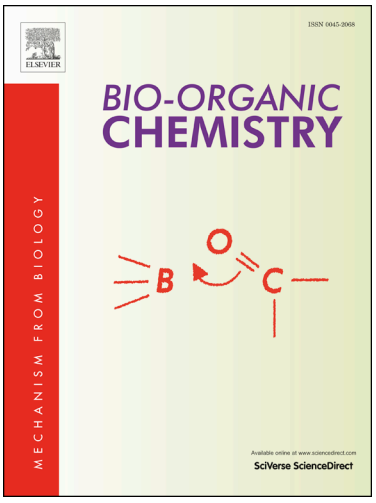

Design, Synthesis, *In-Vitro Thymidine Phosphorylase* Inhibition, *In-Vivo* Antiangiogenic and *In-Silico* Studies of C-6 substituted dihydropyrimidines

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Design, Synthesis, *In-Vitro Thymidine Phosphorylase* Inhibition, *In-Vivo* Antiangiogenic and *In-Silico* Studies of C-6 substituted dihydropyrimidines

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Abstract

Thymidine phosphorylase (TP) is an angiogenic enzyme. It plays an important role in angiogenesis, tumour growth, invasion and metastasis. In current research work, we study the effect of structural modification of dihydropyrimidine-2-ones (DHPM-2-ones) on TP inhibition. A series of eighteen new derivatives of 3,4-dihydropyrimidone-2-one were designed and synthesized through the structural modification at C-6 position. All these new derivatives were then assessed for *in-vitro* inhibition of thymidine phosphorylase (TP) from *E. coli*. Oxadiazole derivatives **4a-e** exhibited excellent TP-inhibition at low micromolar concentration levels better than standard drug 7-deazaxanthine (7-DX). Among all these compounds, **4b** was found to be the most potent with IC_{50} = 1.09±0.004 μM. Anti-angiogenesis potential of representative compounds were also studied in a chorioallantoic membrane (CAM) assay. Here again, compound **4b** was found to be the potent anti-angiogenesis compound in a CAM assay. Docking studies were also performed with Molecular Operating Environment (MOE) to further analyse the mode of inhibition of these compounds. Binding mode analysis of the most active inhibitors showed that these are well accommodated into the binding site of enzyme though stable hydrogen bonding and hydrophobic interactions.

Key words: Dihydropyrimine-2-ones; Thymidine phosphorylase; Chorioallantoic membrane assay; Antiangiogenesis

1. Introduction

Thymidine phosphorylase (TP, EC 2.4.2.4), discovered in 1954, is involved in the reversible catalysis of phosphorylation of thymidine nucleoside to thymine and 2-deoxy-D-ribose-1-phosphate. 2-Deoxy-D-ribose-1-phosphate then de-phosphorylated to 2-deoxy-D-ribose. This catabolised 2-deoxy-D-ribose is basically responsible for angiogenesis by its involvement in the secretion and stimulation of angiogenic factors like vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs). Angiogenesis is the formation of new blood vessels in the replicated cell-lines and thus recognized as the key process in the metastasis of tumour. Human *Thymidine phosphorylase* (*h*TP) is identical to platelet-derived endothelial-cell growth factor (PD-ECGF) or gliostatin (an endothelial cell growth factor derived from platelets) and this factor is responsible for angiogenesis in human cell lines. If uncontrolled, then a complex series of events start, eventually ending up in the tumour growth [1-7]. In addition to its role in cancers, the hyper proliferation due to over-expressed TP have been also been found in diseases like rheumatoid arthritis and psoriasis, which is again attributed to its angiogenic activity [8]. These factors are in turn involved in many sub-processes like proliferation of endothelial cells due to metalloproteinase and formation of new blood vessels in tumour tissues, thus supporting the cancer metastasis. Moreover, TP not only act on thymidine but its poor substrate specificity renders it to recognize and catabolise the deoxyuridine and other related pyrimidine nucleosides. That is why many chemotherapeutics based on 5-substituted pyrimidine deoxyribonucleoside scaffolds remained in-effective *in vitro* despite having real or potential efficacy against tumours [9]. All these facts lead to conclusion that in *vitro* angiogenic activities and hence tumour growth can be inhibited/controlled by inhibiting TP enzyme.

There are evidences where competitive inhibitors of this enzyme have shown blocking of enzymatic activity hence reduced or terminated angiogenesis at the point of solid tumours. Since a large number of potential drug candidates based on mono and multi-substrate, largely pyrimidines or purines-based have been discovered or developed. These drugs were actually designed in such a way that these can effectively interact with the binding site of thymidine. The classical inhibitor 6-amino-5-bromouracil (**1**, 6A5BU, IC_{50} value of 0.035 μM), is typically used as the benchmark for the design of more potent inhibitors. 7-deazaxanthine (**2**, 7-DX, IC_{50} value= 40 μM) was the first purine derivative to display inhibition of both *E. coli* TP and angiogenesis in chorioallantoic membrane. Another compound structurally related to 5-chloro-6-[(2-iminopyrrolidin-1-yl)methyl]uracil (**3**) is also identified as potent TP inhibitor (TPI) [3, 10].

1,3,4-oxadiazoles are an important class of heterocyclic compounds exhibiting a broad spectrum of biological activities in different studies. Shahzad *et al*. reported the synthesis and anti-TP activities of a series of oxadiazoles derivatives [11-12]. The two most active inhibitors of that series were found to be compounds **4** and **5** with IC_{50} values 14.40 ± 2.45 and 17.60±1.07 μM respectively. Similarly, Schiff base derivatives also showed some good activities against *Ec*TP. Khan et al reported Schiff based derivative **6** (**Figure 1**) as a good inhibitor of *Ec*TP with an IC_{50} value of 19.77 μM [18]. Bensaber et al reported pyrazol-3-one Schiff base derivatives of type **7** (**Figure 1**) as inhibitors of *h*TP [13].

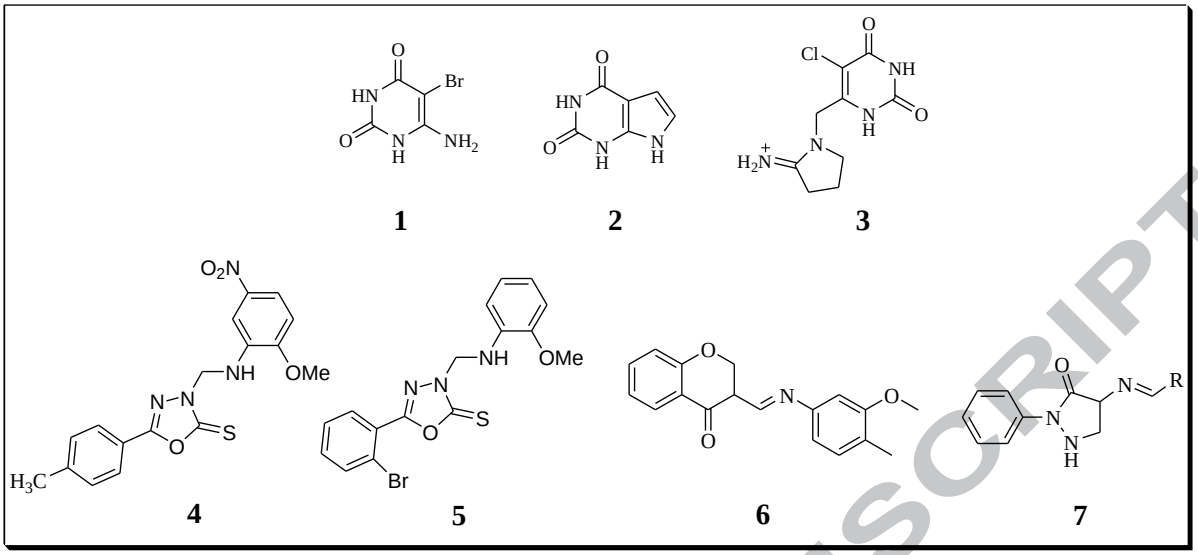


Figure 1: Potent TP inhibitors (**1-3**); Some important 1,3,4-oxadiazoles derivatives as TP inhibitors (**4-5**); Schiff base derivatives **6-7** as TPI.

The classical TP-inhibitors **1-3** share the typical core of pyrimidine-one's moiety, therefore, these are considered as the basic-model for designing new pharmacophores for TP-inhibition. The motivation behind current research is to design and synthesis potent compounds having structural similarities with potent TPI **3 (**IC_{50} = 35 nM**)** [10]. **Figure 2** depicts the design strategy for our new compounds where the points of similarities between the basic cores of clinically administered drug **3** and our compounds is also highlighted. Hence, in this study a series of dihydropyrimidine-2-ones (DHPMs-2-ones) based TP-inhibitors have been designed and synthesized. We selected C-6 position of DHPM-2-one core for the design strategy and synthesized a variety of TP inhibitors by introducing structural units such as Schiff-base and 1,3-oxadiazoles. All these compounds were synthesized *via* 6-bromomethylene and 6-aminomethyl DHPM intermediates.

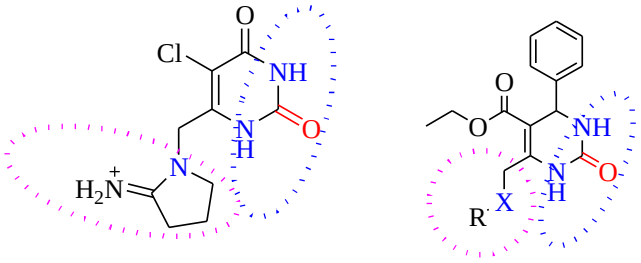
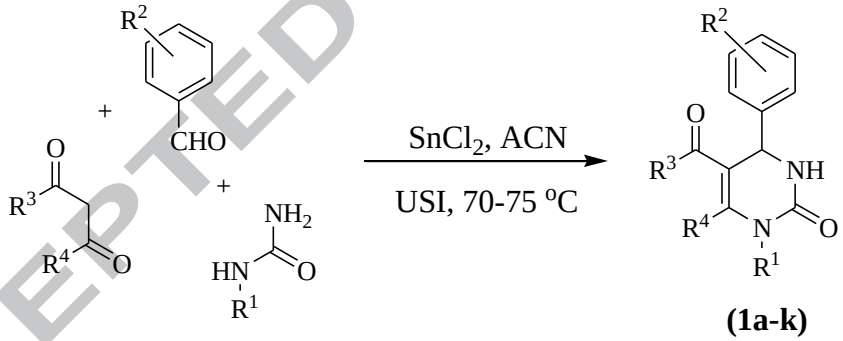


Figure 2: Structural similarities between **3** and proposed design strategy of our work.

2. Results and discussion

2.1. Chemistry

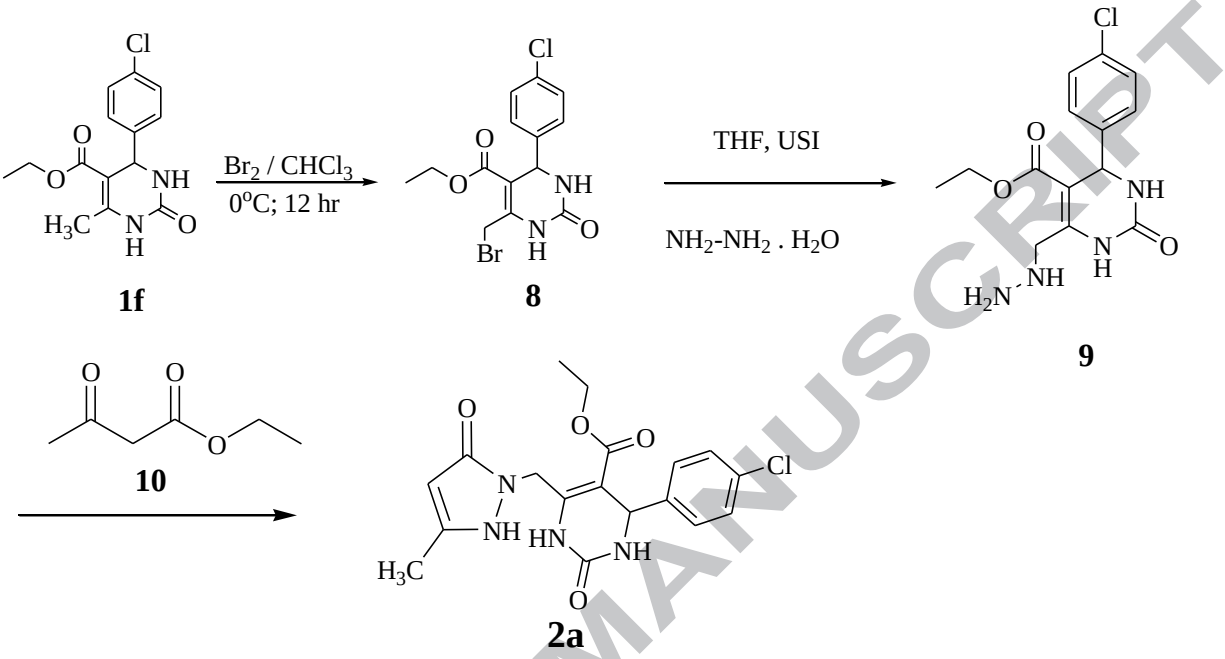
Compounds **1a-k** (**Scheme 1**) were obtained as reported previously by our research group using classical three-component Biginelli reaction under ultrasonic irradiation (USI) [20]. Whereas, compounds **2a-h**, **3a-d** and **4a-e** were synthesized as shown in **Schemes 2-5** by the structural modification of **1f** (**Scheme 2**).



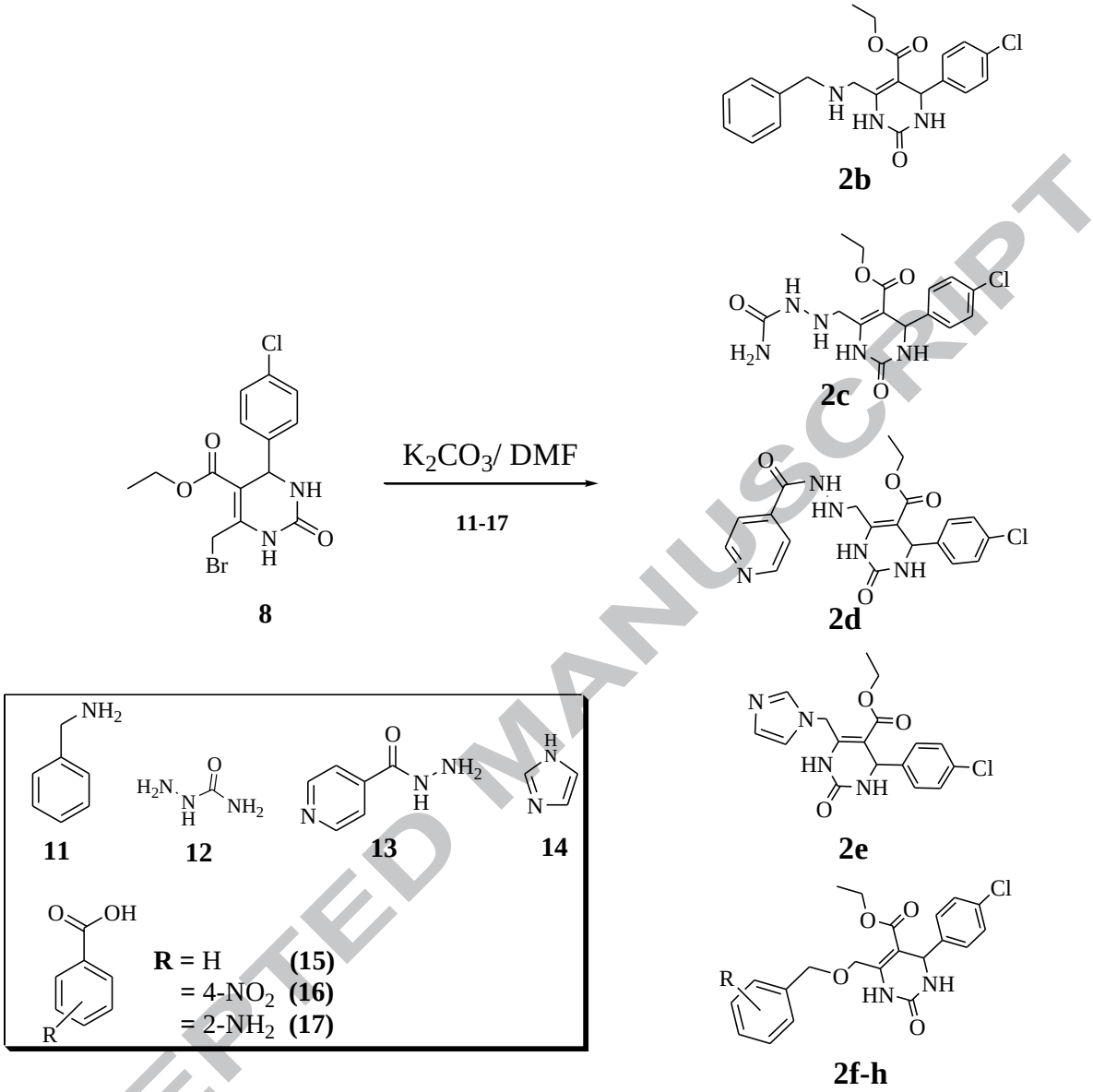
Scheme 1: Synthesis of DHPM-2-one core (**1a-k**)

The structural modification of **1f** was carried out *via* allylic bromination at C-6 position [14]. Allylic bromination of **1f** resulted in 6-bromo derivative **8 (Scheme 2**). The intermediate **8** is vital to our further studies as it can undergo the nucleophilic substitution reactions to yield a series of compounds. Initially, a series of diversely substituted eight compounds (**2a-h)** were synthesized. Pyrazolone ring containing compound **2a** was obtained *via* Paal-Knorr type of reaction by condensing compound **9** with ethyl acetoacetate (**10**). The formation of

regioisomers cannot be ruled out here. Compounds **2b-h** (**Scheme 3**) were synthesized by the reaction of compound **8** with **11-17** in the presence of K_2CO_3 and DMF**.**



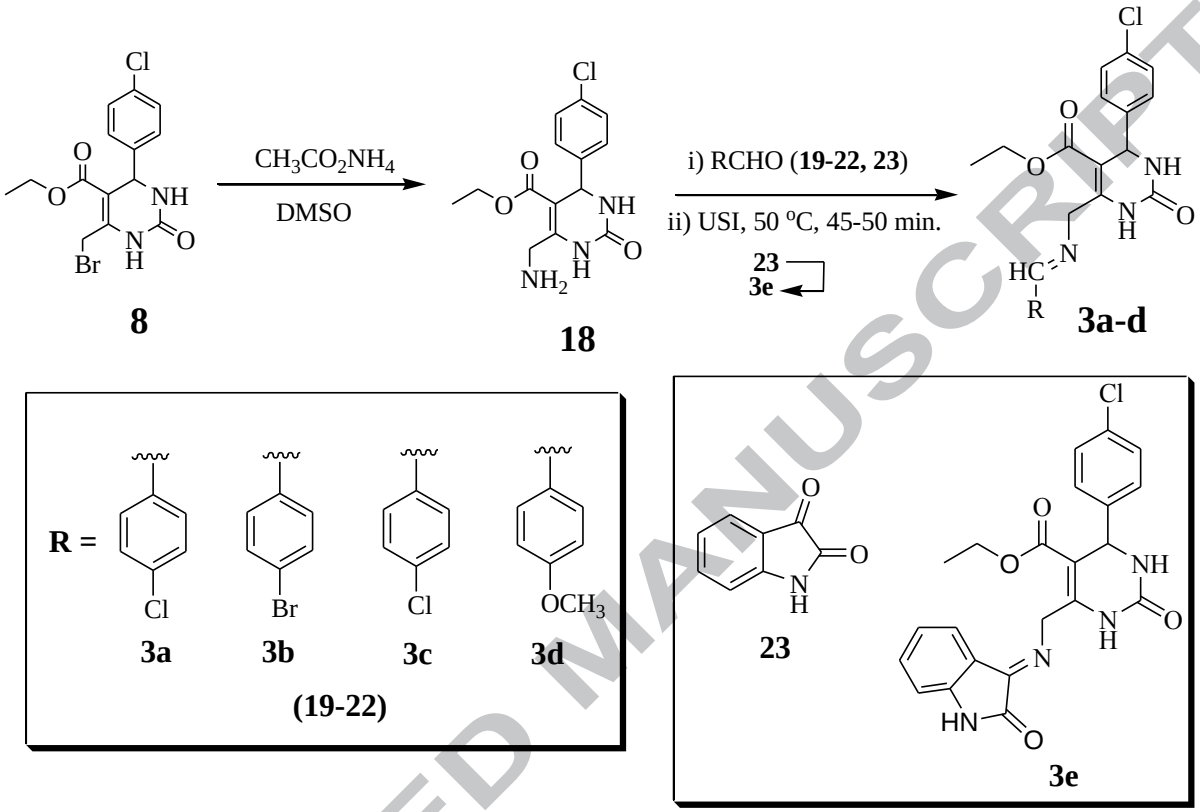
Scheme 2: Synthesis of pyrazolone ring **2a** from 6-bromomethylene derivative (**8**)



Scheme 3: Synthesis of 6-bromomethylene derivatives **2b-h** from compound **8**

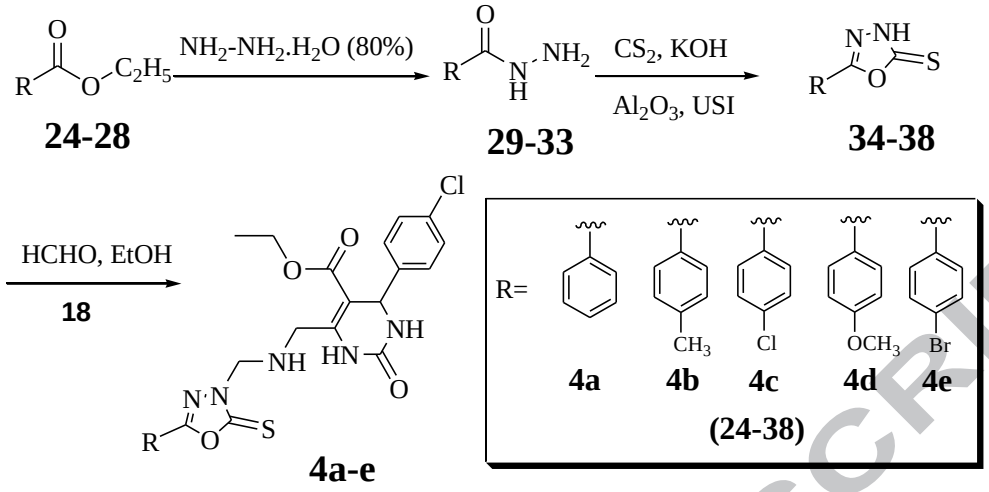
Synthesis of compounds **3a-d** and **4a-e** (**Schemes 4-5**) were commenced *via* the synthesis of intermediate compound 6-aminomethyl derivative (**18).** Intermediate 18 was synthesized by the reaction of ammonium acetate (as a substitute of ammonia) with compound **8** in a good yield (88 %) [15]. This derivative was further used as precursor for the synthesis of Schiff base derivatives (**3a-e**) and 5-substituted-1,3-oxadiazoles (**4a-e**). Schiff-base derivatives **3a-e** were the result of classical condensation between four different aryl aldehydes and isatin with amino group of C-6 in acidic medium (**Scheme 4**). These compounds were identified by the

loss of two NH_2-protons, and appearance of a more de-shielded double bond, characteristic of an imine formation.



Scheme 4: Schiff-base derivatives of compound 1**8**

A series 5-substituted-1,3-oxadiazoles (**4a-e**, **Scheme 5**) containing 6-aminomethyl DHPM derivative (**18**) and 1,3,4-oxadiazole-2(3H)-thiones (**33-37**) were synthesized by using Mannich reaction. Intermediate **34-38**, formalin (40 %) and compound **18** were stirred in ethanolic solution at room temperature to obtain **4a-e.**

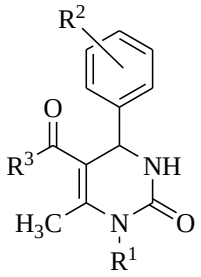


Scheme 5: synthesis of 5-substituted-1,3-oxadiazoles derivatives **(4a-e**)

2.2. Structure-activity relationship (SAR) around hit 1f and *in vitro* activity against *Ec*TP

Initially, a series of diversely substituted 3,4-dihydropyrimidin-ones (**1a-k**) were synthesized and tested for their TP-inhibitory activity (**Table 1)** against ***Ec*TP**. Unlikely as expected, out of these eleven compounds, only compound **1f** showed above 70% inhibition (**Table 1**, IC_{50}= 150.5±0.005 μM) when compared to the standard **7-DX**. Compound **1a** showed above 50% inhibition. Rest of all the compounds showed less than 50% inhibition i.e. insignificant IC_{50} values. With an aim to improve the potential of TP inhibition, we carried out structure-activity relationship (SAR) of this **1f** hit. All the new eighteen derivatives of **1f** are categorized into three different sets i.e. **2a-h (Schemes 2-3)**, **3a-e (Scheme 4)** and **4a-e (Scheme 5).** These derivatives have also been assessed for their *in vitro* enzyme inhibition potential against 7-DX as standard drug. All the tested compounds along with their IC_{50} values are shown in Tables **1-4**.

Table 1: IC_{50} values of some 3,4-DHPM-ones(**1a-k**) as thymidine phosphorylase



(1a-k)

No.	R^1	R^2	R^3	IC_{50}±SEM (μM)
1a	H	Ph	OEt	279.0±0.04
1b	H	Ph	Me	NI
1c	H	3- OMe,4-OH C_6H_3	OEt	NI
1d	H	2- Me.C_6H_4	Me	NI
1e	Me	Ph	Me	NI
1f	H	4-Cl.C_6H_4	OEt	150.5±0.005
1g	Bn	Ph	OEt	NI
1h	H	Ph	OEt	NI
1i	H	Ph	Me	NI
1j	H	3-OH.C_6H_4	OEt	NI
1k	H	3-OH.C_6H_4	Me	NI
7-DX (Standard TP inhibitor)				**38.68±0.42**

[a] NI= No inhibition shown in tested concentration

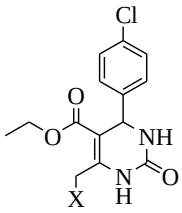
The IC_{50} values of eight compounds **2a-h** is shown in **Table 2**. A close inspection revealed that the presence of amide or carboxyl group at C-6 side chain, strongly affects the activities as evident from the IC_{50} values of **2a**, **2c, 2d** and **2h** with IC_{50}=53.34±1.05, 38.50±0.85, 25.64±1.10 and 40.65±0.85 μM respectively. DHPM with semicarbazide nucleus (**2c**) showed good inhibition with IC_{50} value of 38.50±0.85 μM which is close to standard 7-DX. Replacement of amide $–NH_2$ with pyridine ring (**2d**, using isoniazid **13** as starting material)

showed even better inhibition (IC_{50} =25.64±1.10 μM) than **2c**. Compounds **2b**, **2f** and **2e** showed poor inhibition against target enzyme with an IC_{50} = 123.64±2.44, 95.25±1.54 and 129.14±2.84 μM respectively. Compound **2g** is found to be totally in-active against TP and showed no inhibition at all (**Table 2)**.

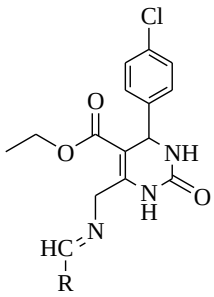
Second series comprises five Schiff-base derivatives (**3a-e**) of 6-aminomethyl derivative (**18)** with four different aldehydes (**19-22**) and one isatin (**23**). These all compounds showed poor inhibition (**Table 2**), except compound **3e**. Compound **3e** with IC_{50} of 32.26±1.20 μM emerged as active compound of this series. This activity may be attributed to the isatin core, where, isatin have shown promising anticancer activities in previous studies [16].

Third series belongs to 5-substituted-1,3-oxadizole derivatives (**4a-e**) of compound **18**. IC_{50} values of all the compounds from this series are tabulated in **Table 2**. It is clear from these values that all of these five compounds proved to be stronger TP-inhibitors, as compared to the standard. Compound **4b** being the strongest inhibitor of this series with an IC_{50}=1.09±0.004 μM. Next more active compound of this series is compound **4c** with an IC_{50}=6.57±0.03 μM. Rest of three also showed excellent inhibition where IC_{50} values range from 9.14±0.06- 14.50 ±0.82 μM.

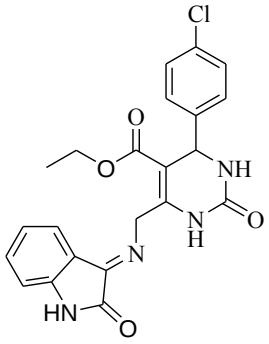
Table 2: IC_{50} values of derivatives of 5-substituted-1,3-0xadiazole derivatives of 3,4-DHPM-ones as thymidine phosphorylase.



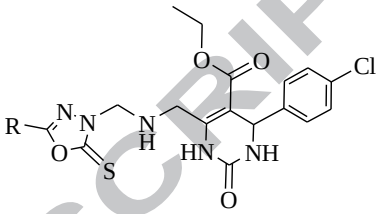
2a-h



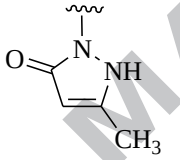
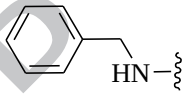
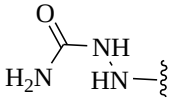
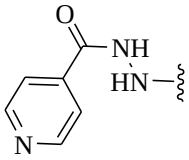
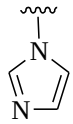
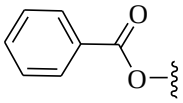
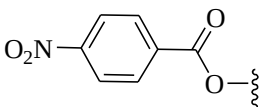
3a-d

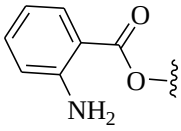
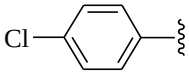
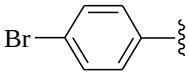
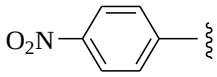
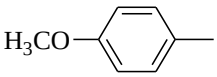
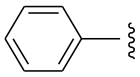
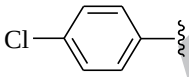
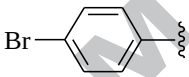
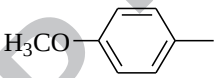
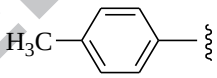


3e



4a-e

Compound No.	X/R	IC_{50}±SEM (μM)
2a		53.34±1.05
2b		123.64±2.44
2c		38.50±0.85
2d		25.64±1.10
2e		129.14+2.84
2f		95.25±1.54
2g		NI

2h		40.65±0.85
3a		87.35±3.14
3b		64.64±2.81
3c		ppt[a]
3d		118.0±3.27
3e	-	32.26±1.20
4a		9.14+0.06
4b		1.09±0.004
4c		6.57±0.03
4d		11.80±0.63
4e		14.50±0.82
7-DX (Standard TP inhibitor)		**38.68±0.42**

[a] Compound was precipitated out in the assay medium

2.3. Antiangiogenic studies

The study has been extended to assess the potential of our synthesized compounds on normal vascular development in chick embryos using chicken chorioallantoic membrane (CAM) model [17]. CAM is a multifaceted, simple and low cost preclinical *in vivo* model for tumour biology and used as alternative to commonly used murine models. It is widely used to study tumour angiogenesis and different malignancies [18]. The chorioallantoic membrane (CAM) is a transient respiratory organ of the chick, which lies just beneath the shell membrane [19].

Tumour growth substantially depends upon the blood supply and the blood supply depends upon the number of blood vessels. So, each tumour requires new blood vessels formation repeatedly due to its significantly rapid growth. Therefore, to inhibit the tumour growth, the inhibition of neovascularization is one of the targets. We employed CAM assay to evaluate the potential of our test compounds on inhibition of new blood vessels formation. The angiogenic inhibitory assay was carried out on fertilized eggs. Dexamethasone and methotrexate was used as positive control. Some of us reported CAM assay for the antitumor and anti-angiogenic potentials of crude extracts of some medicinal plants using dexamethasone as positive control [20]. Dexamethasone has been reported to be anti-angiogenic and have the ability to inhibit the new vascularization [21]. The highest angiogenesis inhibition potential was demonstrated by **4b** with IC_{50} value of 11.34 μM (**Table 3**). Similarly, in the remaining tested compounds the **4a** have also been good activity with IC_{50} value of 13.05 μM. It is clear from the mentioned results that all the other samples will also showed dose dependent response. In a reported CAM assay, 7-DX inhibited partially the TP-induced angiogenesis [22]. In current experiment, 7-DX was found to have IC_{50} value of 9.92±0.65 μM on normal vascular development in chick embryos. Methotrexate showed highest antiangiogenic properties with IC_{50} value of 0.85±0.42 μM. The IC_{50} values for anti-angiogenesis activity are given in **Table 3**. Figures of the CAM results are shown in **Figure 3**.

The anti-angiogenic activity of the synthesized compound different concentration (1000, 500, 250, 125 and 62.5 μg/ml) were based on the number of blood vessels formed in the CAM of chick embryo. **Table S-1** (**in Supporting Information**) showed the average number of blood vessels of each concentration in the CAM of chick embryo using different five concentration of each compound. The IC_{50} value was calculated *via* regression analysis of dose response

curve by adding all the concentrations and percent activities (**Figure S-1** in **Supporting Information**).

Table 3: Results of anti-angiogenic assay of various samples

Samples	IC_{50} ± SEM (μM)
2a	**63.1±0.55**
2e	**503.7±0.85**
3d	**307.1±0.06**
3e	**56.2±1.41**
4a	**13.05±0.58**
4b	**11.34±0.71**
4d	**27.6±0.90**
4e	**25.0±1.15**
7-DX	**9.92±0.65**
Dexamethasone	**30.0±1.05**
Methotrexate	**0.85±0.42**

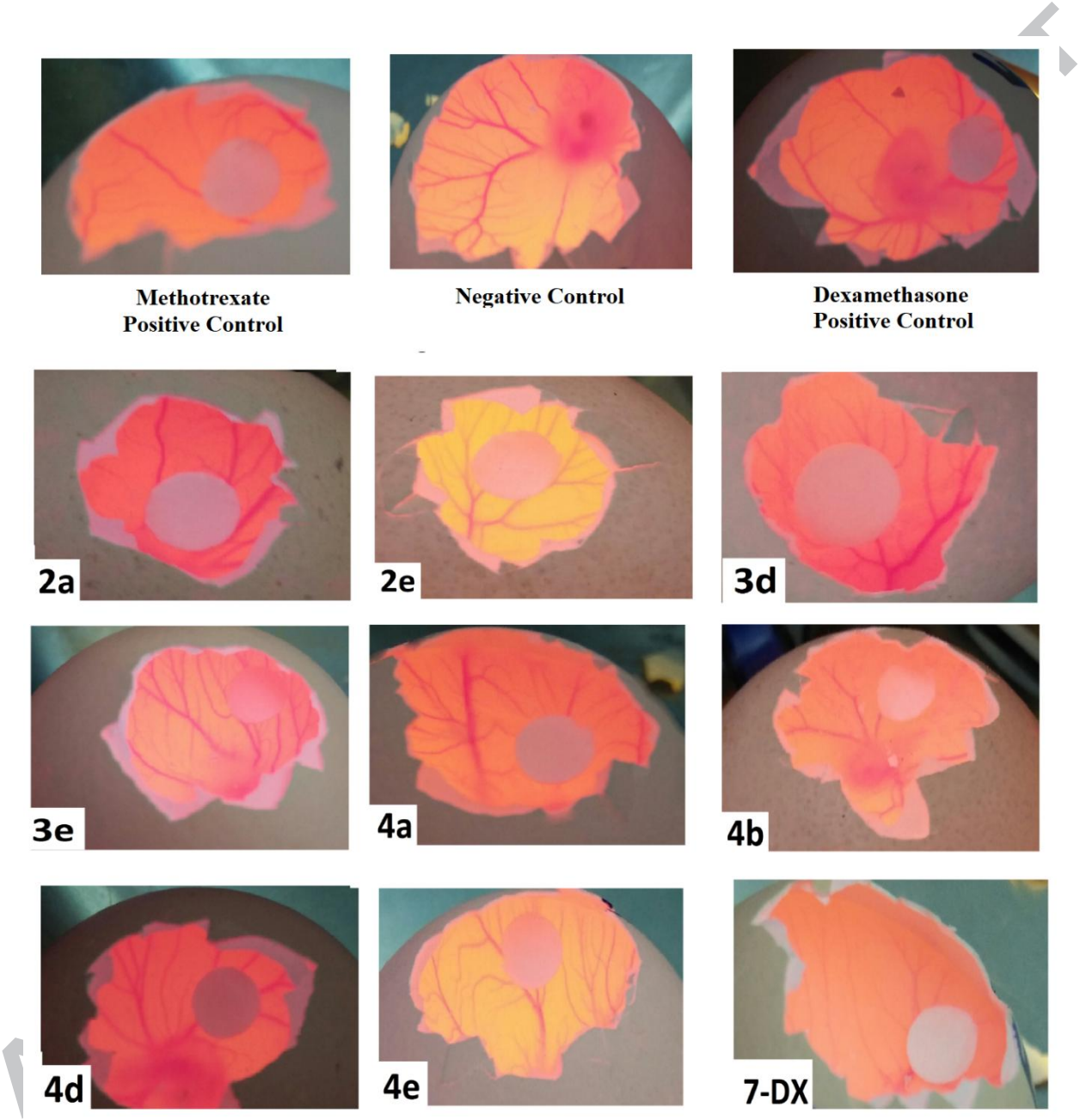


Figure 3: Representative images of CAM results of anti-angiogenic assay

2.4. Docking studies

Many structures of *Thymidine phosphorylase* (TP) are now a day's available, including *Salmonella typhimurium* (4YEK) and *E. coli* (4EAD). Recently, structure of human TP (*h*TP)

has also been solved and available from PDB (1UOU and 2JOF) [23-26]. The IUOU, one of the reported structure of *h*TP is basically co-crystallized structure with uracil derivative at a resolution of 2.1 Å. Omari *et al* deposited another crystal structure of *h*TP in PDB (code: 2J0F), at 2.3 Å resolution. This structure is basically a non-trypsinized *h*TP–thymine complex crystal structure. Structural analysis of enzyme revealed that thymine, thymidine, and phosphate binding sites are situated in a 10 Å deep and 8 Å wide cavity like space.

With most of the conserved α-domain, the TP of *E. Coli* (*Ec*TP) and *h*TP have almost ~42% of sequence similarity overall [24, 27]. This conserved sequence is even similar for the active-site residues with a single exception for Phe210 in *Ec*TP. In detail the active site of *h*TP consists of residues His116, Ser117, Leu148, Arg171, Ser186, Lys190, Arg202, Val208, Ile214, His216, Lys221 and Val241. Where, Val241in *h*TP corresponds to Phe210 in *Ec*TP. His85, His116, Arg171, Ser186, and Lys190 are some of residues of active site that hold key importance in all the catalytic processes.

Owing to the conserved active site residues of *h*TP and *Ec*TP, we checked the propensity of the compounds to bind to *h*TP. We selected 2J0F and docked the compounds into its active site. The reliability of the docking algorithm was validated using RMSD method. The co-crystalized ligand (thymine) was re-docked and the root mean square deviation (RMSD) between co-crystallized and re-docked conformation was determined. The RMSD value of <2.0 Å is considered as accurate in predicting binding orientation of ligand. In order to compare the binding poses of our compounds, we also performed the docking studies on potent TPIs **2** and **3**. The two-dimensional binding pose of **2** and **3** are shown in **Figure S-2** in **Supporting information**.

Visual inspection of the docked poses of all the series of the synthesized compounds into the active site of 2J0F revealed that phenyl rings of the compounds formed extensive π-π stacking interactions with His116, a key residue of α/β domain. Similarly, Ser117, Arg202

also formed hydrogen bonding interactions with carbonyl oxygens and –N/-NH of the compounds. Compounds of Series 2 (**2a-h**) showed variable *in vitro* TP inhibition data. Comparison of two active compounds of this series **2c** and **2d** revealed that semicarbazide derivative **2c** (IC_{50}=38.50±0.85 μM) forms five HB interactions with Ser117, Thr118, Gly120, Ser144 and Thr154 (**Figure 4a)**. While, isoniazid **2d** (IC_{50}=25.64±1.10 μM) forms five HB interactions and one π-stacking interactions with His116 (**Figure 4b)**. Oxygen atom of DHPM ring orients itself towards Ser217 and Lys221. Pyridine nitrogen of isoniazid accepts hydrogen form Ser126 and ring forms π-stacking interactions with His116. The binding poses of compounds **2a-b** are shown in **Figure S-2** (Supporting Information). The poor inhibitor of the series is imidazole derivative **2e** (IC_{50}=129.14±2.84 μM) forms only π-stacking interactions with His116 (**Figure S-3 in Supporting Information)**.

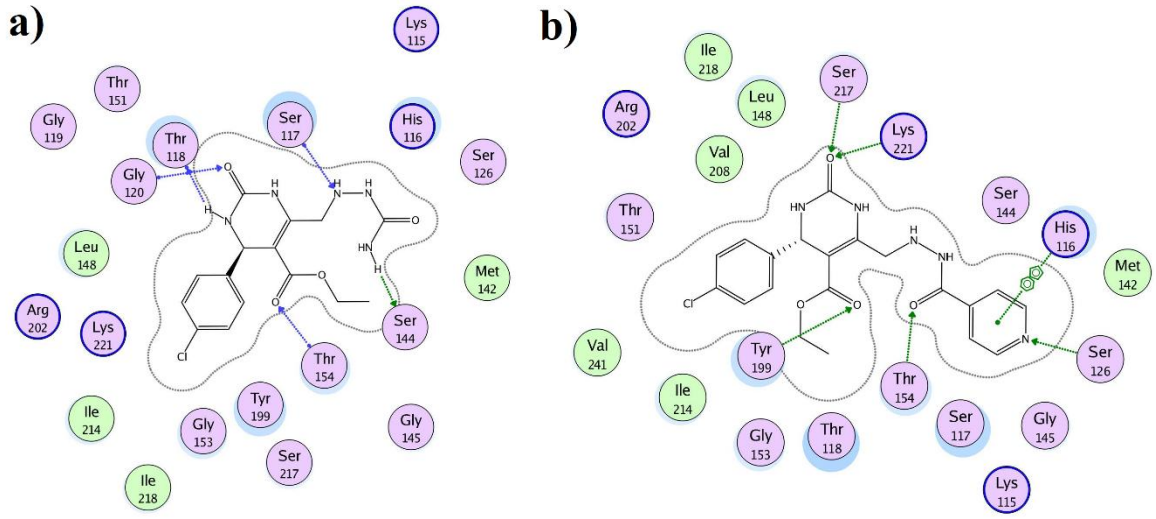


Figure 4: Two-dimensional (2D) ligand interaction map of compound docked into the binding site of 2JOF. a) **2c** and b) **2d**

We have analysed the docking poses of active compound of Schiff base derivative (**3e**) and the most potent TPI studied in present research (**4b,** IC_{50} = 1.09±0.004 μM). The superimposed docking pose of these compounds, TPI and 7-dx is shown in **Figure 5a-b**.

Examination of the best-ranked pose of most active compound of the series **3e,** revealed that isatin nucleus is located close to the key residues Arg202 and Ser217 (**Figure 5c**). DHPM ring forms HB interactions with Tyr199 and Thr154. Chlorobenzene ring at 4-position of DHPM forms π-stacking interactions with His116. These results are in good agreement with bioactivity data. Oxadiazole ring point towards hydrophobic pocket formed by Val208 and Ile214. Chlorobenzene ring at 4-position of DHPM forms π-stacking interactions with His116 and chloro-group interacts with Asp114. DHPM ring itself directed towards Ser117, Thr118, Gly119, Gly120 pocket and forms HB interactions with these residues. Moreover,2-thioxo group displays interactions with Arg202. On the other hand, 4-chlorophenyl at 5-position of oxadiazole ring directed towards phosphate binding site (His150 and Gly239) and forms HB interactions with Gly239 (**Figure 5d**). It is noted that in all the cases, the same amino acids are involved in the binding of our compounds and TPIs **2-3**.

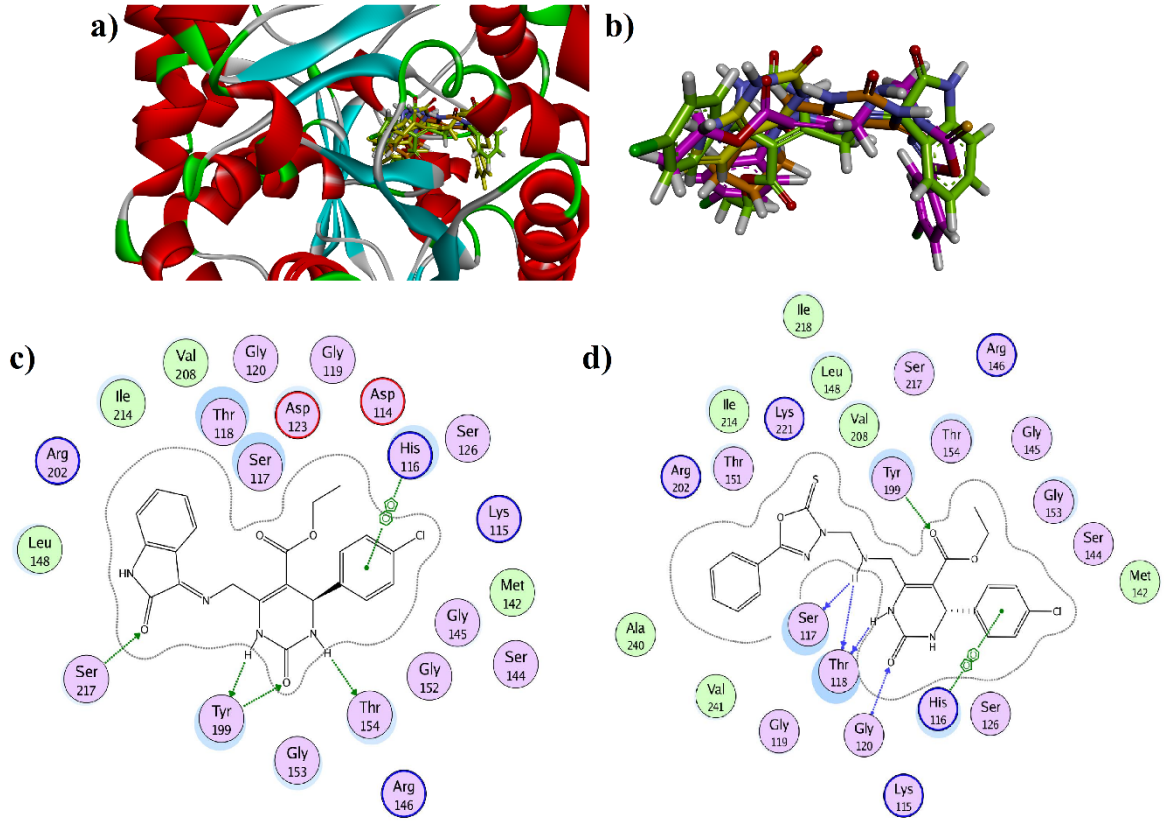


Figure 5: (a-b) Lowest energy superimposed model of **3e** (green)**, 4b** (pink), **TPI** (brown) and 7-dx (yellow) into the binding site of 2JOF. **(c-d)** 2D ligand interaction map of compound **3e (c)** and **4b (d)** docked into the binding site of 2JOF.

3. Conclusions

Three series of compounds were synthesized by the structural modification of initial DHPM hit **1f** at C-6 position. The resulting 18 compounds were evaluated for their in vitro thymidine phosphorylase inhibition potential. A number of compounds showed remarkable activity profile toward TP. Compounds **2c** and **2d** of series-1 were found more active than standard drug. For series-2, only **3e** was able to inhibit TP with IC_{50} value of 32.26 μM. Oxadiazole derivatives **4a-e** exhibited excellent affinity with TP and display activity in low micromolar range (IC_{50} values in μM). Based on these results, we further investigated the *in vivo* antiangiogenic activity of some of the compounds. Compound **4b**, an excellent TP inhibitor of current study, also showed excellent antiangiogenic activity. By using MOE, binding mode analysis of all the compounds into the active site of human TP was explored and explained the results of TP inhibition.

4. Materials and methods

All the reagents and solvents were purchased from standard commercial vendors and were used without any further purification. Isoniazid was taken from Islamabad Pharmaceutical Products (IPP), Islamabad. ^{1}H and ^{13}C-NMR spectra were recorded in DMSO-d_6 on a Bruker spectrometer at 300 and 75 MHz respectively using tetramethylsilane (TMS) as internal reference. Chemical shifts are given in δ scale (ppm). Melting points were determined in open capillaries using Gallenkamp melting point apparatus (MP-D). The progress of all the reactions was monitored by TLC on 2.0 x 5.0 cm aluminium sheets pre-coated with silica gel

60F254 with a layer thickness of 0.25 mm (Merck). LC-MS spectra were obtained using Agilent technologies 1200 series high performance liquid chromatography comprising of G1315 DAD (diode array detector) and ion trap LCMS G2445D SL. Elemental analyses were conducted using a LECO-932 CHNS analyser. Reported results are the mean value of three individual readings for each element (C, H and N).

4.1: Synthesis of 3,4-dihydropyrimidinones (1a-k)

In a pyrex tube equimolar quantities of variety of aldehydes and di-carbonyl compounds (10 mmol each) along with urea (12 mmol) were mixed in acetonitrile (10 mL). $SnCl_2.2H_2O$ (10 mol %) was used as catalyst. The reaction mixture was then sonicated in an ultrasonic bath at 70-75 ^oC. Progress of the reaction was monitored through TLC. Pure product was obtained through silica gel chromatography. All the products were characterized by their melting points, LC/MS and their spectral data. Compounds (**1a-k**) were already synthesized and reported earlier by our research group [20-21].

4.2: Ethyl-6-(bromomethyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (8)

To a solution of 30 mmol of dihydropyrimidine (**1f,** 8.85 g) suspended in 30 ml of $CHCl_3$, added 20 mmol bromine dropwise through a dropping funnel. The reaction mixture was stirred for 12 hrs at 4 ^oC. Solvent was removed under pressure on completion of reaction and pure products were obtained through column chromatography (silica gel; petroleum ether:ethyl acetate) [20]. ^1H NMR (300 MHz, DMSO-d_6): δ 9.22 (s, 1H, *NH*), 7.77 (s, 1H, *NH*), 7.14 (d, 2H *J*= 7.5 Hz, Ar-*H*), 7.02 (d, 2H *J*= 7.5 Hz, Ar-*H*), 5.14 (d, 1H, *J*=3.3 Hz, *CH*), 4.62 (s, 2H, CH_2Br), 3.98 (q, 2H, *J*=7.2 Hz, OCH_2), 1.09 (t, 3H, *J*=7.2 Hz, OCH_2CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 166.6 153.1, 147.26, 144.8, 135.1, 128.3, 127.9, 126.9, 126.7, 106.95, 60.61, 52.83, 37.63, 15.0. LCMS : *m/z* = 373.1 [M+H].

4.2.1: Ethyl-4-(4-chlorophenyl)-6-hydrazinylmethyl)-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylate (**9**)

Compound **8** (10 mmol) and hydrazine hydrate (10 mmol) were mixed in 20 mL of ethanol. Then few drops of H_2SO_4 were added to the reaction mixture. Reaction mixture was then refluxed for 4-5 hrs. After completion of reaction the solvent was removed under vacuum. Pure product **9** was obtained through silica gel column chromatography with 10% methanol (MeOH) in chloroform ($CHCl_3$). R_f =0.39 ($CHCl_3$/MeOH 5:1). Yield 68%. m.p. 208-210°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.11 (s, 1H, *NH*), 7.61 (s, 1H, *NH*), 7.12 (d, 2H *J*= 8.4 Hz, Ar-*H*), 6.88 (d, 2H *J*= 8.4 Hz, Ar-*H*), 5.03 (d, 1H, *J*=2.7 Hz, *CH*), 3.97 (q, 2H, *J*=6.9 Hz, *CH*), 3.57 (s, 2H, *CH*$_2$NH), 3.25 (brs, 2H, NH_2), 2.88 (brs, 1H, NH), 1.11 (t, 3H, *J*=6.9 Hz, OCH_2CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 168.7, 153.02, 142.6, 140.07, 135.3, 128.3, 127.9, 126.7, 126.5, 106.4, 59.87, 52.9, 50.09, 15.03. LCMS : *m/z* = 325.1 [M+H].

4.2.1.1: Synthesis of ethyl-4-(4-chlorophenyl)-6-((3-methyl-5-oxo-2H-pyrazol-1(5H)-yl)methyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2a)

2a was prepared by reacting compound **9** (5 mmol) and ethyl acetoacetate (5 mmol). The reaction mixture was refluxed in acidified methanol. After completion of reaction the solvent was removed under pressure. The crude solid was recrystallized with ethyl acetate to obtain pure product. R_f = 0.43 ($CHCl_3$/MeOH; 6:1). Yield 73%. m.p. 196-198 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.86 (s, 1H, NH), 9.11 (br s, 1H, *NH*), 7.65 (br s, 1H, *NH*), 7.12 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.02 (d, 2H *J*= 8.1 Hz, Ar-*H*), 5.28 (1H, CO-CH), 5.04 (d, 1H, *J*=2.7 Hz, *CH*), 3.99 (q, 2H, *J*=7.2 Hz, *OCH*$_2$), 3.60 (s, 2H, *CH*$_2$N), 1.91 (s, 3H, C=C*CH*$_3$), 1.01 (t, 3H, *J*=7.2 Hz, OCH_2CH_3).^{13}C-NMR (75 MHz, DMSO-d_6): δ 164.9, 162.7, 154.7, 151.4, 143.3,

137.3, 134.3, 130.8, 130.6, 126.4 (2C), 101.9, 96.5, 60.1, 54.7, 53.8, 27.1, 14.9; LCMS : *m/z* = 391.1 [M+H]. Anal. calcd for $C_{18}H_{19}ClN_4O_4$: C 55.32; H 4.90; N 14.34 (found) C 55.28; H 4.92; N 14.32.

4.2.2: Ethyl-6-(bromomethyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives (2b-h):

Equimolar (2 mmol) mixtures of 6-bromo methylene DHPM (**8**) and compounds (**11-17**) each in 5mL of THF was heated in ultrasonic bath at 40 ºC. Reactions were monitored through TLC and LCMS. Crude mixtures were then purified by re-crystallization or column chromatography whichever was applicable.

4.2.3: Ethyl-6-((benzylamino)methyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylate (2b)

2b was prepared according to the general procedure by reacting **8** (2 mmol) and benzyl amine (2 mmol). R_f =0.51 (*n*-hexanes/ethyl acetate; 5:1). Yield 73%. m.p. 181-183°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.97 (br s, 1H, *NH*), 8.19 (br s, 1H, *NH*), 7.21 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.16-1.08 (m, 5H, Ar-H), 7.06 (d, 2H *J*= 8.1 Hz, Ar-*H*), 6.01 (brs, 1H, *J*= 6.9 Hz, NH), 4.97 (d, 1H, *J*=3.3 Hz, *CH*), 4.17 (q, 2H, *J*=7.2 Hz, OCH_2), 4.57 (brs 2H, *J*= 6.9 Hz, CH_2NH), 3.28 (brs, 2H, *J*= 6.9 Hz, CH_2NH), 1.01 (t, 3H, *J*=7.2 Hz, OCH_2CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ168.62, 152.37, 145.21, 138.1, 137.37, 134.01, 130.9, 130.3, 129.9, 126.2, 126.1, 124.6 (2C), 124.2, 124.1, 102.9, 60.8, 53.8, 52.6, 50.9, 14.6. LCMS : *m/z* = 400.2 [M+H]. Anal. calcd for $C_{21}H_{22}ClN_3O_3$: C 63.08; H 5.55; N 10.51 (found) C 63.05; H 5.54; N 10.49.

4.2.4. Ethyl-6-((benzylamino)methyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylate (2c)

2c was prepared according to the general procedure by reacting **8** (2 mmol) and semicarbazide (2 mmol). R_f =0.39 ($CHCl_3$/CH_3OH; 3:1). Yield 76 %. m.p. 182-183°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.33(s, 2H, NH_2), 10.68 (brs, 1H, NH), 9.89 (brs, 1H, NH), 8.19 (br s, 1H, *NH*), 7.18 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.03 (d, 2H *J*= 8.1 Hz, Ar-*H*), 6.68 (br s, 1H, *NH*), 5.14 (d, 1H, *J*=3.3 Hz, *CH*), 4.13 (q, 2H, *J*=7.2 Hz, OCH_2), 3.48 (brs, 2H, CH_2NH), 1.19 (t, 3H, *J*=7.1Hz, OCH_2CH_3).^{13}C-NMR (75 MHz, DMSO-d_6): δ167.87, 155.33, 152.23, 143.86, 137.5, 134.1, 130.9, 130.4, 126.4, 126.2, 103.78, 61.53, 53.21, 51.11, 15.14. LCMS : *m/z* = 368.1 [M+H]. Anal. calcd for $C_{15}H_{18}ClN_5O_4$: C 48.98; H 4.93; N 19.04 (found) C 49.00; H 4.94; N 19.02.

4.2.5 Ethyl 4-(4-chlorophenyl)-2-oxo-6-{[(pyridin-4-yl)formohydrazido]methyl}-1,2,3,4-tetrahydropyrimidine-5-carboxylate (2d)

2d was prepared according to the general procedure by reacting **8** (2 mmol) and isonicotinohydrazide (**13**) (2 mmol). R_f = 0.56 (*n*-hexanes/ethyl acetate; 2:1). Yield 83 %. m.p. 202-204°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.11 (brs, 1H, NH), 9.88 (s, 1H, NH), 8.77 (d, 2H, *J*=7.8 Hz, Ar-H), 8.24 (br s, 1H, *NH*), 7.99 (d, 2H, *J*=7.8 Hz, Ar-H), 7.12 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.04 (d, 2H *J*= 8.1 Hz, Ar-*H*), 6.69 (br s, 1H, *NH*), 5.09 (d, 1H, *J*=3.0 Hz, *CH*), 4.00 (q, 2H, *J*=6.9 Hz, OCH_2), 3.34 (brs, 2H, CH_2NH), 0.93 (t, 3H, *J*=6.9 Hz, OCH_2CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 167.6, 166.2, 153.0, 151.5 (2C), 142.9, 141.6, 139.8, 134.3, 126.8 (2C), 126.6, 126.4, 124.1 (2C), 104.6, 62.0, 53.2, 50.6, 14.4. LCMS: *m/z* = 430.1 [M+H]. Anal. calcd for $C_{20}H_{20}ClN_5O_4$: C 55.88; H 4.69; N 16.29 (found) C 55.86; H 4.70; N 16.32.

4.2.6: Ethyl-6-((1H-imidazol-1-yl)methyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylate (2e)

2e was prepared according to the general procedure by reacting **8** (2 mmol) and 1H-imidazole (2 mmol). R_f =0.47 (*n*-hexanes/ethyl acetate; 2:1); Yield 81 %. m.p. 208-210 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.68 (s, 1H, NH), 8.10 (s, 1H, NH), 7.64 (s, 1H, -N-HC=N), 7.19 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.03 (d, 2H *J*= 8.1 Hz, Ar-*H*), 6.98 (m, 2H, *imidazole-H*), 5.09 (d, 1H, *J*= 3.3 Hz, *CH*), 4.39 (s, 2H, CH_2N), 4.16 (q, 2H, *J*=7.1 Hz, OCH_2), 1.27 (t, 3H, *J*=7.1Hz, OCH_2CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 169.13, 152.6, 142.89, 141.37, 134.9, 134.3, 126.8, 126.7, 126.4 (2C), 126.09, 118.71, 104.93, 61.48, 53.01, 50.17, 15.11. LCMS : *m/z* = 361.1 [M+H]. Anal. calcd for $C_{17}H_{17}ClN_4O_3$; C, 56.59; H, 4.75; N, 15.53 (found) C 56.56; H 4.76; N 15.55.

4.2.7: Ethyl-6-(benzoyloxymethyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylate (2f)

2f was prepared according to the general procedure by reacting **8** (2 mmol) and benzoic acid (2 mmol). R_f = 0.52 (*n*-hexanes/ethyl acetate; 5:1) . Yield 86 %. m.p. 199-200 °C ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.476 (s, 1H, NH), 8.011 (s, 1H, NH), 7.99 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.891-7.583 (m, 1H, Ar-H), 7.55 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.39-7.25 (m, 4H, Ar-H), 5.13 (d, 1H, *J*=3.3 Hz, *CH*), 5.10 (s, 2H, CH_2O), 3.99 (q, 2H, *J*=7.2 Hz, OCH_2), 1.050 (t, 3H, *J*=7.2 Hz, OCH_2CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): 169.6, 168.18, 152.6, 145.79, 139.6, 132.7, 132.03, 131.97, 130.1, 129.4 (2C), 128.1 (2C), 126.8, 126.5, 106.3, 104.1, 67.79, 62.2, 50.4, 15.19. LCMS : *m/z* = 415.1 [M+H]. Anal. calcd for $C_{21}H_{19}ClN_2O_5$: C 60.80; H 4.62; N 6.75 (found) C 60.76; H 4.64; N 6.77.

4.2.8: Ethyl-4-(4-chlorophenyl)-6-((4-nitrobenzoyloxy)methyl)-2-oxo-1,2,3,4-tetra-hydropyrimidine-5-carboxylate (2g)

2g was prepared according to the general procedure by reacting **8** (2 mmol) and 4-nitro benzoic acid (2 mmol). R_f = 0.48 (*n*-hexanes/ethyl acetate; 5:1). Yield 81%. m.p. 207-209°C.

^{1}H NMR (300 MHz, DMSO-d_6): δ 9.98 (s, 1H, NH), 9.81 (s, 1H, NH), 8.33 (d, 2H *J*= 8.7 Hz, *Ar-H*), 8.17 (d, 2H, *J*= 8.7 Hz, *Ar-H*), 7.19 (d, 2H *J*= 7.8 Hz, Ar-*H*), 7.03 (d, 2H *J*= 7.8 Hz, Ar-*H*), 5.17 (d, 1H, *J*=3.3 Hz, *CH*), 5.08 (s, 2H, CH_2O), 4.17 (q, 2H, *J*=7.5 Hz, OCH_2), 1.33 (t, 3H, *J*=7.5 Hz, OCH_2CH_3).^{13}C-NMR (75 MHz, DMSO-d_6): 168.6, 167.9, 154.1, 153.3, 146.36, 139.89, 139.1, 133.4 (2C), 132.6, 130.1 (2C), 129.15, 127.9, 127.3, 126.9, 105.9, 69.01, 62.3, 50.8, 15.21. LCMS: *m/z* = 460.1. Anal. calcd for $C_{21}H_{18}ClN_3O_7$: C, 54.85; H, 3.95; N, 9.14 (found) 54.84; H, 3.97; N, 9.12.

4.2.9. Ethyl-6-((2-aminobenzoyloxy)methyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetra-hydropyrimidine-5-carboxylate (2h)

2h was prepared according to the general procedure by reacting **8** (2 mmol) and 2-aminobenzoic acid (2 mmol). R_f =0.51 (*n*-hexanes/ethyl acetate; 5:1). Yield 79%. m.p. 211-213 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.1 (s, 1H, NH), 9.87 (s, 1H, NH), 7.91 (dd, *J*= 8.4 Hz, *J*= 1.5 Hz, 1H, Ar-H), 7.32 (m, 1H, Ar-H), 7.16 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.06 (d, 2H *J*= 8.1 Hz, Ar-*H*), 6.76 (t, 2H, *J*= 7.5 Hz, Ar-*H*), 5.84 (s, 2H, NH_2), 5.16 (d, 1H, *J*=3.3 Hz, *CH*), 5.10 (s, 2H, CH_2O), 4.14 (q, 2H, *J*=7.2 Hz, OCH_2), 1.22 (t, 3H, *J*=7.2 Hz, OCH_2CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): 169.1, 168.29, 153.5, 152.8, 146.4, 143.7, 142.91, 135.2, 129.9, 126.79 (2C), 126.6, 126.4, 120.6, 118.42, 108.8, 104.6, 67.87, 62.6, 50.31, 15.17. LCMS : *m/z* = 430.1. Anal. calcd for $C_{21}H_{20}ClN_3O_5$: C 58.68; H 4.69; N 9.78 (found) C 56.65; H 4.70; N 9.80.

4.3: Synthesis of ethyl-6-(aminomethyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetrahydro pyrimidine-5-carboxylate (18)

A solution of 6-bromo DHPM (15 mmol) in DMSO (10 mL), was made to react with 30 mmol of ammonium acetate in an ultrasonic bath at 45-50 °C for 35 minutes. Pure product was obtained through silica gel chromatography. R_f = 0.56 (*n*-hexanes/ethyl acetate; 5:2).

Yield 88%. m.p. 212-214 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.79 (br s, 1H, *NH*), 8.09 (br s, 1H, *NH*), 7.16 (d, 2H *J*= 7.8 Hz, Ar-*H*), 7.00 (d, 2H *J*= 7.8 Hz, Ar-*H*), 6.28 (brs, 2H, NH_2), 5.07 (d, 1H, *J*=3.3 Hz, *CH*), 4.10 (q, 2H, *J*=7.2 Hz, OCH_2), 3.66 (brs, 2H, CH_2NH_2), 1.12 (t, 3H, *J*=7.2 Hz, OCH_2CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ168.3, 153.07, 141.8, 139.6, 134.6, 128.1, 127.9, 126.63 (2C), 106.7, 60.59, 50.12, 42.8, 14.99. LCMS : *m/z* = 310.2. Anal. calcd for $C_{14}H_{16}ClN_3O_3$: C 54.29; H 5.21; N 13.57 (found) C 54.31; H 5.22; N 13.54.

Synthesis of 6-aminomethyl DHPM-derivatives (3a-e):

Compounds **3a-e** was synthesized according to the general reported procedure. Compound (**18,** 0.010 mol) was refluxed in ethanol with five different aldehydes (0.010 mol) in acidic medium. Few drops of glacial acetic acid were used as catalyst. After completion of reaction the solvent was evaporated and pure products were obtained through re-crystallization from ethanol. All the products were then fully characterized on the basis of physical and spectral parameters.

4.3.1: (E)-ethyl-6-((4-chlorobenzylideneamino)methyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3a)

3a was prepared according to the general procedure by reacting **18** (2 mmol) and 4-chlorobenzaldehyde (2 mmol). R_f 0.38 (*n*-hexanes/ethyl acetate; 5:1). Yield 81 %. m.p. 193-195 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.11 (br s, 1H, *NH*), 8.31 (s, 1H, CH=N-), 7.97 (br s, 1H, *NH*), 7.47 (d, 2H *J*= 8.4 Hz, Ar-*H*), 7.34 (d, 2H *J*= 8.4 Hz, Ar-*H*), 7.04 (d, 2H, *J*= 8.4 Hz, Ar-*H*), 6.65 (d, 2H *J*= 8.4 Hz, Ar-*H*), 5.03 (d, 1H, *J*=3.0 Hz, *CH*), 4.36 (s, 2H, CH_2N=C), 3.97 (q, 2H, *J*=6.9 Hz, OCH_2), 1.11 (t, 3H, *J*=6.9 Hz, OCH_2CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 168.3, 162.2, 153.1, 141.6, 140.6 (2C), 139.7 (2C), 138.2, 134.4, 131.3,

131.1, 129.3, 128.0, 127.4, 127.2, 105.95, 60.5, 52.9, 50.1, 14.4. LCMS : *m/z* = 432.1. Anal. calcd for $C_{21}H_{19}C_{l2}N_3O_3$: C 58.34; H 4.43; N 9.72 (found) C 58.38; H 4.41; N 9.70.

4.3.2: (E)-ethyl 6-((4-bromobenzylideneamino)methyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3b)

3b was prepared according to the general procedure by reacting **18** (2 mmol) and 4-bromobenzaldehyde (2 mmol). R_f 0.46 (*n*-hexanes/ethyl acetate; 5:1). Yield 86 %. m.p. 201-203°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.79 (br s, 1H, *NH*), 8.34 (s, 1H, CH=N-), 8.10 (br s, 1H, *NH*), 7.71 (d, 2H *J*= 8.4 Hz, Ar-*H*), 7.39 (d, 2H *J*= 8.4 Hz, Ar-*H*), 7.21 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.03 (d, 2H *J*= 8.1 Hz, Ar-*H*), 5.19 (d, 1H, *J*=3.3 Hz, *CH*), 4.56 (s, 2H, *CH*$_2$N=C), 4.13 (q, 2H, *J*=7.1 Hz, *OCH*$_2$), 1.27 (t, 3H, *J*=7.1 Hz, OCH$_2$*CH*$_3$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 168.6, 161.9, 153.0, 142.01, 141.9, 141.6, 140.03, 134.6, 132.6, 132.4, 131.2, 130.3, 129.0, 128.78, 128.72, 128.11, 105.83, 60.71, 52.6, 50.11, 14.69. LCMS : *m/z* = 476.03. Anal. calcd for $C_{21}H_{19}BrClN_3O_3$: C, 52.90; H, 4.02; N, 8.81. (found) C 52.87; H 4.03; N 8.84.

4.3.3: (E)-ethyl-6-((4-nitrobenzylideneamino)methyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3c)

3c was prepared according to the general procedure by reacting **18** (2 mmol) and 4-nitrobenzaldehyde (2 mmol). R_f 0.56 (*n*-hexanes/ethyl acetate; 5:1). Yield 88 %. m.p. 198-201°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.83 (br s, 1H, *NH*), 8.41 (d, 2H *J*= 8.1 Hz, Ar-*H*), 8.29 (s, 1H, CH=N-), 8.11 (br s, 1H, *NH*), 7.79 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.18 (d, 2H *J*= 7.8 Hz, Ar-*H*), 7.01 (d, 2H *J*= 7.8 Hz, Ar-*H*), 5.04 (d, 1H, *J*=3.3 Hz, *CH*), 4.37 (s, 2H, *CH*$_2$N=C), 4.17 (q, 2H, *J*=7.2 Hz, *OCH*$_2$), 1.05 (t, 3H, *J*=7.2 Hz, OCH$_2$*CH*$_3$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 169.9, 162.5, 153.5, 151.7, 148.1, 142.9, 142.2, 142.1, 140.03, 132.7, 132.5, 132.4, 131.89, 129.0, 128.9, 128.2, 105.7, 60.67, 53.7, 50.09, 15.1. LCMS: *m/z* = 443.1

[M+H]. Anal. calcd for $C_{21}H_{19}ClN_4O_5$: C 56.95; H 4.32; N 12.65 (found) C 56.97; H 4.33; N 12.63.

4.3.4: (E)-ethyl 6-((4-methoxybenzylideneamino)methyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3d)

3d was prepared according to the general procedure by reacting **18** (2 mmol) and 4-methoxybenzaldehyde (2 mmol). R_f =0.49 (*n*-hexanes/ethyl acetate; 5:1). Yield 73%. m.p. 189-192 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.76 (br s, 1H, *NH*), 8.35 (s, 1H, CH=N-), 8.13 (br s, 1H, *NH*),), 7.66 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.20 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.04 (d, 2H *J*= 8.4 Hz, Ar-*H*), 6.99 (d, 2H *J*= 8.4 Hz, Ar-*H*), 5.27 (d, 1H, *J*=3.3 Hz, *CH*), 3.99 (s, 3H, OCH_3), 4.46 (s, 2H, CH_2N=C), 4.11 (q, 2H, *J*=7.1 Hz, OCH_2), 1.23 (t, 3H, *J*=7.1 Hz, OCH_2CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 168.1, 164.41, 161.1, 152.7, 142.6, 141.4, 134.2, 131.7, 129.8, 129.6, 128.5, 128.3, 127.9, 127.7, 104.89, 111.1, 111.3, 61.6, 60.6, 52.3, 50.07, 14.47. LCMS : *m/z* =: 428.3 [M+H]. Anal. calcd for $C_{22}H_{22}ClN_3O_4$: C 61.75; H 5.18; N 9.82 (found) C 61.71; H 5.20; N 9.84.

4.3.5: (E)-ethyl 4-(4-chlorophenyl)-2-oxo-6-((2-oxoindolin-3-ylideneamino)methyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (3e)

3e was prepared according to the general procedure by reacting **18** (2 mmol) and (2 mmol). R_f = 0.53 (*n*-hexanes/ethyl acetate; 2:1). Yield 73%. m.p. 189-192 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.13 (br s, 1H, *NH*), 9.77 (br s, 1H, *NH*), 8.13 (br s, 1H, *NH*), 7.69 (d, 2H *J*= 8.4 Hz, Ar-*H*), 7.60-7.53 (m, 1H, Ar-*H*), 7.19-7.09 (m, 6H, Ar-*H*), 5.10 (d, 1H, *J*=3.3 Hz, *CH*), 4.39 (s, 2H, CH_2NH_2), 4.03 (q, 2H, *J*=7.2 Hz, OCH_2), 1.09 (t, 3H, *J*=7.2 Hz, OCH_2CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 168.8, 168.4, 163.9, 152.3, 148.04, 142.2, 141.7, 134.1, 129.3, 129.1, 128.76, 127.2, 126.6, 126.4, 122.23, 118.6, 108.3, 61.3, 60.9,

50.87, 50.10, 14.6. LCMS : *m/z* = 439.1 [M+H]. Anal. calcd for $C_{22}H_{19}ClN_4O_4$: C, 60.21; H, 4.36; N, 12.77 (found) C 60.18; H 4.37; N 12.79.

4.4: Synthesis of acid hydrazides (29-33):

A mixture of an ester **24-28** (15 mmol each) and hydrazine hydrate (15 mmol) were refluxed in ethanol (30 mL), for 48 hours. Reaction was monitored through TLC. After completion of reaction the excess of solvent was distilled out. The resulting precipitates were then filtered and thoroughly washed with solvent.

4.4.1. Benzohydrazide (29)

Hydrazide **29** was prepared according to the general procedure by reacting ethyl benzoate (15 mmol) and hydrazine hydrate (15 mmol). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.09 (br s, 1H, *NH*), 4.21 (br s, 2H, NH_2), 8.01-7.46 (m, 5H, Ar-*H*).

4.4.2. 4-Methylbenzohydrazide (30)

Hydrazide **30** was prepared according to the general procedure by reacting 4-hydroxy ethyl benzoate (15 mmol) and hydrazine hydrate (15 mmol). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.23 (br s, 1H, *NH*), 4.07 (br s, 2H, NH_2), 7.71 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.19 (d, 2H *J*= 8.1 Hz, Ar-*H*), 2.33 (s, 3H, CH_3).

4.4.3: 4-Chlorobenzohydrazide (31)

Hydrazide **31** was prepared according to the general procedure by reacting 4-chloro ethyl benzoate (15 mmol) and hydrazine hydrate (15 mmol). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.13 (s, 1H, *NH*), 4.23 (s, 2H, NH_2), 7.76 (d, 2H *J*= 8.4 Hz, Ar-*H*), 7.51 (d, 2H, *J*= 8.4 Hz, Ar-*H*).

4.4.4: 4-Methoxybenzohydrazide (32)

Hydrazide **32** was prepared according to the general procedure by reacting 4-methoxy ethyl benzoate (20 mmol) and hydrazine hydrate (15 mmol). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.19 (s, 1H, *NH*), 7.69 (d, 2H *J*= 8.4 Hz, Ar-*H*), 6.83 (d, 2H *J*= 8.4 Hz, Ar-*H*), 4.10 (s, 2H, NH_2), 3.81 (s, 3H, OCH_3).

4.4.5: 4-bromobenzohydrazide (33)

Hydrazide **33** was prepared according to the general procedure by reacting 4-bromo ethyl benzoate (20 mmol) and hydrazine hydrate (20 mmol). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.20 (br s, 1H, *NH*), 4.17 (s, 2H, NH_2), 7.93 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.81 (d, 2H *J*= 8.1 Hz, Ar-*H*).

4.5.1. Synthesis of 5-substituted-1,3,4- oxadiazole-2(3H)-thiones (34-38)

Compounds **34-38** were synthesized according to the general procedure [16]. To a solution of hydrazides (**29-33**, 10 mmol) in ethanol, carbon disulphide (10 mmol) and triethylamine were added. The reaction mixture was then irradiated with ultrasounds at 70 °C, for 24 hrs. Reaction mixture was washed with ethyl acetate and slightly acidic brine solution, to remove all the in-organic impurities. Organic layer was then dried over anhydrous magnesium sulphate. Solvent was then removed under vacuum. Pure products were obtained by re-crystallization of the crude products from petroleum ether/ethyl acetate.

4.5.1.1. 5-Phenyl-1,3,4-oxadiazole-2(3H)-thione (34)

34 was synthesized according to the general procedure by condensing benzohydrazide with carbondisulfide, in basic medium over alumina. R_f =0.61 (*n*-hexanes/ethyl acetate 1:1)]. Yield 80 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.54 (s, 1H, NH), 7.90 (d, 2H *J*= 7.3 Hz, Ar-*H*), 7.47-7.59 (m, 3H, Ar-H). LCMS : *m/z* = 179.02 [M+H].

4.5.1.2. 5-(4-Chlorophenyl)-1,3,4-oxadiazole-2(3H)-thione (35)

35 was synthesized according to the general procedure by condensing 4-chlorobenzohydrazide with carbon disulphide, in basic medium over alumina. R_f 0.69 (*n*-hexanes/ethyl acetate 1:1. Yield 92%. m.p. 173-174 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.52 (br s, 1H, NH), 7.88 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.65 (d, 2H *J*= 8.1 Hz, Ar-*H*). LCMS : *m/z* = 212.98 [M+H].

4.5.1.3. 5-(4-Bromophenyl)-1,3,4-oxadiazole-2(3H)-thione (36)

38 was synthesized according to the general procedure by condensing 4-bromobenzohydrazide with carbondisulfide, in basic medium over alumina.

R_f =0.66 (*n*-hexanes/ethyl acetate 1:2). Yield 93%. m.p. 23-231 °C. ^{1}H NMR (300 DMSO-d_6): δ 10.52 (br s, 1H, NH), 7.8 (d, 2H, *J*=8.4 Hz, Ar-H), 7.7 (d, 2H, *J*=8.4 Hz, Ar-H). LCMS : *m/z* = 256.3 [M+H].

4.5.1.4. 5-(4-Methoxyphenyl)-1,3,4-oxadiazole-2(3H)-thione (37)

37 was synthesized according to the general procedure by condensing 4-hydroxybenzohydrazide with carbon disulfide, in basic medium over alumina. Yield 91 %. m.p. 233-235 °C. ^{1}H NMR (300 MHz, DMSO-d_6,): δ 10.53 (br s, 1H, NH), 7.18 (d, 2H, *J*=8.4 Hz, Ar-H), 7.83 (d, 2H, *J*=8.4 Hz, Ar-H), 3.77 (s, 1H, OCH_3). LCMS : *m/z* = 209.03 [M+H].

4.5.1.5. 5-*p*-Tolyl-1,3,4-oxadiazole-2(3H)-thione (38)

35 was synthesized according to the general procedure by condensing 4-methylbenzohydrazide with carbon disulphide, in basic medium over alumina. R_f 0.69 (*n*-hexanes/ethyl acetate 1:2). Yield 98%. m.p. 159-160 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ

10.53 (br s, 1H, NH), 7.74 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.30 (d, 2H *J*= 8.1 Hz, Ar-*H*), 2.38 (s, 3H, CH_3). LCMS : *m/z* = 193.04 [M+H].

4.5.2: Synthesis of 3-substituted oxadiazole (4a-e)

Compounds **4a-e** was synthesized according to the following general procedure. 5 mmol of 1,3,4-oxadiazole (**34-38**) each was stirred in ethanol (30 mL). To this solution formalin 40% (5 mmol) was added and mixture is continuously kept on stirring. Now, ethanolic solution of **18** (5 mmol) was gradually added to the stirring mixture, and mixture was allowed to stir at room temperature for about 5-7 hrs. Reaction was monitored through TLC. The reaction was left in a refrigerator overnight and the precipitates of the crude product were then filtered, washed with ethanol and then dried. Pure product was obtained by re-crystallization using hexane/ethyl acetate.

4.5.2.1: Ethyl-4-(4-chlorophenyl)-2-oxo-6-(((5-phenyl-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)methylamino)methyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4a)

4a was synthesized according to the general procedure by condensing **18** (2 mmol) with **34** (5 mmol) in the presence of formalin (6 mmol). R_f =0.39 (*n*-hexanes/ethyl acetate; 5:1). Yield 82 %. m.p. 220-222 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.71 (br s, 1H, *NH*), 8.15 (br s, 1H, *NH*), 7.64-7.53 (m, 5H, Ar-H), 7.12 (d, 2H *J*= 8.1 Hz, Ar-*H*), 6.90 (d, 2H *J*= 8.1 Hz, Ar-*H*), 6.16 (br s, 1H, NH), 5.09 (d, 1H, *J*=3.3 Hz, *CH*), 4.30 (s, 2H, *CH*$_2$NH), 3.93 (q, 2H, *J*=7.2 Hz, *OCH*$_2$), 3.31 (s, 2H, *CH*$_2$NH), 1.01 (t, 3H, *J*=7.2 Hz, OCH$_2$*CH*$_3$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 180.1, 168.6, 157.7, 153.0, 143.2, 142.8, 134.6, 134.1, 131.6 (2C), 131.3, 131.0, 130.7, 130.3, 128.9, 127.9, 126.7, 106.9, 68.1, 60.6, 50.2, 45.9, 14.7. LCMS : *m/z* = 500.11 [M+H]. Anal. calcd for $C_{23}H_{22}ClN_5O_4S$: C 55.25; H 4.44; N 14.01 (found) C 55.22; H 4.46; N 14.03.

4.5.2.2: ethyl 4-(4-chlorophenyl)-6-(((5-(4-chlorophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)methylamino)methyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4b)

4b was synthesized according to the general procedure by condensing **18** (5 mmol) with **35** (5 mmol) in the presence of formalin 40% (5 mmol). R_f =0.39 (*n*-hexanes/ethyl acetate; 5:1). Yield 86%. m.p. 216-218°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.70 (br s, 1H, *NH*), 8.17 (br s, 1H, *NH*), 7.66 (d, 2H *J*= 8.4 Hz, Ar-*H*), 7.56 (d, 2H *J*= 8.4 Hz, Ar-*H*), 7.15 (d, 2H *J*= 8.1 Hz, Ar-*H*), 6.95 (d, 2H *J*= 8.1 Hz, Ar-*H*), 6.15 (br s, 1H, NH), 5.11 (d, 1H, *J*=3.3 Hz, *CH*), 4.29 (s, 2H, *CH*$_2$NH), 3.98 (q, 2H, *J*=6.9 Hz, *OCH*$_2$), 3.31 (s, 2H, *CH*$_2$NH), 0.93 (t, 3H, *J*=6.9 Hz, OCH$_2$*CH*$_3$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 179.4, 168.8, 157.9, 153.1, 142.3, 138.02, 134.1, 133.4(2C), 131.9, 131.7, 130.5 (2C), 130.3, 130.1, 128.8, 128.5, 106.6, 68.1, 60.19, 50.07, 46.97, 14.39. LCMS : *m/z* = 534.1 [M+H]. Anal. calcd for $C_{23}H_{21}Cl_2N_5O_4S$: C 51.69; H 3.96; N 13.10 (found) C 51.65; H 3.98; N 13.12.

4.5.2.3: Ethyl 6-(((5-(4-bromophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)methylamino) methyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4c)

4c was synthesized according to the general procedure by condensing **18** (5 mmol) with **36** (5 mmol) in the presence of formalin 40% (5 mmol). R_f 0.53 (*n*-hexanes/ethyl acetate; 5:1). Yield 84%. m.p. 204-206°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.79 (br s, 1H, *NH*), 8.13 (br s, 1H, *NH*), 7.76 (d, 2H *J*= 8.4 Hz, Ar-*H*), 7.73 (d, 2H *J*= 8.4 Hz, Ar-*H*), 7.19 (d, 2H *J*= 8.4 Hz, Ar-*H*), 7.01 (d, 2H *J*= 8.4 Hz, Ar-*H*), 6.12 (br s, 1H, NH), 5.09 (d, 1H, *J*=2.7 Hz, *CH*), 4.68 (s, 2H, *CH*$_2$NH), 3.99 (q, 2H, *J*=7.2 Hz, *OCH*$_2$), 3.34 (s, 2H, *CH*$_2$NH), 1.02 (t, 3H, *J*=7.2 Hz, OCH$_2$*CH*$_3$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ178.67, 168.69, 156.89, 152.9, 142.6, 139.6, 134.3, 133.5, 133.3, 130.8 (2C), 130.2, 130.1, 129.9, 129.7, 129.2, 123.9, 106.7, 68.37, 60.23, 50.11, 47.14, 14.31. LCMS: *m/z* = 578.1 [M+H]. Anal. calcd for $C_{23}H_{21}BrClN_5O_4S$: C 47.72; H 3.66; N 12.10 (found) C 47.75; H 3.65; N 12.09.

4.5.2.4: Ethyl 4-(4-chlorophenyl)-6-(((5-(4-methoxyphenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)methylamino)methyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4d)

4d was synthesized according to the general procedure by condensing **18** (5 mmol) with **37** (5 mmol) in the presence of formalin 40% (5 mmol). R_f =0.49 (*n*-hexanes/ethyl acetate; 5:1). Yield 81 %. m.p. 206-208°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.76 (s, 1H, *NH*), 8.19 (s, 1H, *NH*), 7.66 (d, 2H *J*= 7.8 Hz, Ar-*H*), 7.23 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.10 (d, 2H, J=8.1 Hz, Ar-H), 6.89 (d, 2H, *J*= 7.8 Hz, Ar-H), 6.19 (br s, 1H, NH), 5.11 (d, 1H, *J*=3.3 Hz, *CH*), 4.29 (d, 2H, J= 6.6 Hz, *CH*$_2$NH), 3.98 (q, 2H, *J*=6.9 Hz, *OCH*$_2$), 3.76 (s, 3H, OCH_3), 3.31 (d, 2H, *J*= 6.3 Hz, *CH*$_2$NH), 0.97 (t, 3H, *J*=6.9 Hz, OCH_2*CH*$_3$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 178.1, 171.8, 168.6, 162.1, 156.5, 153.0, 141.9, 139.4, 132.9, 132.7, 130.5, 129.7, 129.5, 126.8, 126.6, 114.7, 114.3, 106.4, 68.2, 60.1, 58.0, 50.1, 46.9. 14.3.; LCMS: *m/z* = 530.12 [M+H]; Anal. calcd for $C_{24}H_{24}ClN_5O_5S$: C, 54.39; H, 4.56; N, 13.21. (found) C 54.42; H 4.54; N 13.19.

4.5.2.5: Ethyl-4-(4-chlorophenyl)-2-oxo-6-(((2-thioxo-5-*p*-tolyl-1,3,4-oxadiazol-3(2H)-yl)methylamino)methyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4e)

4d was synthesized according to the general procedure by condensing **18** (5 mmol) with **38** (5 mmol) in the presence of formalin 40% (5 mmol). R_f =0.46 (*n*-hexanes/ethyl acetate; 5:1). Yield 82%. m.p. 217-220°C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.79 (s, 1H, *NH*), 8.13 (br s, 1H, *NH*), 7.69 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.28 (d, 2H *J*= 8.1 Hz, Ar-*H*), 7.19 (d, 2H *J*= 7.8 Hz, Ar-*H*), 7.05 (d, 2H *J*= 7.8 Hz, Ar-*H*), 6.12 (br s, 1H, NH), 5.08 (d, 1H, *J*=3.0 Hz, *CH*), 4.29 (s, 2H, *CH*$_2$NH), 4.01 (q, 2H, *J*=7.2 Hz, *OCH*$_2$), 3.30 (s, 2H,*CH*$_2$NH), 2.20 (s, 3H, CH_3), 1.02 (t, 3H, *J*=7.2 Hz, OCH_2*CH*$_3$). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 179.7, 168.1, 157.9, 153.23, 142.1, 138.79, 134.6, 133.8, 131.01, 130.9, 130.6,130.5 (2C), 130.3, 128.6, 128.3, 128.1,

106.9, 68.6, 60.2, 50.11, 47.3, 25.99, 14.24. LCMS : *m/z* = 514.12 [M+H]. Anal. calcd for $C_{24}H_{24}ClN_5O_4S$: C 56.08; H 4.71; N 13.63 (found) C 56.10; H 4.70; N 13.62.

4.3. Protocol for thymidine phosphorylase inhibition assay

Thymidine phosphorylse (TP/PD-ECGF, *Escherichia coli* origin, Sigma T6632), thymidine and all other required chemical were purchase from sigma. The activity was determined by measuring the absorbance at 290 nm spectrophotometrically by a modification of the previously described method [27-28]. Briefly 200 mM potassium phosphate buffer PH 7.00, 245 µL, TP (0.068U/300µL) 20 µL and test sample in 5 µL DMSO, were mixed in 96-well microplate and pre-incubated for 10 min. at 30º C. the reaction was initiated by adding 30 µL of 1mM of thymidine as the substrate (dissolved in 200mM potassium phosphate buffer PH 7.00). The decreased in Absorbance was monitor spectrophotometrically at 290 nm, with 96-well microplate reader (Molecular device, Spectramax USA) reader using quartz microplates. The IC_{50} values were the average of at least three determinations performed in triplicate.

4.4. Antiangiogenic study: Chick chorioallantoic membrane activity (CAM) assay

The experiment was performed with the approval of ethical committee of Department of Pharmacy, University of Malakand, Pakistan according to the animal By-Law 2008 (Scientific procedure Issue-1).

The fertilized chicken eggs were used in this study to carry out the CAM assay [20, 29]. The fertilized domestic chicken eggs purchased from poultry trader Chakdara, Pakistan. The eggs were washed and cleaned via with methanol at sterilized cotton swab. The eggs were incubated horizontally for 3–5 days at 37 0C with constant humidity (HYSCKorea(BI-81/150/250) and were slowly shacked at least three to four times a day. After the completion of incubation period, the seven-day old eggs were observed under flash light to identify and

encircle the embryo head. A small opening in the egg shell, opposite to the position of the embryo, was done to expose the chorioallantoic membrane for experimental treatment. The slightly dried, treated filter disc was placed directly into the chorioallantoic membrane. After that the different concentration were applied on the discs. We used five different concentration for each compound ranging from 1000, 500, 250, 125 and 62.5 µg/ml and for each sample dose, six different eggs were used for reducing chances of error. The small opening of the 8-day old treated eggs was tightly resealed with adhesive sterilized tape and eggs were returned incubated horizontally for 24 to 48 hours. After incubation, the hard-shell instant to the treatment site was removed, revealing the chorioallantoic membrane surrounding and under the treated filter disc. The number of blood vessels in each egg has been counted by inspecting the actual number of blood vessels in 1 cm^2 area of the CAM adjacent to the sample application site. Vessels radially converging in the direction of the centre were counted under a microscope. The % of increase and inhibition were calculated using formula;

$$\%\ inhibition = \frac{\text{CAMns} - \text{CAMts}}{\text{CAMns}} \times 100$$

CAM ns (number of blood vessels treated with normal saline)

CAM ts (number of blood vessels treated with test samples)

Median inhibitory concentrations (IC_{50}) for anti-angiogenic assay were calculated using Microsoft excel program.

4.5. Docking Studies

Docking experiments were performed *via* Molecular Operating Environment (MOE) docking program version 2016.08 [30]. Crystal structure of *h*TP in (PDB code: 2J0F) in complex with thymine was selected for these studies. All the water molecules were removed from the

protein structure, then hydrogen atoms were added and energy optimization was carried out using default force field. The three-dimensional (3D) structures of compounds were modelled through the builder program implemented in MOE. The geometrical parameters for 3D structures of the compounds were optimized, and partial charges were calculated before docking. The 3D protonation of the 2J0F was done and energy minimization of the retrieved protein molecule was carried out by using default parameters of MOE energy minimization algorithm [gradient: 0.05, Force Field: MMFF94X]. The resulting model was subjected to systematic conformational search at default parameters with RMS gradient of 0.01 kcal/mol using Site Finder. The active site of the enzyme was defined within 10 Å of the native ligand thymine. The lowest energy minimized pose was used for further analysis. Ligand-interaction module of MOE was used to calculate the 2D ligand-enzyme interactions. The view of the docking results and analysis of their surface with graphical representations were done using MOE and discovery studio visualizer [31].

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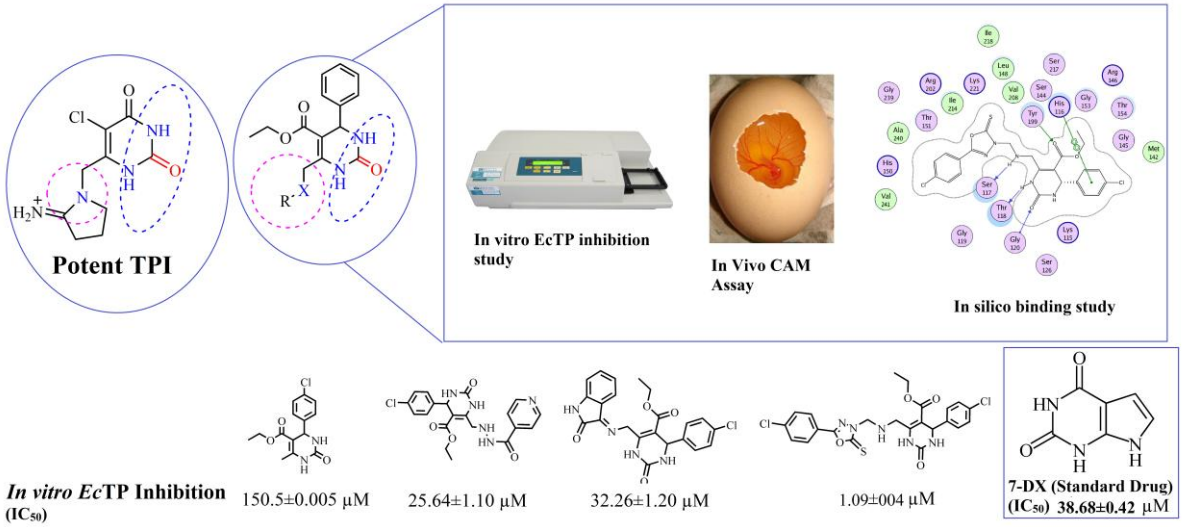
Graphical abstract



Highlights

- Eighteen new derivatives of 3,4-dihydropyrimidone-2-one were synthesized
- In vitro thymidine phosphorylase inhibition potential was determined
- Compounds **4a-e** exhibited TP-inhibition at low micromolar concentration
- In vivo anti-angiogenesis potential of some compounds were also studied