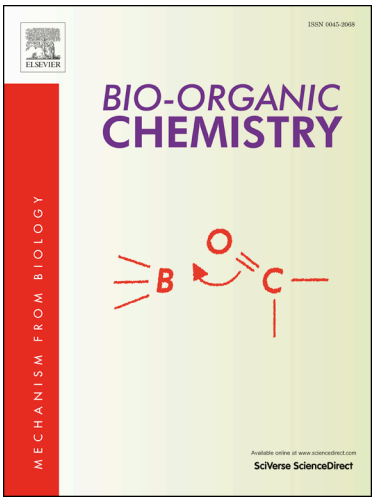

Synthesis, SAR elucidations and molecular docking study of newly designed Isatin based oxadiazole analogs as potent inhibitors of thymidine phosphorylase

Muhammad Tariq Javid, Fazal Rahim, Muhammad Taha, Mohsan Nawaz, Abdul Wadood, Muhammad Ali, Ashik Mosaddik, Syed Adnan Ali Shah, Rai Khalid Farooq

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Synthesis, SAR elucidations and molecular docking study of newly designed Isatin based oxadiazole analogs as potent inhibitors of thymidine phosphorylase

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Abstract

Thymidine phosphorylase is an enzyme involved in pyrimidine salvage pathway that is identical to platelet-derived endothelial cell growth factor (PD-ECGF) and gliostatin. It is enormously up regulated in a variety of solid tumors. Furthermore, surpassing of TP level protects tumor cells from apoptosis and helps cell survival. Thus TP is identified as a prime target for developing novel anticancer therapies. A new class of exceptionally potent isatin based oxadiazole (**1-30**) has been synthesized and evaluated for thymidine phosphorylase inhibitory potential. All analogs showed potent thymidine phosphorylase inhibition when compared with standard 7-Deazaxanthine, 7DX (IC_{50} = 38.68 ± 1.12 μM). Molecular docking study was performed in order to determine the binding interaction of these newly synthesized compounds, which revealed that these synthesized compounds established stronger hydrogen bonding network with active site of residues as compare to the standard compound 7DX.

Keywords: Isatin, Oxadiazole, Synthesis, TP inhibition, SAR, Molecular docking.

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1.0. Introduction

Cancer is the leading cause of premature mortality worldwide [1], which ultimately led vast interest in exploring several potential angiogenic modulators in order to develop new therapies for cancer treatment [2-4]. Among various angiogenic activators, thymidine phosphorylase (TP, EC 2.4.2.4) has been identified as a crucial angiogenic protein [5]. TP is found in many normal tissues and cells present in both cytoplasm and nucleus [6]. TP causes a variety of pathological complications such as psoriasis, rheumatoid arthritis, inflammatory bowel disease and atherosclerosis *etc* [7]. TP is a major contributor in tumor angiogenesis as it facilitates proliferation of endothelial cells during cancer metastasis [7, 8]. TP has been shown to be identical to platelet-derived endothelial cell growth factor (PDECGF) [9-12], which has been implicated in angiogenesis and chemotaxis in human tumors [13, 14]. Increased hypoxia correlates with elevated TP activity, and increased levels of this enzyme have been observed in colorectal, ovarian, pancreatic, and breast tumors [15, 16]. In the last few years, hybrid drug design has emerged as a prime tool for the discovery of innovative anticancer therapies that can potentially overcome most of the pharmacokinetic drawbacks encountered when using conventional anticancer drugs.

Isatin as an indole derivative widely distributed endogenously in human and other mammalian tissues and fluids probably due to tryptophan metabolic pathway. The versatility of isatin molecular architecture makes it as an ideal platform for structural modification and derivatization and many isatin derivatives exhibit a broad range of biological activities such as anticancer[17], antidepressant [18], anticonvulsant [19], antifungal [20], anti-HIV [21] and anti-inflammatory activity [22]. In the last several decades, many researchers have embarked on the development of new isatin-based anticancer agents [23-25].

1,3,4-Oxadiazole have attracted attention due to their remarkable biological and pharmacological properties. Recently, differently substituted 1,3,4-oxadiazoles have been reported for their biological activities, such as antibacterial [26], antifungal [27], insecticidal [28] antiviral [29] inflammatory [30] antitubercular [31] antitumor [32] and analgesic activity [33].

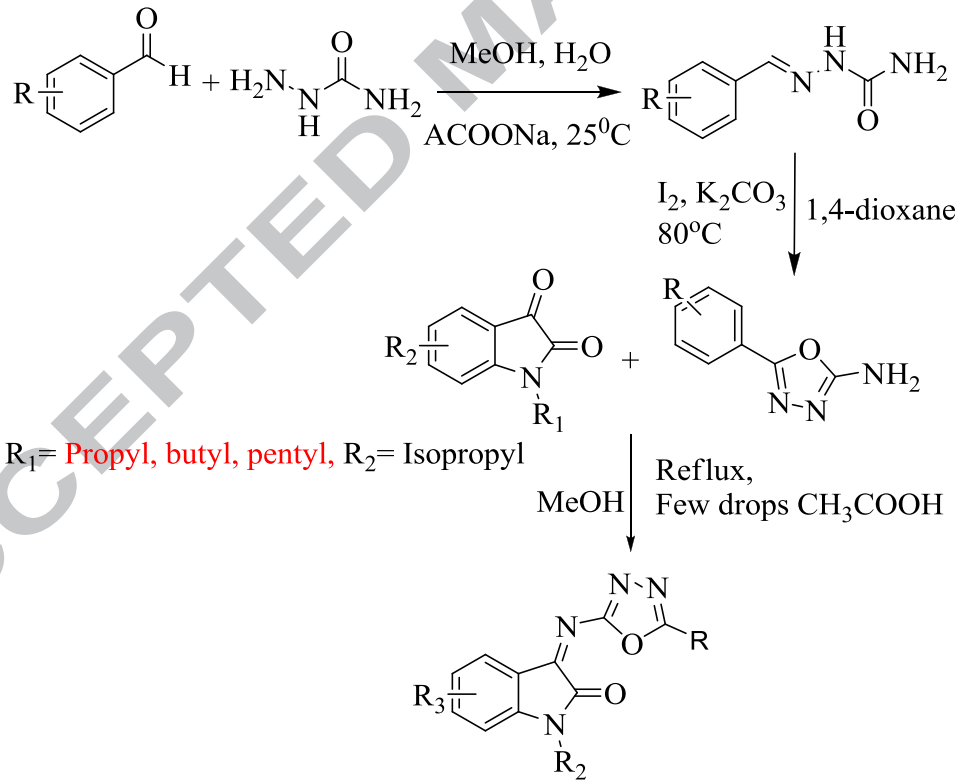
Our research group has been working on synthesis of different heterocycles such as thiazole, oxazole, oxadiazole, thiadiazole, thiazolidenone *etc* and reports various activities of these compounds [34-36]. Currently we are involved in synthesis of heterocyclic hybrid molecules

[37-45]. We have observed that the hybrid compounds are more potent than their individual counterpart. Keeping in view the great biological potential of isatin and oxadiazole, here in this study we are reporting synthesis and thymidine phosphorylase inhibitory potential of isatin based oxadiazole analogs.

2.0. Result and discussions

2.1. Chemistry

Isatin based oxadiazole were synthesized in two steps, first 2-aminooxadiazole was refluxed by reacting semicarbazide with different substituted benzaldehyde in methanol. After completion of reaction, the intermediate was further cyclized by treating it with iodine and potassium carbonate in 1,4-dioxane to form 2-aminooxadiazole. In the second step, synthesized 2-aminooxadiazole was further heated under reflux with different substituted isatin in methanol for 3hrs to obtained desired different isatin based oxadiazole.



Scheme: Synthesis of isatin based oxadiazole derivatives (**1-30**)

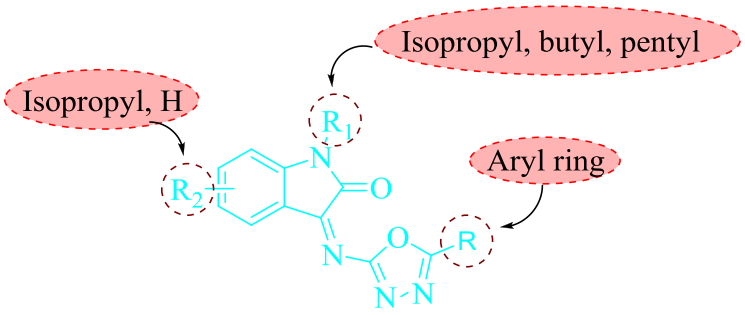
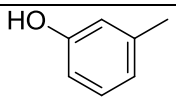
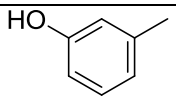
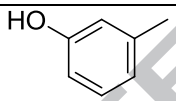
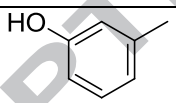
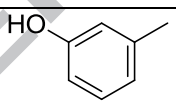
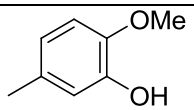
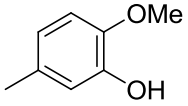
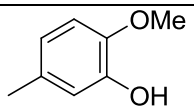
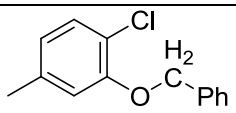
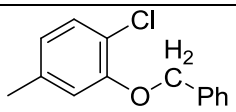
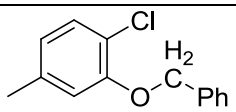
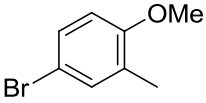
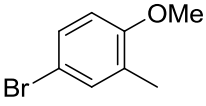
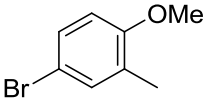
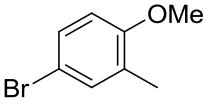
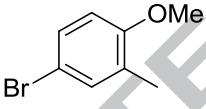
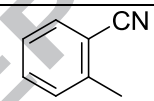
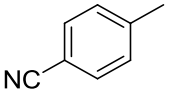
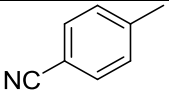
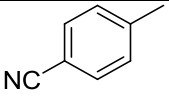
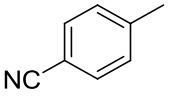
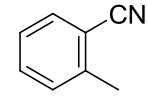
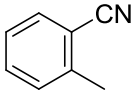
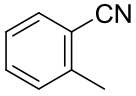
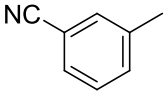
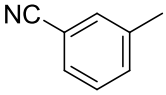
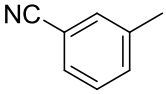
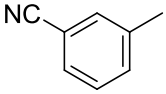
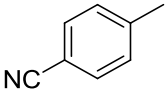
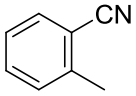
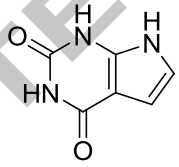


Figure-1: General structure for compounds (**1-30**)

Table-1: Synthesis of various analogs of isatin based oxadiazole derivatives (**1-30**) and its thymidine phosphorylase inhibition

Comp. No.	R	R_1	R_2	IC_{50} (μM ± SEM[a])
Category-A				
1		*N*-Isopropyl	H	7.80 ± 0.20
2		*N*-Butyl	H	9.40 ± 0.20
3		H	5-isopropyl	5.30 ± 0.10
4		*N*-Pentyl	H	16.50 ± 0.30
5		H	H	19.40 ± 0.40
Category-B				
6		H	5-isopropyl	6.20 ± 0.10
7		*N*-Isopropyl	H	4.70 ± 0.10
8		*N*-Butyl	H	5.30 ± 0.10
Category-C				

9		H	5-isopropyl	14.60 ± 0.3
10		*N*-Isopropyl	H	11.30 ± 0.30
11		*N*-Butyl	H	17.60 ± 0.40
Category-D				
12		H	5-isopropyl	48.50 ± 1.20
13		*N*-Isopropyl	H	29.10 ± 0.90
14		*N*-Butyl	H	38.60 ± .90
15		*N*-Pentyl	H	46.20 ± 1.20
16		H	H	49.40 ± 1.30
Category-E				
17		*N*-Isopropyl	H	25.10 ± 0.50
18		*N*-Butyl	H	19.70 ± 0.40
19		*N*-Pentyl	H	34.60 ± 0.60
20		H	5-isopropyl	26.80 ± 0.60
21		*N*-Isopropyl	H	18.40 ± 0.40
22		*N*-Butyl	H	37.30 ± 0.80

23		*N*-Pentyl	H	26.40 ± 0.60
24		H	5-isopropyl	22.10 ± 0.60
25		H	5-isopropyl	34.30 ± 0.60
26		*N*-Pentyl	H	34.60 ± 0.70
27		*N*-Butyl	H	30.10 ± 0.60
28		H	H	18.70 ± 0.40
29		H	H	17.90 ± 0.50
30		H	H	16.40 ± 0.40
Standard  **7-Deazaxanthine[d]**				**38.68 ± 1.12**

2.2. Biological activity

Isatin based oxadiazole derivatives (**1-30)** were substituted at position-5 as R_2 and N-alkylated as R_1 and aryl substituted as R with a diverse range of groups having electron withdrawing and electron donating effect. On the basis of these groups, compounds are classified into five categories A-E in order to simplify the SAR. The SAR was elucidated on the basis of different substitutions. It was assumed that a variation in the inhibitory activities of compounds in a particular category is attributed by different substitutions on isatin part and phenyl part of oxadiazole (Figure-2).

The isatin based oxadiazole a class of heterocycles should be acknowledged as new lead inhibitors with much more pronounced inhibitory potentials against thymidine phosphorylase and angiogenesis as compared to 7DX. While these analogs possess the strong and convincing potential in the future design of new TP inhibitors. All synthesized compounds (**1-30**) were evaluated against thymidine phosphorylase inhibition. All compounds showed potent thymidine phosphorylase inhibition when compared with standard 7-Deazaxanthine, 7dx (IC_{50} = 38.68 ± 1.12 μM). Structure activity relationship has been established for all compounds mainly based on substitution pattern on phenyl ring. In order to simplify the SAR all compounds were categorized in five categories on the basis of substitution on phenyl ring of oxadiazole. Five analogs such as **1-5**are kept in **category A.** The compounds **6-8** were kept in **category B**. Three compounds such as **9-11** were kept in **category C.** Compounds such as **12-16** were kept in **category D.** The remaining compounds **17-30** were kept in **category E.** Analog**1** (IC_{50} = 7.80 ± 0.20 µM) having 3-hydroxyl group on phenyl part and N-alkylated isopropyl, analog **2** (IC_{50} = 9.40 ± 0.20 µM) having 3-hydroxyl group on phenyl part and N-butyl, analog **3** (IC_{50} = 5.30 ± 0.10 µM) having 3-hydroxyl on phenyl part and 5-isopropyl on Isatin phenyl part and analog **4** (IC_{50} = 16.50 ± 0.30 µM) having 3-hydroxyl on phenyl part and N-alkylated pentane showed potent inhibition. Other analog **5** (IC_{50} = 19.40 ± 0.40 µM) having 4-hydroxy on phenyl part showed excellent inhibitory potential due to substitution on phenyl ring and N-alkylation. The greater potential is due to the methoxy group on phenyl ring. Similar pattern was observed like hydroxy analogs.

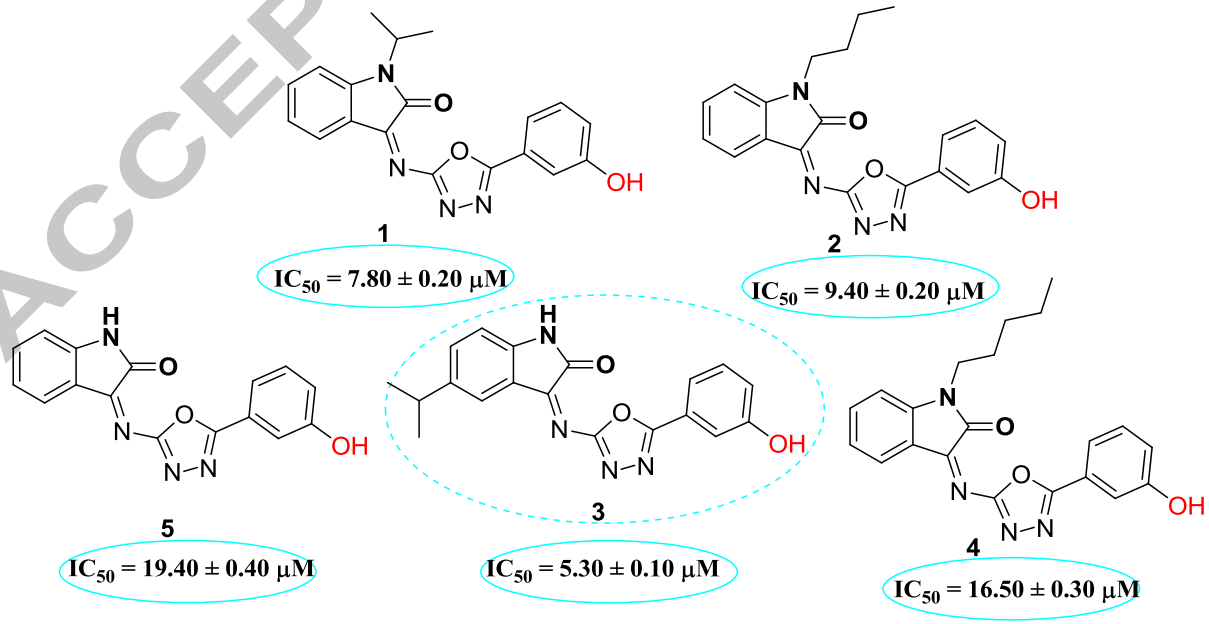


Figure-2: Structure of compounds (**1-5**)

Analog **6** (IC_{50} = 6.20 ± 0.10 µM) a hydroxy, methoxy and isopropyl on isatin phenyl part and analog **7** (IC_{50} = 4.70 ± 0.10 µM) a hydroxy, methoxy and N-isopropyl and analog **8**(IC_{50} = 5.30 ± 0.10 µM) a hydroxy, methoxy and N-beutane showed potent inhibitory potential.

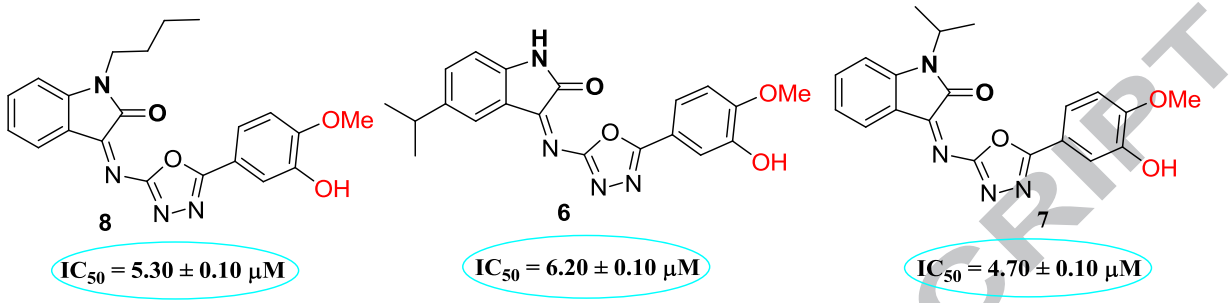


Figure-3: Structure of compounds (**6-8**)

A category-C possessed different substitution on isatin and same substitution on phenyl part. Analog **9** (IC_{50} = 14.60 ± 0.3 µM) a chloro, benzyloxy and 5-isopropyl on isatin phenyl part and analog **10** (IC_{50} = 11.30 ± 0.30 µM) a chloro, benzyloxy and N-isopropyl showed excellent potential. Analog **11**(IC_{50} = 17.60 ± 0.40 µM) having 3-benzyloxy-4-chloro on phenyl part and N-alkylated butane. The slight activity difference among these analogs showed that the position of isopropyl also affect the inhibition. By comparing analog **11**aN-alkylated butane and analog **9** a 5-isopropylwith analog **10**a N-alkylated isopropane, the analog **10** was found superior than these two analogs **9**and **11**.

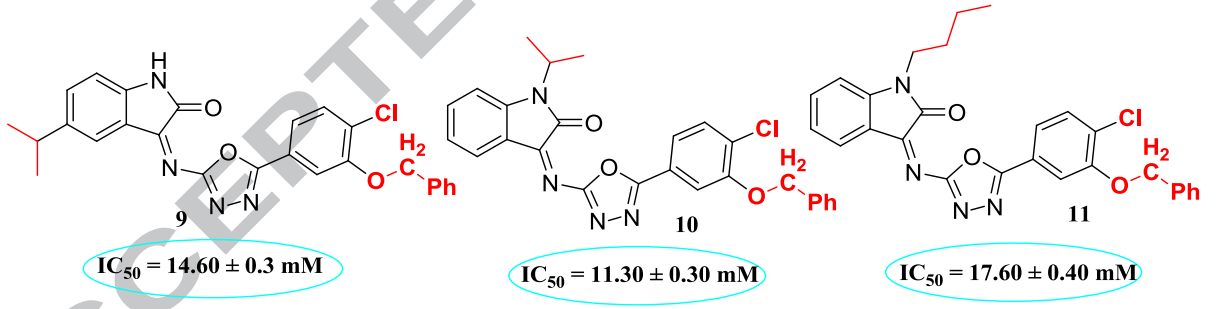


Figure-4: SAR of compounds (**9-11**)

In category-D, analog **16** possess no substitution on isatin part and other analogs in category-C possess different substitutions' on isatin part and same substitution on phenyl part. Analog **13** having 5-bromo-2-methoxy on phenyl part and N-isopropyl and Analog **12, 14**and**15** having 5-bromo2-methoxy phenyl, 5-isopropyl, n-alkylated butane, and pentane showed good to moderate thymidine phosphorylase inhibition. Among these analog **13** showed good inhibition than compared with standard 7DX.

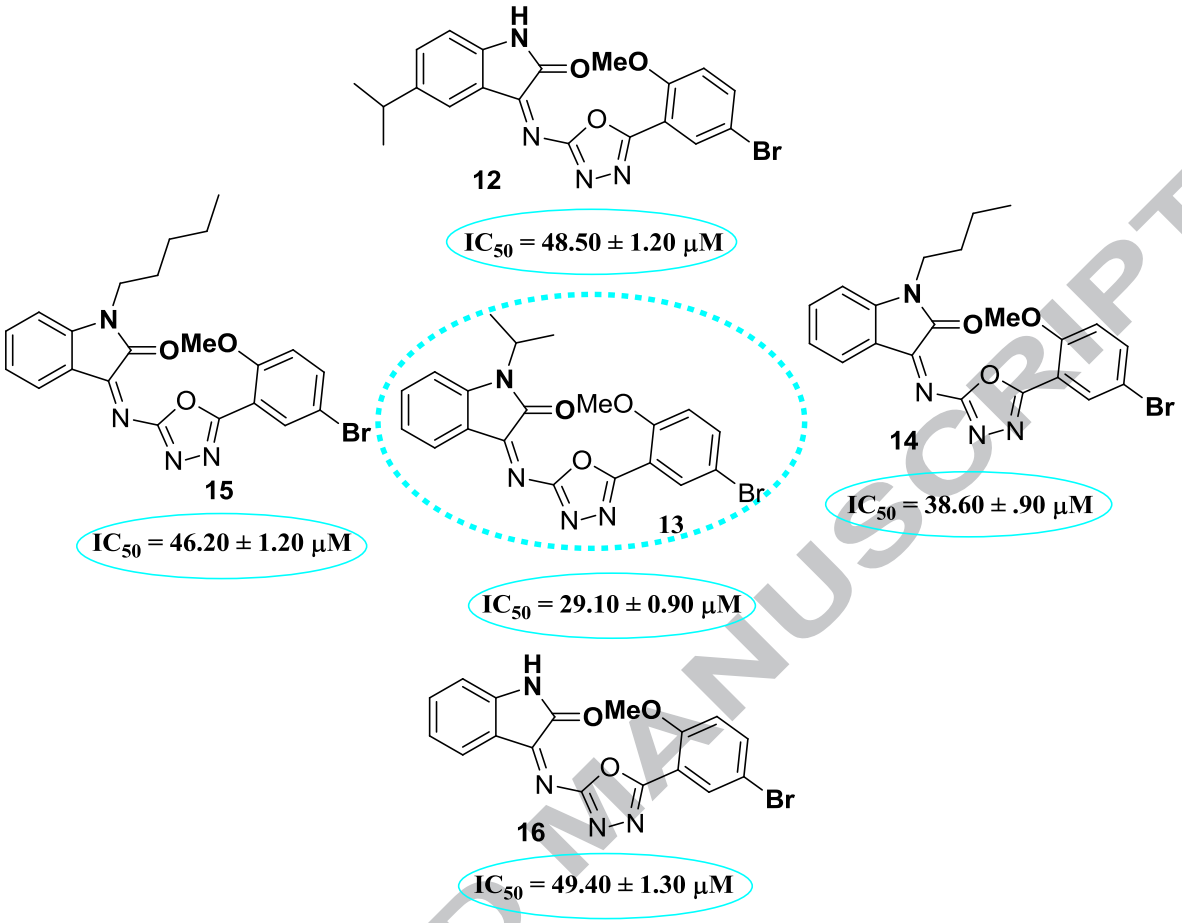


Figure-5: SAR of compounds (**12-16**)

The category-E possessed different substitution on isatin and different substitution on phenyl part. Analog (**17-30**) having nitrile group at *ortho*, *meta* and *para* position on phenyl part and substituted, N-alkylated isatin showed remarkable thymidine phosphorylase inhibition. By comparing analogs **17-30** was found well than the other analogs. The reason for remarkable potential of these fourteen (14) analogs is due to the position difference of nitrile group on phenyl ring and substituted, N-alkylated isatin.

It was concluded from this study that either EWG or EDG on phenyl part exhibited potential but the slight variance in potential was mainly affected by the position of the substituent as well as in some cases the number of substituent also play a role.

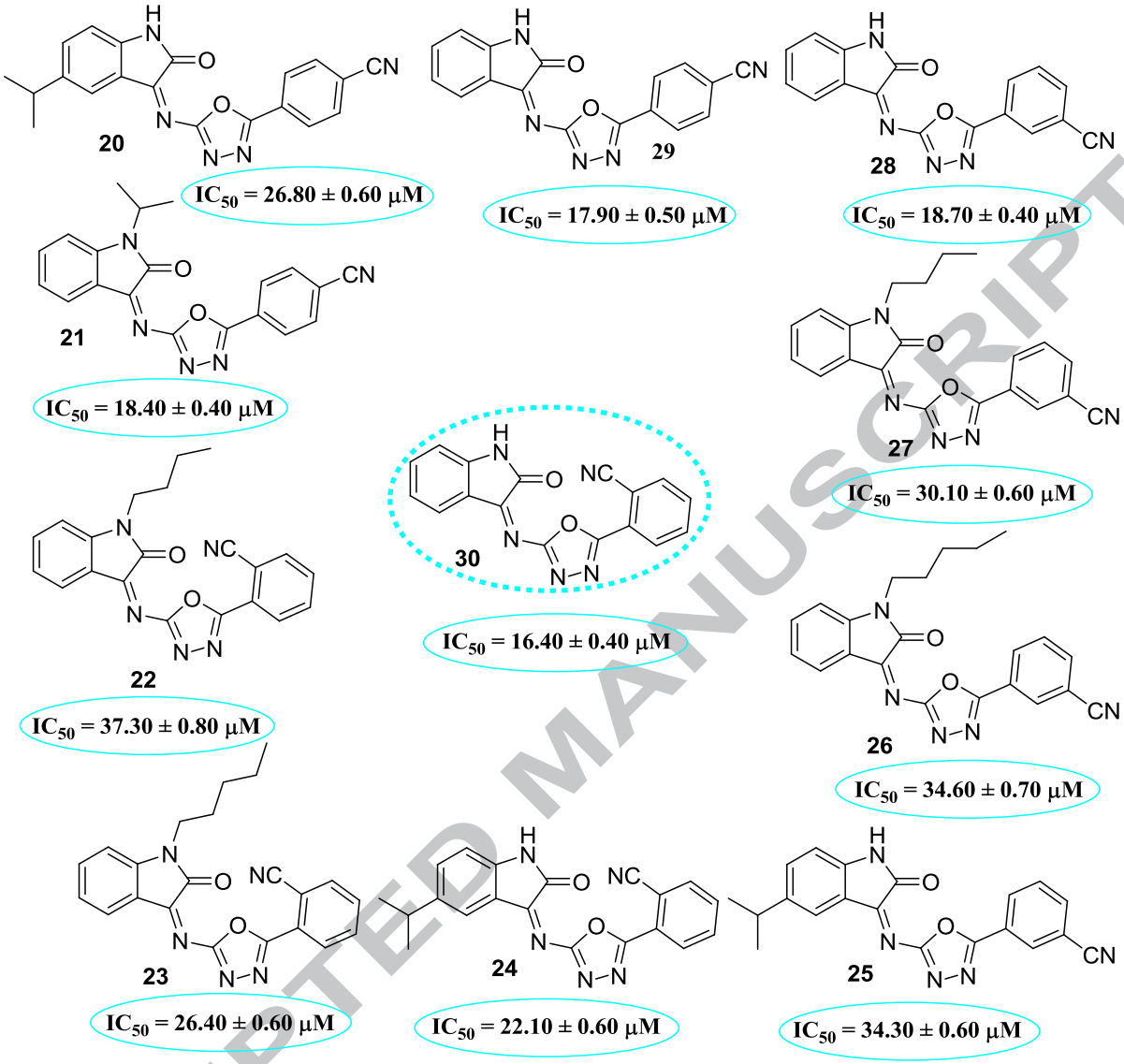


Figure-6: SAR of compounds (**20-30**)

However, the in vitro thymidine phosphorylase inhibition studies and to understand the different structural motifs of the compounds in binding with the active site of thymidine phosphorylase, in silico molecular modeling was also performed.

2.3. Molecular modeling and docking studies

To explore the binding mode of these oxadiazole derivatives, molecular docking was performed. The docking results showed that all the compounds well accommodated in the active site of thymidine phosphorylase. From the docking conformation of the most active compound, compound **7 (IC_{50} = 4.70 ± 0.10)**, it was observed that this compound established six polar interactions and four pi-H interactions with active site residues and good docking score (**-13.1172**) as compare to the reference compound having docking score of **-8.1034** and

biological activity with IC_{50} of **38.68 ± 1.12**. The oxygen and two nitrogen atoms present at the 1,3,4-oxadiazole moiety of the compound formed one H-acceptor with His 85 and two H-acceptor bonds with Lys 190 respectively. Furthermore, carbonyl oxygen atom of the 1-isopropylindolin-2-one moiety formed H-acceptor bonds with active site residue Gln 156 whereas the -OH moiety of the compound formed two H-acceptor interactions with the -NH group of the Arg 171 residue of the enzyme. Arg 115, Tyr 168 and Ile 183 made pi-H linkages with the 5-ring and 6-ring moieties of the compound as shown in **Figure-7a**. This strong bonding network might be one of the reasons for this compound to show good biological activity. The docking conformation of the second most active compound, compound **3** (**IC_{50} = 5.30 ± 0.10**), showed that this compound formed five bonds with the active site residues His 85, Tyr 168, Ser 186 and Lys 190 respectively (**Figure-7b**) and good docking score **(-12.7026).** The nitrogen atom of the compound formed one H-acceptor bond with His 85 while Tyr 168 residue was observed making two hydrogen bonds with nitrogen atoms of the 1,3,4-oxadiazole moiety of the compound. Ser 186 and Lys 190 residues formed polar interactions with the -NH and carbonyl oxygen atom of the 5-isopropylindolin-2-one moiety of the compound respectively. Arg 115, Ile 183 and Phe 210 formed three pi-H linkages with the compound. The potency of this compound might be due to the presence of the electron donating group (-OH). The third most active compound **6 (IC_{50} = 6.20 ± 0.10)** was observed with docking score of **-12.0767** and good interactions with the binding pocket residues (**Figure-7c**). His 85 and Lys 190 formed pi-H linkages with the compound. His 85 and Tyr 168 established polar interactions with the -OH and oxygen atom of the MeO moieties respectively while Arg 115 formed polar bonds with the –NH of the 1,3,4-oxadiazol-2-amine moiety of the ligand respectively. The electronic cloud system of this compound might be the reason of its high potency. The forth most one active compound, compound **1 (IC_{50} = 7.80 ± 0.20** and **docking score = -12.0502)** formed two polar interactions with the His 85 and Lys 190 while Tyr 168 formed two pi-H contacts with the compound as shown in **Figure-7d**.

Overall a good correlation was observed between the docking scores and biological activities of these compounds **Figure-8** (**see S1**). The docking score and report of predicted interaction of dock confirmation was mention in table (**See S2**).

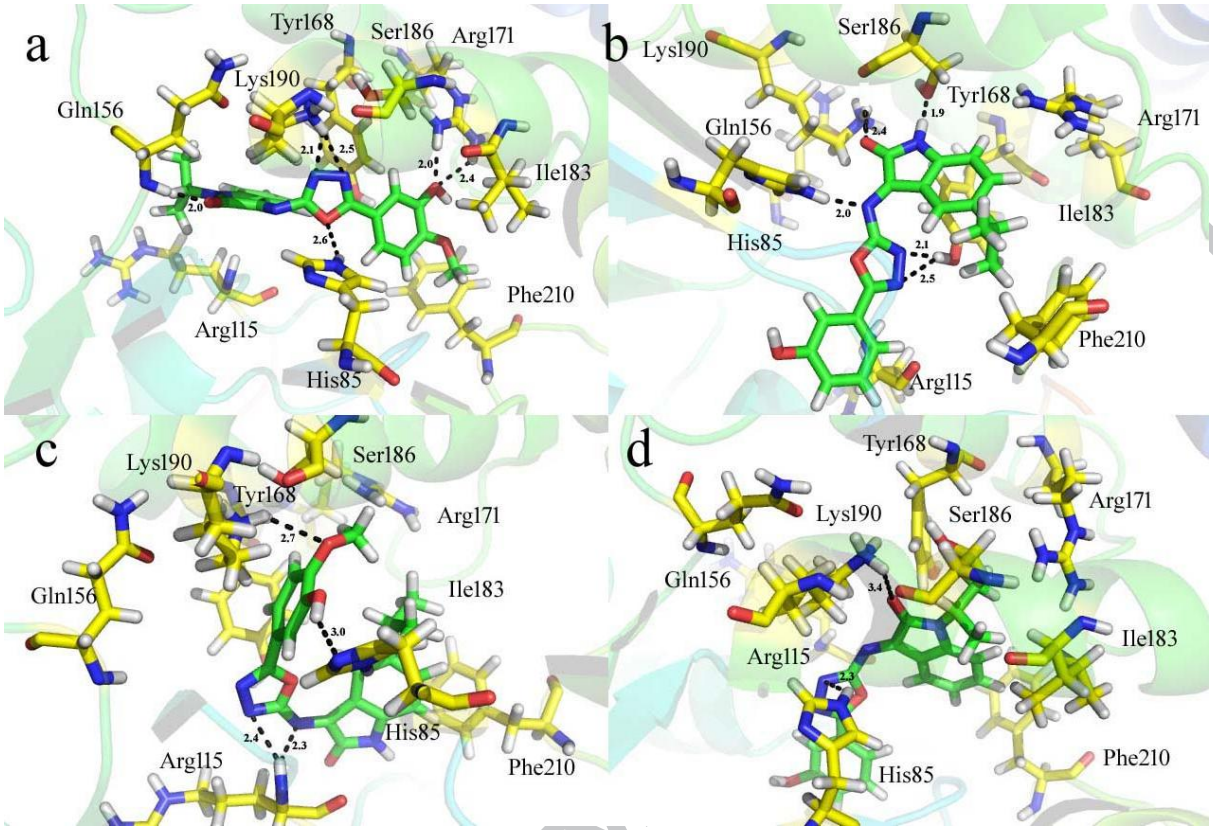


Figure-7: Docking conformations of compounds on thymidine phosphorylase enzyme. **(a)** 3D binding mode of compound **7**as inhibitor of thymidine phosphorylase enzyme. **(b)** 3D binding mode of compound **3**. **(c)** 3Dbinding mode of compound **6**. **(d)** 3D binding mode of compound **1** in binding cavity of thymidine phosphorylase enzyme. Ligands are shown green colour.

Conclusion

In conclusion we have synthesized isatin based oxadiazole analogs **1–30**, characterized by EI-MS and ^{1}H-NMR and checked for thymidine phosphorylase inhibitory potentials. Eight compounds were identified as potent thymidine phosphorylase inhibitor.

3.0. Experimental

3.1. General methods

NMR experiments were performed on Advance Bruker AM 500 MHz machine (France). DMSO was used for accurate NMR analysis. Light of wavelength 254 and 265 nm were used to visualize the chromatogram. Electron impact mass spectra (EI MS) were recorded on a

Finnigan MAT-311A (Germany) mass spectrometer. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany).

3.2. General Procedure for the synthesis of isatin based oxadiazole derivatives (1-30)

Isatin based oxadiazole were synthesized in two steps, first semicarbazide (1mmol, 0.71g) and different substituted benzaldehydes (1mmol) were mixed and reflux in methanol for 2-3 hrs. The completion of reaction was monitored by TLC. After completion of reaction, the intermidiate was further cyclized by treating it with iodine and potassium carbonate in 1, 4-dioxane to give a 2-aminooxadiazole moiety. After drying, it was reacted with 5% $Na_2S_2O_3$ (20 mL) and then it was extracted the desired organic compound from the reaction mixture using CH_2Cl_2/MeOH (10:1, 10 mL × 4). The desired organic compound was dried over anhydrous sodium sulphate and was purified using petroleum ether and ethylacetate mixture as eluent through silica gel column chromatography afford the synthesis of corresponding analogs. In the second step, 1mmol of synthesized 2-aminooxadiazole were refluxed with different substituted isatin in methanol in the presence of few drops of glacial acetic acid for 3hrs. Reaction completion was monitored through TLC. After completion of reaction, reaction mixture was washed with chloroform/hexane to obtained desired different isatin based oxadiazole.

3.2.1. (*E*)-3-(5-(3-hydroxyphenyl)-1,3,4-oxadiazol-2-ylimino)-1-isopropylindolin-2-one (1)

Dark black solid, Yield: 73%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.81 (d, J = 5.6 Hz, 1H, Ar), 7.78 (d, J = 5.7 Hz, 1H, Ar), 7.48 (t, J = 5.9 Hz, 1H, Ar), 7.30 (s, 1H, Ar), 7.58 (d, J = 6.2 Hz, 1H, Ar), 7.31 (t, J = 5.6 Hz, 1H, Ar), 7.20 (t, J = 6.0 Hz, 1H, Ar), 6.86 (d, J = 6.2 Hz, 1H, Ar), 5.32 (s, 1H, -OH), 1.20 (s, 6H, -CH_3), 3.91 (s, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 163.8, 163.1, 162.5, 158.3, 157.1, 147.1, 131.0, 130.2, 129.1, 124.0, 127.4, 120.8, 118.5, 116.4, 113.5, 110.5, 58.1,23.3, 23.2; HREI-MS: *m/z* Calcd for $C_{19}H_{16}N_4O_3$, $[M]^+$ 348.1222; found 348.1208.

3.2.2. (*E*)-1-butyl-3-(5-(3-hydroxyphenyl)-1,3,4-oxadiazol-2-ylimino)indolin-2-one (2)

Light black solid, Yield: 71%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.57 (d, J = 6.4 Hz, 1H,Ar),7.50 (d, J = 5.7 Hz, 1H, Ar), 7.48 (t, J = 7.2 Hz, 1H, Ar), 7.41 (s, 1H, Ar), 7.24 (d, J = 6.3 Hz, 1H, Ar), 7.15 (d, J = 6.9, 1H, Ar), 7.11 (t, J = 6.5 Hz, 1H, Ar), 6.90 (d, J = 6.8, 1H, Ar), 6.70 (s, 1H, -OH), 3.28 (m, 6H, -CH_2), 1.20 (s, 3H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-

d_6): δ 163.8, 163.4, 162.8, 162.3, 157.8, 147.1, 131.5, 130.3, 129.2, 127.1, 124.0, 120.6, 117.9, 116.4, 116.0, 113.1, 43.4, 30.4, 21.4, 16.5; HREI-MS: *m/z* Calcd for $C_{20}H_{18}N_4O_3$, $[M]^+$ 362.1378; found 362.1365.

3.2.3. (*E*)-3-(5-(3-hydroxyphenyl)-1,3,4-oxadiazol-2-ylimino)-5-isopropylindolin-2-one (3)

Light brown solid , Yield: 74%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 9.58 (s, 1H, -NH), 7.53 (s, 1H, Ar), 7.47(d, J = 6.5 Hz, 1H, Ar), 7.34 (d, J = 6.4 Hz, 1H, Ar), 7.27 (s, 1H, Ar), 7.08 (t, J = 6.8 Hz, 1H, Ar), 6.89 (d, J = 6.5 Hz, 1H, Ar), 6.77 (d, J = 6.6 Hz, 1H, Ar), 5.54 (s, 1H, -OH), 2.88 (s, 1H, -CH), 1.82 (s, 6H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.1, 163.3, 163.1, 157.8, 151.2, 146.5, 139.1, 131.2, 129.4, 128.5, 127.3, 122.4, 120.8, 118.2, 116.4, 113.5, 34.5, 24.6, 24.6; HR-EI-MS: *m/z* Calcd for $C_{19}H_{16}N_4O_3$, $[M]^+$ 348.1221; found 348.1211.

3.2.4. (*E*)-3-(5-(3-hydroxyphenyl)-1,3,4-oxadiazol-2-ylimino)-1-pentylindolin-2-one (4)

Light black solid, Yield: 76%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.76 (d, J = 7.0 Hz, 1H, Ar), 7.68 (d, J= 6.7 Hz, 1H, Ar), 7.53 (d, J = 6.4 Hz, 1H, Ar),7.47 (t, J = 5.7 Hz, 1H, Ar), 7.27 (t, J = 6.6 Hz, 1H, Ar), 7.19 (s, 1H, Ar), 7.08 (t, J = 6.5 Hz, 1H, Ar), 6.94 (d, J = 6.7 Hz, 1H, Ar), 5.64 (s, 1H, -OH), 3.84 (m, 8H, -CH_2), 0.92 (s, 3H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.2, 164.5,163.4, 163.1, 157.9, 148.1, 132.1, 131.2, 130.3, 128.1, 125.2, 121.4, 118.6, 116.7, 116.4, 113.5, 43.8, 30.5, 28.4, 23.7, 16.6; HR-EI-MS: *m/z* Calcd for $C_{21}H_{20}N_4O_3$, $[M]^+$ 376.1534; found 376.1520.

3.2.5. (*E*)-3-(5-(3-hydroxyphenyl)-1,3,4-oxadiazol-2-ylimino)indolin-2-one (5)

Light red solid, Yield: 77%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 8.10 (s, 1H, -NH), 7.60 (d, J = 6.4 Hz, 1H, Ar), 7.49 (d, J = 6.6 Hz, 1H, Ar), 7.59 (t, J = 6.7 Hz, 1H, Ar), 7.32 (d, J = 6.1 Hz, 1H, Ar), 7.28 (s, 1H, Ar), 7.12 (t, J = 6.5 Hz, 1H, Ar), 7.08 (t, J = 6.1 Hz, 1H, Ar), 6.95 (d, J = 6.7 Hz, 1H, Ar), 5.55 (s, 1H, Ar); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.0, 163.2, 161.2, 157.8, 151.4, 142.5, 131.4, 129.7, 128.5, 127.8, 125.2, 120.9, 120.2, 117.4, 115.5, 113.5; HR-EI-MS: *m/z* Calcd for $C_{16}H_{10}N_4O_3$, $[M]^+$ 306.0752; found 306.0739.

3.2.6. (*E*)-3-(5-(3-hydroxy-4-methoxyphenyl)-1,3,4-oxadiazol-2-ylimino)-5-isopropylindolin-2-one (6)

Red solid, Yield: 76%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 8.0 (s, 1H, -NH), 7.75 (s, 1H, Ar), 7.53 (d, 6.4 Hz, 1H, Ar), 7.48 (d, *J*= 6.2 Hz, 1H, Ar), 7.27 (s, 1H, Ar), 7.02 (d, *J* = 6.9 Hz,1H, Ar), 6.87 (d, *J* = 6.9 Hz, 1H, Ar), 6.14 (s, 1H, -OH), 3.91 (s, 3H, -CH_3), 2.87 (s, 1H, -CH), 1.85 (s, 6H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 159.3, 156.7, 156.1, 155.9, 154.5,150.6, 149.4, 138.3, 131.0, 127.5, 124.9, 122.7, 117.8, 117.6, 113.8, 111.6, 56.1, 30.6, 25.3, 25.3; HR-EI-MS: *m/z* Calcd for $C_{20}H_{18}N_4O_4$, $[M]^+$ 378.1327; found 378.1315.

3.2.7. (*E*)-3-(5-(3-hydroxy-4-methoxyphenyl)-1,3,4-oxadiazol-2-ylimino)-1-isopropylindolin-2-one (7)

Dark brown solid, Yield: 81%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.81 (d, , *J* = 6.7 Hz, 1H, Ar), 7.52 (d, , *J* = 6.6 Hz, 1H, Ar), 7.45 (d, , *J* = 7.2 Hz, 1H, Ar), 7.31 (d, 6.5 Hz, 1H, Ar), 7.29 (d, *J* = 6.8 Hz, 1H, Ar), 7.17 (s, 1H, Ar), 6.96 (d, *J* = 6.1 Hz, 1H, Ar), 5.54 (s, 1H, -OH), 2.52 (s, 1H, -CH), 2.26 (s, 3H, -CH_3), 1.67 (s, 6H, -CH_3): ^{13}C-NMR (125 MHz, DMSO-d_6): δ 174.8, 156.9, 151.5, 149.0, 147.3, 140.1, 133.9, 133.3, 129.8, 127.6, 118.3, 116.1, 113.9, 112.6, 111.7, 108.9, 56.6, 55.5, 24.6, 24.6; HR-EI-MS: *m/z* Calcd for $C_{20}H_{18}N_4O_4$, $[M]^+$ 378.1328; found 378.1320.

3.2.8. (*E*)-1-butyl-3-(5-(3-hydroxy-4-methoxyphenyl)-1,3,4-oxadiazol-2-ylimino)indolin-2-one (8)

Yellow solid, Yield: 82%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 8.22 (d, *J* = 6.4 Hz, 1H, Ar), 7.87 (d, *J* = 6.6 Hz, 1H, Ar), 7.77 (d, *J* = 6.5 Hz, 1H, Ar), 7.65 (d, *J* = 6.3 Hz, 1H, Ar), 7.47 (s, 1H, Ar), 7.20 (d, *J* = 6.1 Hz, 1H, Ar), 7.03 (d, *J* = 6.7 Hz, 1H, Ar), 5.64 (s, 1H, -OH), 3.91 (m, 2H, -CH_2), 3.82 (s, 3H, -CH_3), 1.74 (m, 4H, -CH_2), 1.36 (m, 3H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.2, 159.9, 156.7, 156.2, 155.7, 150.8, 149.7, 138.4, 131.2, 127.9, 124.8, 122.4, 117.6, 117.3, 113.9, 111.7, 58.2, 54.1, 30.5, 24.5, 18.6; HREI-MS: *m/z* Calcd for $C_{21}H_{20}N_4O_4$, $[M]^+$ 392.1484; found 392.1472.

3.2.9. (*E*)-3-(5-(3-(benzyloxy)-4-chlorophenyl)-1,3,4-oxadiazol-2-ylimino)-5-isopropylindolin-2-one (9)

Red solid, Yield: 78%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 9.8 (s, 1H, -NH), 7.76 (s, 1H, Ar),7.55 (d, *J* = 6.5 Hz, 2H, Ar), 7.47 (m, 3H, Ar), 7.32 (d, *J* = 6.2 Hz, 1H, Ar), 7.36 (s, 1H, Ar), 7.27 (d, *J* = 6.3 Hz, 1H, Ar), 7.13 (d, *J* = 6.0 Hz, 1H, Ar), 6.86 (d, *J* = 6.6 Hz, 1H, Ar), 5.13 (s, 2H, -CH_2), 3.84 (s, 1H, -CH), 1.92 (s, 6H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 164.9, 164.2, 163.7, 151.3, 156.2,146.6, 139.2, 137.4, 131.1, 129.5, 129.5, 127.9, 127.6,

127.6, 127.2, 127.0, 125.7, 123.1, 121.7, 121.4, 118.1, 113.3, 71.4, 34.2, 24.5, 24.5; HR-EI-MS: *m/z* Calcd for $C_{26}H_{21}ClN_4O_3$, $[M]^+$ 472.1302; found 472.1297.

3.2.10. (*E*)-3-(5-(3-(benzyloxy)-4-chlorophenyl)-1,3,4-oxadiazol-2-ylimino)-1-isopropylindolin-2-one (10)

Silver solid, Yield: 75%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.82 (d, J = 6.6 Hz, 1H, Ar), 7.52 (d, J = 6.4 Hz, 1H, Ar),7.42 (d, J = 6.5 Hz, 1H, Ar), 7.38 (d, J = 6.6 Hz, 2H, Ar), 7.35 (t, J = 5.9. Hz, 3H, Ar), 7.23 (s, 1H, Ar), 7.20 (d, J = 6.2 Hz, 1H, Ar), 7.18 (d, J = 6.4 Hz, 1H, Ar), 7.07 (d, J = 6.8 Hz, 1H, Ar), 5.21 (s, 2H, -CH_2), 3.83 (s, 1H, -CH), 2.08 (s, 6H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ163.5, 157.2, 156.8, 149.5, 148.7, 139.4, 136.8, 128.9, 128.4, 128.3, 128.0, 127.9, 127.7, 123.6, 121.1, 120.6, 118.0, 117.2, 113.5, 113.0, 109.4, 108.5, 72.6, 55.6, 24.7, 24.7; HR-EI-MS: *m/z* Calcd for $C_{26}H_{21}ClN_4O_3$, $[M]^+$ 472.1302; found 472.1295.

3.2.11. (*E*)-3-(5-(3-(benzyloxy)-4-chlorophenyl)-1,3,4-oxadiazol-2-ylimino)-1-butylindolin-2-one (11)

Light Yellow solid, Yield: 81%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.85 (d, J = 6.6 Hz, 1H, Ar), 7.64 (d, J = 6.2 Hz, 1H, Ar), 7.50 (t, J = 5.6 Hz, 1H, Ar), 7.45 (t, J = 5.2 Hz, 1H, Ar), 7.44 (m, 3H, Ar), 7.38 (d, J = 6.6 Hz, 1H, Ar), 7.28 (d, J = 6.4 Hz, 1H, Ar), 7.19 (d, J = 6.2 Hz, 2H, Ar), 7.08 (s, 1H, Ar), 5.25 (s, 2H, -CH_2), 3.82 (m, 6H, -CH_2), 1.69 (m, 3H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 162.5, 159.2, 156.9, 151.7, 149.6, 138.4, 137.8, 129.9, 129.4, 128.5, 128.2, 128.1, 127.7, 127.6, 125.2, 124.7, 122.7, 122.1, 118.5, 116.4, 116.4, 113.5, 73.6, 44.7, 31.5, 22.7, 17.6; HR-EI-MS: *m/z* Calcd for $C_{27}H_{23}ClN_4O_3$, $[M]^+$ 486.1458; found 486.1443.

3.2.12. (*E*)-3-(5-(5-bromo-2-methoxyphenyl)-1,3,4-oxadiazol-2-ylimino)-5-isopropylindolin-2-one (12)

Dark Yellow solid, Yield: 65%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 8.10 (s, 1H, -NH), 7.77 (s, 1H, Ar), 7.47 (d, J = 6.6 Hz, 1H, Ar), 7.38 (s, 1H, Ar), 7.36 (d, J = 6.5 Hz, 1H, Ar), 7.01 (d, J = 7.3 Hz,1H, Ar), 6.85 (d, J = 6.5 Hz, 1H, Ar), 3.86 (s, 3H, -CH_3), 2.89 (s, 1H, -CH), 1.26 (s, 6H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 159.4, 156.6, 156.0, 155.9, 148.8, 143.1, 136.5, 134.2, 132.4, 131.0, 127.5, 122.2, 117.7, 117.4, 114.8, 112.7, 56.2, 32.6, 23.6, 23.6; HR-EI-MS: *m/z* Calcd for $C_{20}H_{17}N_4O_3$, $[M]^+$ 440.0484; found 440.0472.

3.2.13. (*E*)-3-(5-(5-bromo-2-methoxyphenyl)-1,3,4-oxadiazol-2-ylimino)-1-isopropylindolin-2-one (13)

Crystal solid, Yield: 78%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 8.09 (d, J = 6.8 Hz, 1H, Ar), 7.84 (d, J = 6.6 Hz, 1H, Ar), 7.57 (d, J = 6.3Hz, 1H, Ar), 7.45 (d, J= 6.1 Hz, 1H, Ar), 7.12 (s, 1H, Ar), 7.02 (d, J = 6.8 Hz, 1H, Ar), 6.74 (d, J = 6.6 Hz, 1H, Ar), 3.34 (s, 1H, -CH), 2.12 (s, 3H, -CH_3), 1.84 (s, 6H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 164.9, 160.9, 156.6, 156.1, 147.5, 133.9, 133.4, 133.0, 131.1, 127.5, 125.0, 118.2, 116.4, 115.6, 113.8, 112.7, 79.1, 55.9, 30.6, 30.6; HR-EI-MS: *m/z* Calcd for $C_{20}H_{17}BrN_4O_3$, $[M]^+$ 440.0484; found 440.0471.

3.2.14. (*E*)-3-(5-(5-bromo-2-methoxyphenyl)-1,3,4-oxadiazol-2-ylimino)-1-butylindolin-2-one (14)

Light Brown solid, Yield: 80%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.80 (d, J = 6.4 Hz, 1H, Ar), 7.53 (d, J = 6.5 Hz, 1H, Ar), 7.48 (d, J= 6.6 Hz, 1H, Ar), 7.36 (s, 1H, Ar), 7.29 (d, J = 6.4 Hz, 1H, Ar), 6.95 (d, J = 6.2 Hz, 1H, Ar), 6.87 (d, J = 6.4 Hz, 1H, Ar), 3.83 (s, 3H, -CH_3), 3.77 (m, 6H, -CH_2), 1.65 (m, 3H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ164.4, 158.6, 157.0, 156.9, 150.8, 143.1, 136.5, 136.2, 133.4, 131.0, 126.5, 123.2, 117.8, 117.5, 114.9, 112.8, 56.2, 45.3, 31.6, 22.6, 17.8; HR-EI-MS: *m/z*Calcd for $C_{21}H_{19}BrN_4O_3$, $[M]^+$ 454.0640; found 454.0628.

3.2.15. (*E*)-3-(5-(5-bromo-2-methoxyphenyl)-1,3,4-oxadiazol-2-ylimino)-1-pentylindolin-2-one (15)

Transparent solid, Yield: 79%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.86 (d, J = 6.3 Hz, 1H, Ar), 7.67 (d, J = 6.1 Hz, 1H, Ar), 7.51 (d, J= 6.0 Hz, 1H, Ar), 7.47 (d, J = 6.6 Hz,1H, Ar), 7.32 (s, 1H, Ar), 7.29 (d, J = 6.5 Hz, 1H, Ar), 7.02 (d, J = 6.4 Hz, 1H, Ar), 3.92 (s, 3H, -CH_3), 2.10 (m, 8H, -CH_2), 1.67 (m, 3H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 164.8, 161.7, 156.8, 156.4, 149.5, 148.9, 133.5, 133.1, 131.3, 129.5, 125.0, 118.4, 116.6, 115.4, 115.8, 113.7, 58.2, 44.9, 30.5, 28.9, 25.4, 18.5; HREI-MS: *m/z* Calcd for $C_{22}H_{21}BrN_4O_3$, $[M]^+$ 468.0796; found 468.0783.

3.3.16. (*E*)-3-(5-(5-bromo-2-methoxyphenyl)-1,3,4-oxadiazol-2-ylimino)indolin-2-one (16)

Light orange solid, Yield: 79%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 8.12 (s, 1H, -NH), 7.87 (d, J = 6.0 Hz, 1H, Ar), 7.77 (d, J = 6.1 Hz, 1H, Ar), 7.67 (t, J = 6.1 Hz, 1H, Ar), 7.59 (d, J = 6.7 Hz, 1H, Ar), 7.35 (s, 1H, Ar), 7.19 (s, 1H, Ar), 7.08 (d, J = 6.6 Hz, 1H, Ar), 3.87 (s, 3H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 163.4, 161.6, 156.2, 151.4, 142.5, 135.6, 134.8, 131.4, 130.4, 125.2, 120.6, 118.6, 118.2, 115.4, 113.8, 56.4, 32.6; HR-EI-MS: *m/z* Calcd for $C_{17}H_{11}BrN_4O_3$, $[M]^+$ 398.0016; found 398.0004.

3.3.17. (*E*)-2-(5-(1-isopropyl-2-oxoindolin-3-ylideneamino)-1,3,4-oxadiazol-2-yl)benzonitrile (17)

Dark Brown solid, Yield: 71%; ^{1}H-NMR:(500 MHz, DMSO-d_6): δ 8.09 (d, J = 6.4 Hz, 1H, Ar), 7.94 (d, J = 6.1 Hz, 1H, Ar), 7.84 (d, J = 6.6 Hz, 1H, Ar), 7.73 (t, J = 6.5 Hz, 1H, Ar), 7.64 (t, J = 6.2 Hz, 1H, Ar), 7.53 (t, J = 6.0 Hz, 1H, Ar), 7.46 (d, J = 6.7 Hz, 1H, Ar), 7.35 (t, J = 5.6 Hz, 1H, Ar), 2.12 (s, 1H, -CH), 1.67S (s, 6H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.4, 164.3, 162.8, 161.7, 148.2, 141.1, 134.3, 133.5, 132.2, 130.5, 130.1, 129.2, 125.4, 118.2, 117.8, 116.1, 105.0, 60.1, 21.6, 21.6; HR-EI-MS: *m/z* Calcd for $C_{20}H_{15}N_5O_2$, $[M]^+$ 357.1225; found 357.1212.

3.3.18. (*E*)-4-(5-(1-butyl-2-oxoindolin-3-ylideneamino)-1,3,4-oxadiazol-2-yl)benzonitrile (18)

Brown solid, Yield: 81%; ^{1}H-NMR:(500 MHz, DMSO-d_6): δ 7.93 (d, J = 5.9 Hz, 1H, Ar), 7.83 (d, J = 6.5 Hz, 1H, Ar), 7.76 (d, J= 6.6 Hz, 2H, Ar), 7.56 (d, J = 6.2 Hz, 2H, Ar), 7.49 (t, J = 6.2 Hz, 1H, Ar), 7.11 (t, J = 5.3 Hz, 1H, Ar), 2.09 (m, 6H, -CH_2), 1.68 (m, 3H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.2, 164.1, 162.5, 161.3, 148.3, 141.1, 134.2, 134.2, 132.1, 130.7, 130.2, 129.1, 129.1, 125.3, 119.5, 118.3, 113.0, 46.1, 30.7, 23.4, 17.5; HR-EI-MS: *m/z* Calcd for $C_{21}H_{17}N_5O_2$, $[M]^+$ 371.1381; found 371.1369.

3.3.19. (*E*)-4-(5-(2-oxo-1-pentylindolin-3-ylideneamino)-1,3,4-oxadiazol-2-yl)benzonitrile (19)

Dark brown solid, Yield: 81%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 7.94 (d, J = 6.4 Hz, 1H, 1H, Ar), 7.87 (d, J= 6.2 Hz, 1H, Ar), 7.65 (d, J= 6.2 Hz, 2H, Ar), 7.55 (d, J = 6.6 Hz, 2H, Ar), 7.46 (t, J = 5.9 Hz, 1H, Ar), 7.11 (t, J = 5.2 Hz, 1H, Ar), 2.18 (m, 8H, -CH_2), 1.76 (m, 3H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.1, 164.3, 162.2, 161.4, 148.4, 141.2, 134.2, 134.5, 132.4, 130.5, 130.6, 129.3, 129.3, 125.5, 119.4, 118.6, 113.2, 46.0, 30.7, 29.6, 24.4, 18.5; HR-EI-MS: *m/z* Calcd for $C_{22}H_{19}N_5O_2$, $[M]^+$ 385.1538; found 385.1530.

3.3.20. (*E*)-4-(5-(5-isopropyl-2-oxoindolin-3-ylideneamino)-1,3,4-oxadiazol-2-yl)benzonitrile (20)

Dark orange solid, Yield: 71%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 8.01 (s, 1H, -NH), 7.93 (d, J = 6.2 Hz, 2H, Ar), 7.83 (d, J= 6.5 Hz, 2H, Ar), 7.49 (s, 1H, Ar), 7.36 (d, J = 6.8 Hz,1H, Ar), 6.88 (d, 1H, J = 6.6 Hz, Ar), 2.88 (s, 1H, -CH), 1.74 (m, 6H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.5, 164.4, 162.9, 161.6, 148.3, 141.2, 134.4, 133.2, 132.5, 130.4, 129.2,127.2, 123.4, 119.2, 117.9, 117.1, 113.0, 37.1, 25.4, 25.4; HR-EI-MS: *m/z* Calcd for $C_{20}H_{15}N_5O_2$, $[M]^+$ 357.1225; found 357.1217.

3.3.21. (*E*)-4-(5-(1-isopropyl-2-oxoindolin-3-ylideneamino)-1,3,4-oxadiazol-2-yl)benzonitrile (21)

Light orange solid, Yield: 72%; ^{1}H-NMR:(500 MHz, DMSO-d_6): δ 7.94 (d, J = 6.2 Hz, 2H, Ar), 7.83 (d, J = 6.6 Hz, 1H, Ar), 7.76 (d, J = 6.5 Hz, 2H, Ar), 7.66 (d, J = 6.2 Hz, 1H, Ar), 7.51 (t, J = 6.1 Hz, 1H, Ar), 7.34 (t, J = 5.8 Hz, 1H, Ar), 3.85 (s, 1H, -CH), 1.68 (m, 6H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.4, 164.3, 162.8, 161.7, 148.2, 133.5, 133.5, 132.2, 131.4, 129.2, 129.2, 129.1, 125.3, 119.2, 117.8, 113.1, 105.0, 60.2, 21.8, 21.8; HR-EI-MS: *m/z* Calcd for $C_{20}H_{15}N_5O_2$, $[M]^+$ 357.1225; found 357.1216.

3.3.22. (*E*)-2-(5-(1-butyl-2-oxoindolin-3-ylideneamino)-1,3,4-oxadiazol-2-yl)benzonitrile (22)

Dark Orange solid, Yield: 81%; ^{1}H-NMR:(500 MHz, DMSO-d_6): δ 8.14 (d, J = 6.6 Hz, 1H, Ar), 8.09 (d, J = 6.5 Hz, 1H, Ar), 7.86 (d, J = 6.5 Hz, 1H, Ar), 7.73 (t, J = 6.6 Hz, 1H, Ar), 7.65 (d, J = 6.4 Hz, 1H, Ar), 7.53 (t, J = 5.6 Hz, 1H, Ar), 7.46 (t, J = 5.2 Hz, 1H, Ar), 7.38 (t, J = 4.8 Hz, 1H, Ar), 3.64 (m, 6H, -CH_2), 2.09 (m, 3H, -CH_3);^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.1, 164.0, 162.4, 161.2, 148.1, 141.0, 134.3, 134.4, 132.3, 130.8, 130.4, 129.6, 129.6, 125.2, 119.6, 118.5, 113.1, 46.2, 30.5, 23.2, 17.4; HR-EI-MS: *m/z* Calcd for $C_{21}H_{17}N_5O_2$, $[M]^+$ 371.1381; found 371.1368.

3.3.23. (*E*)-2-(5-(2-oxo-1-pentylindolin-3-ylideneamino)-1,3,4-oxadiazol-2-yl)benzonitrile (23)

Dark Brown solid, Yield: 81%; ^{1}H-NMR:(500 MHz, DMSO-d_6): δ 8.11 (d, J = 6.6 Hz, 1H, Ar), 7.86 (d, J = 6.4 Hz, 1H, Ar), 7.73 (t, J = 6.6 Hz, 1H, Ar), 7.70 (d, J= 6.4 Hz, 1H, Ar), 7.53 (t, J = 6.1 Hz, 1H, Ar), 7.49 (t, J = 5.6 Hz, 1H, Ar), 7.16 (t, J = 5.4 Hz, 1H, Ar), 6.78 (d,

J = 6.8 Hz, 1H, Ar), 3.44 (m, 8H, -CH_2), 1.64 (m, 3H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.2, 164.1, 162.5, 161.4, 148.6, 140.5, 134.3, 133.5, 132.2, 130.4, 130.4, 129.1, 125.2, 118.4, 117.8, 116.5, 104.2, 45.0, 30.4, 28.6, 24.1, 17.6; HR-EI-MS: *m/z* Calcd for $C_{22}H_{19}N_5O_2$, $[M]^+$ 385.1538; found 385.1524.

3.3.24. (*E*)-2-(5-(5-isopropyl-2-oxoindolin-3-ylideneamino)-1,3,4-oxadiazol-2-yl)benzonitrile (24)

Light Red solid, Yield: 76%; ^{1}H-NMR:(500 MHz, DMSO-d_6): δ 8.12 (s, 1H, -NH), 7.92 (d, *J* = 6.4 Hz, 2H, Ar), 7.86 (d, *J* = 6.1 Hz, 2H, Ar), 7.49 (s, 1H, Ar), 7.37 (d, *J* = 6.8 Hz, 1H, Ar), 6.88 (d, 1H, *J* = 6.6 Hz, Ar), 2.88 (s, 1H, -CH), 1.77 (m, 6H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.4, 164.3, 162.8, 161.7, 148.2, 141.1, 134.3, 133.5, 132.2, 130.5, 130.1, 129.2, 125.4, 118.2, 117.8, 116.1, 105.0, 60.1, 21.6, 21.6; HR-EI-MS: *m/z* Calcd for $C_{20}H_{15}N_5O_2$, $[M]^+$ 357.1225; found 357.1213.

3.3.25. (*E*)-3-(5-(5-isopropyl-2-oxoindolin-3-ylideneamino)-1,3,4-oxadiazol-2-yl)benzonitrile (25)

Light yellow solid, Yield: 71%; ^{1}H-NMR:(500 MHz, DMSO-d_6): δ 8.10 (s, 1H, -NH), 8.09 (d, *J* = 6.6 Hz, 1H, Ar), 7.99 (d, *J* = 5.9 Hz, 1H, Ar), 7.86 (s, 1H, Ar), 7.58 (t, *J* = 6.5 Hz, 1H, Ar), 7.48 (d, *J* = 6.7 Hz, 1H, Ar), 7.38 (s, 1H, Ar), 6.71 (d, *J* = 6.7 Hz, 1H, Ar), 2.89 (s, 1H, -CH), 1.22 (m, 6H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.2, 164.1, 162.9, 161.8, 149.2, 146.1, 139.3, 133.5, 132.3, 130.4, 130.2, 129.2, 127.4, 127.1, 122.6, 119.2, 117.8, 35.6, 25.4, 25.4; HR-EI-MS: *m/z* Calcd for $C_{20}H_{15}N_5O_2$, $[M]^+$ 357.1225; found 357.1213.

3.3.26. (*E*)-3-(5-(2-oxo-1-pentylindolin-3-ylideneamino)-1,3,4-oxadiazol-2-yl)benzonitrile (26)

Light brown solid, Yield: 81%; ^{1}H-NMR:(500 MHz, DMSO-d_6): δ 8.10 (d, *J* = 6.5 Hz, 1H, Ar), 7.98 (s, 1H, Ar), 7.84 (d, *J* = 6.3 Hz, 1H, Ar), 7.77 (d, *J* = 6.3 Hz, 1H, Ar), 7.58 (t, *J* = 6.5 Hz, 1H, Ar), 7.49 (t, *J* = 6.4 Hz, 1H, Ar), 7.16 (t, *J* = 6.0 Hz, 1H, Ar), 6.70 (d, *J* = 6.9 Hz, 1H, Ar), 3.90 (m, 8H, -CH_2), 1.35 (m, 3H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ165.6, 164.1, 162.5, 161.0, 148.1, 133.2, 132.8, 132.2, 131.5, 130.4, 129.5, 127.6, 125.3, 119.3, 118.5, 116.4, 113.6, 46.2, 30.6, 29.5, 24.2, 18.1; HR-EI-MS: *m/z* Calcd for $C_{22}H_{19}N_5O_2$, $[M]^+$ 385.1538; found 385.1529.

3.3.27. (*E*)-3-(5-(1-butyl-2-oxoindolin-3-ylideneamino)-1,3,4-oxadiazol-2-yl)benzonitrile (27)

Light brown solid, Yield: 81%; ^{1}H-NMR:(500 MHz, DMSO-d_6): δ 8.09 (d, J = 6.6 Hz, 1H, Ar), 7.87 (d, J = 6.4 Hz, 1H, Ar), 7.76 (t, J = 6.6 Hz, 1H, Ar), 7.60 (d, J = 6.4 Hz, 1H, Ar), 7.58 (t, J = 6.3 Hz, 1H, Ar), 7.44 (t, J = 5.9 Hz, 1H, Ar), 7.12 (t, J = 5.3 Hz, 1H, Ar), 6.74 (d, J = 6.8 Hz, 1H, Ar), 3.88 (m, 8H, -CH_2), 1.66 (m, 3H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.0, 163.4, 162.1, 161.0, 148.2, 133.0, 132.4, 131.8, 130.9, 130.2, 130.1, 127.6, 125.6, 119.2, 118.6, 116.5, 114.1, 45.2, 30.4, 22.2, 16.4; HR-EI-MS: *m/z* Calcd for $C_{21}H_{17}N_5O_2$, $[M]^+$ 371.1381; found 371.1372.

3.3.28. (*E*)-3-(5-(2-oxoindolin-3-ylideneamino)-1,3,4-oxadiazol-2-yl)benzonitrile (28)

Orange solid, Yield: 71%; ^{1}H-NMR:(500 MHz, DMSO-d_6): δ 8.14 (s, 1H, -NH), 8.10 (d, J = 6.7 Hz, 1H, Ar), 7.99 (d, J = 6.6 Hz, 1H, Ar), 7.85 (s, 1H, Ar), 7.78(d, J = 6.4 Hz, 1H, Ar), 7.60 (t, J = 6.6 Hz, 1H, Ar), 7.51 (t, J = 5.6 Hz, 1H, Ar), 7.15 (t, J = 5.1 Hz, 1H, Ar), 6.92 (d, J = 6.5 Hz, 1H, Ar); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.1, 164.0, 162.4, 150.2, 143.1, 133.0, 133.0, 131.4, 130.3, 130.1, 128.4, 126.6, 125.6, 120.2, 119.4, 118.4, 113.2; HR-EI-MS: *m/z* Calcd for $C_{17}H_9N_5O_2$, $[M]^+$ 315.0755; found 315.0741.

3.3.29. (*E*)-4-(5-(2-oxoindolin-3-ylideneamino)-1,3,4-oxadiazol-2-yl)benzonitrile (29)

Orange solid, Yield: 71%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 8.10 (s, 1H, -NH), 7.97 (d, J = 9.3 Hz, 2H, Ar), 7.84 (d, J = 9.2 Hz, 2H, Ar), 7.60 (d, J = 9.0 Hz, 1H, Ar), 7.52 (d, 1H, J = 8.8 Hz, Ar), 7.50 (t, J = 6.5 Hz, 1H, Ar), 7.08 (t, J = 5.6 Hz, 1H, Ar); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 165.2, 164.1, 162.6, 151.0, 142.2, 133.2, 133.2, 131.7, 130.8, 130.2, 128.6, 126.6, 125.4, 120.3, 119.3, 118.2,113.1; HR-EI-MS: *m/z* Calcd for $C_{17}H_9N_5O_2$, $[M]^+$ 315.0755; found 315.0844.

3.3.30. (*E*)-2-(5-(2-oxoindolin-3-ylideneamino)-1,3,4-oxadiazol-2-yl)benzonitrile (30)

Orange solid, Yield: 71%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 8.11 (s, 1H, -NH), 7.95 (d, J = 6.4 Hz, 1H, Ar), 7.86 (d, J = 6.4 Hz, 1H, Ar), 7.72 (t, J = 6.5 Hz, 1H, Ar), 7.60 (t, J = 6.0 Hz, 1H, Ar), 7.51 (d, 1H, J = 6.2 Hz, Ar), 7.49 (t, J = 5.7 Hz, 1H, Ar), 7.08 (t, J = 5.2 Hz, 1H, Ar), 6.93 (d, J = 6.5 Hz, 1H, Ar); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 164.1, 163.0, 161.2, 151.3, 142.6, 141.4, 134.6, 133.5, 132.4, 130.6, 130.6, 129.4, 125.6, 120.3, 118.6, 117.8, 105.4; HR-EI-MS: *m/z* Calcd for $C_{17}H_9N_5O_2$, $[M]^+$ 315.0755; found 315.0840.

3.4. Thymidine phosphorylase assay

Since human TP is not easily to get, we used commercially available recombinant *E. coli* TP. Main sequence of TP is frequently preserved throughout evolution as mammalian TP that is reported to share 39% sequence resemblance with the TP of E. coli. The mammalian enzyme is also shared up to 70% resemblance with the active site residues, and three-dimensional structure of *E. coli* TP enzyme. The Thymidine phosphorylase/PD-ECGF (*E. coli*) activity was determined by measuring the absorbance at 290 nm spectrophotometrically [46-48]. In brief, total reaction mixture of 200 μL contained 145 μL of potassium phosphate buffer (pH 7.4), 30 μL of enzyme (*E. coli*) at concentration 0.05 and 0.002 U, respectively, were incubated with 5 lL of test materials for 10 min at 25 $^{\circ}$C in microplate reader. After incubation, pre-read at 290 nm was taken to deduce the absorbance of substrate particles. Substrate (thymidine, λ_{max}; 265 nm) 20 μL, 1.5 mM, was dissolved in potassium phosphate buffer, was immediately added to plate and degradation of thymidine continuously read after 10, 20, and 30 min in 96-well ELISA plate reader (spectra max, molecular devices, CA, USA). The 7-Deazaxanthine was used as positive control. All assays were performed in triplicate. The extinction coefficient "e" of product was required to know enzyme activity related to absorbance. Its Beer Lambert Abs = e c l. The l was pathlength of cuvette and c was concentration of product that appeared or substrate that disappeared.

3.5 Statistical analysis/Calculations

Results were processed using SoftMax Pro 4.8 software (Molecular Devices, CA, USA) and then by Microsoft Excel. Percent inhibition for above mentioned biological activities was calculated by following formula:

$$\text{Percent Inhibition} = 100 - (OD_{\text{test compound}} / OD_{\text{control}}) \times 100$$

3.6. Molecular docking

The docking is a significant tool to explore the interactions between an inhibitor and the target [49]. To find the binding interactions of these compounds in the active sites of the thymidine phosphorylase, the MOE-Dock program (www.chemcomp.com) was used to perform molecular docking. The 3D crystal structure of the thymidine phosphorylase (4EAD) was retrieved from the Protein Databank (PDB). The synthesized compounds were docked into the active site of the target enzyme in MOE (www.chemcomp.com) by the default

parameters i-e Placement: Triangle Matcher, rescoring 1: London dG, Refinement: Forcefield, Rescoring 2: London dG. For each ligand ten conformations were generated, and the top ranked conformation based on docking score was selected for further studies in molecular docking. After the molecular docking, we analyzed the best poses having polar, H-pi and pi-H interactions by Pymol software.

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Synthesis, SAR elucidations and molecular docking study of newly designed Isatin based oxadiazole analogs as potent inhibitors of thymidine phosphorylase

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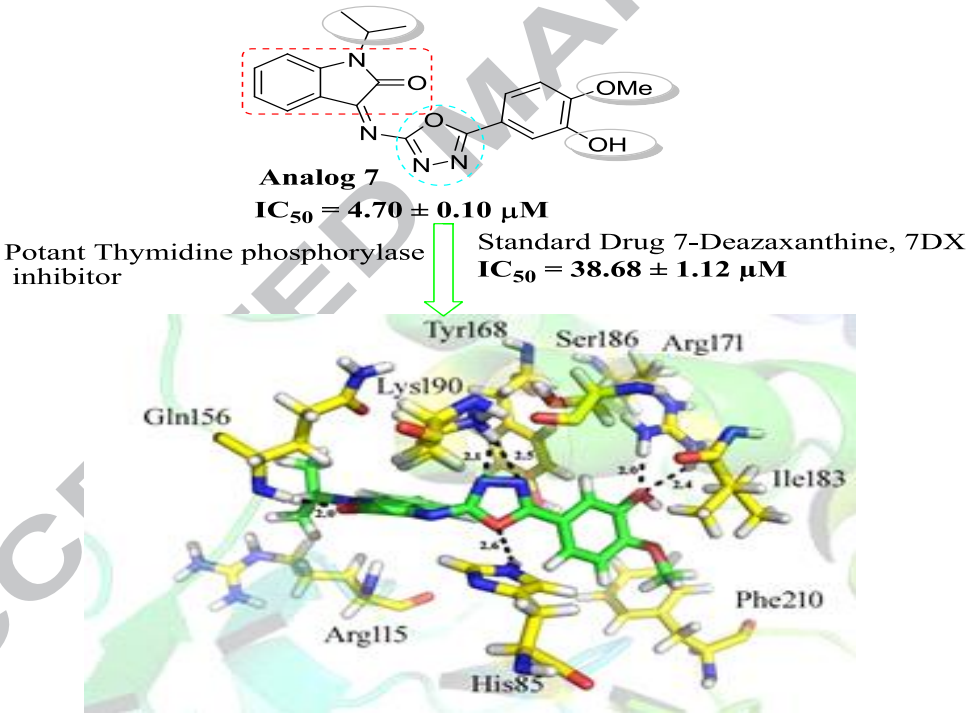

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Highlights:

- Synthesis of isatin based oxadiazole analogs
- *In vitro* thymidine phosphorylase
- Identification of a new of *thymidine phosphorylase* inhibitor
- Structure Activity Relationship established
- Molecular docking