



# CHEMISTRY & BIOLOGY INTERFACE

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## Synthesis and evaluation of antimicrobial and antitubercular activity of arylidene hydrazines of indeno[2,3-d]pyrimidine

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**Abstract:** A series of new 6,7-dimethoxy-9*H*-indeno[3',2':4,5]thieno[2,3-*d*]pyrimidines have been synthesized in good yields and were screened for *in vitro* antimicrobial activity against the bacteria such as *Staphylococcus aureus* MTCC 96, *Streptococcus pyogenes* MTCC 442, *Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 1688 and fungi *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282 and *Aspergillus clavatus* MTCC 1323. These compounds showed significant to moderate antibacterial activities against tested bacteria. The compounds **3**, **4**, **6**, **7b**, **7c**, **7d**, **7l**, **7m**, **7o** showed significant antibacterial activity compared to Ampicillin and showed poor activity as compared to Gentamycin, Chloramphenicol, Ciprofloxacin and Norfloxacin. Also the compounds **5**, **7b**, **7c**, **7f**, **7h**, **7k**, **7n**, **7o**, **7q**, **7r** showed significant antifungal activity compared to Greseofulvin and poor activity compared to Nystatin and Isoniazid. These compounds were also tested for inhibition of mycobacterium tuberculosis H37Rv.

**Keywords:** Thienopyrimidine, Thienopyrimidine hydrazone derivatives, Antimicrobial activity, Antitubercular activity

### 1. Introduction

Infectious diseases are the main cause of mortality in the world. The rapid increase in antibiotic resistance amongst pathogenic bacteria has become a serious public health problem. These bacteria replicate rapidly and obtain mutation to survive in presence of antibiotic drugs. Eventually the microbial population has become predominant throughout.

Antimicrobial resistance become a factor in virtually all hospital-acquired infections and physicians are anxious that several bacterial infections are untreatable [1, 2]. Therefore, new efficacious antimicrobial drugs are required. Thienopyrimidine derivatives are the one of outcome of the research in this field.

Tuberculosis (TB) and cancer are called the big killer and intractable diseases; Antimicrobial

resistance (AMR) is one of the most important factors contributing to the failure of current therapies for TB and cancer. Hence, there is an indispensable need to develop potent, fast-acting, new classes of agents likely to be unaffected by existing resistance mechanisms with low toxicity. Heterocyclic compounds play a vital role in untiring efforts aiming to the development of new antimicrobial and antitumor agents with new mechanism of action [3].

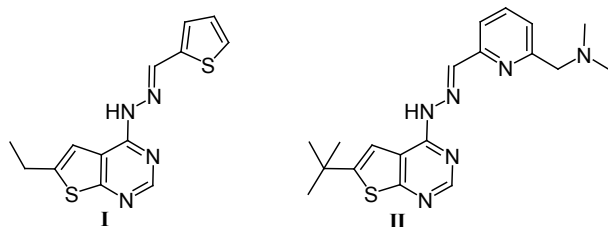


**Figure 1:** Compounds I and II have been identified as potent, selective CDK4 inhibitors.

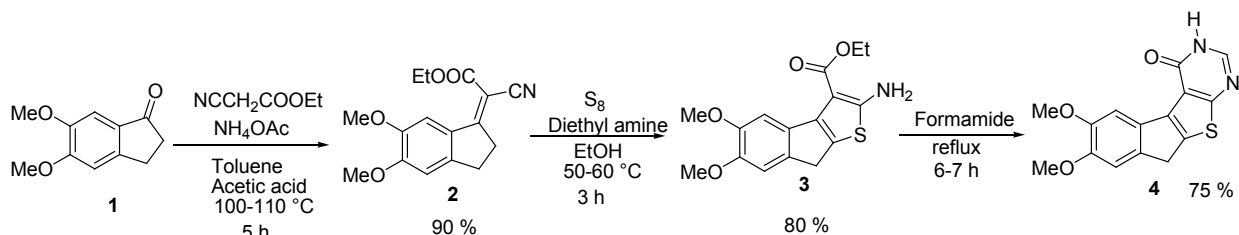
Amongst the thieno[2,3-*d*]pyrimidin-4-ylhydrazones, 1-(6-ethylthieno[2,3-*d*]pyrimidin-4-yl)-2-((thiophen-2-yl)methylene)hydrazine **I** and 2-(6-tert-butylthieno[2,3-*d*]pyrimidin-4-yl)-1-((6-(dimethylamino)methylpyridin-2-yl)methylene)hydrazine **II** have been identified as potent, selective cyclin-dependent kinase CDK4 inhibitors [4, 5] (**Figure 1**) having sufficient cytotoxic activities against HCT116 human colon carcinoma cell line with ability to prevent cell progression. Since in the last two decades, thienopyrimidines has become biologically important heterocyclic moiety in medicinal chemistry research due to their diverse biological activities such as anti-

inflammatory [6], antimicrobial [6, 7-14], antiviral, antihypertensive, antihistaminic, neurotropic [15], antidepressant, analgesic [16-18], inhibition of cancer cell proliferation [19],  $\alpha_1$  antagonism [20] adrenoceptors and prevention of cartilage destruction in articular diseases [21]. Thienopyrimidines are also reported as *antitubercular* drug [22, 23]. The hydrazones containing azomethine [-NH-N=CH-] have become an important class in the new drug development [24]. Many researchers have been synthesized these targeted structures and evaluated their biological activities. Hydrazones are reported to possess antimicrobial [25], antitubercular [26], anticonvulsant [27], analgesic [28], anti-inflammatory and anti-platelet [29], antiviral [30], antitumor [31] and anti-malarial activities [32]. Inspired with these results and our ongoing research on thienopyrimidines as molluscicidal agents [33] and antimicrobial agents [34]; herein we report the synthesis and biological activity of new thienopyrimidines and their hydrazone derivatives and their antimicrobial and antitubercular activities.

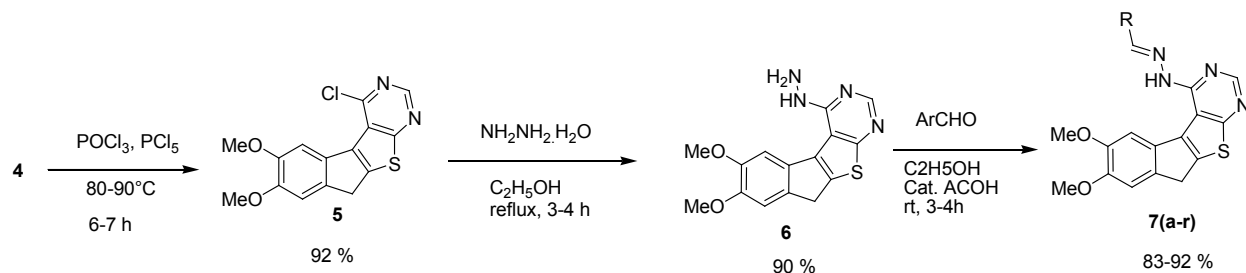
## 2. Results and Discussion

### 2.1. Chemistry

The  $\alpha$ -aminocarboxylate is the key precursor used for the synthesis of thienopyrimidines [23]. The reaction of 2, 3-dihydro-5, 6-dimethoxyinden-1-one **1** and ethylcyanoacetate in presence of ammonium acetate and acetic acid in toluene furnished ethyl 2-cyano-2-(1,2-dihydro-5,6-dimethoxyinden-3-ylidene)



**Scheme 1:** Synthetic route for indeno[2,3-*d*]pyrimidine



**Scheme 2:** Synthetic route for new arylidene hydrazines of indeno[2,3-*d*]pyrimidine

acetate **2** in 90% yield. Gewald reaction of compound **2**, elemental sulphur and diethyl amine in ethanol furnish precursor ethyl-2-amino-5,6-dimethoxy-8*H*-indeno[2,1-*b*]thiophene-3-carboxylate **3** in 80% yield.

The synthesis of 6,7-dimethoxy-3*H*-indeno[3',2':4,5]thieno[2,3-*d*]pyrimidin-4(9*H*)-one, **4** and 4-chloro-6,7-dimethoxy-9*H*-indeno [3',2':4,5]thieno[2,3-*d*]pyrimidine **5** [35] by series of reactions on 2,3-dihydro-5,6-dimethoxyinden-1-one **1** was reported. The indeno [2,1-*b*]thiophene-3-carboxylate **3** on reaction with formamide yields 75% thieno[2,3-*d*]pyrimidin-4(3*H*)-one **4**. The compound **4** was converted to 4-chloro thieno [2,3-*d*]pyrimidine **5** in 92% yield by refluxing in

POCl<sub>3</sub> with catalytic amount of PCl<sub>5</sub>. (**Scheme 1**).

It was observed that in absence of PCl<sub>5</sub> compound **4** underwent decomposition causes low yield of compound **5**, as the reaction with is POCl<sub>3</sub> is exothermic. The role of PCl<sub>5</sub> is to initiate the reaction at lower temperature 80-90 °C, which consequently avoids decomposition. Compound **5** on refluxing with hydrazine monohydrate in ethanol furnish 90% yield of 4-hydrazinyl-6,7-dimethoxy-9*H*-indeno[3',2':4,5]thieno[2,3-*d*]pyrimidine **6**. Further, hydrazine **6** on reaction with substituted aromatic aldehydes was converted to series of arylidene hydrazine derivatives **7** in ethanol using catalytic amount of acetic acid (**Scheme**

**Table 1:** Time and yield of arylidene hydrazines of indeno[2,3-*d*]pyrimidine

| Entry | R                     | Time in hour(h) | Yield (%) |
|-------|-----------------------|-----------------|-----------|
| 7a    | m-BrPh                | 3.0             | 90        |
| 7b    | m-MeOPh               | 3.0             | 91        |
| 7c    | Thiophene-2-          | 3.5             | 85        |
| 7d    | 4-FPh                 | 3.0             | 92        |
| 7e    | 2,3,4-MeOPh           | 4.0             | 89        |
| 7f    | 3,4-MeOPh             | 4.0             | 87        |
| 7g    | 3-NO <sub>2</sub> Ph  | 3.0             | 84        |
| 7h    | 4-MePh                | 3.0             | 91        |
| 7i    | Ph                    | 3.0             | 90        |
| 7j    | 2-HOPh                | 3.5             | 89        |
| 7k    | 1-Napthalenyl         | 3.5             | 83        |
| 7l    | 4-Pyridinyl           | 3.5             | 86        |
| 7m    | 4-MeOPh               | 3.5             | 89        |
| 7n    | Isobutyryl            | 3.0             | 85        |
| 7o    | Cinnamyl              | 3.5             | 84        |
| 7p    | 3,4-MeOPh             | 3.2             | 92        |
| 7q    | 4-ClPh                | 3.0             | 91        |
| 7r    | 4-NMe <sub>2</sub> Ph | 4.0             | 83        |

2). The reaction time and yield are given in **Table 1**. The synthesized arylidene hydrazines were tested for their antimicrobial and anti-tubercular activities. The structures of all new compounds **2**, **3**, **6** and **7** were confirmed from their IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and Mass.

### 3. Biology

The MICs of synthesized compounds were carried out by broth micro dilution method as described by Rattan [36]. Antibacterial activity was screened against two Gram positive (*Staphylococcus aureus* MTCC 96,

*Streptococcus pyogenes* MTCC 442) and two Gram negative (*Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 1688) bacteria by using gentamycin, ampicillin, chloramphenicol, ciprofloxacin, antimicrobial and anti-tubercular screening (**Table 2**). All MTCC cultures were norfloxacin as the standard antibacterial agents. Antifungal activity was screened against three fungal species *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 and nystatin and greseofulvin was used as a standard antifungal agent and were collected from Institute of Microbial Technology, Chandigarh. Mueller

**Table 2: In vitro antimicrobial and Anti tuberculosis Activity of newly synthesized compounds 2 to 7r MICs (µg/mL)**

| Comp.           | Gram Negative Bacteria |           | Gram Positive Bacteria |          | Fungi    |          |           | M. tuberculosis H37Rv (% Inhibition) |
|-----------------|------------------------|-----------|------------------------|----------|----------|----------|-----------|--------------------------------------|
|                 | E. C.                  | P. A.     | S. A.                  | S. P.    | C. A.    | A. N.    | A. C.     |                                      |
|                 | MTCC 443               | MTCC 1688 | MTCC 96                | MTCC 442 | MTCC 227 | MTCC 282 | MTCC 1323 |                                      |
| <b>2</b>        | 200                    | 125       | 200                    | 250      | >1000    | >1000    | >1000     | 500                                  |
| <b>3</b>        | 100                    | 200       | 250                    | 250      | >1000    | >1000    | >1000     | 250                                  |
| <b>4</b>        | 100                    | 200       | 100                    | 62.5     | >1000    | 250      | 250       | 1000                                 |
| <b>5</b>        | 250                    | 250       | 100                    | 100      | 500      | >1000    | >1000     | 1000                                 |
| <b>6</b>        | 62.5                   | 200       | 125                    | 200      | 1000     | 1000     | 1000      | 1000                                 |
| <b>7a</b>       | 200                    | 200       | 125                    | 250      | 1000     | >1000    | >1000     | 500                                  |
| <b>7b</b>       | 125                    | 250       | 100                    | 250      | 250      | >1000    | >1000     | 500                                  |
| <b>7c</b>       | 125                    | 250       | 125                    | 250      | 500      | 1000     | 1000      | 100                                  |
| <b>7d</b>       | 100                    | 200       | 200                    | 200      | 1000     | >1000    | >1000     | 500                                  |
| <b>7e</b>       | 200                    | 250       | 200                    | 125      | 1000     | 1000     | 1000      | 62.5                                 |
| <b>7f</b>       | 100                    | 250       | 250                    | 200      | 500      | >1000    | >1000     | 100                                  |
| <b>7g</b>       | 200                    | 100       | 125                    | 62.5     | >1000    | >1000    | >1000     | 1000                                 |
| <b>7h</b>       | 250                    | 100       | 250                    | 200      | 500      | >1000    | >1000     | 1000                                 |
| <b>7i</b>       | 250                    | 100       | 200                    | 100      | >1000    | >1000    | >1000     | 500                                  |
| <b>7j</b>       | 200                    | 62.5      | 125                    | 62.5     | >1000    | >1000    | >1000     | 1000                                 |
| <b>7k</b>       | 250                    | 125       | 200                    | 100      | 500      | >1000    | >1000     | 250                                  |
| <b>7l</b>       | 100                    | 125       | 500                    | 250      | >1000    | 1000     | 1000      | 1000                                 |
| <b>7m</b>       | 100                    | 250       | 200                    | 250      | >1000    | 250      | 1000      | 250                                  |
| <b>7n</b>       | 200                    | 200       | 250                    | 500      | 250      | 500      | 500       | 500                                  |
| <b>7o</b>       | 125                    | 250       | 200                    | 250      | 250      | 500      | 500       | 1000                                 |
| <b>7p</b>       | 200                    | 100       | 125                    | 200      | 1000     | 1000     | 1000      | 500                                  |
| <b>7q</b>       | 250                    | 200       | 62.5                   | 100      | 500      | 1000     | >1000     | 1000                                 |
| <b>7r</b>       | 200                    | 250       | 100                    | 125      | 500      | 1000     | 500       | 250                                  |
| Gentamycin      | 0.05                   | 1         | 0.25                   | 0.5      | n.t.     | n.t.     | n.t.      | n.t.                                 |
| Ampicilin       | 100                    | --        | 250                    | 100      | n.t.     | n.t.     | n.t.      | n.t.                                 |
| Chloramphenicol | 50                     | 50        | 50                     | 50       | n.t.     | n.t.     | n.t.      | n.t.                                 |
| Ciprofloxacin   | 25                     | 25        | 50                     | 50       | n.t.     | n.t.     | n.t.      | n.t.                                 |
| Norfloxacin     | 10                     | 10        | 10                     | 10       | n.t.     | n.t.     | n.t.      | n.t.                                 |
| Nystatin        | n.t.                   | n.t.      | n.t.                   | n.t.     | 100      | 100      | 100       | n.t.                                 |
| Greseofulvin    | n.t.                   | n.t.      | n.t.                   | n.t.     | 500      | 100      | 100       | n.t.                                 |
| Isoniazid       | n.t.                   | n.t.      | n.t.                   | n.t.     | n.t.     | n.t.     | n.t.      | 0.20                                 |

E.C.: *Escherichia coli* Coli, P.A.: *Pseudomonas aeruginosa*, S.A.: *Staphylococcus aureus*, S.P.: *Streptococcus pyogenes*, C.A.: *Candida albicans*, A.N.: *Aspergillus niger*, A.C.: *Aspergillus clavatus*  
n.t.: not tested/l;

**Table 3:** *In vitro* Anti tuberculosis Activity table of newly synthesized compounds **2** to **7r** MICs ( $\mu\text{g/mL}$ )

| Comp.     | Mycobacterium; Tuberculosis;H37 | Comp.     | Mycobacterium ;Tuberculosis H37 |
|-----------|---------------------------------|-----------|---------------------------------|
| <b>2</b>  | 500                             | <b>7h</b> | 1000                            |
| <b>3</b>  | 250                             | <b>7i</b> | 500                             |
| <b>4</b>  | 1000                            | <b>7j</b> | 1000                            |
| <b>5</b>  | 1000                            | <b>7k</b> | 250                             |
| <b>6</b>  | 1000                            | <b>7l</b> | 1000                            |
| <b>7a</b> | 500                             | <b>7m</b> | 250                             |
| <b>7b</b> | 500                             | <b>7n</b> | 500                             |
| <b>7c</b> | 100                             | <b>7o</b> | 1000                            |
| <b>7d</b> | 500                             | <b>7p</b> | 500                             |
| <b>7e</b> | 62.5                            | <b>7q</b> | 1000                            |
| <b>7f</b> | 100                             | <b>7r</b> | 250                             |
| <b>7g</b> | 1000                            | Isoniazid | 0.20                            |

Hinton broth was used as nutrient medium to grow and dilute the drug suspension for the test. Inoculum size for test strain was adjusted to 108 CFU (Colony Forming Unit) per millilitre by comparing the turbidity. DMSO was used as diluent to get desired concentration of drugs to test standard bacterial strains. In addition the MIC of compounds was determined against *M. tuberculosis* H37Rv strain (**Table 3**) by using Lowenstein-Jensen medium (conventional method) as described by Rattan [36].

#### 4. Conclusion

New 6,7-dimethoxy-9*H*-indeno[3',2':4,5]thieno[2,3-*d*]pyrimidine and their arylidene hydrazine derivatives were synthesized from ethyl 2-cyano-2-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-ylidene)acetate by series of reactions. The indeno[3',2':4,5]thieno[2,3-*d*]pyrimidine derivatives screened for antimicrobial and *anti-tuberculosis* activity showed moderate to significant activity against standard drugs.

#### 5. Experimental

Melting points were determined on a Gallenkamp melting point apparatus. The  $^1\text{H}$  (300 MHz) and  $^{13}\text{C}$  (75 MHz) NMR spectra were recorded on a Varian NMR Mercury 300 spectrometer. Chemical shifts were reported in ppm relative to tetramethylsilane (TMS), and multiplicities are given as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), or m (multiple). Infrared spectra were recorded as KBr pellets on a Shimadzu FTIR-408 spectrophotometer. Mass spectra were recorded on a Shimadzu LC-MS: EI QP 2010A mass spectrometer with an ionization potential of 70 eV. Elemental analysis (C, H and N) were performed on Thermo Finnigan Eager 300 EA 1112 series analyzer. Reactions were monitored by thin layer chromatography (TLC), carried out on 0.2 mm silica gel 60 F<sub>254</sub> (Merck) plates using UV light (254 and 366 nm) for detection and compounds were purified by column chromatography using silica gel of 5-20  $\mu\text{m}$  (Merck, 60-120 mesh). Column dimension was 39 x 2 cm<sup>2</sup> and elution volume used was about 200-400 mL for each product. Common reagent grade chemicals were either commercially available and were used without further purification or were prepared by

standard literature procedures.

### 5.1 Ethyl 2-cyano-2-(5,6-dimethoxy-2,3-dihydro-1H-inden-1-ylidene)acetate (2)

The reaction flask fitted with a Dean-Stark trap containing 2,3-dihydro-5,6-dimethoxyinden-1-one, **1** (1.92g, 0.01 mol), ethyl cyanoacetate (1.16 g, 0.0103 mol), acetic acid (5 mL), ammonium acetate (2.03 g, 0.034 mol) and toluene (20 mL) was refluxed for 5 h (TLC check, chloroform: methanol 9:1). The equivalent amount of water collected in Dean-Stark trap indicate completion of reaction. The excess of toluene was removed under reduced pressure and product was filtered off, dried and recrystallized from ethanol/DMF (3:1 v/v). White amorphous solid, yield 2.58g (90%); mp. 180-182 °C; IR: 2974 (CH), 2214 (CN), 1708 (C=O), 1600 (C=C), 1253 (C-O)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300MHz,  $\text{CDCl}_3$ ):  $\delta$  1.38 (t, 3H,  $J = 6.9\text{Hz}$ ,  $\text{CH}_3$ ), 3.05 (t, 2H,  $J = 6.6\text{Hz}$ ,  $\text{CH}_2$ ), 3.50 (t, 2H,  $J = 6.6\text{Hz}$ ,  $\text{CH}_2$ ), 3.98(s, 6H,  $2 \times \text{OCH}_3$ ), 4.31(q, 2H,  $J = 6.9\text{Hz}$ ,  $\text{OCH}_2$ ), 6.90(s, 1H, ArH), 8.19(s, 1H, ArH);  $^{13}\text{C}$ NMR:  $\delta$  14.20, 30.06, 34.96, 56.013, 50.07, 61.23, 91.302, 106.71, 107.06, 117.52, 129.56, 148.73, 149.34, 154.58, 163.908, 175.76; Mass 287(100%) ; Anal. calcd. For  $\text{C}_{16}\text{H}_{17}\text{NO}_4$  (287.32): C, 66.89; H, 5.96; N, 4.87. Found C 67.15; H, 6.11; N, 5.01.

### 5.2 Ethyl 2-amino-5,6-dimethoxy-8H-indeno[2,1-b]thiophene-3-carboxylate (3)

Compound **2** (2.87 g, 0.01 mol) was added to the flask containing elemental sulfur (0.32 g, 0.01 mol), diethyl amine (2.5 mL) and absolute ethanol (25 mL). The reaction mixture was refluxed for 3 h (TLC check in chloroform: methanol 9:1). After cooling the reaction mixture to room temperature, the solid precipitated was filtered, washed with ethanol ( $2 \times 3$  mL) dried and purified by column chromatography eluting with chloroform. White amorphous solid, yield 2.55g (80%); mp: 208-210 °C; IR: 3408, 3300 ( $\text{NH}_2$ ), 2985 (CH), 1705 (C=O), 1654(C=C),

1276(C-O)  $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR (300 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  1.38 (t,  $J = 7.2\text{Hz}$ , 3H,  $\text{CH}_3$ ), 3.56 (s, 2H,  $\text{CH}_2$ ), 3.79 (s, 3H,  $\text{OCH}_3$ ), 3.81 (s, 3H,  $\text{OCH}_3$ ), 4.36 (q,  $J = 7.2\text{Hz}$ , 2H,  $\text{OCH}_2$ ), 7.13(s, 1H, ArH), 7.49(bs, 2H,  $\text{NH}_2$ ), 7.79(s, 1H, ArH); Mass: 319 (80%), 273(100%); Anal. calcd. For  $\text{C}_{16}\text{H}_{17}\text{NO}_4\text{S}$  (319.38): C, 60.17; H, 5.37; N, 4.39. Found C, 60.41; H, 5.49; N, 4.28.

### 5.3 6, 7-Dimethoxy-3H-indeno[3',2':4,5]thieno[2,3-d]pyrimidin-4(9H)-one (4)

Compound **3** (3.19 g, 0.01 mol) was reflux in formamide (25 mL) for 6-7 h (TLC check, chloroform: methanol 9:1). After completion of reaction, the excess of formamide was removed under reduced pressure and the reaction mixture was poured in ice cold water (150 mL). The precipitated solid was then filtered and washed with cold methanol (3 mL) and recrystallized from ethanol. Pale brown crystalline solid, yields 2.25g (75%), m.p.:283-285 [Lit [35]. m.p.: 284-286 °C].

### 5.4 4-Chloro-6,7-dimethoxy-9H-indeno[3',2':4,5]thieno[2,3-d]pyrimidine (5)

The reaction flask containing compound **4** (3.0g, 0.01 mole) was stirred in  $\text{POCl}_3$  (15 mL) and  $\text{PCl}_5$  (0.5 g) at 80-90 °C for 3-4 h (TLC check, chloroform). After completion of reaction, the excess of  $\text{POCl}_3$  was removed under reduced pressure. The reaction mixture was poured on crushed ice and neutralized with 10%  $\text{NaHCO}_3$ . The precipitated solid was filtered, washed with water, dried and purified by column chromatography eluting with chloroform to yield brown crystalline solid. Yield 2.92g (92%); m.p. 292-294 °C [Lit. [35]. m.p. 290-293 °C].

### 5.5 4-Hydrazinyl-6,7-dimethoxy-9H-indeno[3',2':4,5]thieno[2,3-d]pyrimidine (6)

The reaction flask containing 4-chloro-6,7-dimethoxy-9H-indeno[3',2':4,5]thieno[2,3-d]pyrimidine **5** (3.18g, 0.01 mole) and hydrazine

hydrate (99% solution in H<sub>2</sub>O, 0.02 mole, 1 ml approximately) was refluxed in ethanol for 4 h (TLC check, chloroform). After completion of reaction, the reaction mixture cooled to room temperature. The precipitated product was filtered, washed with cold ethanol and dried. The solid obtained was recrystallized from ethanol yield White crystalline solid. Yield 2.82g (90%); m.p. 200-202 °C; IR: 3392, 3352 (NH<sub>2</sub>), 3350 (NH), 2954 (C-H), 1620 (C=C), 1213 (C-O) cm<sup>-1</sup>; <sup>1</sup>HNMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 3.78(s, 2H, CH<sub>2</sub>), 3.84(s, 3H, OCH<sub>3</sub>), 3.89(s, 3H, OCH<sub>3</sub>), 4.52(bs, 1H, NH), 7.22(s, 1H, ArH), 7.55(bs, 2H, NH<sub>2</sub>), 7.72(s, 1H, ArH), 8.65(s, 1H, ArH); Mass 315(M+1, 100 %); Anal. calcd. For C<sub>15</sub>H<sub>14</sub>N<sub>4</sub>O<sub>2</sub>S (314.37): C, 57.31; H, 4.49; N, 17.82. Found C, 57.52; H, 4.65; N, 17.97.

#### General procedure for the synthesis of arylidene hydrazine derivatives 7(a-r)

The reaction mixture of 4-hydrazinyl-6,7-dimethoxy-9H-indeno[3',2':4,5]thieno[2,3-*d*]pyrimidine **6** (3.14 g, 0.01 mol) and aromatic aldehyde (0.01 mol) and catalytic amount of acetic acid (0.5 mL) was stirred in ethanol for 3-4 h, (TLC check, toluene : acetone (3:1 v/v)). The solid precipitated after cooling to room temperature was filtered to yield hydrazones **7** in 83-92% yield. The obtained solid was purified by column chromatography eluting with chloroform. The exact reaction time and yield of each product is given in table 1.

##### 5.6.1 4-(2-(3-Bromobenzylidene)hydrazinyl)-6, 7-dimethoxy-9H-indeno[3',2':4,5]thieno[2,3-*d*]pyrimidine (7a)

Brown color amorphous solid, yield 4.33g (90%); m.p.: 270-272 °C; IR: 3247 (NH), 2993 (C-H) 1625(C=N), 1209(C-O) cm<sup>-1</sup>; <sup>1</sup>HNMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 3.76 (s, 2H, CH<sub>2</sub>), 3.85(s, 3H, OCH<sub>3</sub>), 3.89(s, 3H, OCH<sub>3</sub>), 7.15(s, 1H, ArH), 7.39(t, 1H, J= 8.1Hz, ArH), 7.53(d, 1H, J=8.1Hz, ArH), 7.86(d, 1H, J=8.1Hz, ArH),

7.88(s, 1H, ArH), 8.29(s, 1H, ArH), 8.40(s, 1H, CH), 8.67(s, 1H, ArH), 12.18(bs, 1H, NH); <sup>13</sup>CNMR (75 Hz, DMSO-*d*<sub>6</sub>): δ 34.92, 55.53, 55.60, 107.54, 108.80, 114.73, 122.18, 127.31, 129.51, 130.59, 131.34, 132.21, 137.64, 137.70, 138.57, 140.19, 143.90, 146.74, 147.56, 149.53, 150.88, 163.59; Mass 480(M<sup>+</sup>, 100%), 484(M+4, 33%); Anal. calcd. For C<sub>22</sub>H<sub>17</sub>BrN<sub>4</sub>O<sub>2</sub>S (481.36): C, 54.89; H, 3.56; N, 11.64. Found C, 55.07; H, 3.43; N, 11.77.

##### 5.6.2 6,7-Dimethoxy-4-(2-(3-methoxybenzylidene)hydrazinyl)-9H-indeno[3',2':4,5]thieno[2,3-*d*]pyrimidine (7b)

Brown color amorphous solid, yield 3.93g (91%); m.p.: 246-248 °C; IR: 3299(NH), 2935 (C-H), 1627(C=N), 1209 (C-O) cm<sup>-1</sup>; <sup>1</sup>HNMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 3.79 (s, 2H, CH<sub>2</sub>), 3.85 (s, 3H, OCH<sub>3</sub>), 3.88 (s, 3H, OCH<sub>3</sub>), 3.92 (s, 3H, OCH<sub>3</sub>), 7.01 (d, 1H, J = 7.5Hz, ArH), 7.18 (s, 1H, ArH), 7.38 (t, 1H, J = 7.5Hz, ArH), 7.50 (d, 1H, J = 7.5Hz, ArH), 7.61 (s, 1H, ArH), 7.88 (s, 1H, ArH), 8.46 (s, 1H, CH), 8.73 (s, 1H, ArH), 12.03 (bs, 1H, NH); <sup>13</sup>CNMR (75 Hz, DMSO-*d*<sub>6</sub>): δ 34.88, 55.19, 55.44, 55.57, 107.57, 108.78, 112.64, 114.78, 115.39, 120.85, 129.55, 131.37, 136.56, 137.64, 138.41, 140.21, 143.96, 146.71, 147.52, 149.03, 152.49, 159.45, 163.24. Mass 432(M<sup>+</sup>); Anal. calcd. For C<sub>23</sub>H<sub>20</sub>N<sub>4</sub>O<sub>3</sub>S (432.49): C, 63.87; H, 4.66; N, 12.95. Found C, 64.03; H, 4.79; N, 12.83.

##### 5.6.3 6,7-Dimethoxy-4-(2-(thiophen-2-ylmethylene)hydrazinyl)-9H-indeno[3',2':4,5]thieno[2,3-*d*]pyrimidine (7c)

Yellow amorphous solid, yield 3.47g (85%); m.p.: 256-258 °C; IR: 3350 (NH), 3068(C-H), 1623(C=N), 1211(C-O) cm<sup>-1</sup>; <sup>1</sup>HNMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 3.79 (s, 2H, CH<sub>2</sub>), 3.89 (s, 3H, OCH<sub>3</sub>), 3.91 (s, 3H, OCH<sub>3</sub>), 7.16 (d, 1H, J = 4.2Hz, ArH), 7.18 (s, 1H, ArH), 7.55 (d, 1H, J = 3.0Hz, ArH), 7.69 (d, 1H, J = 5.1Hz,

ArH), 7.88 (s, 1H, ArH), 8.67 (s, 1H, CH), 8.68 (s, 1H, ArH), 11.73 (bs, 1H, NH);  $^{13}\text{C}$ NMR (75 Hz, DMSO- $d_6$ ):  $\delta$  35.42, 56.03, 56.12, 108.07, 109.37, 115.26, 128.28, 129.46, 131.36, 131.88, 138.22, 138.96, 140.45, 140.66, 144.67, 147.27, 147.70, 148.05, 148.79, 163.61; Mass (408  $M^+$ ); Anal. calcd. For  $\text{C}_{20}\text{H}_{16}\text{N}_4\text{O}_2\text{S}_2$  (408.5): C, 58.80; H, 3.95; N, 13.72. Found C, 59.05; H, 4.10; N, 13.83.

**5.6.4 4-(2-(4-Fluorobenzylidene)hydrazinyl)-6,7-dimethoxy-9H-indeno[3',2':4,5]thieno[2,3-d] pyrimidine (7d)**

Pale yellow amorphous solid, yield 3.86g (92%); m.p.: 230-232 °C; IR: 3390(NH), 3074(C-H), 1629(C=N), 1224(C-O)  $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  3.84 (s, 2H,  $\text{CH}_2$ ), 3.90 (s, 3H,  $\text{OCH}_3$ ), 3.92 (s, 3H,  $\text{OCH}_3$ ), 7.21 (s, 1H, ArH), 7.29-7.32 (dd, 2H,  $J = 9.1$  & 8.4Hz, ArH), 7.90 (s, 1H, ArH), 8.04-8.07(dd, 2H,  $J = 8.4\text{Hz}$  & 5.2Hz, ArH), 8.50 (s, 1H, CH), 8.74 (s, 1H, ArH), 12.10 (bs, 1H, NH); Mass 421 ( $M+1$ , 100%); Anal. calcd. For  $\text{C}_{22}\text{H}_{17}\text{FN}_4\text{O}_2\text{S}$  (420.46): C, 62.84; H, 4.08; N, 13.33. Found C, 63.09; H, 3.89; N, 13.53.

**5.6.5 6,7-Dimethoxy-4-(2-(2,3,4-trimethoxybenzylidene)hydrazinyl)-9H-indeno[3',2':4,5]thieno[2,3-d]pyrimidine (7e)**

Yellow amorphous solid, yield 4.38g (89%); m.p.: 220-222 °C; IR: 3357(NH), 2987(C-H), 1622, 1240, 1211, 1193, 1172  $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  3.77 (s, 2H,  $\text{CH}_2$ ), 3.82 (s, 3H,  $\text{OCH}_3$ ), 3.84 (s, 3H,  $\text{OCH}_3$ ), 3.86 (s, 3H,  $\text{OCH}_3$ ), 3.89 (s, 3H,  $\text{OCH}_3$ ), 3.94 (s, 3H,  $\text{OCH}_3$ ), 6.92 (d, 1H,  $J = 8.1\text{Hz}$ , ArH), 7.20 (s, 1H, ArH), 7.85 (s, 1H, ArH), 8.06 (d, 1H,  $J = 8.1\text{Hz}$ , ArH), 8.70 (s, 1H, CH), 8.83 (s, 1H, ArH), 11.96 (bs, 1H, NH); Mass 493 ( $M+1$ , 100%); Anal. calcd. For  $\text{C}_{25}\text{H}_{24}\text{N}_4\text{O}_5\text{S}$  (492.55): C, 60.96; H, 4.91; N, 11.37. Found C, 61.21; H, 5.13; N, 11.08.

**5.6.6 4-((2-(6,7-Dimethoxy-9H-indeno[3',2':4,5]thieno[2,3-d]pyrimidin-4-yl)hydrazono)methyl)-2-methoxyphenol (7f)**

Brown amorphous solid, yield 3.90g (87%); m.p. 168-170 °C; IR: 3527(NH), 3340(OH), 3076(C-H), 1622(C=N), 1209(C-O)  $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  3.78 (s, 2H,  $\text{CH}_2$ ), 3.88 (s, 6H, 2x $\text{OCH}_3$ ), 3.90 (s, 3H,  $\text{OCH}_3$ ), 6.82 (d, 1H,  $J = 8.0\text{Hz}$ , ArH), 7.18 (s, 1H, ArH), 7.26 (d, 1H,  $J = 8.0\text{Hz}$ , ArH), 7.62 (s, 1H, ArH), 7.85 (s, 1H, ArH), 8.38 (s, 1H, CH), 8.73 (s, 1H, ArH), 9.47 (bs, 1H, OH), 11.86 (bs, 1H, NH); Mass 449 ( $M+1$ , 100%); Anal. calcd. For  $\text{C}_{23}\text{H}_{20}\text{N}_4\text{O}_4\text{S}$  (448.49): C, 61.59; H, 4.49; N, 12.49. Found C, 61.80; H, 4.25; N, 12.58.

**5.6.7 6,7-Dimethoxy-4-(2-(3-nitrobenzylidene)hydrazinyl)-9H-indeno[3',2':4,5]thieno[2,3-d] pyrimidine (7g)**

Brown amorphous solid, yield 3.75g (84%); m.p. 236-238 °C; IR: 3338(NH), 3074(C-H), 1627(C=N), 1348( $\text{NO}_2$ ), 1209(C-O)  $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  3.77 (s, 2H,  $\text{CH}_2$ ), 3.92 (s, 3H,  $\text{OCH}_3$ ), 3.94 (s, 3H,  $\text{OCH}_3$ ), 7.22 (s, 1H, ArH), 7.76 (t, 1H,  $J = 7.5\text{Hz}$ , ArH), 7.90 (s, 1H, ArH), 8.25 (d, 1H,  $J = 7.5\text{Hz}$ , ArH), 8.42 (d, 1H,  $J = 7.5\text{Hz}$ , ArH), 8.62 (s, 1H, ArH), 8.73 (s, 1H, CH), 8.85(s, 1H, ArH), 12.31 (bs, 1H, NH); Mass 448 ( $M+1$ , 100%); Anal. calcd. For  $\text{C}_{22}\text{H}_{17}\text{N}_5\text{O}_4\text{S}$  (447.47): C, 59.05; H, 3.83; N, 15.65. Found C, 59.28; H, 3.99; N, 15.79.

**5.6.8 6,7-Dimethoxy-4-(2-(4-methylbenzylidene)hydrazinyl)-9H-indeno[3',2':4,5] thieno[2,3-d] pyrimidine (7h)**

off white amorphous solid, yield 3.79g (91%); m.p. 250-250 °C; IR: 3288, 3024, 2989, 2939, 1627, 1608, 1244, 1207, 1184  $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  2.15 (s, 3H,  $\text{CH}_3$ ), 3.76 (s, 2H,  $\text{CH}_2$ ), 3.88 (s, 6H, 2x $\text{OCH}_3$ ), 7.15 (s, 1H, ArH), 7.25 (d,  $J = 8.2\text{Hz}$ , 2H, ArH), 7.77 (d,  $J$

= 8.2Hz, 2H, ArH), 7.84 (s, 1H, ArH), 8.42 (s, 1H, CH), 8.70 (s, 1H, ArH), 11.95 (bs, 1H, NH); Mass 416 (M<sup>+</sup>); Anal. calcd. For C<sub>23</sub>H<sub>20</sub>N<sub>4</sub>O<sub>2</sub>S (416.5): C, 66.33; H, 4.84; N, 13.45. Found C, 66.58; H, 5.02; N, 13.57.

**5.6.9 4-(2-Benzylidenehydrazinyl)-6,7-dimethoxy-9H-indeno[3',2':4,5]thieno[2,3-d]pyrimidine (7i)**

Yellow amorphous solid; yield 3.62g (90%); m.p. 238-240 °C; IR: 3353, 3074, 3002, 2954, 1625, 1209, 1191 cm<sup>-1</sup>; <sup>1</sup>HNMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 3.78 (s, 2H, CH<sub>2</sub>), 3.90 (s, 3H, OCH<sub>3</sub>), 3.92 (s, 3H, OCH<sub>3</sub>), 7.20 (s, 1H, ArH), 7.44-7.46 (m, 3H, ArH), 7.89 (s, 1H, ArH), 8.01-8.05 (m, 2H, ArH), 8.50 (s, 1H, CH), 8.75 (s, 1H, ArH), 12.09 (bs, 1H, NH); Mass 403 (M+1, 100%); Anal. calcd. For C<sub>22</sub>H<sub>18</sub>N<sub>4</sub>O<sub>2</sub>S (402.47): C, 65.65; H, 4.51; N, 13.92. Found C, 65.91; H, 4.68; N, 14.07.

**5.6.10 2-((2-(6,7-Dimethoxy-9H-indeno[3',2':4,5]thieno[2,3-d]pyrimidin-4-yl)hydrazono) phenomethyl) (7j)**

Pale yellow amorphous solid, yield 3.72g (89%); m.p. 242-244 °C; IR: 3441 (NH), 3353(OH), 2991(C-H), 1625(C=N), 1209(C-O), cm<sup>-1</sup>; <sup>1</sup>HNMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 3.78 (s, 2H, CH<sub>2</sub>), 3.90 (s, 6H, 2xOCH<sub>3</sub>), 6.90-7.92 (m, 4H, ArH), 7.22 (s, 1H, ArH), 7.85 (s, 1H, ArH), 8.79 (s, 1H, CH), 8.84 (s, 1H, ArH), 10.16 (bs, 1H, OH), 11.98 (bs, 1H, NH); Mass 419 (M+1, 100%); Anal. calcd. For C<sub>22</sub>H<sub>18</sub>N<sub>4</sub>O<sub>3</sub>S (418.47): C, 63.14; H, 4.34; N, 13.39. Found C, 63.37; H, 4.46; N, 13.23.

**5.6.11 6,7-Dimethoxy-4-(2-(naphthalen-1-ylmethylene)hydrazinyl)-9H-indeno[3',2':4,5]thieno[2,3-d]pyrimidine (7k)**

Brown amorphous solid, yield 3.75g (83%); m.p. 224-226 °C; IR: 3363(NH), 3070(C-H), 1627(C=N), 1207(C-O) cm<sup>-1</sup>; <sup>1</sup>HNMR (300

MHz, DMSO-*d*<sub>6</sub>): δ 3.79 (s, 2H, CH<sub>2</sub>), 3.91 (s, 3H, OCH<sub>3</sub>), 3.97 (s, 3H, OCH<sub>3</sub>), 7.18 (s, 1H, ArH), 7.50-8.22 (m, 8H, ArH), 8.45 (s, 1H, CH), 8.80 (s, 1H, ArH), 11.86 (bs, 1H, NH); Mass 453 (M+1, 100%); Anal. calcd. For C<sub>26</sub>H<sub>20</sub>N<sub>4</sub>O<sub>2</sub>S (452.53): C, 69.01; H, 4.45; N, 12.38. Found C, 69.29; H, 4.60; N, 12.25.

**5.6.12 6,7-Dimethoxy-4-(2-(pyridin-4-ylmethylene)hydrazinyl)-9H-indeno[3',2':4,5]thieno[2,3-d]pyrimidine (7l)**

Brown amorphous solid, yield 3.46g (86%); m.p. 210-212 °C; IR: 3392(NH), 3080(C-H), 1623(C=N), 1211(C-O) cm<sup>-1</sup>; <sup>1</sup>HNMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 3.76 (s, 2H, CH<sub>2</sub>), 3.80 (s, 3H, OCH<sub>3</sub>), 3.84 (s, 3H, OCH<sub>3</sub>), 7.21 (s, 1H, ArH), 7.51 (d, 2H, *J* = 8.5Hz, ArH), 7.79 (s, 1H, ArH), 8.01 (d, 2H, *J* = 8.5Hz, ArH), 8.55 (s, 1H, CH), 8.75 (s, 1H, ArH), 11.98 (bs, 1H, NH); Mass 404 (M+1, 100%); Anal. calcd. For C<sub>21</sub>H<sub>17</sub>N<sub>5</sub>O<sub>2</sub>S (403.46): C, 62.52; H, 4.25; N, 17.36. Found C, 62.79; H, 4.11; N, 17.22.

**5.6.13 6,7-Dimethoxy-4-(2-(4-methoxybenzylidene)hydrazinyl)-9H-indeno[3',2':4,5]thieno[2,3-d]pyrimidine (7m)**

Pale yellow amorphous solid, yield 3.84g (89%); m.p. 228-230 °C; IR: 3290(NH), 3072(C-H), 1627(C=N), 1207(C-O) cm<sup>-1</sup>; <sup>1</sup>HNMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 3.77 (s, 2H, CH<sub>2</sub>), 3.83 (s, 3H, OCH<sub>3</sub>), 3.86 (s, 3H, OCH<sub>3</sub>), 3.90 (s, 3H, OCH<sub>3</sub>), 7.01 (d, 2H, *J* = 8.4Hz, ArH), 7.18 (s, 1H, ArH), 7.77 (d, 2H, *J* = 8.4Hz, ArH), 7.80 (s, 1H, ArH), 8.43 (s, 1H, CH), 8.60 (s, 1H, ArH), 11.95 (bs, 1H, NH); Mass 432 (M<sup>+</sup>, 100%); Anal. calcd. For C<sub>23</sub>H<sub>20</sub>N<sub>4</sub>O<sub>3</sub>S (432.49): C, 63.87; H, 4.66; N, 12.95. Found C, 64.14; H, 4.81; N, 12.81.

**5.6.14 6,7-Dimethoxy-4-(2-(2-methylpropylidene)hydrazinyl)-9H-indeno[3',2':4,5]thieno[2,3-d]pyrimidine**

**(7n)**

Off white amorphous solid, yield 3.13g (85%); m.p.: 178-180 °C; IR: 3361(NH), 2956(C-H), 1643(C=N), 1207(C-O)  $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  1.17 (d, 6H, 2x  $\text{CH}_3$ ), 1.26 (m, 1H, CH), 3.77 (s, 2H,  $\text{CH}_2$ ), 3.85 (s, 3H,  $\text{OCH}_3$ ), 3.88 (s, 3H,  $\text{OCH}_3$ ), 7.12 (s, 1H, ArH), 7.75 (s, 1H, ArH), 8.18 (s, 1H, CH), 8.60 (s, 1H, ArH), 11.56 (bs, 1H, NH); Mass 369 (M+1, 100%); Anal. calcd. For  $\text{C}_{19}\text{H}_{20}\text{N}_4\text{O}_2\text{S}$  (368.45): C, 61.94; H, 5.47; N, 15.21. Found C, 62.13; H, 5.63; N, 15.34.

**5.6.15 6,7-Dimethoxy-4-((E)-2-((E)-3-phenylallylidene)hydrazinyl)-9H-indeno[3',2':4,5]thieno [2,3-d]pyrimidine (7o)**

Yellow amorphous solid, yield 3.59g (84%); m.p. 248-250 °C; IR: 3350(NH), 3059(C-H), 1620(C=C), 1635(C=N), 1208(C-O)  $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  3.79 (s, 2H,  $\text{CH}_2$ ), 3.86 (s, 3H,  $\text{OCH}_3$ ), 3.91 (s, 3H,  $\text{OCH}_3$ ), 7.14 (m, 3H, ArH), 7.17 (s, 1H, ArH), 7.34 (d, 1H,  $J = 16.2\text{Hz}$ , CH), 7.40 (dd, 1H,  $J = 16.2$  &  $7.2\text{Hz}$ , CH), 7.60 (m, 2H, ArH), 7.81 (s, 1H, ArH), 8.32 (d, 1H,  $J = 7.2\text{Hz}$ , CH), 8.67 (s, 1H, ArH), 11.97 (bs, 1H, NH); Mass 429 (M+1, 100%); Anal. calcd. For  $\text{C}_{24}\text{H}_{20}\text{N}_4\text{O}_2\text{S}$  (428.51): C, 67.27; H, 4.70; N, 13.07. Found C, 67.49; H, 4.85; N, 12.91.

**5.6.16 4-(2-(3,4-Dimethoxybenzylidene)hydrazinyl)-6,7-dimethoxy-9H-indeno[3',2':4,5]thieno [2,3-d]pyrimidine (7p)**

Faint brown amorphous solid, yield 4.25g (92%); m.p. 260-62 °C; IR: 3328(NH), 2948(C-H), 1623(C=N), 1263(C-O)  $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  3.77 (s, 2H,  $\text{CH}_2$ ), 3.80 (s, 3H,  $\text{OCH}_3$ ), 3.84 (s, 3H,  $\text{OCH}_3$ ), 3.86 (s, 3H,  $\text{OCH}_3$ ), 3.90 (s, 3H,  $\text{OCH}_3$ ), 7.02 (d, 1H,  $J = 8.1\text{Hz}$ , ArH), 7.18 (s, 1H, ArH), 7.37 (d, 1H,  $J = 8.1\text{Hz}$ , ArH), 7.66 (s, 1H, ArH), 7.86 (s, 1H,

ArH), 8.42 (s, 1H, CH), 8.73 (s, 1H, ArH), 11.93 (bs, 1H, NH); Mass 463 (M+1, 100%); Anal. calcd. For  $\text{C}_{24}\text{H}_{22}\text{N}_4\text{O}_4\text{S}$  (462.52): C, 62.32; H, 4.79; N, 12.11. Found C, 62.57; H, 4.93; N, 12.23.

**5.6.17 4-(2-(4-Chlorobenzylidene)hydrazinyl)-6,7-dimethoxy-9H-indeno[3',2':4,5]thieno[2,3-d] pyrimidine (7q)**

Yellow amorphous solid, yield 3.97g (91%); m.p. 250-252 °C; IR: 3348(NH), 3072(C-H), 1633(C=N), 1207(C-O)  $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  3.79 (s, 2H,  $\text{CH}_2$ ), 3.89 (s, 3H,  $\text{OCH}_3$ ), 3.92 (s, 3H,  $\text{OCH}_3$ ), 6.75 (d, 2H,  $J = 8.4\text{Hz}$ , ArH), 7.21 (s, 1H, ArH), 7.77 (s, 1H, ArH), 7.80 (d, 2H,  $J = 8.4\text{Hz}$ , ArH), 8.39 (s, 1H, CH), 8.77 (s, 1H, ArH), 11.86 (bs, 1H, NH); Mass 436 (M+, 100%), 438 (M+2, 33.5%); Anal. calcd. For  $\text{C}_{22}\text{H}_{17}\text{ClN}_4\text{O}_2\text{S}$  (436.91): C, 60.48; H, 3.92; N, 12.82. Found C, 60.75; H, 4.09; N, 12.97.

**5.6.18 4-((2-(6,7-Dimethoxy-9H-indeno[3',2':4,5]thieno[2,3-d]pyrimidin-4-yl)hydrazono)methyl)-N,N-dimethylaniline (7r)**

Yellow amorphous solid, yield 3.69g (83%); m.p. 280-282 °C; IR: 3301(NH), 3004(CH), 1620(C=N), 1209(C-O)  $\text{cm}^{-1}$ ;  $^1\text{H}$ NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  3.79 (s, 2H,  $\text{CH}_2$ ), 3.80 (s, 3H,  $\text{NCH}_3$ ), 3.82 (s, 3H,  $\text{NCH}_3$ ), 3.91 (s, 3H,  $\text{OCH}_3$ ), 3.93 (s, 3H,  $\text{OCH}_3$ ), 7.03 (d, 1H,  $J = 8.1\text{Hz}$ , ArH), 7.22 (s, 1H, ArH), 7.44 (d, 1H,  $J = 8.1\text{Hz}$ , ArH), 7.69 (d, 1H,  $J = 8.1\text{Hz}$ , ArH), 7.77 (s, 1H, ArH), 7.89 (d, 2H,  $J = 8.1\text{Hz}$ , ArH), 8.45 (s, 1H, CH), 11.96 (bs, 1H, NH); Mass 445 (M+, 100%); Anal. calcd. For  $\text{C}_{24}\text{H}_{23}\text{N}_5\text{O}_2\text{S}$  (445.54): C, 64.70; H, 5.20; N, 15.72. Found C, 64.96; H, 5.38; N, 15.85.

**6. Biological assay**

**6.1 In vitro evaluation of antimicrobial activity**

The MICs of synthesized compounds were carried out by broth micro dilution method. DMSO was used as diluents to get desired concentration of drugs to test with standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately sub cultured (before inoculation) by spreading a loop full evenly over a quarter of plate of medium suitable for the growth of the test organisms and put for incubation at 37 °C overnight. The tubes were then incubated overnight. The MIC of the control organism was read to check accuracy of the drug concentrations. The lowest concentration inhibiting growth of organism was recorded as the MIC. The tubes not showing visible growth (in the same manner as control tube described above) was sub cultured and incubated overnight at 37 °C. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show similar number of colonies indicating bacteriostatic. The reduced number of colonies indicates partial or slow bactericidal activity and no growth if whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. Each synthesized molecule was diluted to obtain 2000 µg/ml concentration, as stock solution. In primary screening 500, 250 and 125 µg/ml concentrations of the synthesized drugs were taken. The active synthesized molecules found in this primary screening were further tested in a second set of dilution against all microorganisms. Some molecules found active in primary screening were similarly diluted to obtain 100, 50, 25, 12.5, 6.250, 3.125 and 1.5625 µg/ml concentrations. The highest dilution showing at least 99% inhibition was taken as MIC. Compounds **3**, **4**, **6**, **7b**, **7c**, **7d**, **7l**, **7m**, **7o** showed significant antibacterial activity compared with Ampicilin and **5**, **7b**, **7c**, **7f**, **7h**, **7k**, **7n**, **7o**, **7q**, **7r** showed significant antifungal activity compared with Greseofulvin (**Table 2**). These compounds were also tested

for inhibition of mycobacterium tuberculosis H37Rv. (**Table 3**).

## 6.2 In vitro evaluation of antitubercular activity

The preliminary antitubercular screening for test compounds was obtained for M. tuberculosis H37Rv, the MIC of each drug was determined by broth dilution assay by L. J. agar (MIC) method [36] where primary 1000, 500 and 250 mg/ml and secondary 200, 100, 62.5, 50, 25, 12.5, 6.25 and 3.25 µg/ml dilutions of each test compound were added liquid L. J. Medium and then media were sterilized by inspissation method. A culture of M. tuberculosis H37Rv growing on L. J. Medium was harvested in 0.85% saline in bijoux bottles. All test compound makes first stock solution of 2000 µg/ml concentration of compounds was prepared in DMSO. These tubes were then incubated at 37 °C for 24 h followed by streaking of M. tuberculosis H37Rv (5 x 10<sup>4</sup> bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 12 days, 22 days and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with M. tuberculosis H37Rv. The concentration at which no development of colonies occurred or <20 colonies was taken as MIC concentration of test compound. The standard strain M. tuberculosis H37Rv was tested with known drug Isoniazid. All the tested compounds shows poor antitubercular activity compared with standard isoniazid (**Table 3**).

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