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# Synthesis and biological evaluation of novel oxadiazole derivatives: A new class of thymidine phosphorylase inhibitors as potential anti-tumor agents

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ABSTRACT

Based on the fact that the thymidine phosphorylase inhibitors are considered potential anti-tumor agents, a range of novel oxadiazole derivatives **3a**–**3u** was designed and synthesized by a simple and facile synthetic route. The biological assay revealed that majority of compounds displayed modest inhibitory activity against thymidine phosphorylase at low micromolar concentrations ($IC_{50}$ 173.23 ± 3.04 to 14.40 ± 2.45 μM). In the current study the most active compounds were **3h** and **3q** with $IC_{50}$ values 14.40 ± 2.45 and 17.60 ± 1.07 μM, respectively. Molecular docking studies were performed on the most active compounds (**3h**, **3k**, **3o**–**3q**) to show their binding mode.



## 1. Introduction

Angiogenesis is the formation of new blood vessels from pre-existing vessels and it is essential for organ growth and repair. However, it is well known that this is a vital step in the process of cancer growth.[1,2] Thus, angiogenesis inhibitors are believed to be potential candidates for blocking cancer growth. In particular, thymidine phosphorylase (TP) is a pro-angiogenic factor which catalyzes the reversible phosphorolysis of thymidine into thymine and 2′-deoxy-D-ribose 1-phosphate.[3,4] The 2′-deoxy-D-ribose 1-phosphate undergoes further dephosphorylation to produce 2′-deoxy-D-ribose which stimulates the secretion of vascular endothelial growth factor (VEGF). VEGF activates a number of processes including endothelial cells for secretion of matrix metalloproteinases, proliferation, and migration of endothelial cells to tumor tissue. These actions result in fast generation of new blood vessels and cancer metastasis.[5]

TP inhibitors affect the production of 2′-deoxy-D-ribose and in turn suppress tumor growth.[5,6] Therefore, there is an urgent need to develop new and potent thymidine phosphorylase inhibitors which have the ability to suppress the formation of new blood vessels and stop tumor growth. A number of efforts have been reported on the development of TP inhibitors.[5,7–14] The pyrimidine derivative 5-chloro-6-[1-(2-iminopyrrolidinyl)methyl] uracil

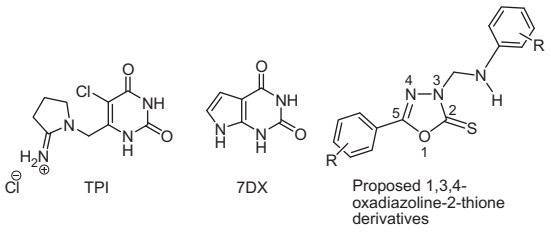


**Figure 1.** Chemical structure of known TP inhibitors—TPI and **7DX**, and proposed 1,3,4-oxadiazole-2-thione derivatives as new class of TP inhibitors.

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hydrochloride (TPI) is the most potent inhibitor of human TP known till date and 7-deazaxanthine (**7DX**) is the first purine derivative identified as TP inhibitor (Fig. 1).[38–40] Moreover, TPI is currently being evaluated in phase II clinical trials for solid tumors.

1,3,4-Oxadiazole ring is well known due to its number of biological properties such as anti-inflammatory, antitumor, anticancer, antimicrobial, antibacterial, anti-trypanosomacruzi, antifungal and anti-HIV activities.[15–23] 1,3,4-Oxadiazole derivatives are also identified as potent inhibitors of dengue and West Nile virus NS2B/NS3 proteases, immunosuppressive agents, anti-hepatitis B virus compounds and also urease inhibitors.[24–28]

In addition, certain Mannich bases derived from 1,3,4-oxadiazoles are reported to possess anti-inflammatory, analgesic activity, antifungal activity, antibacterial activity, anti-HIV activity, anti-tuberculosis and anti-cancer activities.[29–34] Such broad spectrum of therapeutic applications of oxadiazoles and structural resemblance with potent known inhibitors have prompted us to synthesize and evaluate the new derivatives of 1,3,4-oxadiazole-2-thiones as potent TP inhibitors (Fig. 1). The proposed new oxadiazoles contain N–H, C═N, O and CS groups and R substituents which may interact to the enzyme active sites (His85, Ser186, Lys190, Arg171, Tyr168, Phe210) in a similar pattern like TPI. **7DX** was used and considered as a reference compound in the current in vitro TP inhibition studies. In this paper we report the synthesis, TP inhibition, structure activity relationship (SAR) and docking studies of new 1,3,4-oxadiazole derivatives.

## 2. Results and discussion

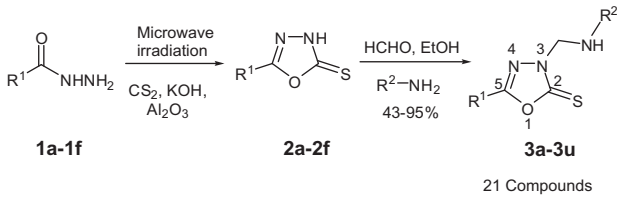
### 2.1. Chemistry

In our current research, a series of new 1,3,4-oxadiazoline-2-thione derivatives **3a**–**3u** bearing different level of substituents were prepared from compounds **2a**–**2f** according to the procedure given in Scheme 1.[18] The precursors 5-substitituted 1,3,4-oxadiazole-2-thiones **2a**–**2f** were prepared according to a previously reported method from the reaction of acid hydrazide with carbon disulfide in the presence of KOH under microwave irradiation.[28,35] A variety of *N*-2′ substituted derivatives **3a**–**3u** were synthesized in good to excellent yields (43–95%) from formalin and a selected range of primary amines (Scheme 1). All final compounds were structurally characterized by IR, NMR, EIMS and elemental analysis.

### 2.2. Biological activities

Twenty one compounds (**3a**–**3u**) were evaluated for TP inhibitory activity. Interestingly, all compounds were active and showed TP inhibition with $IC_{50}$ values ranged between 14.40 ± 2.45 and 173.23 ± 3.04 μM. Enzyme inhibition data is presented in Table 1. 7-Deazaxanthine was used as a standard inhibitor.

In order to address the structure–activity relationship and to achieve potent TP inhibitors various degrees of substituents on phenyl have been introduced. These substituents include electron donating group such as methoxy and electron withdrawing group like nitro. Hydrophobic moieties are also of our interest, for instance, alkyl groups and halogens such groups as Cl and Br. All these groups in conjunction can show important contributions towards TP inhibition activity.

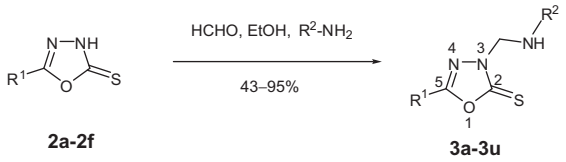



**Scheme 1.** Synthetic protocol of 1,3,4-oxadiazoline-2-thione derivatives **3a**–**3u**.

Compound **3h** showed the lowest $IC_{50}$ value of 14.40 ± 2.45 μM compared to the standard inhibitor 7-deazaxanthine (38.68 ± 4.42 μM). With the lowest $IC_{50}$ value, it was the most active compound against the enzyme and proved better than the standard inhibitor. From TP inhibitory model studies,[37] we may conclude that either core moiety or side chain group –$CH_2$NH– may interact and inhibit the activity. Compounds **3a**–**3u** were prepared in order to determine the effect of varying $R^2$ substituents at 5 ($R^1$)-substituted-1,3,4-oxadiazole for biological activity (see chemical structures in Table 1). A selection of $R^2$ was designed to explore the effect of the substituents on the phenyl ring in the compounds **3a**–**3u**. Therefore selected primary amines (Table 1) were used to synthesize the corresponding Mannich bases **3a**–**3u**. The compound **3a** ($R^1$ = phenyl; $R^2$ = 2″-methoxyphenyl) with a methoxy group at *ortho* position exhibited $IC_{50}$ value 87.24 ± 2.08 μM, which was improved to $IC_{50}$ 40.64 ± 0.23 in the case of its analogue having methoxy group at *meta* position (entry 2, Table 1). Similarly in compound **3d** with $R^2$ = 3″,4″-dimethylphenyl showed good activity with $IC_{50}$ value 40.61 ± 3.17 μM. The enhancement in enzyme inhibitory activity which was observed may be due to positive inductive effect of two methyl groups. A significant decrease in enzyme inhibition was observed in the case of compound **3c** ($R^1$ = phenyl; $R^2$ = 2″-chlorophenyl) with $IC_{50}$ value 117.70 ± 3.70 μM and however is structurally similar to compound **3o** ($R^1$ = 2′-bromophenyl; $R^2$ = 2″-chlorophenyl) which has $IC_{50}$ value 21.83 ± 3.95 μM. This lower activity is probably due to lack of bromine group at *ortho* position of benzene moiety in compound **3c**. Compound **3e** with $IC_{50}$ value 117.19 ± 2.38 μM was found to be less potent than **3f** ($IC_{50}$ 69.32 ± 0.43 μM) possibly it is due to unfavorable interactions with TP. Possibly increased inhibitory activity of **3f** ($R^1$ = 3′,4′,5′-trimethoxyphenyl; $CH_2CH_2$–Ph) was observed as a result of the presence of flexible $CH_2CH_2$–Ph chain in **3f** (Table 1, entries 5 & 6). It seems enzyme inhibition is also dependent on flexibility of side chain. In compound **3g**, the presence of $R^1$ = 2′-hydroxyphenyl and $R^2$ = 2″-chlorophenyl resulted in substantial decrease in inhibitory activity ($IC_{50}$ = 130.50 ± 0.87) (Table 1, entry 7). In compound **3h** the presence of strongly electron donating methoxy group at postion-2″ and the electron withdrawing nature of polar nitro group at position-5″ of phenyl ring has improved potency of the compound which is in fact greater than the standard used in this study. Typically, a nitro substituent at position-5″ in compound **3h** appeared to be important for activity (Table 1, entry 8). Based on this activity, and selecting **3h** as a lead from our first library, we set about probing the chemical space around the NH–Ar by including a variety of substituents of varying size at aromatic ring, whilst retaining the 4'-methylphenyl substituent at C-5 in compounds **3h**–**3n** (Table 1, entries 8–14).). In comparison with compound **3m** ($IC_{50}$ 83.19 ± 0.50), compound **3l** (133.77 ± 0.54) has been observed to exhibit lower activity which clearly states that dimethyls– are better substituents as compare to dichloro– (Table 1, entries 12 and 13). Compound **3h** was found to be most active in the tested series with $IC_{50}$ 14.40 ± 2.45 μM, in this case especially 5″-nitrophenyl is considered to be an important group which has maximally increased the activity (see Section 2.3 for details). In fact substituents in the $R^2$ has greater impact on activity, since weak enzyme inhibitory activity ($IC_{50}$ = 104.12 ± 0.75 μM) was unveiled by compound **3j** with un-substituted phenyl group (Table 1, entry 10) as compare to other compounds bearing substituted phenyl group (Table 1 entries 8, 9 and 11–14).

The nature and position of halogen groups in the $R^1$ and $R^2$ have been found to be very important in enzyme inhibition activity. For example, compound **3q** with 2′-bromophenyl and 2″-methoxyphenyl

**Table 1**
TP inhibitory activities of Mannich bases of 5-substituted-1,3,4-oxadiazole-2-thiones **3a**–**3u** in term of $IC_{50}$ values (μM)




| Entry | Compounds | $R^1$ | $R^2$ | Yield (%) | Temperature (°C) | Time (min) | $IC_{50}$[a] (μM) |
|---|---|---|---|---|---|---|---|
| 1 | **3a** | Phenyl | 2″-Methoxyphenyl | 95 | RT | 5 | 87.24 ± 2.08 |
| 2 | **3b** | Phenyl | 3″-Methoxyphenyl | 88 | RT | 4 | 40.64 ± 0.23 |
| 3 | **3c** | Phenyl | 2″-Chlorophenyl | 90 | RT | 10 | 117.70 ± 3.70 |
| 4 | **3d** | Phenyl | 3″,4″-Dimethylphenyl | 83 | RT | 8 | 40.61 ± 3.17 |
| 5 | **3e** | 3′,4′,5′-Trimethoxyphenyl | 2″-Methylphenyl | 80 | RT | 2 | 117.19 ± 2.38 |
| 6 | **3f** | 3′,4′,5′-Trimethoxyphenyl | 2″-phenylethyl | 78 | RT | 5 | 69.32 ± 0.43 |
| 7 | **3g** | 2′-Hydroxyphenyl | 2″-Chlorophenyl | 77 | RT | 4 | 130.50 ± 0.87 |
| 8 | **3h** | 4′-Methylphenyl | 2″-Methoxy-5″-nitrophenyl | 43 | 30 | 30 | 14.40 ± 2.45 |
| 9 | **3i** | 4′-Methylphenyl | 4″-Methylphenyl | 83 | RT | 9 | 54.72 ± 0.47 |
| 10 | **3j** | 4′-Methylphenyl | Phenyl | 81 | RT | 8 | 104.12 ± 0.75 |
| 11 | **3k** | 4′-Methylphenyl | 2″,3″-Dimethyl | 62 | RT | 7 | 23.70 ± 0.97 |
| 12 | **3l** | 4′-Methylphenyl | 3″,4″-Dichlorophenyl | 71 | 30 | 12 | 133.77 ± 0.54 |
| 13 | **3m** | 4′-Methylphenyl | 3″,4″-Dimethylphenyl | 74 | RT | 6 | 83.19 ± 0.50 |
| 14 | **3n** | 4′-Methylphenyl | 3″-Methoxyphenyl | 76 | RT | 2 | 84.40 ± 1.38 |
| 15 | **3o** | 2′-Bromophenyl | 2″-Chlorophenyl | 93 | RT | 3 | 21.83±3.95 |
| 16 | **3p** | 2′-Bromophenyl | 4″-Bromophenyl | 68 | RT | 7 | 26.33±3.28 |
| 17 | **3q** | 2′-Bromophenyl | 2″-Methoxyphenyl | 94 | RT | 4 | 17.60 ± 1.07 |
| 18 | **3r** | 4′-Chlorophenyl | 2″-Methylphenyl | 73 | RT | 10 | 58.21 ± 5.01 |
| 19 | **3s** | 4′-Chlorophenyl | 4″-Bromophenyl | 51 | RT | 13 | 173.23 ± 3.04 |
| 20 | **3t** | 4′-Chlorophenyl | 2″-Chlorophenyl | 82 | 30 | 9 | 46.38 ± 3.42 |
| 21 | **3u** | 4′-Chlorophenyl | 2″-Methoxyphenyl | 87 | RT | 7 | 88.73 ± 2.81 |
| | | | Standard inhibitor 7-Deazaxanthine | — | — | — | 38.68 ± 4.42 |

[a] Enzyme inhibition $IC_{50}$ values are means of three independent experiments (mean ± SEM, $n = 3$).

showed quite high activity ($IC_{50}$ 17.60 ± 1.07 μM) and similarly, compound **3o** having 2′-bromophenyl and 2″-chlorophenyl also exhibited good activity with $IC_{50}$ 21.83 ± 3.95 μM. However, Mannich bases **3r**–**3u** where $R^1$ is 4-chlorophenyl displayed reduced activity with $IC_{50}$ values (46.38 ± 3.42 μM to 173.23 ± 3.04 μM).

## 2.3. Molecular modelling

PDB database contains a few three-dimensional structures of enzyme from two different sources: human and *Escherichia coli*.[41] Among them 4EAD complex was selected for the analysis of the binding mode of the most active derivative **3h** and the assay reference compound—7-deazaxanthine (**7DX**). This one, recently published in PDB, was *E. coli* TP with high resolution of 1.50 Å—enzyme of the same source as used in biological experiments. TP contains important α and α/β domains which are essential for the enzymatic activity as they are necessary for closing the active site cleft for action to take place.[42] This kind of change facilitates the nucleophilic attack of phosphate onto the sugar ring.[43] There are three forms of enzyme: the most open, transition and the most closed.[42] Complexes with inhibitors—substrate analogues usually occur in the most closed form such as 4EAD which contains 3′-azido-2′-fluoro-dideoxyuridine (**ONP**). The inhibitor binds in very characteristic way. The pyrimidine base part creates some hydrogen bonds with Arg171, Ser186, Lys190 and a few water molecules, it also stacks to Tyr168, meanwhile the sugar part interacts with Phe210 and water molecules. The crystal structure also contains a sulfate ion in the phosphate binding site because of crystallization conditions. Before any calculations, the sulfate ion was replaced by phosphate in its dihydrogen form as suggested literature.[44,45] Docking of novel compounds was preceded by validation process which was based on two reference inhibitors: 3′-azido-2′-fluoro-dideoxyuridine (**ONP**) and 5-chloro-6-[1-(2-iminopyrrolidinyl)methyl] uracil (**TPI**). Both of them were crystalized with TP (PDB codes: 4EAD and 1UOU, respectively). Docking procedure reproduced original arrangement of native ligand **ONP** in *E. coli* TP (4EAD) very well with low rmsd value (rmsd = 0.8). In case of docking **TPI** to the same enzyme, all interactions from its native complex (with human TP, 1UOU) occurred in complex with *E. coli* TP. To calculate rmsd value both enzymes (*E. coli* and human TP) were superimposed in respect of the most important amino acids from the active site (His85, Ser186, Lys190, Arg171, Tyr168, Phe210) and then docked pose was fully compared with original state. Comparison showed good fit with relatively low rmsd. Validation confirmed that docking parameters were set properly and enabled to start docking of 1,3,4-oxadiazole-2-thione derivatives and **7DX**.

Structure of 7-deazaxanthine can be treated as two condensed parts—uracil (pyrimidine-dione) and pyrrol. Due to high similarity to substrate, **7DX** interacted in a similar manner. The NH and CO groups created hydrogen bonds with Arg171, Ser186 and Lys190, and the ring of molecule formed π-π stacking with Tyr168. The strength of binding was assessed by GoldScore function and for 7-deazaxanthine obtained value 63.78 ($IC_{50}$ 38.68 μM).

The most active compounds from the whole series (**3h**, **3k**, **3o**–**3q**) represented similar general binding mode. $R^2$ substituent was located in pyrimidine base region, oxadiazoline-thione in the sugar region (middle part) and $R^1$ substituent projected towards phosphate binding site. The docking runs for them were better converged in comparison with other derivatives. The differences in the binding mode concerned the substituents present in both phenyl rings from $R^1$ and $R^2$. The most active compound **3h** was analyzed in detail. It was assessed by GoldScore better than **7DX**. The score value obtained 82.60 ($IC_{50}$ 14.40 μM) which stayed in accordance with experimental results. The schematic and detailed binding mode of this derivative is presented in the Figure 2. The highest activity of inhibitor **3h** was provided by the presence of

nitro group which could form H-bonds with Arg171 and Ser186. The phenyl ring from $R^2$ substituent interacted with protonated amine group of Lys190 and with side chain of Tyr168. The oxygen atom from oxadiazoline ring formed hydrogen bond with HO(Thr120). Thione and methoxy groups were directed towards Leu117 and created hydrophobic interactions. $R^1$ substituent could provide some less important 'aromatic' hydrogen bonds (CH—O=C(Gly89), CH—phosphate).

Comparing **7DX** and **3h**, the reference compound due to the smaller molecule occupied only area where methoxy-nitrophenyl moiety of derivative **3h** was located.

## 3. Conclusion

Twenty one new Mannich bases possessing the 1,3,4-oxadiazole core have been effectively synthesized and most of them have been identified as modest TP inhibitors. A number of derivatives have been found to be more active as compared to reference compound **7DX**. SAR demonstrated that the increase or decrease in TP inhibitory activities is very much dependent on the nature and position of substituents in the $R^1$ and $R^2$. Compound **3h** bearing the nitro moiety was the most potent among the tested oxadiazoles having an $IC_{50}$ value 14.40 ± 2.45 μM which is about 2.5 fold more active than **7DX**. Molecular modelling not only explained the binding mode of more active compounds (**3h**, **3k**, **3o**–**3q**) among the series but it has also exhibited the importance of nitro group in the most active compound **3h,** which can possibly generate H-bonds with Arg171 and Ser186. Thus, the current research opens a new direction for design and development of new and more potent TP inhibitors with promising anti-tumor activity.

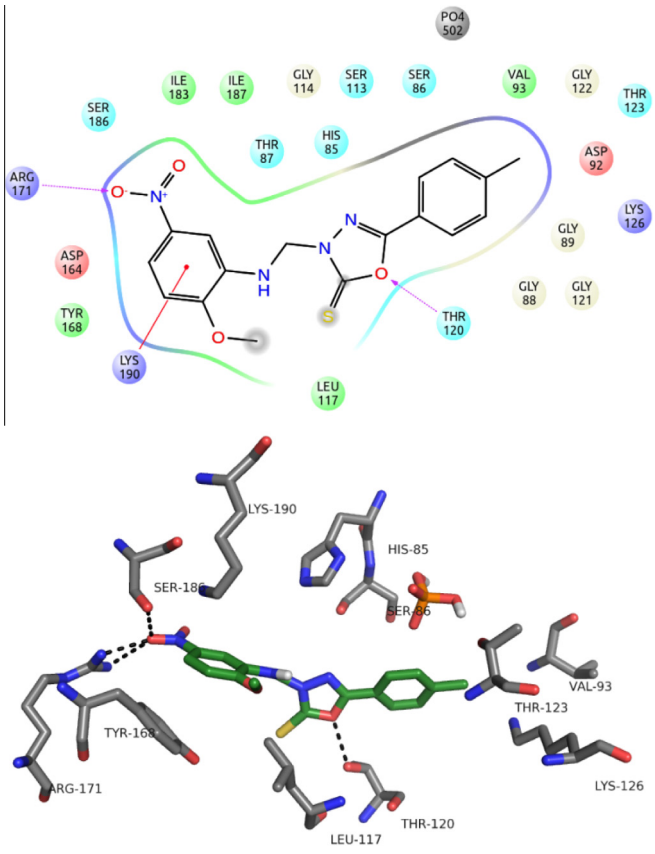



**Figure 2.** Schematic and detailed representation of binding mode for compound **3h**. Upper part shows the most important interactions (2 H-bonds and cation-π interaction), meanwhile lower one presents all H-bonds.

## 4. Experimental

### 4.1. General methods

The ultraviolet spectra were measured in chloroform on a Lambda 5 UV/vis. spectrophotometer (Perkin–Elmer). IR spectra (KBr discs) were recorded on a Bruker FT-IR IFS48 spectrophotometer. EI mass spectra data were recorded with various MAT 711 (70 eV) spectrophotometers and data are tabulated as *m*/*z*. $^1$H NMR spectra were recorded in $CD_3COCD_3$, $CDCl_3$ and DMSO-$d_6$ using Bruker AC (300 and 400, 500 MHz) spectrometers, respectively. Splitting patterns were as follows s (singlet), d (doublet), dt (doublet of triplet), dd (double doublets), t (triplet), and m (multiplet). Chemical shifts are reported in $\delta$ (ppm) and coupling constants are given in Hz. The progress of all reactions was monitored by TLC, which was performed on 2.0 × 5.0 cm aluminum sheets precoated with silica gel $60F_{254}$ to a thickness of 0.25 mm (Merck). The chromatograms were visualized under ultraviolet light (254–366 nm) or iodine vapors. All the reagents were commercially available (Flulka, Aldrich, and Wako).

Compounds **2a**–**2f** were prepared according to our previously reported procedures (**29**)

### 4.2. General procedure for the synthesis of 5-substituted-1,3,4-oxadiazole-2-thione (2a–2f)

A mixture of respective hydrazide (10 mmol), potassium hydroxide (0.56 g, 10 mmol) and alumina were finely ground in a glove box with a mortar and pestle. Carbon disulfide (1.2 ml, 20 mmol) was added to this mixture in a pyrex glass vial, which was placed in a screw-capped thick-walled Teflon® vessel. Microwave-irradiation (MW domestic type oven 900 W with a frequency 2450 MHz, Dawlance, Pakistan) was applied for 3–7 min. After the completion of reaction (TLC analysis), ethanol was added into reaction mixture and filtered. Filtrate was evaporated; distilled water was added to semi-solid material and acidified with hydrochloric acid to pH = 4. Precipitates so obtained were filtered and dried to afford off white solid and then recrystallized from ethanol/water (50:50) mixture to afford compounds **2a**–**2f**. The compounds **2a**–**2f** were completely characterized and data was in good match with previous reports.[28,35]

### 4.3. General procedure for the preparation of 3-(substituted amino methyl)-5-(substituted)-1,3,4-oxadiazoline-2-thione (3a–3u)

Formalin 40% (0.75 ml, 0.01 mol) was added to a stirred solution of 5-substituted 1,3,4-oxadiazole-2-thione **2a**–**2f** (0.01 mol) in absolute ethanol (25 ml). An ethanolic solution (10 ml) of appropriate amine (0.01 mol) was added portion wise to the reaction mixture, stirred for 3–6 h at room temperature, and left overnight in a refrigerator. The precipitate formed was filtered, washed with cold ethanol, dried, and recrystallized from the suitable solvent.[18]

#### 4.3.1. 3-(2-Methoxyphenylaminomethyl)-5-(phenyl)-1,3,4-oxadiazoline-2-thione (3a)

Yield: 2.97 g (95%); mp: 127 °C; $R_f$ = 0.60 (ethyl acetate/hexane/chloroform = 1:1:0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1616 (C=N), 1595 (C=C), 1438 (C=S), 1249 and 1186 (C–O–C); $^1$H NMR (300 MHz, $CD_3COCD_3$): $\delta$ 7.70–7.51 (m, 5H, H–Ar), 7.28 (dd, 1H, *J* = 6.0, *J* = 1.5 Hz, H-6″), 7.05 (m, 1H, H-5″), 6.94 (d, 1H, *J* = 5.8, H-3″), 6.89 (dd, 1H, *J* = 6.0, 5.8 Hz, H-4″), 5.79 (bt, 1H, *J* = 7.4 Hz, *NH*), 5.62 (d, 2H, *J* = 7.4 Hz, $CH_2$), 3.91 (s, 3H, $OCH_3$); EI MS: *m*/*z* (rel abund.%): 177 (25), 136 (15), 122 (7), 121 (6), 117 (100), 107 (3), 105 (25), 103 (41), 92 (24), 91 (38), 77 (81), 51 (82); Anal. Calcd

for $C_{16}H_{15}O_2N_3S$ (313.37): C, 61.32; H, 4.82, N, 13.41. Found: C, 61.34; H, 4.79, N, 13.44.

#### 4.3.2. 3-(3″-Methoxyphenylaminomethyl)-5-(phenyl)-1,3,4-oxadiazoline-2-thione (3b)

Yield: 2.75 g (88%); mp: 156–158 °C; $R_f$ = 0.64 (ethyl acetate/hexane/chloroform = 1:1:0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1606 (C=N), 1590 (C=C), 1445 (C=S), 1250 and 1176 (C–O–C); $^1H$ NMR (300 MHz, $CD_3COCD_3$): $\delta$ 7.71–7.62 (m, 5H, Ar–H′), 7.29 (1H, d, $J$ = 7.3 Hz, H-6″), 7.37 (t, 1H, $J$ = 7.3 Hz, H-5″), 6.97 (d, 1H, $J$ = 7.3 Hz, H-4″), 6.89 (s, 1H, H-2″), 6.1 (t, 1H, $J$ = 7.2 Hz, NH), 5.57 (d, $J$ = 7.2 Hz, 2H, $CH_2$), 3.91 (s, 3H, $OCH_3$); EI MS: $m/z$ (rel abund.%): 177 (11), 136 (8), 122 (34), 108 (12), 105 (33), 103 (100), 91 (10), 77 (18); Anal. Calcd $C_{16}H_{15}O_2N_3S$ (313.37): C, 61.32; H, 4.82, N, 13.41. Found: C, 61.36; H, 4.78, N, 13.45.

#### 4.3.3. 3-[2″-Chlorophenylaminomethyl]-5-{phenyl}-1,3,4-oxadiazoline-2-thione (3c)

Yield: 2.86 g (90%); mp: 122 °C; $R_f$ = 0.58 (ethyl acetate/hexane/chloroform = 1:1:0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1626 (C=N), 1585 (C=C), 1448 (C=S), 1249 and 1196 (C–O–C); $^1H$ NMR (300 MHz, $CD_3COCD_3$): $\delta$ 7.69–7.61 (m, 5H, Ar–H′), 7.39 (d, 1H, $J$ = 6.4 Hz, H-3″), 7.33 (dd, 1H, $J$ = 6.5, $J$ = 6.1 Hz, H-5″), 6.86 (m, 1H, H-4″), 6.83 (dd, 1H, $J$ = 6.6, 1.2 Hz, H-3″), 6.08 (bt, 1H, $J$ = 6.8 Hz, NH), 5.60 (d, $J$ = 6.8 Hz, 2H, $CH_2$); EI MS: $m/z$ (rel abund.%): 177 (5), 142 (18), 140 (40), 128 (7), 126 (19), 113 (8), 111 (22), 105 (14), 103 (10), 77 (20), 51 (100) Anal. Calcd for $C_{15}H_{12}N_3OSCl$ (317.79): C, 56.69; H, 3.81; N, 13.22. Found; $C_{15}H_{12}N_3OSCl$: C, 56.65; H, 3.84; N, 13.26.

#### 4.3.4. 3-(3″,4″-Dimethylphenylaminomethyl)-5-(phenyl)-1,3,4-oxadiazoline-2-thione (3d)

Yield: 2.58 g (83%); mp: 175 °C; $R_f$ = 0.68 (ethyl acetate/hexane/chloroform = 1:1:0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1650 (C=N), 1599 (C=C), 1448 (C=S), 1269 and 1198 (C–O–C);

$^1H$ NMR (300 MHz, $CD_3COCD_3$): $\delta$ 7.78–7.64 (m, 5H, Ar–H′), 7.33 (d, 1H, $J$ = 7.4 Hz, H-5″), 7.18 (d, 1H, $J$ = 7.4 Hz, H-6″), 6.91 (s, 1H, H-2″), 5.90 (t, 1H, $J$ = 7.5 Hz, -NH), 5.56 (d, 2H, $J$ = 7.5 Hz, $CH_2$), 2.76 (s, 6H, $2CH_3$ EI MS: $m/z$ (rel abund.%): 177 (23), 134 (36), 120 (62), 106 (6), 105 (41), 103 (100), 90 (22), 77 (24), 75 (53); Anal. Calcd for $C_{17}H_{17}ON_3S$ (311.40): C, 65.57; H, 5.50; N, 13.49. Found: C, 65.59; H, 5.45; N, 13.54.

#### 4.3.5. 3-(2-Methylphenylaminomethyl)-5-(3,4,5-trimethoxy phenyl)-1,3,4-oxadiazoline2-thione (3e)

Yield: 3.09 g (80%); mp: 129 °C; $R_f$ = 0.62 (ethyl acetate/hexane/chloroform = 1:1: 0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1636 (C=N), 1575 (C=C), 1438 (C=S), 1239 and 1166 (C–O–C); $^1H$ NMR (400 MHz, $CD_3COCD_3$): $\delta$ 7.06-7.04 (m, 4H, Ar–H), 6.66 (ddd, overlapped, 1H, H-4″), 6.65 (ddd, 1H, $J$ = 6.1, 5.9, 3.7 Hz, H-6″), 5.83 (t, 1H, $J$ = 7.1 Hz, $NH$), 5.58 (d, 2H, $J$ = 7.1 Hz, $CH_2$), 3.83 (s, 9H, $OCH_3$), 2.78 (s, 3H, $CH_3$); EI MS: $m/z$ (rel abund.%): 281 (6), 267 (100), 208 (32), 195 (45), 193 (70), 151, 150 (18), 136, 135 (13), 121, 120 (16), 107 (3), 92 (4), 91 (10), 76 (16). Anal. Calcd for: $C_{19}H_{21}N_3OS$ (387.45): C, 58.90; H, 5.46; N, 10.85. Found: C, 58.88; H, 5.45; N, 10.86.

#### 4.3.6. 3-[2″-Phenylethylaminomethyl]-5-{3′,4′,5′-trimethoxy phenyl}-1,3,4-oxadiazoline-2-thione (3f)

Yield: 3.12 g (78%); mp: 132 °C; $R_f$ = 0.64 (ethyl acetate/hexane/chloroform = 1:1:0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1612 (C=N), 1592 (C=C), 1418 (C=S), 1241 and 1179 (C–O–C), 755; $^1H$ NMR (400 MHz, $CD_3OCD_3$): $\delta$ 7.28 (dd, 1H, $J$ = 7.2, $J$ = 3.1 Hz, H-3″), 7.28 (dd, 1H, $J$ = 6.2, $J$ = 3.3 Hz, H-6″), 7.24 (d, 1H, $J$ = 7.7 Hz, H-2″), 7.18 (m, 2H, Ar–H), 6.92 (s, 2H, H-2′/6′), 5.4 (s, 2H, $H_a$), 3.60 (t, 2H, $J$ = 6.7 Hz, $H_b$, $CH_2$), 3.98 (s, 1H, NH), 3. 81 (s, 9H, $OCH_3$), 3.05 (t, 2H, $J$ = 6.7 Hz, $CH_2$); EI MS: $m/z$ (rel abund.%): 281 (7), 267 (26), 193 (100), 150 (11), 134 (10), 133 (6), 120 (2), 105 (17), 104 (10), 77 (10); Anal. Calcd for: $C_{20}H_{23}N_3O_4S$ (401.48): C, 59.83; H, 5.77; N, 10.47. Found: C, 59.85; H, 5.71; N, 10.46.

#### 4.3.7. 3-[2″-Chlorophenylaminomethyl]-5-(2′-hydroxyphenyl)-1,3,4-oxadiazoline-2-thione (3g)

Yield: 2.56 g (77%); mp: 141 °C; $R_f$ = 0.57 (ethyl acetate/hexane/chloroform = 1:1:0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1621 (C=N), 1572 (C=C), 1431 (C=S), 1243 and 1146 (C–O–C), 755; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 8.84 (br s, 1H, OH), 7.6 (m, 2H, Ar–H), 7.90 (ddd, 1H, $J$ = 8.1, $J$ = 5.0, $J$ = 1.5 Hz, H-6′), 7.56 (d, 1H, $J$ = 8.9 Hz, H-3″), 7.07 (dd, 1H, $J$ = 8.1, $J$ = 6.4 Hz, H-5′), 7.08 (d, 1H, $J$ = 8.2 Hz, H-3′), 6.66 (ddd, 1H, $J$ = 7.2, 2.13, 1.8 Hz, H-5″), 6.61 (dd, 1H, $J$ = 7.2, $J$ = 1.7 Hz, H-6″), 5.84 (bt, 1H, $J$ = 6.4 Hz, $NH$), 5.66 (d, 2H, $J$ = 6.4 Hz, $CH_2$); EI MS: $m/z$ (rel abund.%): 193 (3), 176 (55), 142 (3), 140 (10), 133 (29), 128, 126, 113, 111, 121 (5), 119 (16), 105 (3), 91 (47), 76 (15); Anal. Calcd $C_{15}H_{12}ClN_3O_4S$ (333.79): C, 53.97; H, 3.62; N, 12.59. Found: C, 53.96; H, 3.60; N, 12.52.

#### 4.3.8. 3-[2″-Methoxy-5″-nitrophenylaminomethyl]-5-{4′-methylphenyl}-1,3,4-oxadiazoline-2-thione (3h)

Yield: 1.59 g (43%); mp: 167 °C; $R_f$ = 0.56 (ethyl acetate/hexane/chloroform = 1:1:0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1610 (C=N), 1590 (C=C), 1422 (C=S), 1243 and 1178 (C–O–C), 767; $^1H$ NMR (300 MHz, $CD_3COCD_3$): $\delta$ 7.89 (s, 1H, H-6″), 7.84 (d, 1H, $J$ = 7.9 Hz, H-4″), 7.51 (d, 1H, $J$ = 7.9 Hz, H-3″), 7.34 (d, 2H, $J$ = 7.7 Hz, H-2′/6′), 7.19 (d, 2H, $J$ = 7.7 Hz, H-3′/5′), 5.55 (s, 2H, $CH_2$), 3.91 (s, 3H, $OCH_3$), 2.33 (s, 3H, $CH_3$); EI MS: $m/z$ (rel abund.%): 191 (52), 150 (27), 136 (18), 132 (100), 121 (34), 119 (30), 117 (10), 108 (11), 104 (15), 102 (1), 94 (13), 91 (47), 90 (38), 65 (17), 62 (6); Anal. Calcd for: $C_{17}H_{16}N_4O_4S$ (372.40): C, 54.83; H, 4.33; N, 15.04. Found: C, 54.86; H, 4.37; N, 15.00.

#### 4.3.9. 3-[4″-Methylphenylaminomethyl]-5-{4-methylphenyl}-1,3,4-oxadiazoline-2-thione (3i)

Yield: 2.58 g (83%); mp: 110 °C; $R_f$ = 0.63 (ethyl acetate/hexane/chloroform = 1:1:0.5); FTIR (KBr) $\nu_{max}$: 3409 (NH), 1627 (C=N), 1342 (C=S), 1018 (C–O); $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 7.61 (d, 2H, $J$ = 7.6 Hz, H-3″/5″), 7.74 (d, 2H, $J$ = 8.1 Hz, H-2′/6′), 7.49 (d, 2H, $J$ = 8.1 Hz, H-3′/5′), 7.30 (d, 2H, $J$ = 7.6 Hz, H-2″/6″), 5.56 (br d, 2H, $J$ = 6.0 Hz, $CH_2$), 5.54 (bt, 1H, $J$ = 6.0 Hz, $NH$), 2.38 (s, 3H, $CH_3$), 2.29 (s, 3H, $CH_3$); EI MS: $m/z$ (rel abund.%): 191 (51), 132 (100), 120 (7), 119 (20), 117 (9), 105 (6), 104 (12), 102 (1), 91 (45), 65 (15), 91 (50); Anal. Calcd for: $C_{17}H_{17}N_3OS$ (311.40); C, 65.57; H, 5.50, N,13.49. Found: C, 65.59; H, 5.54; N,13.51.

#### 4.3.10. 3-[Phenylaminomethyl]-5-{4-methylphenyl}-1,3,4-oxadiazoline-2-thione (3j)

Yield: 2.40 g (81%); mp: 119 °C; $R_f$ = 0.66 (ethyl acetate/hexane/chloroform = 1:1:0.5); FTIR (KBr) $\nu_{max}$: 3409 (NH), 1636 (C=N), 1333 (C=S), 1016 (C–O); $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 7.74 (d, 2H, $J$ = 8.1 Hz H-2′/6′), 7.66–7.50 (m, 5H, Ar–H″), 7.30 (d, 2H, $J$ = 8.1 Hz, H-3′/5′), 5.71 (t, 1H, $J$ = 6.7 Hz, NH), 5.53 (d, 2H, $J$ = 6.7 Hz, $CH_2$), 2.38 (s, 3H, $CH_3$); EI MS: $m/z$ (rel abund.%): 191 (51), 132 (100), 119 (20.25), 117 (9), 106 (13), 104 (12), 102 (2), 92 (3), 91 (45), 77 (21), 65 (15), 51 (10); Anal. Calcd for $C_{16}H_{15}N_3OS$ (297.37): C, 64.62; H, 5.08; N, 14.13. Found: C, 64.55; H, 5.1, N, 14.16.

#### 4.3.11. 3-[2″,3″-Dimethylphenylaminomethyl]-5-{4′-methylphenyl}-1,3,4-oxadiazoline-2-thione (3k)

Yield: 2.01 g (62%); mp: 144 °C; $R_f$ = 0.69 (ethyl acetate/hexane/chloroform = 1:1:0.5); FTIR (KBr) $\nu_{max}$: 3409 (NH), 1635 (C=N), 1331 (C=S), 1026 (C–O); $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 7.74 (d, 2H, $J$ = 8.1 Hz, H-2′/6′), 7.51 (t, 1H, $J$ = 7.7 Hz, H-5″), 7.37–7.42

(m, 2H, H-6″/4″), 7.30 (d, 2H, *J* = 8.1 Hz, H-3′/5′), 6.83 (bt, 1H, *J* = 6.8 Hz, *NH*), 5.60 (br d, 2H, *J* = 6.8 Hz, $CH_2$), 2.38 (s, 3H, $CH_3$), 2.34 (s, 3H, $CH_3$), 2.32 (s, 3H, $CH_3$); EI MS: *m*/*z* (rel abund.%): 191 (48), 132 (100), 120 (6), 119 (24), 117 (11), 105 (7) (14), 104 (8), 102 (3), 91 (41), 76 (3), 65 (13); Anal. Calcd for: $C_{18}H_{19}N_3OS$ (325.43): C, 66.43; H, 5.88; N, 12.91. Found: C, 66.48; H, 5.84; N, 12.95.

#### 4.3.12. 3-[3″,4″-Dichlorophenylaminomethyl]-5-(4′-methyl phenyl)-1,3,4-oxadiazoline-2-thione (3l)

Yield: 2.59 g (71%); mp: 137–139 °C; $R_f$ = 0.59 (ethyl acetate/hexane/chloroform = 1:1:0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1622 (C=N), 1599 (C=C), 1448 (C=S), 1239 and 1166 (C–O–C), 766; $^1$HNMR ($CDCl_3$): $\delta$ 7.78–7.05 (m, 3H, H-4″/5″/6″), 7.36 (d, 2H, *J* = 8.9 Hz, H-2′/6′), 6.60 (d, 2H, *J* = 8.9 Hz, H-3′/5′), 5.67 (bt, 1H, *NH*), 5.00 (br d, 1H, $CH_2$), 2.4 (s, 3H, $CH_3$); EI MS: *m*/*z* (rel abund.%): 191 (39), 178 (16), 176 (15), 174 (48), 164 (6), 162 (7), 160 (21), 141 (10), 139 (33), 132 (100), 119 (16), 117 (7), 104 (11), 102 (5), 91 (45), 90 (2) 65 (22), 91 (50), 76 (3); Anal. Calcd for: $C_{16}H_{13}C_{l2}N_3OS$ (366.26): C, 52.47; H, 3.58; N, 11.47. Found: C, 52.49; H, 3.54; N, 11.50.

#### 4.3.13. 3-[3″,4″-Dimethylphenylaminomethyl]-5-(4′-methyl phenyl)-1,3,4-oxadiazoline-2-thione (3m)

Yield: 2.40 g (74%); mp: 158 °C; $R_f$ = 0.67 (ethyl acetate/hexane/chloroform = 1:1:0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1619 (C=N), 1596 (C=C), 1435 (C=S), 1250 and 1190 (C–O–C), 760; $^1$H NMR (300 MHz, $CD_3COCD_3$): $\delta$ 7.72 (d, 2H, *J* = 8.1 Hz, H-2′/6′), 7.22 (d, 2H, *J* = 8.1 Hz, H-3′/5′), 7.12 (s, 1H, H-2″), 7.39 (d, 1H, *J* = 7.7 Hz, H-5″), 7.15 (d, 1H, *J* = 7.7 Hz, H-6″), 5.71 (bt, 1H, *NH*), 5.55 (br d, 2H, $CH_2$), 2.39 (s, $CH_3$), 2.31 (s, 6H, $2CH_3$); EI MS: *m*/*z* (rel abund.%): 191 (44), 134 (4), 132 (100), 120 (7), 119 (20), 117 (12), 105 (11), 104 (15), 102 (6), 91 (6), 76 (5), 65 (19); Anal. Calcd for $C_{18}H_{19}N_3OS$ (325.42): C, 66.43; H, 5.88; N, 12.91. Found C, 66.48; H, 5.84; N, 12.94.

#### 4.3.14. 3-[3″-Methoxyphenylaminomethyl]-5-(4′-methyl phenyl)-1,3,4-oxadiazoline-2-thione (3n)

Yield: 2.48 g (76%); mp: 140–142 °C; $R_f$ = 0.61 (ethyl acetate/hexane/chloroform = 1:1:0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1633 (C=N), 1597 (C=C), 1430 (C=S), 1244 and 1180 (C–O–C), 763; $^1$HNMR (400 MHz, $CDCl_3$): $\delta$ 7.72 (d, 2H, *J* = 8.5 Hz, H-2′/6′), 7.22 (d, 2H, *J* = 8.5 Hz, H-3′/5′), 7.19-7.15 (m, 2H, H-4″/6″), 7.18 (s, 1H, H-2″), 7.39 (t, 1H, *J* = 7.8 Hz, H-5″), 5.71 (t, 1H, *J* = 6.6 Hz, *NH*), 5.55 (d, 2H, *J* = 6.6 Hz, $CH_2$), 3.46 (s, 3H, $OCH_3$), 2.39 (s, 3H, $CH_3$); EI MS: *m*/*z* (rel abund.%): 191 (45), 136 (41, 132 (100), 122 (12), 121 (29), 119 (16), 117 (6), 107 (23), 104 (14), 102 (9), 91 (47), 65 (28), 76 (3), 65 (13)); Anal. Calcd for $C_{17}H_{17}N_3O_2S$ (327.40): C, 62.36; H, 5.23; N, 12.83. Found: C, 62.39; H, 5.22; N, 12.81.

#### 4.3.15. 3-[2″-Chlorophenylaminomethyl]-5-(2'-bromophenyl)-1,3,4-oxadiazoline-2-thione (3o)

Yield: 3.62 g (93%); mp: 143 °C; $R_f$ = 0.71 (ethyl acetate/hexane/chloroform = 1:1:0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1609 (C=N), 1574 (C=C), 1411 (C=S), 1250 and 1166 (C–O–C), 740; $^1$H NMR (500 MHz, $CDCl_3$ + $CD_3OD$): $\delta$ 7.85(dd, 1H, *J* = 7.9, 1.9 Hz, H-6′), 7.79 (d, 1H, *J* = 6.5 Hz, H-3″), 7.78 (d, 1H, *J* = 7.5 Hz, H-3′), 7.52 (t, 1H, *J* = 7.5, 7.5 Hz, H-4′), 7.44 (m, 1H, H-5′), 7.42–7.37 (m, 3H, H-4″/5″/6″), 6.01 (s, 2H, $CH_2$), 5.57 (br s, 1H, NH); EI MS: *m*/*z* (rel abund.%): 257 (60), 255 (59), 197 (47), 195 (46), 185 (9), 183 (15), 181 (6), 157 (7), 155 (8), 142 (34), 140 (11), 128 (65), 126 (22), 113 (18), 111 (7), 105 (45), 91 (53), 76 (27), 50 (8); $C_{15}H_{11}BrClN_3OS$ (396.69): Anal. Calcd for C, 45.42; H, 2.79; N, 10.59. Found: C, 45.40; H, 2.76; N, 10.55.

#### 4.3.16. 3-[4″-Bromophenylaminomethyl]-5-(2'-bromophenyl)-1,3,4-oxadiazoline-2-thione (3p)

Yield: 2.99 g (68%); mp: 157 °C; $R_f$ = 0.65 (ethyl acetate/hexane/chloroform = 1:1:0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1619 (C=N), 1590 (C=C), 1437 (C=S), 1266 and 1199 (C–O–C), 783; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.83 (dd, 1H, *J* = 7.6 Hz, 1.3 Hz, H-6′), 7.76 (d, 1H, *J* = 7.2 Hz, H-4′,), 7.53 (t, 1H, *J* = 7.2, Hz, H-3′), 7.47 (m, 1H, H-5′); 7.36 (d, 2H, *J* = 7.7 Hz, H-2″/6″), 7.53 (d, 2H, *J* = 7.7 Hz, H-3″/5″), 5.53 (s, 2H, $CH_2$), 5.33 (br s, 1H, *NH*); EI MS: *m*/*z* (rel abund.%): 257 (100), 255 (99), 197 (92), 195 (93), 186 (30), 185 (64), 184 (33), 183 (78), 181 (13), 172 (12), 170 (10), 157 (41), 155 (44), 76 (19), 50 (13); Anal. Calcd for $C_{15}H_{11}Br_2N_3OS$ (441.14): C, 40.84; H, 2.51; N, 9.53 Found: C, 40.84; H, 2.51; N, 9.53.

#### 4.3.17. 3-(2-Methoxyphenylaminomethyl)-5-(2-bromophenyl)-1,3,4-oxadiazoline-2-thione (3q)

Yield: 3.66 g (94%); mp: 112 °C; $R_f$ = 0.70 (ethyl acetate/hexane/chloroform = 1:1:0.5); FTIR (KBr) $\nu_{max}$: 3219 (NH), 1649 (C=N), 1063 (C–O–C); $^1$H NMR (500 MHz, $CD_3OD$): $\delta$ 7.82 (dd, 1H, *J* = 7.7, 1.6 Hz, H-6′), 7.77 (d, 1H, *J* = 7.5 Hz, H-4′), 7.52 (t, 1H, *J* = 7.5, *J* = 7.5 Hz, H-3′), 7.41 (m, IH, H-5′); 7.31–7.36 (m, 2H, H-6″/4″), 7.63 (d, 1H, *J* = 7.9 Hz, H-3″), 7.56 (t, 1H, *J* = 7.9 Hz, H-5″), 5.60 (s, 2H, $CH_2$), 5.51 (br s, 1H, *NH*), 3.91 (s, 3H, $OCH_3$); EI MS: *m*/*z* (rel abund.%): 257 (100), 255 (99), 197 (92), 195 (93), 185 (64), 183 (78), 181 (13), 157 (21), 155 (24), 76 (19), 50 (13); 136 (24), 122 (29), 121 (34), 107 (2), 92 (6) 91 (5); Anal. Calcd for $C_{16}H_{14}BrN_3O_2S$ (392.27): C, 48.99; H, 3.60; N, 10.71. Found: C, 48.95; H, 3.65; N, 10.75.

#### 4.3.18. 3-[2″-Methylphenylaminomethyl]-5-{4′-chlorophenyl}-1,3,4-oxadiazoline-2-thione (3r)

Yield: 2.41 g (73%); mp: 140 °C; $R_f$ = 0.60 ((ethyl acetate/hexane/chloroform = 1:1:0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1641 (C=N), 1590 (C=C), 1455 (C=S), 1272 and 1161 (C–O–C), 785; $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.88 (d, 2H, *J* = 8.6 Hz, H-2′/6′), 7.65 (d, 2H, *J* = 8.6 Hz, H-3′/5′), 7.31-7.44 (m, 4H, H-6″/5″/4″/3″), 5.51 (br s, 1H, *NH*), 5.40 (t, 2H, $CH_2$), 2.35 (s, 3H, $CH_3$); EI MS: *m*/*z* (rel abund.%): 213 (19), 211 (62), 179 (6), 153 (15), 151 (47), 141 (4), 139 (32), 137 (100), 120 (44), 117 (11), 106 (29), 102 (16), 90 (21), 76 (13); Anal. Calcd for $C_{16}H_{14}ClN_3OS$ (331.82): C, 57.91; H, 4.25; N, 12.66. Found C, 57.95; H, 4.20; N, 12.69.

#### 4.3.19. 3-(4-Bromophenylaminomethyl)-5-(4-chlorophenyl)-1,3,4-oxadiazoline-2-thione (3s)

Yield: 2.01 g (51%); mp: 143 °C; $R_f$ = 0.63 (ethyl acetate/hexane/chloroform = 1:1:0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1651 (C=N), 1586 (C=C), 1445 (C=S), 1263 and 1180 (C–O–C), 750; $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 8.09 (d, 2H, *J* = 7.4 Hz, H-2′/6′), 7.75 (d, 2H, *J* = 7.4 Hz, H-3′/5′), 7.55 (d, 2H, *J* = 7.9 Hz, H-2″/6″), 7.66 (d, 2H, *J* = 7.9 Hz, H-3″/5″), 6.01 (bt, 1H, *NH*), 5.53 (d, 2H, *J* = 7.2 Hz, $CH_2$); EI MS: *m*/*z* (rel abund.%): 213 (33), 211 (100), 179 (5), 186 (59), 184 (62), 172 (100), 170 (98), 167 (6), 165 (8), 153 (27), 151 (82), 141 (4), 139 (14), 137 (14), 117 (5), 102 (11), 76 (17); Anal. Calcd for $C_{15}H_{11}Cl\ Br\ N_3OS$ (396.69): C, 45.42; H, 2.79; N, 10.59. Found: C, 45.46; H, 2.74; N, 10.63.

#### 4.3.20. 3-[2-Chlorophenylaminomethyl]-5-{4-chlorophenyl}-1,3,4-oxadiazoline-2-thione (3t)

Yield: 2.88 g (82%); mp: 156 °C; $R_f$ = 0.62 (ethyl acetate/hexane/chloroform = 1:1:0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1643 (C=N), 1592 (C=C), 1440 (C=S), 1239 and 1169 (C–O–C), 767; $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.78 (d, 2H, *J* = 8.0 Hz, H-2′/6′), 7.61 (d, 2H, *J* = 8.0 Hz, H-3′/5′), 7.56–7.30 (d, 4H, *J* = 7.8 Hz, H-6″/ 5″/4″/ 3″), 5.57 (bt, 1H, *NH*), 5.22 (bt, 2H, *J* = 7.0 Hz, $CH_2$); EI MS: *m*/*z* (rel abund.%): 213 (35), 211 (100), 179 (3), 153 (30), 151 (82), 142 (48), 141 (4), 140 (15), 139 (14), 137 (14), 126 (2), 117 (5), 111 (4), 109 (14), 105 (17), 102 (8), 76 (17); Anal. Calcd for $C_{15}H_{11}Cl_2N_3OS$ (352.23): C, 51,15; H, 3.15; N, 11.93. Found: C, 51,19; H, 3.11; N, 11.89.

#### 4.3.21. 3-[2-Methoxyphenylaminomethyl]-5-{4-chlorophenyl}-1,3,4-oxadiazoline-2-thione (3u)

Yield: 3.01 g (87%); mp: 149 °C; $R_f$ = 0.66 (ethyl acetate/hexane/chloroform = 1:1:0.5); IR ($\nu_{max}$, KBr, $cm^{-1}$): 1608 (C=N), 1581 (C=C), 1427 (C=S), 1252 and 1176 (C–O–C), 742; $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.71 (d, 2H, $J$ = 8.5 Hz, H-2′/6′), 7.65 (d, 2H, $J$ = 8.5 Hz, H-3′/5′), 7.41–7.46(m, 2H, H-6″/4″), 7.31–7.37 (m, 2H, H-3″/5″), 5.58 (br s, 1H, $J$ = 7.0 Hz, *NH*), 5.30 (bt, 2H, $J$ = 7.0 Hz, $CH_2$), 3.91 (s, 3H, $OCH_3$);i EI MS: $m/z$ (rel abund.%): 213 (35), 211 (100), 179 (3), 153 (26), 151 (82), 141 (4), 139 (14), 137 (14), 117 (5), 102 (8), 76(7) 136 (9), 122 (21), 107 (13), 91 (11), 76 (41); Anal. Calcd for $C_{16}H_{14}ClN_2OS$ (347.82): C, 55.25; H, 4.06; N, 12.08. Found: C, 55.29; H, 4.00; N, 12.1.

### 4.4. Thymidine phosphorylase inhibition

TP/PD-ECGF (*E. coli* TP (Sigma T6632)) activity was determined by measuring the absorbance at 290 nm spectrophotometrically. The original method reported by Krenitsky (Krenitsky et al., 1979)[36] was modified. Briefly, in 96 wells, flat bottom, microplate with each well capacity 200 µl, reaction mixture of 200 µl was prepared which contained 145 µl of potassium phosphate buffer (pH 7.4), 20 µl of 1.5 mM Thymidine 5′ mono phosphate solution as substrate, 30 µl of enzyme (*E. coli* TP (Sigma T6632) at concentration 0.05 and 0.002 U, respectively, were incubated with 5 µl of test materials for 10 min at 25 °C in temperature controlled incubator before taking readings by microplate reader (SpectraMax Plus$^{384}$, USA) at 290 nm. The wells containing reaction mixture devoid of substrate were blank and the mean OD of these blank wells was substracted from wells containing reaction mixture with substrate. The readings were taken continuously after 10, 20, and 30 min by microplate reader. All assays were performed in triplicate.

### 4.5. Docking studies

Corina online tool[46] was applied to creating three-dimensional structure of compounds. Gasteiger–Marsili charges were assigned by Sybyl-X 1.1[47] following check of atom types and protonation states of the ligands. Finally, analyzed structures were saved in the mol2 format.

*Escherichia coli* TP from 4EAD crystal structure was prepared in two steps. Initially, sulfate ion was replaced by phosphate in its dihydrogen form, the N- and C-terminal amino acids were set as charged and hydrogen atoms were added to the protein, water and ligands using Sybyl-X 1.1. Then, all histidine residues were protonated at Nε, ligand molecules except phosphate removed, and binding site defined as all amino acid residues within 10 Å from ONP using Hermes 1.5.[48] The presence of water molecules within 5 Å from ONP was also taken into account. They were set as toggle.

Docking was performed using Gold 5.1 program.[49] A standard set of genetic algorithm with population size 100 and number of operations 100,000 was applied. As a result, 20 poses for each ligand were obtained and sorted according to GoldScore values. Results were visualized by PyMOL and Maestro.[50,51]

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