1H, N=CH), 10.96 (br s, 1H, OH); ESI-MS, m/z: 382.03 $[M]^+$, 383.94 $[M+2]^+$; Anal. calcd. for $C_{15}H_9ClFN_3O_4S$: C, 47.19; H, 2.38; N, 11.01. Found: C, 47.28; H, 2.37; N, 11.04.

*4-((7-chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-ylimino)methyl) phenol* **8c:**

*Yield:* 73%; m.p.: 232-234°C; reddish powder; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3371 (OH), 1656 (N=CH), 1538, 1349 ($NO_2$), 1242 (C-F), 1087 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 7.54-7.57 (m, 4H, ArH), 7.93-7.95 (d, 1H, ArH J = 7.59 Hz), 8.79 (s, 1H, N=CH), 10.90 (br s, 1H, OH); ESI-MS, m/z: 351.75 $[M]^+$, 353.42 $[M+2]^+$; Anal. calcd. for $C_{14}H_7ClFN_3O_3S$: C, 47.81; H, 2.01; N, 11.95. Found: C, 47.94; H, 2.03; N, 11.94.

*2-((7-chloro-6-fluoro-5-nitrobenzo[d]thiazol-2-ylimino)methyl) phenol* **8d:**

*Yield:* 73%; m.p.: 221-223°C; reddish crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3378 (OH), 1662 (N=CH), 1542, 1347 ($NO_2$), 1241 (C-F), 1086 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 7.53-7.56 (m, 4H, ArH), 7.95-7.97 (d, 1H, ArH J = 7.68 Hz), 8.82 (s, 1H, N=CH), 10.97 (br s, 1H, OH); ESI-MS, m/z: 351.13 $[M]^+$, 353.04 $[M+2]^+$; Anal. calcd. for $C_{14}H_7ClFN_3O_3S$: C, 47.81; H, 2.01; N, 11.95. Found: C, 48.02; H, 2.02; N, 11.98.

*7-chloro-6-fluoro-N-(4-methoxybenzylidene)-5-nitrobenzo[d]thiazol-2-amine* **8e:**

*Yield:* 73%; m.p.: 227-229°C; light yellow crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1643 (N=CH), 1535, 1349 ($NO_2$), 1256, 1045 (C-O-C), 1233 (C-F), 1072 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.83 (s, 3H, $OCH_3$), 7.52-7.56 (m, 4H, ArH), 7.95-7.96 (d, 1H, ArH J = 7.19 Hz), 8.77 (s, 1H, N=CH); ESI-MS, m/z: 365.37 $[M]^+$, 367.01 $[M+2]^+$; Anal. calcd. for $C_{15}H_9ClFN_3O_3S$: C, 49.26; H, 2.48; N, 11.49;. Found: C, 49.58; H, 2.47; N, 11.42.

*7-chloro-N-(4-chlorobenzylidene)-6-fluoro-5-nitrobenzo[d]thiazol-2-amine* **8f:**

*Yield:* 73%; m.p.: 214-216°C; yellowish crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1641 (N=CH), 1536, 1349 ($NO_2$), 1241 (C-F), 1094 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 7.50-7.53 (m, 4H, ArH), 7.93-7.95 (d, 1H, ArH J = 7.86 Hz), 8.76 (s, 1H, N=CH); ESI-MS, m/z: 369.57 $[M]^+$, 371.18 $[M+2]^+$; Anal. calcd. for $C_{14}H_6Cl_2FN_3O_2S$: C, 45.42; H, 1.63; N, 11.35. Found: C, 45.83; H, 1.67; N, 11.33.

*General procedure for the preparation of 9-chloro-8-fluoro-7-nitro-2-substitutedphenyl-2H-[1,3,5]thiadiazino[4,3-b][1,3]benzothiazole 3,3-dioxide* **9a-f:**

To a solution of **8a-f** (0.002 mol) in dry 1,4-dioxane (15 mL) was added dry triethylamine (0.555 mL, 0.004 mol) and to this solution was added methanesulfonyl chloride (0.232 mL, 0.003 mol) in 1,4-dioxane (10 mL) was added drop wise at 0-5°C under stirring for 30 min. The stirring was continued for a period of 8 h at the same temperature. The reaction mixture was then poured in to crashed ice (100 g) and extracted with chloroform (3×20 mL) three times, then dried over anhydrous sodium sulphate. Removal of the solvent under reduced pressure gave the product. The product was washed with cold dichloromethane.

*N-[4-(9-chloro-8-fluoro-7-nitro-3,3-dioxido-2H-[1,3,5]thiadiazino[4,3-b][1,3] benzothiazol-2-yl)phenyl]-N,N-dimethylamine* **9a:**

*Yield:* 73%; m.p.: 275-277°C; buff coloured crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1550, 1346 ($NO_2$), 1362, 1151 ($SO_2$), 1240 (C-F), 1089 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.02 (s, 6H, $N(CH_3)_2$), 3.27 (s, 2H, $CH_2$), 3.58 (s, 1H, CH), 7.56-7.60 (m, 4H, ArH), 7.95-7.97 (d, 1H, J = 7.63 Hz, ArH); ESI-MS, m/z: 456.26 $[M]^+$, 458.06 $[M+2]^+$; Anal. calcd. for $C_{17}H_{14}ClFN_4O_4S_2$: C, 44.69; H, 3.09; N, 12.26. Found: C, 44.75; H, 3.12; N, 12.28.

*4-(9-chloro-8-fluoro-7-nitro-3,3-dioxido-2H-[1,3,5]thiadiazino[4,3-b] [1,3]benzothiazol-2-yl)-2-methoxyphenol* **9b:**

*Yield:* 73%; m.p.: 293-295°C; pale crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3376 (OH), 1542, 1341 ($NO_2$), 1367, 1164 ($SO_2$), 1234 (C-F), 1090 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.28 (s, 2H, $CH_2$), 3.69 (s, 1H, CH), 3.89 (s, 3H, $OCH_3$), 7.61-7.65 (m, 3H, ArH), 7.97-7.99 (d, 1H, J = 7.81 Hz, ArH), 9.06 (br s, 1H, OH); ESI-MS, m/z: 459.26 $[M]^+$, 461.18 $[M+2]^+$; Anal. calcd. for $C_{16}H_{11}ClFN_3O_6S_2$: C, 41.79; H, 2.41; N, 9.14. Found: C, 41.95; H, 2.44; N, 9.18.

*4-(9-chloro-8-fluoro-7-nitro-3,3-dioxido-2H-[1,3,5]thiadiazino[4,3-b] [1,3]benzothiazol-2-yl)phenol* **9c:**

*Yield:* 73%; m.p. 283-285°C; colourless crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3380 (OH), 1546, 1342 ($NO_2$), 1359, 1161 ($SO_2$), 1230 (C-F), 1088 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.32 (s, 2H, $CH_2$), 3.68 (s, 1H, CH), 7.55-7.59 (m, 4H, ArH), 7.92-7.94 (d, 1H, J = 7.76 Hz, ArH), 8.95 (br s, 1H, OH); ESI-MS, m/z: 429.31 $[M]^+$, 431.14 $[M+2]^+$; Anal. calcd. for $C_{15}H_9ClFN_3O_5S_2$: C, 41.91; H, 2.11; N, 9.78. Found: C, 42.05; H, 2.12; N, 9.73.

*2-(9-chloro-8-fluoro-7-nitro-3,3-dioxido-2H-[1,3,5]thiadiazino[4,3-b] [1,3]benzothiazol-2-yl)phenol* **9d:**

*Yield:* 73%; m.p.: 269-270°C; black coloured crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3393 (OH), 1542, 1349 ($NO_2$), 1368, 1172 ($SO_2$), 1237 (C-F), 1087 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.26 (s, 2H, $CH_2$), 3.66 (s, 1H, CH), 7.57-7.54 (m, 4H, ArH), 7.93-7.90 (d, 1H, J = 8.02 Hz, ArH), 8.98 (br s, 1H, OH); ESI-MS, m/z: 429.16 $[M]^+$, 431.09 $[M+2]^+$; Anal. calcd. for $C_{15}H_9ClFN_3O_5S_2$: C, 41.91; H, 2.11; N, 9.78. Found: C, 42.03; H, 2.13; N, 9.76.

*9-chloro-8-fluoro-2-(4-methoxyphenyl)-7-nitro-2H-[1,3,5]thiadiazino [4,3-b][1,3] benzothiazole-3,3-dioxide* **9e:**

*Yield:* 73%; m.p.: 299-300°C; brown crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1533, 1330 ($NO_2$), 1374, 1182 ($SO_2$), 1241 (C-F), 1094 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.26 (s, 2H, $CH_2$), 3.78 (s, 1H, CH), 3.84 (s, 3H, $OCH_3$), 7.46-7.49 (m, 4H, ArH), 7.88-7.90 (d, 1H, J = 7.89 Hz, ArH); ESI-MS, m/z: 443.23 $[M]^+$, 445.14 $[M+2]^+$; Anal. calcd. for $C_{16}H_{11}ClFN_3O_5S_2$: C, 43.30; H, 2.50; N, 9.47. Found: C, 43.48; H, 2.54; N, 9.45.

*9-chloro-2-(4-chlorophenyl)-8-fluoro-7-nitro-2H-[1,3,5]thiadiazino [4,3-b][1,3]benzothiazole-3,3-dioxide* **9f:**

*Yield:* 73%; m.p.: 269-271°C; buff coloured crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1538, 1323 ($NO_2$), 1366, 1170 ($SO_2$), 1243 (C-F), 1099 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.31 (s, 2H, $CH_2$), 3.75 (s, 1H, CH), 7.64-7.68 (m, 4H, ArH), 7.91-7.95 (d, 1H, J = 7.69 Hz, ArH); ESI-MS, m/z: 447.49 $[M]^+$, 449.34 $[M+2]^+$; Anal. calcd. for $C_{15}H_8Cl_2FN_3O_4S_2$: C, 40.19; H, 1.80; N, 9.37. Found: C, 40.28; H, 1.39; N, 9.34.

*7-chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-amine* **10:**

Compound **6** (1 g, 0.004040 mole), in 70% $H_2SO_4$ 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 with DMF- water mixture.

*Yield:* 94%; m.p.: 193-194˚C; yellowish needle shaped crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3452 (N-H), 1550, 1333 ($NO_2$) 1247 (C-F), 1087 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 4.29 (s, 2H, $NH_2$), 8.40-8.42 (d, 1H, J = 9.42, ArH); ESI-MS, m/z: 247.28 $[M]^+$, 249.14 $[M+2]^+$; Anal. calcd. for $C_7H_3ClFN_3O_2S$: C, 33.95; H, 1.22; N, 16.97. Found: C, 34.01; H, 1.23; N, 16.91.

*General procedure for the preparation of N-substitutedbenzylidene-7-chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-amine* **11a-f:**

A mixture of compound **10** (0.01 mole), substituted aromatic aldehydes (0.01 mole) and 2-3 drops of glacial acetic acid in dry benzene was refluxed for 36 h in Dean-Stark apparatus. The reaction was monitored by TLC. After completion, the reaction mixture was kept in the refrigerator overnight, the solid product obtained was filtered and recrystallized from benzene.

*7-chloro-N-(4-(dimethylamino)benzylidene)-6-fluoro-4-nitrobenzo [d]thiazol-2-amine* **11a:**

*Yield:* 73%; m.p.: 210-211˚C; yellow crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1650 (N=CH), 1545, 1352 ($NO_2$), 1238 (C-F), 1091 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.04 (s, 6H, $N(CH_3)_2$), 7.49-7.53 (m, 4H, ArH), 8.43-8.46 [d, 1H, ArH J = 9.32 Hz (C-F)], 8.76 (s, 1H, N=CH); ESI-MS, m/z: 378.15 $[M]^+$, 379.97 $[M+2]^+$; Anal. calcd. for $C_{16}H_{12}ClFN_4O_2S$: C, 50.73; H, 3.19; N, 14.79; Found: C, 50.63; H, 3.34; N, 14.72.

*4-((7-chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-ylimino)methyl)-2-methoxyphenol* **11b:**

*Yield:* 73%; m.p.: 218-220˚C; yellow crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3383 (OH), 1663 (N=CH), 1550, 1336 ($NO_2$), 1254, 1040 (C-O-C), 1232 (C-F), 1069 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.88 (s, 3H, $OCH_3$), 7.57-7.61 (m, 3H, ArH), 8.46-8.48 [d, 1H, ArH J = 9.34 Hz (C-F)], 8.84 (s, 1H, N=CH), 11.25 (br s, 1H, OH); ESI-MS, m/z: 381.03 $[M]^+$, 382.85 $[M+2]^+$; Anal. calcd. for $C_{15}H_9ClFN_3O_4S$: C, 47.19; H, 2.38; N, 11.01. Found: C, 47.72; H, 3.79; N, 10.97.

*4-((7-chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-ylimino)methyl) phenol* **11c:**

*Yield:* 73%; m.p.: 212-214˚C; reddish crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3373 (OH), 1658 (N=CH), 1535, 1323 ($NO_2$), 1239 (C-F), 1081 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 7.50-7.54 (m, 4H, ArH), 8.38-8.42 [d, 1H, ArH J = 9.23 Hz (C-F)], 8.81 (s, 1H, N=CH), 10.95 (br s, 1H, OH); ESI-MS, m/z: 351.05 $[M]^+$, 352.97 $[M+2]^+$; Anal. calcd. for $C_{14}H_7ClFN_3O_3S$: C, 47.81; H, 2.01; N, 11.95. Found: C, 47.95; H, 2.05; N, 11.87.

*2-((7-chloro-6-fluoro-4-nitrobenzo[d]thiazol-2-ylimino)methyl) phenol* **11d:**

*Yield:* 73%; m.p.: 230-232˚C; yellow crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3375 (OH), 1659 (N=CH), 1538, 1332 ($NO_2$), 1239 (C-F), 1086 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 7.69-7.73 (m, 4H, ArH), 8.42-8.44 [d, 1H, ArH J = 9.24 Hz (C-F)], 8.85 (s, 1H, N=CH), 11.23 (br s, 1H, OH); ESI-MS, m/z: 351.07 $[M]^+$, 352.99 $[M+2]^+$; Anal. calcd. for $C_{14}H_7ClFN_3O_3S$: C, 47.81; H, 2.01; N, 11.95. Found: C, 47.87; H, 2.14; N, 11.87.

*7-chloro-6-fluoro-N-(4-methoxybenzylidene)-4-nitrobenzo[d]thiazol-2-amine* **11e:**

*Yield:* 73%; m.p.: 205-206˚C; light yellow crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1649 (N=CH), 1542, 1334 ($NO_2$), 1250, 1042 (C-O-C), 1248 (C-F), 1076 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.86 (s, 3H, $OCH_3$), 7.47-7.51 (m, 4H, ArH), 8.43-8.45 [d, 1H, ArH J = 9.29Hz (C-F)], 8.76 (s, 1H, N=CH); ESI-MS, m/z: 365.28 $[M]^+$, 367.13 $[M+2]^+$; Anal. calcd. for $C_{15}H_9ClFN_3O_3S$: C, 49.26; H, 2.48; N, 11.49. Found: C, 49.08; H, 2.45; N, 11.37.

*7-chloro-N-(4-chlorobenzylidene)-6-fluoro-4-nitrobenzo[d]thiazol-2-amine* **11f:**

*Yield:* 73%; m.p.: 235-237˚C; yellow crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1649 (N=CH), 1524, 1343 ($NO_2$), 1244 (C-F), 1086 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 7.54-7.58 (m, 4H, ArH), 8.49-8.51 [d, 1H, ArH J = 9.38 Hz (C-F)], 8.79 (s, 1H, N=CH); ESI-MS, m/z: 368.82 $[M]^+$, 370.06 $[M+2]^+$; Anal. calcd. for $C_{14}H_6Cl_2FN_3O_2S$: C, 45.42; H, 1.63; N, 11.35. Found: C, 45.51; H, 1.67; N, 11.31.

*General procedure for the preparation of 9-chloro-8-fluoro-6-nitro-2-substitutedphenyl-2H-[1,3,5]thiadiazino[4,3-b][1,3]benzothiazole 3,3-dioxide* **12a-f:**

To a solution of **11a-f** (0.002 mol) in dry 1,4-dioxane (15 mL) was added dry triethylamine (0.555 mL, 0.004 mol) and to this solution was added methanesulfonyl chloride (0.232 mL, 0.003 mol) in 1,4-dioxane (10 mL) was added drop wise at 0-5˚C under stirring for 30 min. The stirring was continued for a period of 10 h at the same temperature. The reaction mixture was then poured in to crashed ice (100 g) and extracted with chloroform (3×20 mL) three times, then dried over anhydrous sodium sulphate. Removal of the solvent under reduced pressure gave the product. The product was washed with cold dichloromethane.

*N-[4-(9-chloro-8-fluoro-6-nitro-3,3-dioxido-2H-[1,3,5]thiadiazino[4,3-b][1,3] benzothiazol-2-yl)phenyl]-N,N-dimethylamine* **12a:**

*Yield:* 73%; m.p.: 284-285˚C buff coloured crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1545, 1339 ($NO_2$), 1369, 1158 ($SO_2$), 1243 (C-F), 1084 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.05 (s, 6H, $N(CH_3)_2$), 3.31 (s, 2H, $CH_2$), 3.59 (s, 1H, CH), 7.54-7.57 (m, 4H, ArH), 8.47-8.49 (d, 1H, J = 9.13 Hz, ArH); ESI-MS, m/z: 456.00 $[M]^+$, 457.94 $[M+2]^+$; Anal. calcd. for $C_{17}H_{14}ClFN_4O_4S_2$: C, 44.69; H, 3.09; N, 12.26. Found: C, 44.61; H, 3.13; N, 12.19.

*4-(9-chloro-8-fluoro-6-nitro-3,3-dioxido-2H-[1,3,5]thiadiazino[4,3-b] [1,3]benzothiazol-2-yl)-2-methoxyphenol* **12b:**

*Yield:* 73%; m.p.: 297-299˚C; light yellow crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3387 (OH), 1548, 1334 ($NO_2$), 1370, 1162 ($SO_2$), 1231 (C-F), 1087 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.34 (s, 2H, $CH_2$), 3.63 (s, 1H, CH), 3.88 (s, 3H, $OCH_3$), 7.59-7.62 (m, 3H, ArH), 8.51-8.53 (d, 1H, J = 9.44 Hz, ArH), 9.04 (br s, 1H, OH); ESI-MS, m/z: 458.86 $[M]^+$, 460.54 $[M+2]^+$; Anal. calcd. for $C_{16}H_{11}ClFN_3O_6S_2$: C, 41.79; H, 2.41; N, 9.14. Found: C, 41.52; H, 2.53; N, 9.17.

*4-(9-chloro-8-fluoro-6-nitro-3,3-dioxido-2H-[1,3,5]thiadiazino[4,3-b] [1,3]benzothiazol-2-yl)phenol* **12c:**

*Yield:* 73%; m.p.: 289-290˚C; buff coloured crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3382 (OH), 1532, 1335 ($NO_2$), 1358, 1161 ($SO_2$), 1238 (C-F), 1086 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.28 (s, 2H, $CH_2$), 3.64 (s, 1H, CH), 7.45-7.48 (m, 4H, ArH), 8.41-8.43 (d, 1H, J = 9.33 Hz, ArH), 8.91 (br s, 1H, OH); ESI-MS, m/z: 429.86 $[M]^+$, 431.31 $[M+2]^+$; Anal. calcd. for $C_{15}H_9ClFN_3O_5S_2$: C, 41.91; H, 2.11; N, 9.78. Found: C, 41.98; H, 2.13; N, 9.77.

*2-(9-chloro-8-fluoro-6-nitro-3,3-dioxido-2H-[1,3,5]thiadiazino[4,3-b] [1,3]benzothiazol-2-yl)phenol* **12d:**

*Yield:* 73%; m.p.: 284-286˚C; buff crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 3388 (OH), 1544, 1346 ($NO_2$), 1372, 1169 ($SO_2$), 1239 (C-F), 1083 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.30 (s, 2H, $CH_2$), 3.66 (s, 1H, CH), 7.42-7.46 (m, 4H, ArH), 8.45-8.47 (d, 1H, J = 9.36 Hz, ArH), 8.95 (br s, 1H, OH); ESI-MS, m/z: 429.52 $[M]^+$, 431.31 $[M+2]^+$; Anal. calcd. for $C_{15}H_9ClFN_3O_5S_2$: C, 41.91; H, 2.11; N, 9.78. Found: C, 41.72; H, 2.15; N, 9.72.

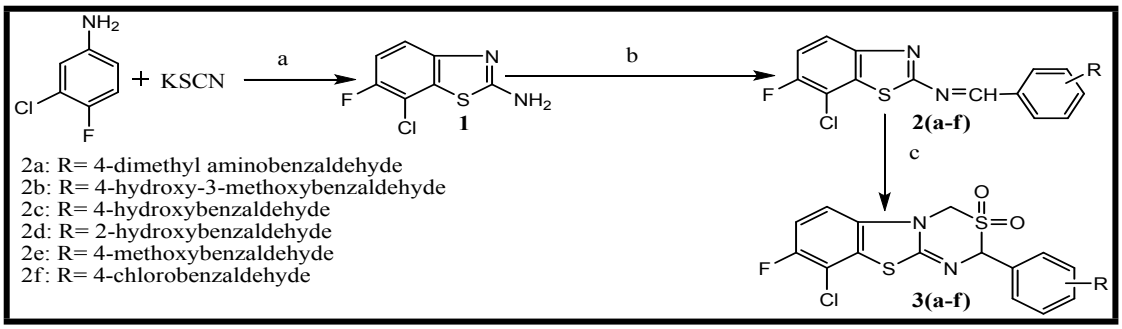
*9-chloro-8-fluoro-2-(4-methoxyphenyl)-6-nitro-2H-[1,3,5]thiadiazino [4,3-b][1,3] benzothiazole 3,3-dioxide* **12e:**

*Yield:* 73%; m.p.: 308-310˚C; yellow crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1537, 1334 ($NO_2$), 1377, 1181 ($SO_2$), 1247 (C-F), 1091 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.28 (s, 2H, $CH_2$), 3.62 (s, 1H, CH), 3.86 (s, 3H, $OCH_3$), 7.37-7.42 (m, 4H, ArH), 8.42-8.43 (d, 1H, J = 9.29 Hz, ArH); ESI-MS, m/z: 443.87 $[M]^+$, 445.24 $[M+2]^+$; Anal. calcd. for $C_{16}H_{11}ClFN_3O_5S_2$: C, 43.30; H, 2.50; N, 9.47. Found: C, 43.54; H, 2.52; N, 9.42.

*9-chloro-2-(4-chlorophenyl)-8-fluoro-6-nitro-2H-[1,3,5]thiadiazino [4,3-b][1,3] benzothiazole 3,3-dioxide* **12f:**

*Yield:* 73%; m.p.: 294-295˚C; light yellow crystalline solid; IR ($\upsilon_{max}$, $cm^{-1}$, KBr): 1542, 1331 ($NO_2$), 1361, 1168 ($SO_2$), 1238 (C-F), 1087 (C-Cl); $^1H$ NMR (400MHz, DMSO-$d_6$), δ (ppm): 3.34 (s, 2H, $CH_2$), 3.73 (s, 1H, CH), 7.56-7.59 (m, 4H, ArH), 8.52-8.54 (d, 1H, J = 9.55 Hz, ArH); ESI-MS, m/z: 447.82 $[M]^+$, 449.04 $[M+2]^+$; Anal. calcd. for $C_{15}H_8Cl_2FN_3O_4S_2$: C, 40.19; H, 1.80; N, 9.37. Found: C, 40.27; H, 1.82; N, 9.40.

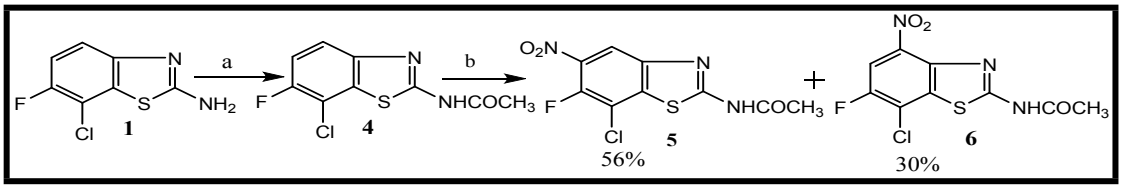
**Scheme 1:**




2a: R= 4-dimethyl aminobenzaldehyde
2b: R= 4-hydroxy-3-methoxybenzaldehyde
2c: R= 4-hydroxybenzaldehyde
2d: R= 2-hydroxybenzaldehyde
2e: R= 4-methoxybenzaldehyde
2f: R= 4-chlorobenzaldehyde

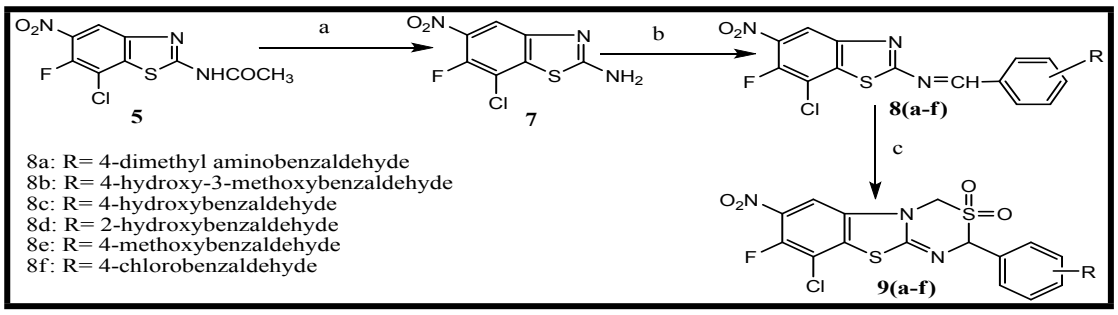
**Reagents and conditions**: (a) $Br_2$ in glacial acetic acid, 5°C, 2 h, r.t. 12 h; (b) Substituted aromatic aldehydes, benzene in Dean-Stark apparatus, reflux; (c) $CH_3SO_2Cl$, $(C_2H_5)_3N$, 1,4-Dioxane, 0-5°C.

**Scheme 2:**




**Reagents and conditions**: (a) $(CH_3CO)_2O$, reflux, 1 h; (b) conc. $H_2SO_4$, conc. $HNO_3$, r.t.

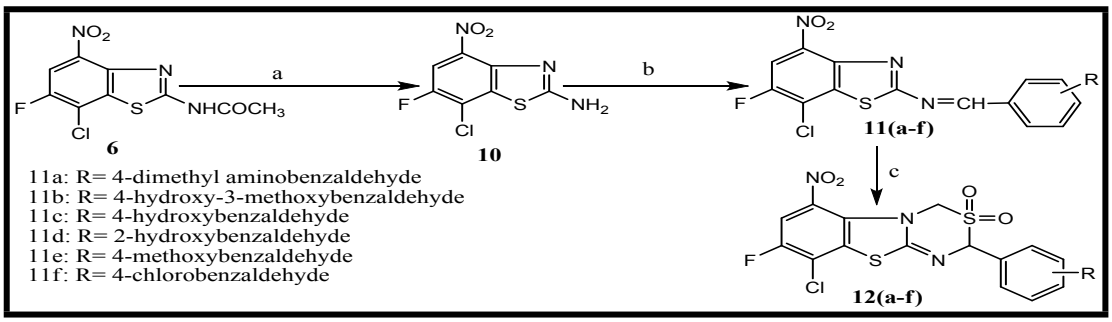
**Scheme 3:**




8a: R= 4-dimethyl aminobenzaldehyde
8b: R= 4-hydroxy-3-methoxybenzaldehyde
8c: R= 4-hydroxybenzaldehyde
8d: R= 2-hydroxybenzaldehyde
8e: R= 4-methoxybenzaldehyde
8f: R= 4-chlorobenzaldehyde

**Reagents and conditions**: (a) 70% $H_2SO_4$, reflux, 30 min; (b) Substituted aromatic aldehydes, benzene in Dean-Stark apparatus, reflux; (c) $CH_3SO_2Cl$, $(C_2H_5)_3N$, 1,4-Dioxane, 0-5°C.

**Scheme 4:**




11a: R= 4-dimethyl aminobenzaldehyde
11b: R= 4-hydroxy-3-methoxybenzaldehyde
11c: R= 4-hydroxybenzaldehyde
11d: R= 2-hydroxybenzaldehyde
11e: R= 4-methoxybenzaldehyde
11f: R= 4-chlorobenzaldehyde

**Reagents and conditions**: (a) 70% $H_2SO_4$, reflux, 30 min; (b) Substituted aromatic aldehydes, benzene in Dean-Stark apparatus, reflux; (c) $CH_3SO_2Cl$, $(C_2H_5)_3N$, 1,4-Dioxane, 0-5°C.

**Pharmacological activity:**

***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 10 µL suspension of *Mycobacterium tuberculosis* strain (10,0000 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 ($CTC_{50}$) THP-1 cell lines [11].

***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 x $10^5$ 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 on to 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 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% ($CTC_{50}$) values is generated from the dose-response curves for each cell line.

$$\% \text{ Growth Inhibition} = 100 - \left[ \frac{\text{Mean absorbance of individual test group}}{\text{Mean absorbance of control group}} \right] X\ 100$$

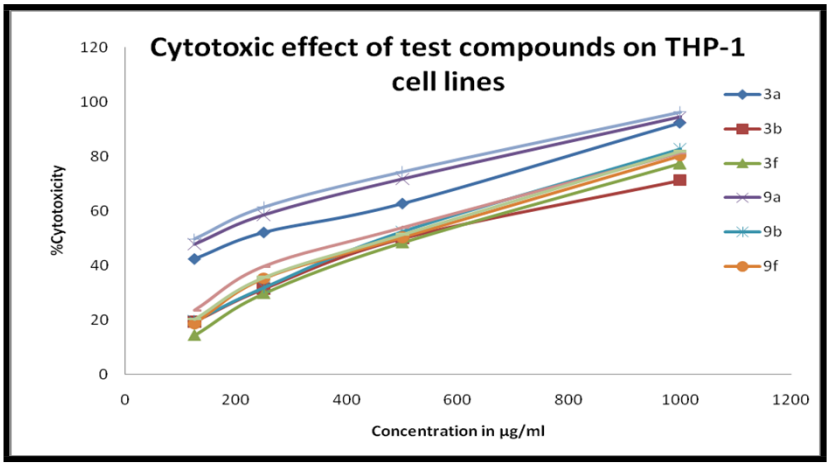



**Fig. 1: Cytotoxic effect of test drugs on THP-1 cells after 72 h of drug treatment. Cell viability was determined by MTT assay.**

**Table No. 1: *In vitro* anti-tubercular evaluation**

| SL. No. | Comp. No. | R | Anti-tubercular activity (MIC) | | | Min. Conc. in nM showed activity |
|---|---|---|---|---|---|---|
| | | | 25µg/ml | 50µg/ml | 100µg/ml | |
| 1 | 3a | 4-$N(CH_3)_2$ | R | S | S | 121.388 |
| 2 | 3b | 4-OH-3-$OCH_3$ | S | S | S | 60.261 |
| 3 | 3c | 4-OH | S | S | S | 64.963 |
| 4 | 3d | 2-OH | R | R | S | 259.855 |
| 5 | 3e | 4-$OCH_3$ | R | S | S | 125.357 |
| 6 | 3f | 4-Cl | S | S | S | 61.991 |
| 7 | 9a | 4-$N(CH_3)_2$ | R | R | S | 280.190 |
| 8 | 9b | 4-OH-3-$OCH_3$ | R | S | S | 108.728 |
| 9 | 9c | 4-OH | R | S | S | 116.325 |
| 10 | 9d | 2-OH | R | R | R | -------- |
| 11 | 9e | 4-$OCH_3$ | R | R | S | 225.296 |
| 12 | 9f | 4-Cl | S | S | S | 55.768 |
| 13 | 12a | 4-$N(CH_3)_2$ | R | R | S | 280.190 |
| 14 | 12b | 4-OH-3-$OCH_3$ | R | S | S | 108.728 |
| 15 | 12c | 4-OH | R | R | S | 116.325 |
| 16 | 12d | 2-OH | R | R | R | -------- |
| 17 | 12e | 4-$OCH_3$ | R | R | S | 225.296 |
| 18 | 12f | 4-Cl | R | S | S | 111.537 |
| 19 | Pyrazinamide | | | 7.5 µg/ml | | 60.095 |
| 20 | Streptomycin | | | 7.5 µg/ml | | 14.387 |

R= resistant; S= sensitive

**Table No. 2: *In vitro* cytotoxicity evaluation on THP-1 cell line**

| Sl. No. | Comp. No. | R | $CTC_{50}$ in µg/ml | $CTC_{50}$ in nM |
|---|---|---|---|---|
| **1** | **3a** | 4-$N(CH_3)_2$ | 241.00 | 585.093 |
| **2** | **3b** | 4-OH-3-$OCH_3$ | 502.50 | 1211.251 |
| **3** | **3f** | 4-Cl | 542.00 | 1343.979 |
| **4** | **9a** | 4-$N(CH_3)_2$ | 149.50 | 327.205 |
| **5** | **9b** | 4-OH-3-$OCH_3$ | 471.00 | 1049.324 |
| **6** | **9f** | 4-Cl | 506.00 | 1128.758 |
| **7** | **12a** | 4-$N(CH_3)_2$ | 129.50 | 283.343 |
| **8** | **12b** | 4-OH-3-$OCH_3$ | 435.00 | 945.940 |
| **9** | **12f** | 4-Cl | 492.00 | 1097.528 |

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