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2-Arylquinazolin-4(3*H*)-ones: A novel class of thymidine phosphorylase inhibitors

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ABSTRACT

Thymidine phosphorylase (TP) over expression plays an important role in several pathological conditions, such as rheumatoid arthritis, chronic inflammatory diseases, psoriasis, and tumor angiogenesis. In this regard, a series of twenty-five 2-arylquinazolin-4(3*H*)-one derivatives **1**–**25** were evaluated for thymidine phosphorylase inhibitory activity. Six compounds **5**, **6**, **20**, **2**, **23**, and **3** were found to be active against thymidine phosphorylase enzyme with IC_{50} values in the range of 42.9–294.6 μM. 7-Deazaxanthine (IC_{50} = 41.0 ± 1.63 μM) was used as a standard inhibitor. Compound **5** showed a significant activity (IC_{50} = 42.9 ± 1.0 μM), comparable to the standard. The enzyme kinetic studies on the most active compounds **5**, **6**, and **20** were performed for the determination of their modes of inhibition, and dissociation constants K_i. All active compounds were found to be largely non-cytotoxic against the mouse fibroblast 3T3 cell line. This study identifies a novel class of thymidine phosphorylase inhibitors which may be further investigated as leads to develop therapeutic agents.

1. Introduction

Thymidine phosphorylase (abbreviated as dThdPase or simply TP) (EC 2.4.2.4) is a pyrimidine based nucleoside phosphorylase, exists in both eukaryotic and prokaryotic domains [1,2]. This enzyme catalyzes the initial step in the catabolism of thymidine (dThd) nucleoside into thymine and 2-deoxy-D-ribose-1-phosphate by reversible phosphorolysis, cleaving glycosidic bond [3,4] (Scheme 1).

The dephosphorylated intermediate, 2-deoxy-D-ribose, plays a key role in triggering the tumor angiogenesis and hence endorses cancer metastasis [5–7]. TP is similar to platelet derived endothelial cell growth factor (PD-ECGF) which is an angiogenic molecule [1,8,9]. Primary sequence of TP is mostly conserved throughout the evolution as mammalian TP which shares 39% sequence similarity with TP of *E. coli*. The mammalian enzyme also shares 65% similarity with the active site residues of *E. coli* enzyme [10]. Various human tumors including colorectal [11], breast [12], bladder [13], and oesophageal cancers [14], showed increased level of PD-ECGF/TP. Inhibition of TP enzyme is thus an important anti-angiogenic/cancer approach. For the suppression of the angiogenic and chemotactic activities of thymidine phosphorylase (dThdPase) [15–19], different inhibitors of TP have been designed as potential anticancer agents [20–24]. Currently only one TP inhibitor (tipiracil), in combination of trifluridine (a cytotoxin), is recently approved by U.S. FDA for clinical use for the treatment of metastatic colorectal cancer. This combination drug (trifluridine/tipiracil), marketed as Lonsurf, is also associated with severe side effects, such as myelosuppression, anemia, and neutropenia. Therefore there is need to develop new TP inhibitory agents [25]. 7-Deazaxanthine (7-DX) and 6-amino-5-bromouracil (6A5BU) are the two classical inhibitors of TP, which are widely used as reference compounds for *in vitro* studies against this enzyme [26,27].

Our research group has been working for the discovery of thymidine phosphorylase inhibitors. In this regard, we have already reported 3-formylchromones [28] and 1,3,4-oxadiazoles [29] as thymidine phosphorylase inhibitors. Recently triazines and their fused pyrazolo and triazolo analogs have been reported for their thymidine phosphorylase inhibitory activities [30–32]. Based on broad medicinal importance of 2-arylquinazolin-4(3*H*)-ones, and their structural resemblance with 7-deazaxanthine

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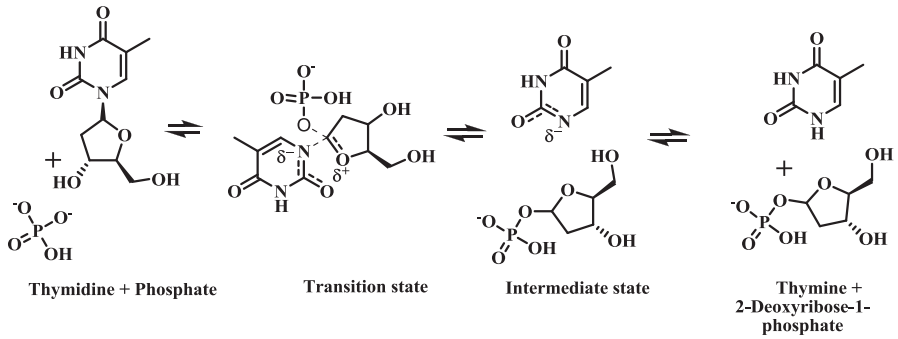
0045-2068/



Scheme 1. Mechanism of thymidine phosphorylase catalyzed reaction.

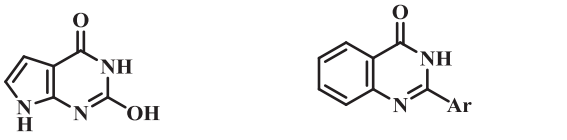


Fig. 1. Structures of 7-deazaxanthine and 2-arylquinazolin-4(3*H*)-ones.

(Fig. 1), a library of 2-arylquinazolin-4(3*H*)-ones was synthesized [33], and subjected to thymdine phosphorylase inhibition studies. The results are presented in Table 1.

In addition to this, all active compounds were also screened through MTT 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazo lium bromide) spectrophotometric assay for the evaluation of their cytoxicity (Table 2). In these studies, reduction of MTT dye to formazan by mitochondrial enzyme was measured. The reduction of MTT can only occur in metabolically active cell, so the activity level is actually a measure of cell viability. These studies gave an idea about the effect of test compounds on the cell viability [34].

2. Results and discussion

2.1. Chemistry

All twenty-five derivatives of 2-arylquinazolin-4(3*H*)-ones (**1**–**25**) were re-synthesized [33] for the evaluation thymidine phosphorylase inhibition (Scheme 2). Structures of the synthetic compounds were deduced by ^{1}H NMR and EI mass spectrometry. All compounds furnished satisfactory HR-EIMS and elemental analyses. Melting points and R_f values were also recorded.

2.2. Enzyme inhibition studies

Differently substituted 2-arylquinazolin-4(3*H*)-ones (**1**–**25**) were evaluated for thymidine phosphorylase inhibition *versus* the standard, 7-deazxanthine (IC_{50} = 41.0 ± 1.63 μM). Among twenty-five compounds, six compounds (**2**, **3**, **5**, **6**, **20**, and **23**) were found to have TP inhibitory activity (Table 1). Compounds **5** and **6** with IC_{50} values of 42.9 ± 1.0 and 59.5 ± 1.9 μM, respectively showed significant TP inhibitory activity in comparison to the standard. Compounds **20** (IC_{50} = 168.8 ± 1.8 μM), **2** (IC_{50} = 234.0 ± 1.5 μM), **23** (IC_{50} = 256.0 ± 2.0 μM), and **3** (IC_{50} = 294.6 ± 2.9 μM) were found to have moderate levels of TP inhibitory activity.

Structure-activity relationship (SAR) studies suggested that TP inhibitory activity depends mainly on the nature of substitution on the phenyl moiety, attached to C-2 of the quinazolin-4(3*H*)-one nucleus, as compound **1** (parent compound) without substitution on phenyl ring was found to be inactive.

Apparently substitution of hydroxyl groups to phenyl ring made compounds active against TP. Compounds **2** and **3** substituted with single hydroxyl group at *para* and *meta* position on phenyl ring showed a weak TP inhibitory activity with IC_{50} values of 234.0 ± 1.5 and 294.6 ± 2.9 μM, respectively. However, compound **4** with hydroxyl substitution at *ortho* position was found to be inactive. Among the di-hydroxyl substituted derivatives, compound **5** with hydroxyl groups at *para* and *meta* positions of phenyl ring showed an IC_{50} = 42.9 ± 1.0 μM, and compound **6** with two hydroxyls at *ortho* and *meta* position of phenyl ring gave an IC_{50} = 59.5 ± 1.9 μM. TP inhibitory activity of compounds **5** and **6** were found comparable to the standard compound *i.e.*, 7-deazaxanthine (IC_{50} = 41.0 ± 1.63 μM).

SAR proposed that hydroxyl substitution on phenyl ring at different positions, plays an important role in inducing TP inhibition. The ability of inhibiting TP was found to be more in di-hydroxylated analogs than mono-hydroxylated analogs. Better activity of di-hydroxylated analogs than the mono-hydroxylated analogs has been linked with the possible increase in polar interactions with enzyme. Hydroxyl group, being strong electron donating, enhances the ability of phenyl rings to undergo π–π interactions with the aromatic amino acid residues, present in active site or hydrophobic pocket of the TP enzyme. In addition to this, hydroxyl groups could also form hydrogen bond with the amino acid residues of the active site or hydrophobic pocket of enzyme.

Alkoxylated derivatives (compounds **8**–**12**) did not show any inhibitory activity but when integrated with hydroxyl group, as in compounds **20** (IC_{50} = 168.8 ± 1.8 μM), and **23** (IC_{50} = 256.0 ± 2.0 μM), were found to be active. SAR proposed that position of hydroxyl and alkoxy groups on phenyl ring affects the ability of compounds to inhibit enzyme, as revealed by the TP inhibitory activity of compounds **20**–**23** (Table 1). Hydroxyl and alkoxy groups being electron donating were proposed to be involved in hydrogen bonding and/or hydrophobic interactions with amino acid residues present in the active site or hydrophobic pocket of TP.

Compounds with electron withdrawing groups such as chloro (compounds **13**–**16**) or nitro (compounds **17**–**19**) groups, did not show any activity. Metyhylated, and di-aminomethylated derivatives (compounds **7**, and **25**, respectively), were also found to be inactive.

2.3. Cytotoxicity evaluation

All active compounds were found to be non-cytotoxic against the mouse fibroblast 3T3 cell lines, except compound **6** which showed a weak cytotoxic activity, in comparison to the standard *i.e.* cycloheximide (Table 2).

Table 1
Thymidine phosphorylase inhibitory activity of 2-arylquinazoline-4-(3*H*)-ones (**1**–**25**).

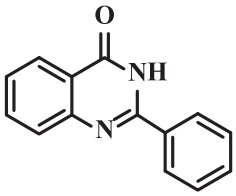
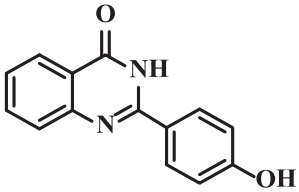
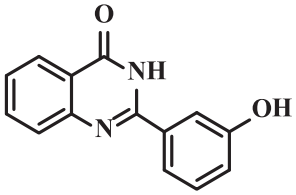
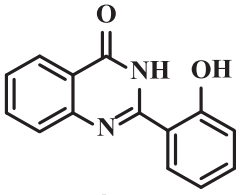
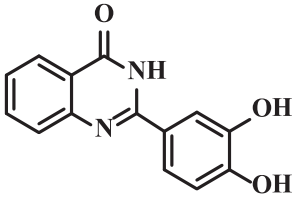
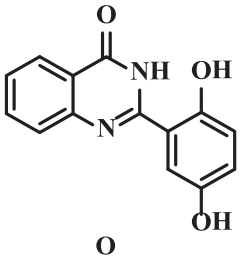
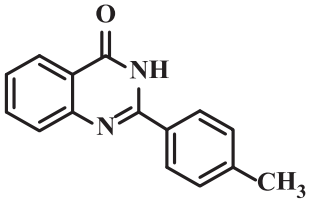
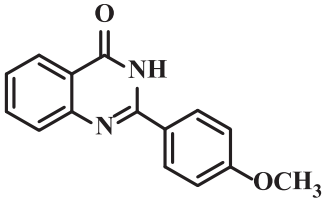
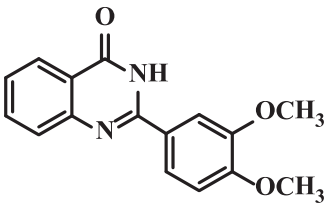
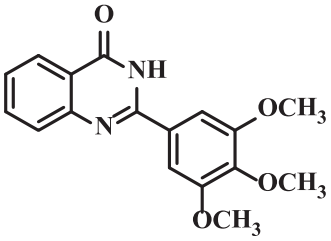
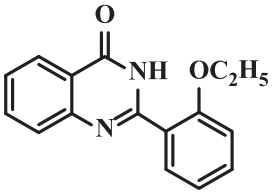
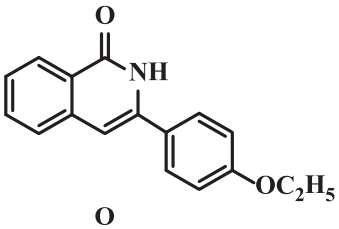
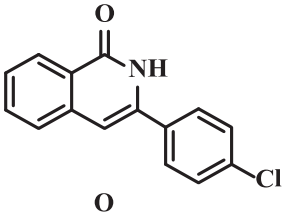
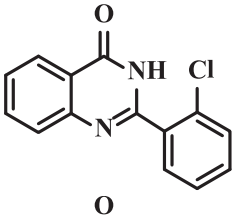
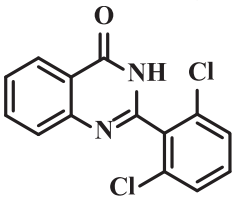
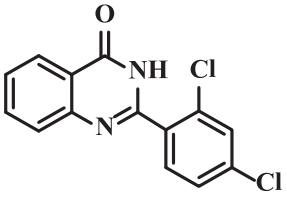
Compound	Structure	IC_{50} ± SEM[a] (μM)
1		N.A.[b]
2		234.0 ± 1.5
3		294.6 ± 2.9
4		N.A.[b]
5		42.9 ± 1.0
6		59.5 ± 1.9
7		N.A.[b]
8		N.A.[b]

Table 1 (*continued*)

Compound	Structure	IC_{50} ± SEM[a] (μM)
9		N.A.[b]
10		N.A.[b]
11		N.A.[b]
12		N.A.[b]
13		N.A.[b]
14		N.A.[b]
15		N.A.[b]
16		N.A.[b]

(*continued on next page*)

Table 1 (*continued*)

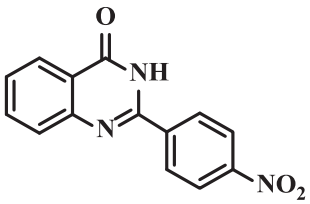
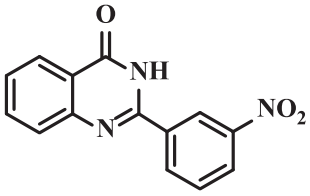
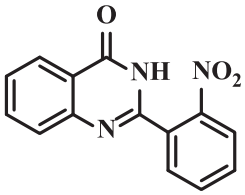
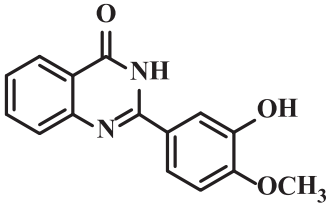
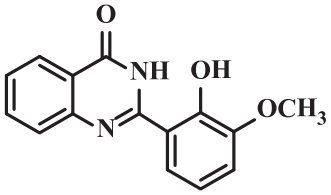
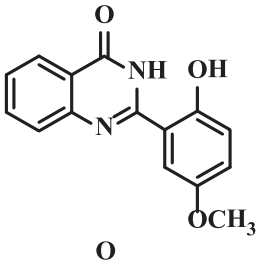
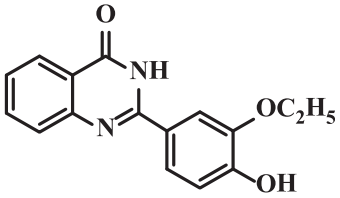
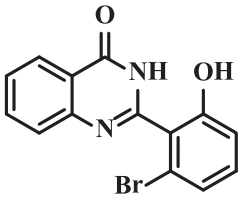
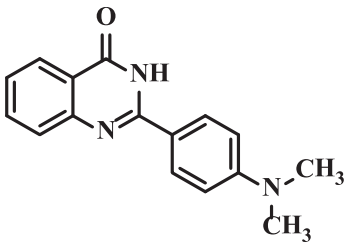
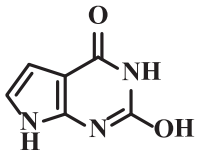
Compound	Structure	IC_{50} ± SEM[a] (μM)
17		N.A.[b]
18		N.A.[b]
19		N.A.[b]
20		168.8 ± 1.8
21		N.A.[b]
22		N.A.[b]
23		256.0 ± 2.0
24		N.A.[b]

Table 1 (*continued*)

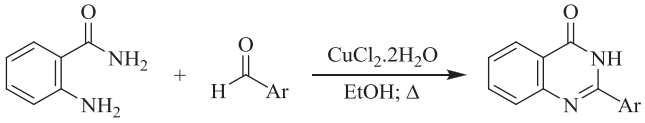
Compound	Structure	IC_{50} ± SEM[a] (μM)
25		N.A.[b]
7-Deazaxanthine (Standard)		41.0 ± 1.63

[a] SEM is the Standard Error of the Mean.
[b] N.A. means Not Active.

Table 2
Cytotoxic data of active 2-arylquinazoline-4-(3*H*)-ones.

Compound	IC_{50} ± SEM[a] (μM)
2	N.C.[b]
3	N.C.[b]
5	N.C.[b]
6	22.80 ± 0.34
20	N.C.[b]
23	N.C.[b]
Cycloheximide	0.20 ± 0.05

[a] SEM: Standard Error Mean.
[b] N.C.: Non-Cytotoxic.



Scheme 2. Synthesis of 2-aryl-4(3*H*)-quinazolinones (**1**–**25**).

Table 3
Kinetic data of active 2-arylquinazoline-4-(3*H*)-ones (**5**, **6**, and **20**).

Compound	K_i[a] ± SEM[b] (μM)	Type of inhibition
5	52.33 ± 0.001	Non-competitive
6	172.0 ± 0.0006	Non-competitive
20	140.0 ± 0.001	Competitive
7-Deazaxanthine	49.1 ± 0.002	Non-competitive

[a] K_i is Dissociation constant.
[b] SEM is the Standard Error of the Mean.

2.4. Kinetic studies

Mechanism of inhibition of most active compounds was determined by kinetic studies, using thymidine as a substrate. Inhibition type was deduced by Lineweaver–Burk plot, where the reciprocal of the rate of the reaction were plotted against the reciprocal of substrate concentration, to monitor the effect of inhibitor on both K_m and V_{max}. The K_i values were determined by plotting the slope of each line in the Lineweaver–Burk plots against the different concentrations of compounds. The K_i values were re-confirmed by Dixon plot in which the reciprocal of the rate of reaction were plotted against the different concentrations of compounds. Kinetic studies on compounds **5** and **6** revealed that they inhibit the TP in non-competitive manner. These two compounds may interact either with the hydrophobic pocket or at allosteric site of enzyme. Compound **20** showed a competitive mode of inhibition, indicating its interaction with the amino acid residues of the enzyme's active site (Table 3 and Figs. 2–4). In competitive-type of inhibition, the V_{max} of enzyme was not affected, while the K_m increased, in case of non-competitive type of inhibition, the K_m of enzyme was not affected, while the V_{max} of enzyme decreased (Figs. 2–4).

3. Conclusion

In conclusion, 2-arylquinazolin-4(3*H*)-one derivatives (**1**–**25**) were evaluated for thymidine phosphorylase inhibitory activity. Six compounds **5**, **6**, **20**, **2**, **23**, and **3** with IC_{50} values in the range of 42.9–294.6 μM, were found to be active against thymidine phosphorylase. 7-Deazxanthine (IC_{50} = 41.0 ± 1.63 μM) was used as a standard in the study. Further studies, based on mode of action of our designed inhibitors, may help in development of therapeutic agents including anticancer drugs, based on quinazolinone skeleton.

4. Experimental

4.1. Method and materials

Thymidine phosphorylase enzyme (from *E. coli*, EC Number 2.4.2.4), thymidine, and potassium phosphate monobasic were purchased from Sigma Aldrich, USA, 7-Deazaxanthine (2,4-Dihydroxypyrrolo[2,3-*d*]pyrimidine,7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diol) was purchased from Santa Cruz Biotechnology Inc., USA, dimethylsulfoxide (DMSO) was purchased from Fisher Scientific, Germany.

4.2. Thymidine phosphorylase inhibition assay

Since human TP is not easily accessible, we used commercially available recombinant *E. coli* TP (expressed in *Escherichia coli*) enzyme. Primary sequence of TP is mostly conserved throughout evolution as mammalian TP is reported to share 39% sequence similarity with the TP of *E. coli*. The mammalian enzyme also shared 65–70% similarity with the active site residues, and three dimensional structure of *E. coli* TP enzyme [10].

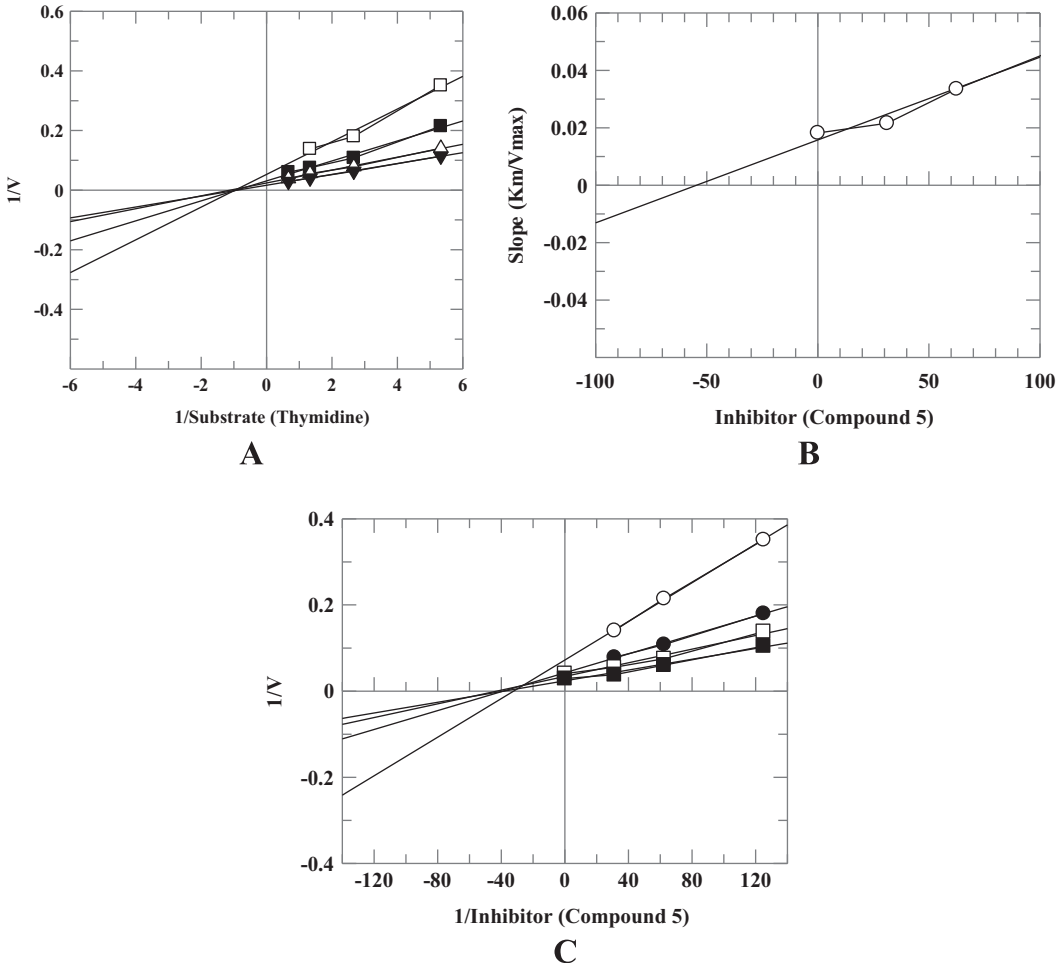


Fig. 2. The inhibition of thymidine phosphorylase by compound **5**. (A) is the Lineweaver–Burk plot of reciprocal rate of reaction (velocities) *versus* reciprocal of substrate (thymidine) in the absence (▼), and in the presence of 31.25 μM (Δ), 62.5 μM (■), and 125 μM (□) of compound **5**. (B) is secondary replot of Lineweaver–Burk plot between the slopes of each line on Lineweaver–Burk plot *versus* different concentrations of compound **5**. (C) is the Dixon plot of reciprocal of rate of reaction (velocities) *versus* different concentrations of compound **5**.

TP inhibition assay was performed spectrophotometrically. The method of Bera et al. was followed with slight modifications [35]. Reaction mixture of 200 μL, contained 20 μL of enzyme (0.058 unit/well), 150 μL of potassium phosphate buffer (pH 7.0, 50 mM), and 10 μL of test compound (0.5 mM). The reaction mixture was then incubated for 10 min at 30 °C. After 10 min, substrate (thymidine, λ_{max}; 265 nm) (20 μL, 1.5 mM) was added, and change in absorbance was observed for 10 min at 290 nm in 96-well ELISA plate reader (Spectramax, Molecular Devices, CA, USA). Every experiment was run in triplicate. 7-Deazaxanthine was used as positive control.

4.3. Kinetic studies

Kinetic studies were carried out to deduce the mechanism of inhibition. Potential inhibitor could interact with enzyme in competitive, non-competitive, mixed or uncompetitive manner. In kinetic assay, the enzyme (0.058U/200 μL) was incubated with different concentrations of inhibitor for 10 min at 30 °C. The reaction was then initiated by adding different concentrations (0.1875–1.5 mM) of substrate (thymidine). Degradation of thymidine was measured continuously at 290 nm for 10 min on a ELISA plate reader [35].

4.4. Cytotoxicity studies

MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) spectrophotometric assay in 96-well plate was used [33], to evaluate the cytotoxic activity of active compounds.

4.5. Statistical analysis

Reactions for above mentioned biological activities were carried out in triplicate. Results were then 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:

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

where OD is the optical density.

Results were presented as means ± standard error mean from triplicate ($n = 3$) observation. IC_{50} values were determined by using EZ-FIT, Enzyme kinetics software by Perrella Scientific, Inc., USA. Grafit 7.0 version was used to determine the kinetics parameter. The software was purchased from the Erithacus Software Ltd., Wilmington House, High Street, East Grinstead, West Sussex RH19 3AU, UK.

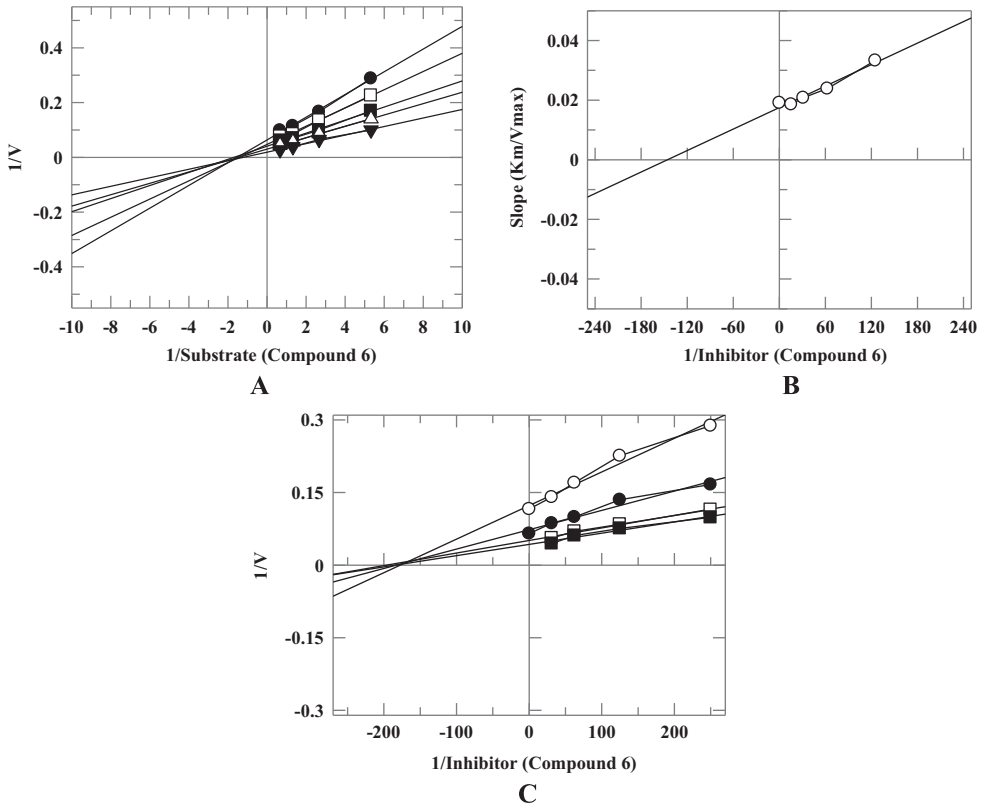


Fig. 3. The inhibition of thymidine phosphorylase by compound **6**. (A) is the Lineweaver–Burk plot of reciprocal rate of reaction (velocities) versus reciprocal of substrate (thymidine) in the absence (▼), and in the presence of 31.25 μM (Δ), 62.5 μM (■), 125 μM (□), 250 μM (●) of compound **6**. (B) is secondary replot of Lineweaver–Burk plot between the slopes of each line on Lineweaver–Burk plot versus different concentrations of compound **6**. (C) is Dixon plot of reciprocal rate of reaction (velocities) verses different concentrations of compound **6**.

4.6. General procedure for the synthesis of compounds 1-25

2-Aminobenzamide (1 eq.), substituted benzaldehydes (1.1 eq) and $CuCl_2{\cdot}2H_2O$ (4 eq.) were mixed in ethanol (15 mL) and refluxed for 16 h. The reaction was periodically monitored by thin layer chromatography. Water was added to the reaction mixtures until the appearance of precipitates. These precipitates were filtered, washed with water, and dried under vacuum. Good yields of title compounds were obtained [33].

*4.7. Characterization data of the active compounds **2**, **3**, **5**, **6**, **20**, and **23***

*4.7.1. 2-(4′-Hydroxyphenyl)quinazolin-4(3H)-one (**2**)*

Yield: 99%; m.p. >300 °C; R_f: 0.15 (ethyl acetate/hexane, 3:7); ^{1}H NMR: (400 MHz, DMSO-d_6): δ_H 12.29 (s, 1H, NH), 10.14 (s, 1H, 4′-OH), 8.11 (m, 1H, H-5), 8.03 (d, 2H, $J_{2',3'} = J_{6',5'} = 8.4$ Hz, H-2′, H-6′), 7.80 (t, 1H, $J_{7(6,8)} = 7.2$ Hz, H-7), 7.67 (d, 1H, $J_{8,7} = 8.0$ Hz, H-8), 7.47 (t, 1H, $J_{6(5,7)} = 7.2$ Hz, H-6), 6.89 (d, 2H, $J_{3',2'} = J_{5',6'} = 8.8$ Hz, H-3′, H-5′); EI-MS: *m*/*z* (rel. abund.%), 238 $[M]^+$ (100), 237 (5), 221 (5), 119 (83); HREI-MS: *m*/*z* calcd for $C_{14}H_{10}N_2O_2$ $[M]^+$ 238.0742; found 238.0738; Anal. Calcd for $C_{14}H_{10}N_2O_2$: C, 70.58; H, 4.23; N, 11.76; O, 13.43; found: C, 70.57; H, 4.24; N, 11.77 [33].

*4.7.2. 2-(3′-Hydroxyphenyl)quinazolin-4(3H)-one (**3**)*

Yield: 88%; m.p. 260–262 °C; R_f: 0.21 (ethyl acetate/hexane, 3:7); ^{1}H NMR: (400 MHz, DMSO-d_6): δ_H 12.42 (s, 1H, NH), 9.74 (s, 1H, 3′-OH), 8.14 (m, 1H, H-5), 7.84 (m, 1H, H-7), 7.72 (d, 1H, $J_{8,7} = 8.0$ Hz, H-8), 7.59 (m, 2H, H-6, H-2′), 7.52 (m, 1H, H-6′), 7.34 (m, 1H, H-5′), 6.97 (m, 1H, H-4′); EI-MS: *m*/*z* (rel. abund.%), 238 $[M]^+$ (82), 237 (9), 221 (7), 119 (100); HREI-MS: *m*/*z* calcd for $C_{14}H_{10}N_2O_2$ $[M]^+$ 238.0742; found 238.0746; Anal. Calcd for $C_{14}H_{10}N_2O_2$: C, 70.58; H, 4.23; N, 11.76; O, 13.43; found: C, 70.59; H, 4.25; N, 11.76 [33].

*4.7.3. 2-(3′,4′-Dihydroxyphenyl)quinazolin-4(3H)-one (**5**)*

Yield: 96%; m.p. 230 °C; R_f: 0.10 (ethyl acetate/hexane, 3:7); ^{1}H NMR: (400 MHz, DMSO-d_6): δ_H 12.21 (s, 1H, NH), 9.64 (s, 1H, 4′-OH), 9.29 (s, 1H, 3′-OH), 8.10 (d, 1H, $J_{5,6} = 8.0$ Hz, H-5), 7.78 (d, 1H, $J_{8,7} = 7.2$ Hz, H-8), 7.66 (m, 2H, H-7, H-2′), 7.54 (d, 1H, $J_{6',5'} = 8.0$ Hz, H-6′), 7.46 (t, 1H, $J_{6(5,7)} = 7.6$ Hz, H-6), 6.83 (d, 1H, $J_{5',6'} = 8.0$ Hz, H-5′); EI-MS: *m*/*z* (rel. abund.%), 254 $[M]^+$ (8), 146 (100), 119 (26); HREI-MS: *m*/*z* calcd for $C_{14}H_{10}N_2O_3$ $[M]^+$ 254.0691; found 254.0680; Anal. calcd for $C_{14}H_{10}N_2O_3$: C, 66.14; H, 3.96; N, 11.02; O, 18.88; found: C, 66.12; H, 3.93; N, 11.05 [33].

*4.7.4. 2-(2′,5′-Dihydroxyphenyl)quinazolin-4(3H)-one (**6**)*

Yield: 95%; m.p. >300 °C; R_f: 0.18 (ethyl acetate/hexane, 3:7); ^{1}H NMR: (300 MHz, DMSO-d_6): δ_H 12.60 (s, 1H, NH), 12.29 (s, 1H, 2′-OH), 9.08 (s, 1H, 5′-OH), 8.14 (d, 1H, $J_{5,6} = 7.5$ Hz, H-5), 7.86 (t, 1H, $J_{7(6,8)} = 7.5$ Hz, H-7), 7.73 (d, 1H, $J_{8,7} = 8.1$ Hz, H-8), 7.60 (s, 1H, H-6′), 7.54 (t, 1H, $J_{6(5,7)} = 7.2$ Hz, H-6), 6.92 (m, 2H, H-3′, H-4′); EI-MS: *m*/*z* (rel. abund.%), 254 $[M]^+$ (100), 119 (36); HREI-MS: *m*/*z* calcd for $C_{14}H_{10}N_2O_3$ $[M]^+$ 254.0691; found 254.0692; Anal. Calcd for $C_{14}H_{10}N_2O_3$: C, 66.14; H, 3.96; N, 11.02; O, 18.88; found: C, 66.16; H, 3.95; N, 11.03 [33].

*4.7.5. 2-(3′-Hydroxy-4′-methoxyphenyl)quinazolin-4(3H)-one (**20**)*

Yield: 100%; m.p. 202–204 °C; R_f: 0.10 (ethyl acetate/hexane, 3:7); ^{1}H NMR: (400 MHz, DMSO-d_6): δ_H 12.30 (s, 1H, NH), 9.35 (s, 1H, 3′-OH), 8.09 (s, 1H, H-5), 7.77 (m, 5H, H-7, H-8, H-6, H-6′, H-5′), 7.04 (s, 1H, H-2′), 3.85 (s, 3H, 4′-OCH_3); EI-MS: *m*/*z*

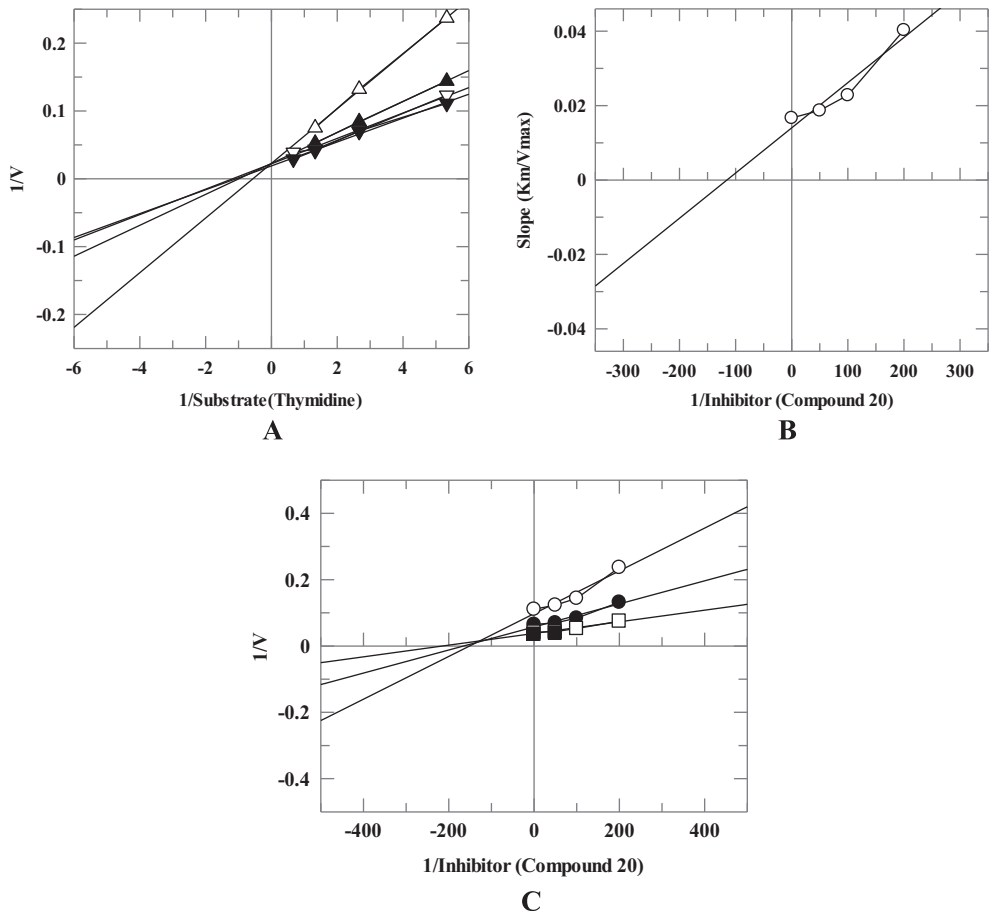


Fig. 4. The inhibition of thymidine phosphorylase by compound **20**. (A) is the Lineweaver–Burk plot of reciprocal rate of reaction (velocities) *versus* reciprocal of substrate (thymidine) in the absence (▼), and in the presence of 50 μM (▽), 100 μM (▲), and 200 μM (Δ) of compound **20**. (**B**) is secondary replot of Lineweaver–Burk plot between the slopes of each line on Lineweaver–Burk plot *versus* different concentrations of compound **20**. (**C**) is the Dixon plot of reciprocal of rate of reaction (velocities) *versus* different concentrations of compound **20**.

(rel. abund.%), 268 $[M]^+$ (100), 253 (26), 119 (82); HREI-MS: *m*/*z* calcd for $C_{15}H_{12}N_2O_3$ $[M]^+$ 268.0848; found 268.0845; Anal. Calcd for $C_{15}H_{12}N_2O_3$: C, 67.16; H, 4.51; N, 10.44; O, 17.89; found: C, 67.15; H, 4.52; N, 10.44.

*4.7.6. 2-(3′-Ethoxy-4′-hydroxyphenyl)quinazolin-4(3H)-one (**23**)*

Yield: 98%; m.p. 229–230 °C; R_f: 0.21 (ethyl acetate/hexane, 3:7); ^{1}H NMR: (400 MHz, DMSO-d_6): δ_H 12.32 (s, 1H, NH), 9.67 (s, 1H, 4′-OH), 8.11 (d, 1H, $J_{5,6}$ = 7.6 Hz, H-5), 7.77 (m, 3H, H-7, H-8, H-2′), 7.68 (d, 1H, $J_{6',5'}$ = 8.0 Hz, H-6′), 7.47 (t, 1H, $J_{6(5,7)}$ = 7.2 Hz, H-6), 6.91 (d, 1H, $J_{5',6'}$ = 8.4 Hz, H-5′), 4.16 (q, 2H, $J_{(CH2,CH3)}$ = 6.8 Hz, CH_2), 1.39 (t, 3H, $J_{(CH3,CH2)}$ = 7.2 Hz, CH_3); EI-MS: *m*/*z* (rel. abund.%), 282 $[M]^+$ (100), 267 (29), 254 (81), 238 (12), 119 (47); HREI-MS: *m*/*z* calcd for $C_{16}H_{14}N_2O_3$ $[M]^+$ 282.1004; found 282.1005; Anal. Calcd for $C_{16}H_{14}N_2O_3$: C, 68.07; H, 5.00; N, 9.92; O, 17.00; found: C, 68.05; H, 5.04; N, 9.94 [33].

Acknowledgments

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