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Synthesis, thymidine phosphorylase, angiogenic inhibition and molecular docking study of isoquinoline derivatives

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ABSTRACT

Isoquinoline analogues (KA-**1** to **16**) have been synthesized and evaluated for their *E. coli* thymidine phosphorylase inhibitory activity. Except compound **11**, all other analogs showed outstanding thymidine inhibitory potential ranging in between 4.40 ± 0.20 to 69.30 ± 1.80 μM when compared with standard drug **7-Deazaxanthine** (IC_{50} = 38.68 ± 4.42 μM**)**. Structure Activity Relationships has been established for all compounds, mainly based on substitution pattern on phenyl ring. All analogs were characterized by various spectroscopic techniques such as ^{1}H NMR, ^{13}C NMR and EI-MS. The binding interactions of isoquinoline analogues with the active site of TP enzyme, the molecular docking studies were performed. Furthermore, the angiogenic inhibitory potentials of isoquinoline analogues **(KA-1-9, 14, 12 and 16)** were determined in the presence of standard drug **Dexamethasone** based on percentage inhibitions at various concentrations. Herein this work analogue **KA-12**, **14** and **16** emerged with most potent angiogenic inhibitory potentials among the synthesized analogues.

1. Introduction

Thymidine phosphorylase (TP) is the key enzyme of the pyrimidine salvage pathway, which speedup the conversion of thymidine and 2́-deoxyuridine to their respective bases and 2-deoxy-D-ribose 1-phosphate [1,2]. Furthermore, the dephosphorylations of 2-deoxy-D-ribose 1-phosphate yield 2-deoxy-D-ribose that stimulates the discharge of vascular endothelial growth factor (VEGF). VEGF motivates a number of processes including endothelial cells for discharge of matrix metalloproteinases, proliferation and migration of endothelial cells to tumor tissue. These accomplishments cause the formation of new blood vessel and cancer metastasis [3]. Thymidine phosphorylase inhibitors hinder the formation of 2-deoxy-D-ribose and destroy tumor growth [4]. Therefore it is very crucial to develop effective and novel TP inhibitors that have the potential to cease tumor growth [5]. In this regard a number of struggles concerned to development of thymidine phosphorylase inhibitors have been described [6-8]. The most effective inhibitor of human TP known till date is the pyrimidine analog 5-chloro-6-[1-(2-iminopyrrolidinyl)methyl] uracil hydrochloride (TPI) and the first purine analog 7-deazaxanthine (7DX) recognized as TP inhibitor (Fig. 1) [9] (see. Scheme 1.).

Isoquinoline is a heterocyclic moiety found in various natural products, especially in alkaloids [10,11]. Isoquinoline alkaloids were found in some botanical families and marine species [12,13] that shows significant pharmacological activities like antibiotic, antitumor [14], α-glucosidase in hibition [15], β-adrenergic [16]. Numerous synthetic isoquinoline derivatives have been reported as anti-malarial agent [17], anti-convulstant agent [18], anti-cancer agent [19], and anti-proliferative agent [20]. Isoquinoline analogs have been also reported as a topoisomerase I catalytic inhibitors [21], acetylcholinesterase

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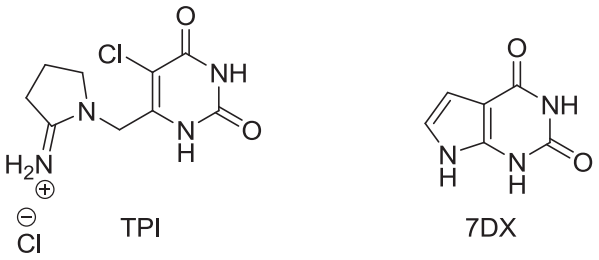


Fig. 1. Structure of known TP inhibitors TPI and 7DX.

inhibitors [22] and as α-Glucosidase Inhibitors [23].

Our research group had reported various heterocyclic analogs with pharmacological potentials [24-26], and also published thiadiazole, oxadiazole and piperazine derivatives as potential inhibitors of thymidine phosphorylase enzyme [27,28]. We have observed in our previous study that nitrogen containing heterocyclic compounds have great biological potential [29-33]. Keeping in view the great biological potential of nitrogen heterocyclic compounds, here in this study we have plan to synthesize the isoquinoline analogs in search of potent thymidine phosphorylase inhibitor. We have obtained outstanding results which support our hypothesis. Here in this study we are going to report new analogs of isoquinoline as potent TP and angiogenesis inhibitor.

2. Results and discussion

2.1. Chemistry

Methyl isoquinoline-3-carboxylate was treated and refluxed with hydrazine hydrate in ethanol as solvent to obtain isoquninoline-3-carbohydrazide as intermediate. Furthermore, the intermediate was treated with various aryl aldehyde/acetophenone in ethanol in the presence of few drops of glacial acetic acid and reflux for 3–4 hrs to obtain *N*-benzyliden-isoquinoline-3-carbohydrazide analogues **(KA-1-16)**. After completion of reaction, the reaction mixture was filtered and washed with *n*-hexane/ethanol to purify the final product.

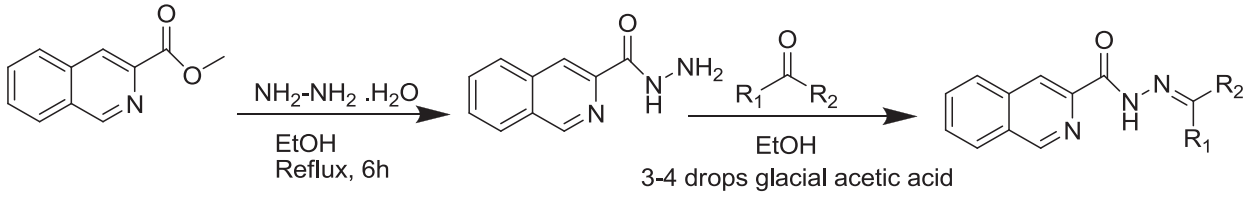
2.2. In vitro study of thymidine phosphorylase inhibition

Sixteen analogs of *N*-benzyliden-isoquinoline-3-carbohydrazide **(KA-1-16)** were synthesized and evaluated their inhibitory activity against thymidine phosphorylase enzyme. All analogues asserted varying degree of inhibitory potential in the range of 4.40 ± 0.20 to 69.30 ± 1.80 μM, under positive control of standard drug 7-Deazaxanthine having an IC_{50} value 38.68 ± 4.42 μM (Table 1**)**. The screened analogs of the series, analog11 was found inactive, while the remaining all analogues **KA-1–10** and **KA-12–16** displayed outstanding TP inhibitory potential with IC_{50} values 11.30 ± 0.30, 8.10 ± 0.30, 39.40 ± 1.10, 69.30 ± 1.80 , 44.70 ± 1.30, 16.10 ± 0.40, 18.40 ± 0.40, 27.60 ± 0.60, 4.40 ± 0.20, 8.10 ± 0.20, 8.60 ± 0.30, 36.10 ± 0.70, 18.40 ± 0.60, 22.20 ± 0.50 and 21.40 ± 0.60 μM respectively. SAR study has been established for all analogues of the whole series which shows that by bringing about slight change in the position, nature as well as the number of substituent on phenyl ring may greatly influenced the inhibitory potential of our synthesized analogs. Analogue **KA-9** (IC_{50} value 4.40 ± 0.20) was found with very healthy inhibitory potential having two methoxy group at the 3,5 position and one hydroxyl group at position-4 of phenyl ring. The excellent potential of this compound seems due to electron donating group *i.e.* the methoxy groups and the interaction of hydroxyl group through hydrogen bond with the active site of enzyme. If analogue **KA-9** was compared with analogue **KA-6** (IC_{50} value 16.10 ± 0.40 μM) having one hydroxyl and one methoxy group at *meta* position to each other, the analog **9** is superior. The greater inhibitory potential of analogue KA-**9** compared to analogue **KA-6** may be due to hydroxyl group at the *para* position and one additional methoxy group. The second utmost active analog of the series is analogue **KA-2** with IC_{50} value 8.10 ± 0.30 μM bearing hydroxyl group at the *ortho* position and chlorine atom at the *meta* position of the phenyl ring. The greater potential endorsed by analog 2 is seem due to EWG the chloro group and hydroxyl group. If it is compared with the third utmost active analog of the series, the analogue **KA-10** (IC_{50} value 8.10 ± 0.20 μM) have hydroxy group at the *para* position and Cl atom at the *meta* position of phenyl ring. The slight difference in their inhibitory activity may be due to the position change of hydroxyl group. It may be seen in SAR study that di-chlroinated phenyl moiety have shown much better inhibitory potential than that of mono-chlorinated phenyl moiety, may be due to chlorine that activates phenyl ring through resonating effect toward binding interaction with enzyme pocket. That is why we observed that analogue **KA-12** (IC_{50} = 8.60 ± 0.30) have two chlorine atom at 2, 4-position was found with many fold better inhibitory activity than analogue **KA-7** (IC_{50} = 18.40 ± 0.40) having only one chlorine atom at *para* position. The positional change of identical substituent around the phenyl ring may influence the inhibitory activity. Therefore analogues KA-**13, KA-14** and **KA-15** with IC_{50} values 36.10 ± 0.70, 18.40 ± 0.60 and 22.20 ± 0.50 have cyano-group at various positions of phenyl ring, but among these three analogs, analog **KA-14** have cyano-group at *ortho* position shown better inhibitory potential. It seems that by changing the position of substituent greatly affect the potential inhibition of these analogues. As the compound **KA-11** has bulky benzyloxy moiety on phenyl ring. The presence of this bulky moiety might be one of the reasons for this compound to be inactive. It was cleared from this study that the little bit difference in IC_{50} values of different analogs might be due to the position, nature as well as the number of substituents on phenyl ring. The binding interactions of the most active analogs with the active site of enzyme were confirmed through molecular docking study.

2.3. Docking study

The docking analysis of synthesized compounds has provided good information about the nature of binding mode and their correlation with experimental results. The top ranked analogs confirmation was soundly coordinated inside the exertive position of thymidine phosphorylase enzyme, which were proven by docking calculation. It was found that thymidine phosphorylase enzyme shown various types of interactions with synthesized analogs through Arg 115, Thr 123, Tyr 168, Arg 171, Ile 183, Ser 186, Lys 190 etc. The docking score and their interactions detail are listed in Table 2**.** The nature of most active analogs and their structure feature show that presence of electro-negative groups like −OH and halogen groups.

The structural aspects interpretation concerns to this group for the active nature of compounds are the manifestation of electro-negative



Scheme 1. Synthesis of isoquinoline analogs **(KA-1–16)**.

Table 1
Different isoquinoline analogs and their TP inhibition.

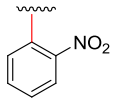
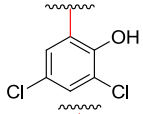
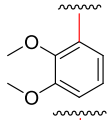
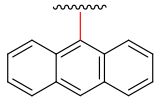
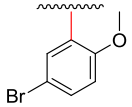
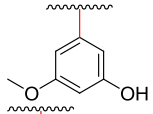
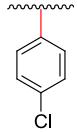
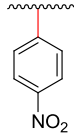
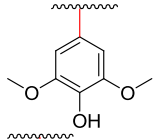
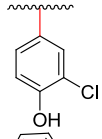
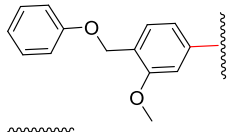
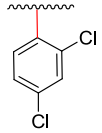
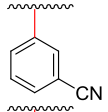
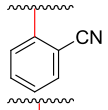
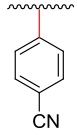
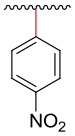
S. No	R_2	R_1	IC_{50} ± SEM[a]
KA-1		H	11.30 ± 0.30
KA-2		H	8.10 ± 0.30
KA-3		H	39.40 ± 1.10
KA-4		H	69.30 ± 1.80
KA-5		H	44.70 ± 1.30
KA-6		H	16.10 ± 0.40
KA-7		H	18.40 ± 0.40
KA-8		CH_3	27.60 ± 0.60
KA-9		H	4.40 ± 0.20
KA-10		H	8.10 ± 0.20
KA-11		H	N.A.
KA-12		H	8.60 ± 0.30
KA-13		H	36.10 ± 0.70
KA-14		H	18.40 ± 0.60
KA-15		H	22.20 ± 0.50

Table 1 (*continued*)

S. No	R_2	R_1	IC_{50} ± SEM[a]
KA-16		H	21.40 ± 0.60
7-Deazaxanthine (7DX)			38.68 ± 1.12 μM

7-Deazaxanthine (7DX) is the standard inhibitor for TP.
[b] NA = not active.
[a] SEM is the standard error mean.

groups like −OH and halogen groups. Analogs of the series having halogen like Cl found with better potential than analog having Br supported hydroxyl group.

Fig. 2(a,b) showing the binding interactions of enzyme with most active analogs among these docked conformations. After docking, the confirmations obtained showed good docking score, results and healthy demonstration in-silico inhibition of the thymidine phosphorylase enzyme. The overall result and correlation concern to biological evaluation and docking study was found very well in (Fig. 3).

2.4. In vitro anti-angiogenesis activity of isoquinoline analogues

In the present study isoquinoline analogues **(KA-1–9, 12, 14** and **16)** at various concentrations were screened for their percentage anti-angiogenic activity in the presence of Dexamethasone as standard drug. All analogues of the series displayed more than 50% anti-angiogenic activity at 500 μg/ml concentration except analogue **KA-4**. Similarly at the 250 μg/ml concentration the anti-angiogenic potentials of some analogues like **KA-2, KA-5** and **KA-9** were decreased than 50%. Subsequently, when analogues were evaluated for their angiogenic inhibitory potentials at 125 μg/ml concentrations, so only analogues **KA-3, KA-6, KA-7, KA-8, KA-12, KA-14** and **KA-16** were found with more than 50% angiogenic inhibition. Regarding the same trends and the concentrations further decrease to 62.2 μg/ml, so the angiogenic inhibitory potential of most analogues ultimately decreased to less than 50%, only analogues **KA-16, KA-8,** and **KA-12** exhibited more than 50% angiogenic inhibition. Additionally, when the concentration decreased to 31.25 μg/ml, only analogue **KA-16** displayed more than 50% angiogenic inhibition. The details of percent angiogenic inhibitions of all analogues at various concentrations are present in Table 3. The relative angiogenic inhibitory activities of analogues with +ive/−ive control are depicted in Fig. 4. Overall results show that analogue **KA-12, KA-14** and **KA-16** emerged with most potent anti-angiogenic activity among the series Fig. 5.

2.5. Conclusion

A series of *N*-benzyliden-isoquinoline-3-carbohydrazide analogs (KA-**1** to **16**) were synthesized and evaluated for their *E. coli* thymidine phosphorylase inhibitions. All screened analogues displayed uneven degree of thymidine phosphorylase inhibition with IC_{50} values ranging between of 4.40 ± 0.20 to 69.30 ± 1.80 μM, under positive control of reference drug 7-Deazaxanthine having IC_{50} value 38.68 ± 4.42 μM. Twelve analogues, analogue **KA-1, 2, 6, 7, 8, 9, 10, 12, 13, 14, 15** and **KA-16** among the entire series were found with most potent inhbitory activity as compared to standard. Some analogues were found in a range good to modorates thyimidine phosphorylase inhbitions like **KA-3, 4** and **5**. Further to this, analogue **KA-11** shown no inhibitory potential. The SAR study was focus mainly on substituents of phenyl part. The docking analysis was carried out to investigate binding interaction between screened analogs and active site enzyme. Herein this work analogue **KA-1 to 9, 11, 12** and **16** was further screened for their

Table 2
Docking scores and report of predicted interactions of docked conformations.

Comp.No	Docking score	Interaction detail (Ligands/thymidine phosphorylase)				
		Ligand	Receptor	Interaction	Distance	E(kcal/mol)
KA-1	−10.0001	O 35	NH1ARG171(A)	H-acceptor	2.88	−3.3
		O 35	NH2ARG171 (A)	H-acceptor	3.00	−1.8
		O 35	CG1 ILE 183 (A)	H-acceptor	3.71	−0.1
		O 36	NH2ARG171 (A)	H-acceptor	2.96	−2.6
		6-ring	CB SER 86 (A)	pi-H	4.39	−0.3
		6-ring	CA GLY 88 (A)	pi-H	3.44	−0.1
		6-ring	CA GLY 88 (A)	pi-H	4.30	−0.1
		6-ring	CG2 ILE 183 (A)	pi-H	3.88	−0.2
		6-ring	CD1 ILE 187 (A)	pi-H	4.83	−0.1
KA-2	−11.2107	C 28	O SER 113 (A)	H-donor	2.54	−0.1
		N 13	CD1 ILE 187 (A)	H-acceptor	2.88	−0.2
		O 21	NH2 ARG171(A	H-acceptor	4.08	−0.7
		6-ring	CD2 PHE 210(A)	pi-H	3.96	−0.3
KA-3	−7.6729	C 1	O SER 113 (A)	H-donor	2.81	−0.1
		C 27	O SER 86 (A)	H-donor	3.12	−0.1
		N 20	NH2 ARG171(A)	H-acceptor	3.42	−0.2
		N 20	CA ILE 183 (A)	H-acceptor	3.79	3.79
KA-4	−6.3209	C 35	O LYS 84 (A)	H-donor	3.87	−0.1
		N 20	CD LYS 190 (A)	H-acceptor	3.24	−0.1
		6-ring	CA HIS 85 (A)	pi-H	4.89	−0.1
		6-ring	CG1 ILE 187(A)	pi-H	3.16	−0.1
KA-5	−7.2301	N 20	NH2 ARG171(A)	H-acceptor	2.87	−1.4
		O 21	NH2 ARG171(A)	H-acceptor	2.88	−0.8
		6-ring	CD LYS 190(A)	pi-H	4.02	−0.6
		6-ring	NZ LYS 190(A)	pi-cation	4.04	−2.5
		6-ring	NZ LYS 190(A)	pi-cation	4.52	−0.8
KA-6	−9.8963	C 34	OD1 ASP 172(A)	H-donor	2.65	−0.8
		N 13	NE2 HIS 85 (A)	H-acceptor	3.34	−0.1
		N 13	CG1 ILE 187(A)	H-acceptor	3.70	−0.1
		O 21	CD1 ILE 187(A)	H-acceptor	3.70	−0.8
		6-ring	6-ringTYR168(A)	pi-pi	3.14	−0.0
		6-ring	6-ringPHE210(A)	pi-pi	3.50	−0.0
KA-7	−9.3210	C 3	SD MET 211 (A)	H-donor	4.35	−0.3
		CL 34	OG SER 113 (A)	H-donor	3.63	−0.4
		N 13	CB SER 186 (A)	H-acceptor	3.37	−0.6
		C 15	6-ringTYR168(A)	H-pi	4.20	−0.1
		6-ring	CE1 HIS 85 (A)	Pi-H	4.73	−0.1
		6-ring	N ARG 115 (A)	pi-H	4.14	−0.1
		6-ring	NH2ARG171(A)C	pi-cation	3.90	−0.2
		6-ring	G1 ILE 187 (A)	pi-H	4.89	−0.2
KA-8	−8.1021	N 13	NH1 ARG171(A)	H-acceptor	2.96	−2.0
		N 13	CG1 VAL 177(A)	H-acceptor	3.87	−0.1
		O 21	NH2ARG 171(A)	H-acceptor	2.66	−2.0
		O 38	N GLN 156 (A)	H-acceptor	3.55	−0.9
		O 39	CD2 TYR 168(A)	H-acceptor	3.37	−0.2
		6-ring	6-ringTYR168(A)	pi-pi	3.12	−0.0
KA-9	−15.9448	C 5	OG SER 186 (A)	H-donor	3.47	−0.2
		O	OH THR 123(A)	H-acceptor	1.20	−0.2
		6-ring	OG1 THR 123(A)	pi-H	3.86	−0.1
		6-ring	CE LYS 190(A)	pi-H	4.09	−0.4
		6-ring	CE LYS 190(A)	pi-H	4.98	−0.4
		6-ring	6-ringTYR168(A)	pi-pi	3.99	−0.0
		O	OG TYR 168(A)	H-acceptor	3.50	−0.0
KA-10	−11.4618	O 21	NH LYS 190 (A)	H-acceptor	2.41	−0.4
		6-ring	N ARG 115 (A)	pi-H	4.73	−0.1
		6-ring	N ARG 115 (A)	pi-H	4.55	−0.1
		6-ring	CB ARG 115(A)	pi-H	4.20	−0.2
		6-ring	CE1 TYR 168(A)	pi-H	4.52	−0.2
		6-ring	OH TYR 168(A)	pi-H	4.00	−1.0
		6-ring	CG2 ILE 183(A)	pi-H	4.55	0.1
		6-ring	6-ring TY 168(A)	pi- pi	3.63	−0.0
KA-11	NA	NA	NA	NA	NA	NA

(*continued on next page*)

Table 2 (*continued*)

Comp.No	Docking score	Interaction detail (Ligands/thymidine phosphorylase)				
		Ligand	Receptor	Interaction	Distance	E(kcal/mol)
KA-12	−11.2309	C 1	O THR 87 (A)	H-donor	3.40	−0.1
		C 3	SD MET 211(A)	H-donor	3.39	−0.1
		C 11	O VAL 177(A)	H-donor	3.16	−0.1
		C 27	O ARG 115 (A)	H-donor	3.38	−0.1
		O 21	NH2 ARG171(A)	H-acceptor	2.81	−5.9
		CL 33	CG2 ILE 112(A)	H-acceptor	4.46	−0.1
		C 25	6-ringTYR168(A)	H- pi-	4.53	−0.3
		6-ring	CA GLY 114(A)	pi-H	3.51	−0.1
		6-ring	CG1 ILE 183(A)	pi-H	3.87	−0.5
		6-ring	CG2 ILE 183(A)	pi-H	3.86	−0.1
KA-13	−7.9982	C 25	O THR 87 (A)	H-donor	3.10	−0.4
		N 35	NH1 ARG171(A)	H-acceptor	3.14	−0.8
		N 35	CA ILE 183(A)	H-acceptor	3.39	−1.0
		N 35	CB SER 186 (A)	H-acceptor	3.36	−0.5
		6-ring	CE LYS 190 (A)	pi-H	3.58	−0.5
		6-ring	NZ LYS 190(A)	pi-cation	3.76	−0.9
KA-14	−9.2319	N 13	CE1 TYR 168(A)	H-acceptor	3.55	−0.1
		N 35	NH2 ARG171(A)	H-acceptor	2.53	−1.4
		N 35	CA ILE 183(A)	H-acceptor	3.34	−0.1
		N 35	CG1 ILE 183(A)	H-acceptor	3.62	−0.1
		N 35	CG2 ILE 183(A)	H-acceptor	3.79	−0.2
		N 35	CB SER 186(A)	H-acceptor	3.70	−0.3
		6-ring	6-ringTYR168(A)	pi-pi	3.63	−0.0
KA-15	−8.5310	N 13	CG2 ILE 183(A)	H-acceptor	3.88	−0.2
		N 13	CD1 ILE 187(A)	H-acceptor	3.99	−0.1
		N 15	CB THR 123 (A)	H-acceptor	2.86	−0.4
		N 15	N LEU 124(A)	H-acceptor	2.51	−0.1
		6-ring	NE2 HIS 85 (A)	pi-H	4.51	−0.2
		6-ring	NE2 HIS 85 (A)	pi-H	4.21	−0.2
		6-ring	N THR 123(A)	pi-H	4.42	−0.1
		6-ring	CG2 ILE 183(A)	pi-H	4.86	−0.1
KA-16	−8.1002	C 30	SD MET 211(A)	H-donor	4.50	−0.4
		N 20	CB SER 186(A)	H-acceptor	3.02	−0.3
		6-ring	NE2 HIS 85 (A)	pi-H	3.71	−0.9
		6-ring	CE1 HIS 85 (A)	pi-H	3.56	−0.5
		6-ring	NH1 ARG 171(A)	pi-cation	3.80	−0.3
Standard 7-Deazaxanthine	−7.8230	N 1	OG SER 186 (A)	H-donor	3.18	−2.3
		O 7	NZ LYS 190 (A)	H-acceptor	2.61	−10.4
		O 8	NH1 ARG171(A)	H-acceptor	3.28	−2.3
		O 8	NH2 ARG171(A)	H-acceptor	3.16	−4.0
		6-ring	NE2 HIS 85 (A)	Pi-H	4.64	−1.9

angiogenic inhibitions to check its anti-tumor potentials. In this regard analogue **KA-12, 14** and **16** were found with most potent therapeutic anti-angiogenic potentials and may act as lead candidates for advance research in tumor drug.

3. Materials and methods

3.1. Synthesis of isoquinoline-3-carbohydrazide.

Methyl isoquinoline-3-carboxylate (1 mmol) was treated with

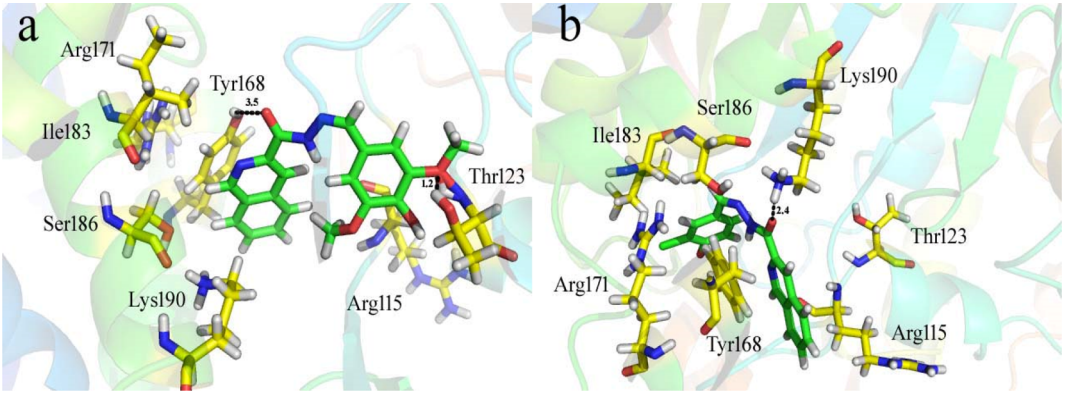


Fig. 2. The most active analogs of the series and their Docking conformations on thymidine phosphorylase enzyme. **(a)** 3D binding mode of interaction of analogue **KA-9** as potential inhibitor of thymidine phosphorylase enzyme. **(b)** 3D binding mode of interaction of analogue **KA-10** in respective binding cavity of thymidine phosphorylase enzyme. Ligands are shown green color.

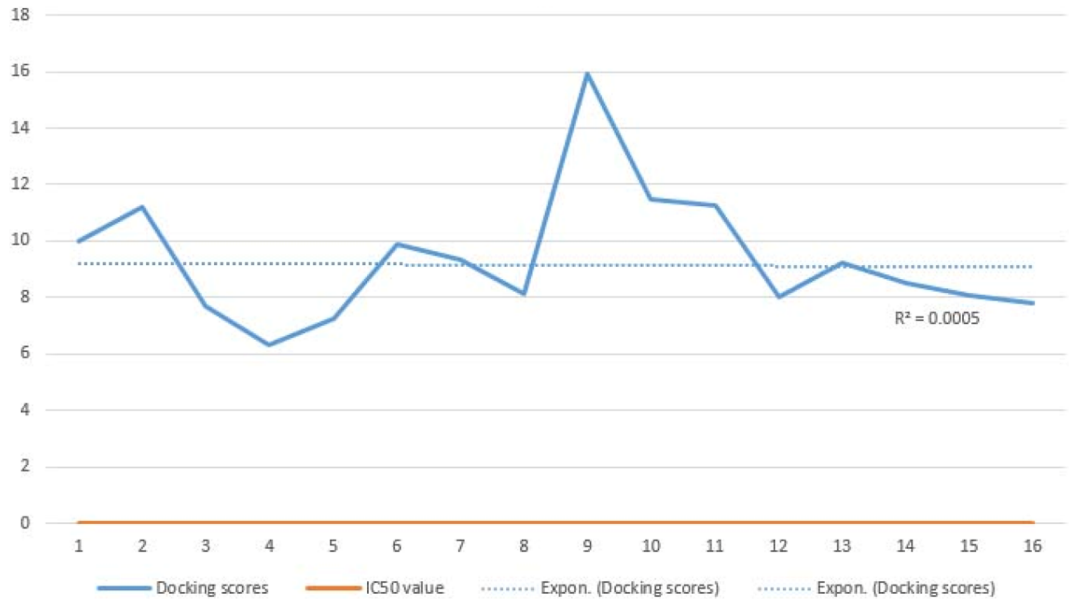


Fig. 3. The docking predicting activity and correlation graph for IC_{50} values. The X-axis represent the IC50 values of the compounds whereas the Y-axis showed the compounds number.

Table 3
The details of percent inhibition of inhibition of isoquinoline analogues (KA-1 to KA-9, KA-12, KA-14 and KA-16).

Samples	Percent inhibition of angiogenesis at various concentrations				
	500	250	125	62.5	31.25
KA-1	66.77 ± 1.24	61.64 ± 1.60	46.43 ± 1.15	39.96 ± 1.01	25.26 ± 1.24
KA-2	54.90 ± 1.55	43.32 ± 3.50	41.46 ± 2.43	28.10 ± 1.10	19.95 ± 2.01
KA-3	69.80 ± 1.50	58.00 ± 2.90	51.83 ± 1.36	41.67 ± 0.88	37.26 ± 1.24
KA-4	45.56 ± 1.06	21.50 ± 0.86	19.96 ± 1.01	15.33 ± 1.20	NA
KA-5	56.42 ± 1.89	44.00 ± 1.15	21.33 ± 0.33	NA	NA
KA-6	66.93 ± 0.67	59.61 ± 1.70	52.83 ± 1.36	48.67 ± 0.67	38.58 ± 1.12
KA-7	69.72 ± 1.01	58.22 ± 0.23	51.96 ± 2.66	41.00 ± 1.00	33.70 ± 1.63
KA-8	67.90 ± 0.96	62.87 ± 0.26	58.43 ± 0.97	52.33 ± 0-0.88	39.67 ± 0.88
KA-9	58.25 ± 0.20	44.70 ± 1.60	21.96 ± 1.01	NA	NA
KA-12	78.00 ± 1.50	72.66 ± 0.89	68.03 ± 0.82	54.00 ± 1.15	51.90 ± 0.52
KA-14	71.93 ± 0.94	65.33 ± 0.68	61.93 ± 1.21	49.50 ± 1.04	42.87 ± 1.27
KA-16	74.50 ± 0.58	69.50 ± 0.58	62.33 ± 0.88	53.00 ± 0.00	46.64 ± 1.60
Dexamethasone	92.74 ± 0.687	86.10 ± 1.15	84.33 ± 0.33	77.32 ± 1.33	68.08 ± 1.04

Dexamethasone is the standard inhibitor for angiogenesis.
NA = not active.

hydrazine hydrate in absolute ethanol as solvent and refluxed the reaction mixture for six hrs and then poured into crush ice after completion the reaction. Isoquinoline-3-carbohydrazide Intermediate obtained was filtered and washed with hexane to remove un-reacted methyl isoquinoline-3-carboxylate. Then equimolar (1 mmol) of isoquinoline-3-carbohydrazide and different aryl aldehyde/acetophenone (1 mmol) was mixed in absolute ethanol, followed by addition of few drops of glacial acetic acid as catalyst and refluxed for 3–4 hrs. The reaction completion was achieved by TLC (Ethyl acetate/hexane; ratio: 3:7, Rf: 0.32–0.5). In-order to obtained pure *N*-benzyliden-isoquinoline-3-carbohydrazide analogs, filter the reaction mixture, dried and then washed with *n*-hexane/ethanol system.

3.1.1. (E)-N' (2-nitrobenzylidene) isoquinoline-3-carbohydrazide (KA-1)

Yield: 80%, ^{1}H NMR (500 MHz, DMSO- d_6): δ 12.7(s, IH, NH), 9.4 (s, 1H, Aromatic-H), 9.1 (s, 1H, CH = N), 8.5 (s, 1H, Aromatic-H), 8.3 (d, J = 5.6 Hz, 1H. Aromatic-H), 8.2 (d, J = 5.7 Hz, 1H. Aromatic-H), 7.9 (t, J = 6 Hz, 1H, Aromatic-H), 7.5 (dd, J = 8 Hz, 1 Hz, 1H, Aromatic-H), 7.3 (dd, J = 1.5 Hz, 8 Hz, 1H, Aromatic-H), 7.2 (dt, J = 0.5 Hz, 7.5 Hz, 1H, Aromatic-H), 7.1 (dt, J = 1.5 Hz, 8.5 Hz, 1H, Aromatic-H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 206.3, 160.2, 155.4, 151.4, 147.8, 146.5, 142.8, 140.7, 135.3, 131.5, 129.5, 129.5, 128.1, 128.1, 127.8, 124.1, 121.1; HR-ESI-MS: *m/z* calcd for $C_{17}H_{14}N_2O_3$ $[M]^+$ 320.09094; Found 320.09084.

3.1.2. (E)-N' (3, 5-dichloro-2–hydroxybenzylidene) isoquinoline-3-carbohydrazide (KA-2)

Yield: 65% ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.9(s, 1H, NH), 9.4 (s, 1H, Aromatic-H), 8.8 (s, 1H, CH = *N*-Ar), 8.7 (s, 1H, Aromatic-H), 8.3 (d, $J_{8,\ 7}$ = 6.7 Hz, 1H, Aromatic-H), 8.2 (d, $J_{5,\ 6}$ = 6.7 Hz, 1H, Aromatic-H), 7.9 (t, $J_{7/6,\ 8}$ = 6.2 Hz, 1H, Aromatic-H), 7.8 (t, $J_{6/5,\ 7}$ = 6.7 Hz, 1H, Aromatic-H), 7.6 (s, 1H, Aromatic-H), 7.5 (s, 1H, Aromatic-H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 206.3, 160.2, 55.4, 151.4, 148.3, 142.3, 135.2, 131.5, 130.2, 129.6, 129.5, 128.3, 128.1, 127.8, 121.5, 121.3, 120.7. HR-ESI-MS: *m/z* calcd for $C_{17}H_{11}Cl_2N_3O_2$ $[M]^+$ 359.02283; Found 359.02273.

3.1.3. (E)-N' (2, 3-dimethoxybenzylidene) isoquinoline-3-carbohydrazide (KA-3)

Yield: 70%, ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.4 (s, 1H, NH), 9.4 (s, 1H, Aromatic-H), 9 (s, 1H, CH = *N*-Ar), 8.7 (s, 1H, Aromatic-H), 8.3 (d, $J_{8,\ 7}$ = 6.7 Hz, 1H, Aromatic-H), 8.2 (d, $J_{5,\ 6}$ = 8.5 Hz, 1H, Aromatic-),

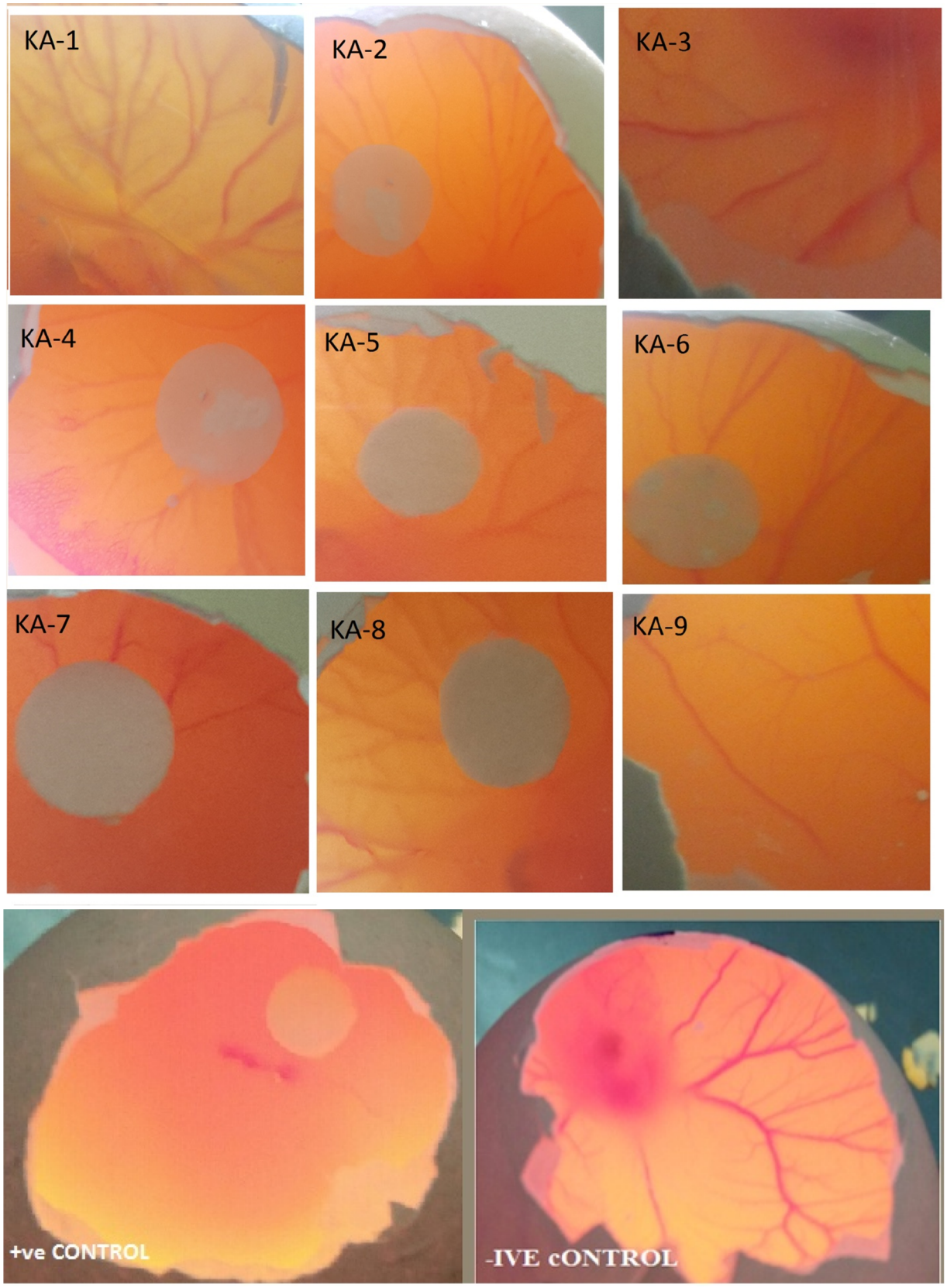


Fig. 4. The relative percent angiogenic inhibitory activities of analogues with positive and negative control.

7.9 (t, $J_{7/6,\ 8}$ = 6.1 Hz, 1H, Aromatic-H), 7.8 (t, $J_{6/5,\ 7}$ = 6.1 Hz, 1H, Aromatic-H), 7.5 (d, $J_{5',\ 6'}$ = 6.1 Hz, 1H, Aromatic-H), 7.1(m, 2H, Aromatic-H), 3.8 (s, 6H, 2-OMe). ^{13}C NMR (125 MHz, DMSO-d_6): δ206.3, 160.6, 152.7, 151.4, 148.2, 144.7, 143.3, 135.3, 131.3, 129.3, 129.3, 128.1, 127.9, 127.7, 124.2, 120.7, 117.1, 56.1, 55.7. HR-ESI-MS: *m/z* calcd for $C_{19}H_{17}N_3O_3$ $[M]^+$ 335.12699; Found 335.12689.

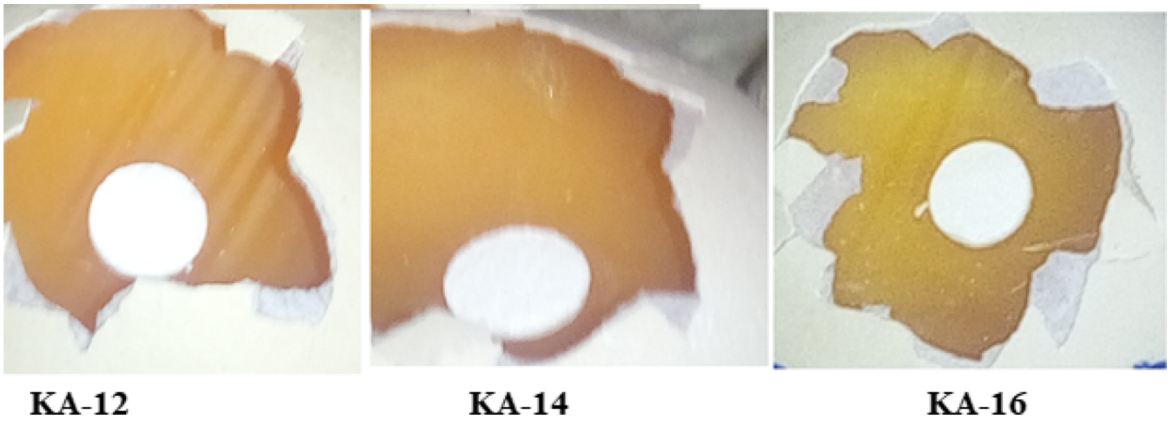


Fig. 5. The most potent angiogenic inhibitory activities of analogues KA-12, KA-14 and KA-16.

3.1.4. (E)-N' (anthracen- 9-ylmethylen) isoquinoline-3-carbohydrazide (KA-4)

Yield: 78%, ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.6 (s, 1H, NH), 10.8 (s, 1H, Aromatic-H), 9.9 (s, 1H, CH = *N*-Ar), 9.5 (s, 1H, Aromatic-H), 8.3 (d, $J_{8,7}$ = 6.7 Hz, 1H, Aromatic-H), 8.2 (d, $J_{5,\ 6}$ = 8.5 Hz, 1H, Aromatic-H), 7.9 (t, $J_{7/6,\ 8}$ = 6.1 Hz, 1H, Aromatic-H), 7.8 (t, $J_{6/5,\ 7}$ = 6.1 Hz, 1H. Aromatic-H), 7.5 (d, $J_{5',\ 4'/7',\ 8'}$ = 8.1 Hz, 2H, Aromatic-H), 7.3 (m, 4H, Aromatic-H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 206.3, 160.6, 151.5, 148.2, 143.2, 135.3, 131.8, 131.6, 131.1, 130.9, 130.8, 130.2 129.6, 129.6, 129.4, 129.3, 128.8, 128.5, 128.1, 127.8, 127.1, 125.4, 125.2, 125.1, 120.7. HR-ESI-MS: *m/z* calcd for $C_{25}H_{17}N_3O$ [M]$^+$ 375.13716; Found 375.13707.

3.1.5. (E)-N' (5-bromo-2-methoxybenzlidene) isoquinoline-3-carbohydrazide (KA-5)

Yield: 62%, ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.4 (s, 1H, NH), 9.4 (s, 1H, Aromatic-H), 8.9 (s, 1H, CH = *N*-Ar), 8.6 (s, 1H, Aromatic-H), 8.3 (d, $J_{8,\ 7}$ = 6.7 Hz, 1H, Aromatic-H), 8.2 (d, $J_{5,\ 6}$ = 6.8 Hz, 1H, Aromatic-H), 8 (d, $J_{6',\ 4'}$ = 2.1 Hz, 1H, Aromatic-H) 7.9 (t, $J_{7/6,\ 8}$ = 6.1 Hz, 1H, Aromatic-H), 7.8 (t, $J_{6/5,\ 7}$ = 6.1 Hz, 1H, Aromatic-H), 7.6 (dd, $J_{4',\ 6'}$ = 2.1 Hz, $J_{4',\ 3'}$ = 7.3 Hz, Aromatic-H), 7.1 (d, $J_{3',\ 4'}$ = 7.4 Hz, Aromatic-H), 3.8 (s, 3H, 1-OMe). ^{13}C NMR (125 MHz, DMSO-d_6): δ 206.4, 160.8, 156.9, 151.5, 149.5, 143.5, 143.3, 143.1, 142.8, 135.3, 133.6, 131.4, 129.4, 128.8, 127.8, 124.8, 120.8, 56.1. HR-ESI-MS: *m/z* calcd for $C_{18}H_{14}BrN_3O_2$ [M]$^+$ 383.02694; Found 383.02684.

3.1.6. (E)-N' (3-hydroxy-5-methoxybenzylidene) isoquinoline-3-carbohydrazide (KA-6)

Yield: 60%, ^{1}H NMR (500 MHz, DMSOd$_6$): δ 12 (s, 1H, NH), 9.4 (s, 1H, Aromatic-H), 8.7 (s, 1H, CH = *N*-Ar), 8.5 (s, 1H, Aromatic-H), 8, 3 (d, $J_{8,\ 7}$ = 6.7 Hz, 1H, Aromatic-H), 8.2 (d, $J_{5,\ 6}$ = 6.4 Hz, 1H, Aromatic-H), 7.9 (t, $J_{7/6,\ 8}$ = 6.8 Hz, 1H, Aromatic-H), 7.8 (t, $J_{6/5,\ 7}$ = 6.1 Hz, 1H, Aromatic-H), 7.3 (d, $J_{6'\ 2'}$ = 1.5 Hz, 1H, Aromatic-H), 7.2 (d, $J_{2',\ 6'}$ = 1.5 Hz, 1H, Aromatic-H), 7.1 ((dd, $J_{4',6'}$ = 1.5, $J_{4',2'}$ = 7, 1H, Aromatic-H), 3.8 (s, 3H, 1-OMe). ^{13}C NMR (125 MHz, DMSO- d_6): δ 206.4, 160.2, 151.6, 149.8, 149.1, 146.8, 143.3, 142.8, 135.3, 135.2, 131.4, 129.8, 127.9, 127.8, 127.2, 124.3, 120.3, 55.7. HR-ESI-MS: *m/z* calcd for $C_{18}H_{15}N_3O_3$ [M]$^+$ 321.11134; Found 321.11126.

3.1.7. (E)-N' (4-chlorobenzylidene) isoquinoline-3-carbohydrazide (KA-7)

Yield: 77%, ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.3 (s, IH, NH), 9.4 (s, IH, Aromatic-H), 8.7 (s, 1H, CH = *N*-Ar), 8.5 (s, IH, Aromatic-H), 8.3 (d, $J_{8,7}$ = 6.7 Hz, 1H, Aromatic-H), 8.2 (d, $J_{5,\ 6}$ = 6.5 Hz, 1H, Aromatic-H), 7.9 (t, $J_{7/6,8}$ = 5.8 Hz, 1H, Aromatic-H), 7.8 (t, $J_{6/5,\ 7}$ = 5.8 Hz, 1H, Aromatic-H), 7.7 (d, $J_{2',\ 3'/6',\ 5'}$ = 7 Hz, 2H, Aromatic-H), 7.5 ($J_{3',\ 2,\ /5',\ 6'}$ = 7 Hz, 2H, Aromatic-H). ^{13}C- NMR (125 MHz, DMSO-d_6): δ 206.4, 160.6, 151.5, 147.6, 143.1, 135.3, 134.5, 133.3, 131.4, 129.4, 129.4 128.9, 128.9, 128.7, 128.0, 127.8, 120.8. HR-ESI-MS: *m/z* calcd for $C_{17}H_{12}ClN_3O$,[M]$^+$ 309.06689; Found 309.06679.

3.1.8. (Z)-N'-(1-(4-nitrophenyl) ethylidene) isoquinoline-3-carbohydrazide (KA-8)

Yield: 78%, ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 12.5(s, 1H, NH), 9.5 (s, 1H, Aromatic-H), 8.8(s, IH, CH = *N*-Ar), 8.5 (s, 1H, Aromatic-H), 8.3 (d, $J_{8,\ 7}$ = 7.3 Hz, 1H, Aromatic-H), 8.2 (d, $J_{5,\ 6}$ = 6.8 Hz, 1H. Aromatic-H), 8.2 (d, $J_{3',2'\ /5',6'}$ = 6,8 Hz, 2H, Aromatic-H), 7.9 (d, $J_{2',3'/6',5'}$ = 7,3 Hz, 2H, Aromatic-H) 7.9 (t, $J_{7/6,8}$ = 5.8 Hz, Aromatic-H), 7.8 (t, $J_{6/5,\ 7}$ = 5.8 Hz, 1H, Aromatic-H), 2.6 (s, 3H, CH3), ^{13}C NMR (125 MHz, DMSO-d_6): δ206.4, 160.9, 151.6, 146.5, 142.8, 140.7, 135.3, 131.5, 129.5, 129.5, 128.2, 128.1, 127.8, 124.1, 121.1, 23.5. HR-ESI-MS: *m/z* calcd for $C_{18}H_{14}N_4O_3$, [M]$^+$ 334.10659; Found 334.10649.

3.1.9. (Z)-N' (4-hydroxy-3, 5-dimethoxybenzylidene) isoquinoline-3-carbohydrazide (KA-9)

Yield: 55%, ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.1 (s, 1H, NH), 9.4 (s, 1H, Aromatic-H), 8.6 (s, 1H, CH = *N*-Ar), 8.5 (s, 1H, Aromatic-H), 8.3 (d, $J_{8,\ 7}$ = 6.7 Hz, 1H, Aromatic-H), 8.2 (d, $J_{5,\ 6}$ = 6.7 Hz, 1H, Aromatic-H), 7.9 (t, $J_{7/6,\ 8}$ = 5.8 Hz, 1H, Aromatic-), 7.8 (t, $J_{6/5,\ 7}$ = 5.8 Hz, 1H, Aromatic-H), 7.1(s, 2H, Aromatic-H), 3.8 (s, 6H, H-2xOMe). ^{13}C NMR (125 MHz, DMSO-d_6): δ 206.4, 160.2, 151.5, 149.6, 148.1, 143.3, 138.0, 135.3, 131.4, 129.3, 129.2, 127.9, 127.8, 124.6, 120.5, 104.7, 104.7, 56.02, 56.02. HR-ESI-MS: *m/z* calcd for $C_{19}H_{17}N_3O_4$, [M]$^+$ 351.12191; Found 351.12182.

3.1.10. (Z)-N' (3-chloro-4-hydroxybenzylidene) isoquinoline-3-carbohydrazide (KA-10)

Yield: 70%, ^{1}H NMR (500 MHz, DMSO-d_6): δ *12.1*(s, 1H, NH), 9.4 (s, 1H, Aromatic-H), 8.6 (s, 1H, CH = *N*-Ar), 8.5 (s, 1H, Aromatic-H), 8.3 (d, $J_{8,\ 7}$ = 6.7 Hz, 1H, Aromatic-H), 8.2 (d, $J_{5,\ 6}$ = 6.8 Hz, 1H, Aromatic-H), 7.9 (t, $J_{7/6,\ 8}$ = 6 Hz, 1H, Aromatic-H), 7.8 (t, $J_{6/5,\ 7}$ = 6.7 Hz, 1H, Aromatic-H), 7.7 (d, $J_{2',\ 5'}$ = 1.5 Hz, 1H, Aromatic-H), 7.5 (dd, $J_{5',\ 2'}$ = 1.5, $J_{5',\ 4'}$ = 7, 1H, Aromatic-H), 7.0 (d $J_{4',\ 5'}$ = 6.9 Hz, 1H, Aromatic-H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 206.3, 171.9, 160.3, 154.8, 151.4, 147.7, 143.2, 135.3, 131.3, 129.3, 129.2, 128.2, 127.9, 127.7, 127.3, 120.6, 55.9. HR-ESI-MS: *m/z* calcd for $C_{17}H_{12}ClN_3O_2$, [M]$^+$ 325.06180; Found 325.06171.

3.1.11. (Z)-N'-(3-methoxy-4-(phenoxymethyl)benzylidene)isoquinoline-3-carbohydrazide (KA-11)

Yield: 85%, ^{1}H NMR (500 MHz, DMSO- d$_6$): δ 12.1(s, 1H, NH), 9.4 (s, 1H, Aromatic-H), 8.6 (s, 1H, CH = *N*-Ar), 8.5 (s, 1H, Aromatic-H), 8.3 (8.3 (d, $J_{8,\ 7}$ = 6.7 Hz, 1H, Aromatic-H), 8.2 (d, $J_{5,\ 6}$ = 6.7 Hz, 1H, Aromatic-H), 7.9 (t, $J_{7/6,\ 8}$ = 6.2 Hz, 1H, Aromatic-H), 7.8 (t, $J_{6/5,\ 7}$ = 6.8 Hz, 1H, Aromatic-H), 5.1 (s, 2H, OCH2–), 3.8 (s, 3H, OMe).^{13}C

NMR (125 MHz, DMSO-d_6): δ 206.3, 160.2, 151.4, 149.7, 149.3, 149.0, 143.2, 136.6, 135.3, 135.3, 129.5, 129.2, 129.2, 128.3, 127.9, 127.8, 127.7, 127.7, 127.3, 121.8, 120.5, 113.0, 108.4, 69.81, 55.4. HR-ESI-MS: *m/z* calcd for $C_{25}H_{21}N_3O_3$, $[M]^+$ 411.15829; Found 411.15820

3.1.12. (E)-N'-(2,4-dichlorobenzylidene)isoquinoline-3-carbohydrazide (KA-12)

Yield: 78%, ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.6 (s, 1H, NH), 9.4 (s, 1H, Aromatic-H), 9.1 (s, 1H, CH = *N*-Ar), 8.7 (s, 1H, Aromatic-H), 8.3 (8.3 (d, $J_{8,\ 7}$ = 6.7 Hz, 1H, Aromatic-H), 8.2 (d, $J_{5,\ 6}$ = 6.8 Hz, 1H, Aromatic-H), 8.1 (d, $J_{6',\ 5'}$ = 7.1 Hz, 1H, Aromatic-H) 7.9 (t, $J_{7/6,\ 8}$ = 6.2 Hz, 1H. ArH), 7.8 (t, $J_{6/5,\ 7}$ = 6.8 Hz, 1H, Aromatic-H), 7.7 (d, $J_{3',\ 5'}$ = 1.2 Hz, 1H, Aromatic-H), 7.5 (dd, $J_{5'/3',\ 6}$ 1H, Aromatic-H). ^{13}C NMR (125 MHz, DMSO-d_6): δ206.4, 160.9, 151.5, 143.9, 143.0, 135.3, 135.0, 134.0, 131.4, 131.0, 129.4, 129.3, 128.1, 128.0, 127.9, 127.9, 121.0. HR-ESI-MS: *m/z* calcd for $C_{17}H_{11}C_{l2}N_3O$, $[M]^+$ 343.02792; Found 343.02783.

3.1.13. (E)-N'-(3-cyanobenzylidene)isoquinoline-3-carbohydrazide (KA-13)

Yield: 48%, ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.4 (s, 1H, NH), 9.4 (s, 1H, Aromatic-H), 8.8 (s, 1H, CH = *N*-Ar), 8.5 (s, 1H, Aromatic-H),), 8.3 (d, $J_{8,7}$ = 6.7 Hz, 1H, Aromatic-H), 8.2 (d, $J_{5,6}$ = 6.7 Hz, 1H, Aromatic-H), 8.1(Hs, 1H, Aromatic-H), 8.2 (d, $J_{6',\ 5'}$ = 6.7 Hz, 1H, Aromatic-H), 8.1 (d, $J_{6',\ 5'}$ = 6.7 Hz, 1H, Aromatic-H), 7.9 (t, $J_{7/6,\ 8}$ = 6.5 Hz, 1H, Aromatic-H), 7.8 (t, $J_{6/5,\ 7}$ = 6.7 Hz, 1H, Aromatic-H), 7.5 (dd, $J_{4',\ 2'/4',\ 5'}$ = 8.1 Hz, 1H, Aromatic-H), 7.3 (t, $J_{5',\ 4',\ 6'}$ = 8.1 Hz, 1H, Aromatic-H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 206.4, 160.8, 151.5, 146.7, 143.0, 135.7, 135.3, 133.2, 131.5, 131.1, 130.5, 130.1, 129.4, 129.4, 128.0, 127.8, 120.9, 118.3, 112.0. HR-ESI-MS: *m/z* calcd for $C_{18}H_{12}N_4O$, $[M]^+$ 300.10111; Found 300.10102.

3.1.14. (E)-N'-(2-cyanobenzylidene)isoquinoline-3-carbohydrazide (KA-14)

Yield: 50, % ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.8(s, 1H, NH), 9.4 (s, 1H, Aromatic-H), 9.1 (s, 1H, CH = *N*-Ar), 8.5 (s, 1H, Aromatic-H), 8.3 (d, $J_{8,\ 7}$ = 6.7 Hz, 1H. Aromatic-H), 8.2 (d, $J_{5,\ 6}$ = 6.8 Hz, 1H, Aromatic-H), 8.1(d, $J_{3,\ 4'}$ = 6.6 Hz, 1H, Aromatic-H) 7.9 d, $J_{6,\ 5'}$ = 6.6 Hz, 1H, Aromatic-H) ^{13}C NMR (125 MHz, DMSO-d_6): δ 206.3, 161.1, 151.6, 144.3, 143.0, 137.2, 135.3, 133.4, 133.4, 131.4, 130.3, 130.3, 128.0, 127.8, 125.7, 121.0, 121.0, 111.0. HR-ESI-MS: *m/z* calcd for $C_{18}H_{12}N_4O$, $[M]^+$ 300.10111; Found 300.10102.

3.1.15. (E)-N'-(4-cyanobenzylidene)isoquinoline-3-carbohydrazide (KA-15)

Yield: 62%, ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.5(s, 1H, NH), 9.4 (s, 1H, Aromatic-H), 8.8 (s, 1H, CH = *N*-Ar), 8.5 (s, 1H, Aromatic-H), 8.3 (d, $J_{8,\ 7}$ = 6.7 Hz, 1H, Aromatic-H), 8.2 (d, $J_{5,\ 6}$ = 6.8 Hz, 1H. Aromatic-H), 8.2 (d, $J_{3,\ 2'/5,\ 6'}$ = 6.8 Hz, 2H, Aromatic-H), 7.9 (d, $J_{2,\ 3'/6,\ 5'}$ = 7, 3 Hz, 2H, Aromatic-H), 7.9 (t, $J_{7/6,\ 8}$ = 5.9 Hz, 1H, Aromatic-H), 7.8 (t, $J_{6/5,\ 7}$ = 5.8 Hz, 1H, Aromatic-H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 206.3, 160.8, 151.5, 146.9, 142.8, 138.8, 135.2, 132.6, 131.4, 129.4, 129.4, 128.0, 127.7, 127.6, 120.9, 118.5, 111.8. HR-ESI-MS: *m/z* calcd for $C_{18}H_{12}N_4O$, $[M]^+$ 300.10111; Found 300.10103.

3.1.16. (E)-N'-(4-nitrobenzylidene)isoquinoline-3-carbohydrazide (KA-16)

Yield: 85%, ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.5(s, 1H, NH), 9.5 (s, 1H, Aromatic-H), 8.7 (s, 1H, CH = *N*-Ar), 8.5 (s, 1H, Aromatic-H), 8.3 (d, $J_{8,\ 7}$ = 7.3 Hz, 1H, Aromatic-H), 8.2 (d, $J_{5,\ 6}$ = 6.8 Hz, 1H, Aromatic-H), 8.2 (d, $J_{3',\ 2'/5',6'}$ = 6,8 Hz, 2H, Aromatic-H), 7.9 (d, $J_{2',3'/6',5'}$ = 7,3 Hz, 2H, Aromatic-H 7.9 (t, $J_{7/6,8}$ = 5.8 Hz, 1H, Aromatic-H), 7.8 (t, $J_{6/5,\ 7}$ = 5.8 Hz, 1H, Aromatic-H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 206.4, 160.9, 151.6, 147.8, 146.5, 142.8, 140.7, 135.3, 131.5, 129.5, 129.5, 128.2, 128.1, 127.8, 124.1, 121.1. HR-ESI-MS: *m/z* calcd for $C_{17}H_{14}N_2O_3$ $[M]^+$ 320.09094; Found 320.09083.

3.2. Thymidine phosphorylase assay protocol

TP/PD-ECGF (E. coli TP (Sigma T6632)) activity was determined by measuring the absorbance at 290 nm spectrophotometric ally. The innovative protocol reported by Krenitsky (Krenitsky et al., 1979) [34] was revised. Briefly, in 96 wells, flat bottom, microplate with each well capacity 200 µl, reaction mixture of 200 µl was prepared which contained 145 µl of potassium phosphate buffer (pH 7.4), 20 µl of 1.5 mM Thymidine 5′ mono phosphate solution as substrate, 30 µl of enzyme (E. coli TP (Sigma T6632) at concentration 0.05 and 0.002 U, respectively, were incubated with 5 µl of test materials for 10 min at 25 °C in temperature controlled incubator before taking readings by microplate reader (SpectraMax Plus^{384}, USA) at 290 nm. The wells containing reaction mixture devoid of substrate were blank and the mean OD of these blank wells was substracted from wells containing reaction mixture with substrate. The readings were taken continuously after 10, 20, and 30 min by microplate reader. All assays were performed in triplicate.

3.3. Assessment of chick chorioallantoic membrane using CAM assay

Herein the current CAM assay fresh fertilized chicken eggs were employed [36]. So, reserved this fresh eggs in incubator at 37 °C (BOD incubator HYSC korea, model: Bl-81/150/250) with narrow end down. On daily basis, all this fresh eggs were moved three to four times. All eggs at fourth day examined the head of embryo were encircled employing a torch. From the narrow end of the eggs one ml of albumen was sucked out using 18-gauge hypodermic needle due to reason moveaway the yolk sac and CAM from the shell. Subsequently, the shell adjacent of the air sac was punched and stripped peeled away. Through various concentrations the thermanox cover-slip is already loaded with testing sample, kept on the CAM surface in order to come in contact with sample. After preparation the samples the eggs were again put back in incubator. Furthermore, small quantity of methanol and acetone (1:1) will add in to chorioallantois via 33-gauge needle. After that carefully detached from egg and then observed the vessels through microscope, followed by counting the vessels which congregated toward the Centre. Herein these study eighteen eggs were used for each sample. For percent angiogenic inhibitions Dexamethasone was used as +ive control and normal saline was employed as −ive control. Angiogenic percent inhibition was determined through using the following formula:

$$\%\text{Inhibition} = \frac{\text{CAM}_{ns} - \text{CAM}_{ts}}{\text{CAM}_{ns}} \times 100$$

where: CAMns is the number of blood vessels in CMA with normal saline, while CAMts is the number of blood vessels in CMA treated with test samples.

3.4. Molecular docking

The docking is a significant tool to explore the interactions between an inhibitor and the target [35]. 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 already validated protocol [28]: Triangle Matcher, Rescoring 1: London dG, Refinement: Force field, 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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