Synthesis of Thymidine Phosphorylase Inhibitor Based on Quinoxaline Derivatives and Their Molecular Docking Study

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Abstract:

We have synthesized quinoxaline analogs **(1-25),** characterized by ^{1}HNMR and HREI-MS and evaluated for thymidine phosphorylase inhibition. Among the series, nineteen analogs showed better inhibition when compared with the standard inhibitor 7-Deazaxanthine (IC_{50} = 38.68 ± 4.42 μM**).** The most potent analog among the series is analog **25** with IC_{50} value 3.20 ± 0.10 μM. Sixteen analogs **1, 2, 3, 4, 5, 6,** 7**, 12, 13, 14, 15, 16, 17, 18, 21**and **24** showed outstanding inhibition which is many folds better than the standard 7-Deazaxanthine. Two analogs **8** and **9** showed moderate inhibition. A structure-activity relationship has been established mainly based upon the substitution pattern on the phenyl ring. The binding interactions of the active compounds were confirmed through molecular docking studies.

Keywords: Quinoxaline Analogs, Synthesis, Thymidine phosphorylase inhibition, Molecular docking

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1.0. Introduction:

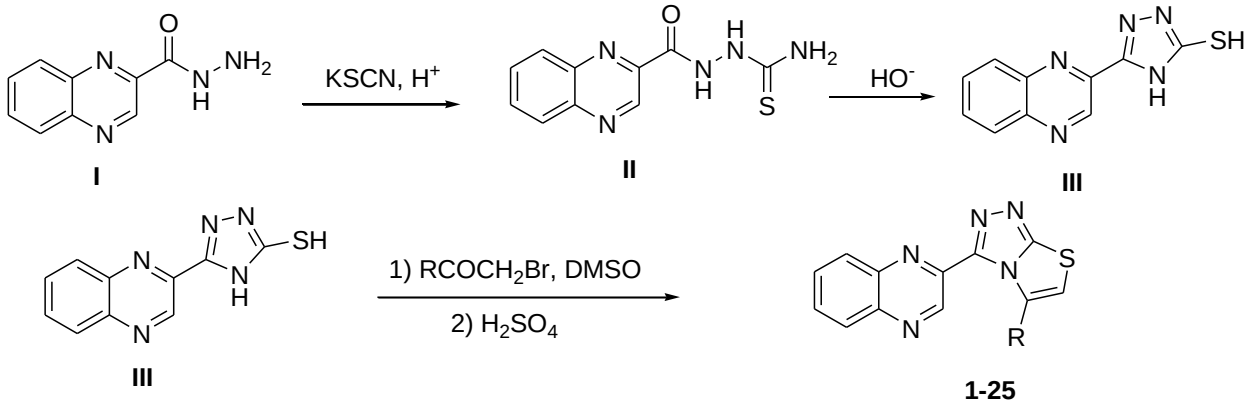
Thymidine phosphorylase (TP), an enzyme involved in catabolism, exists in both prokaryotic and eukaryotic organisms [1-3]. TP speeds up the initial step in catabolism and converts thymidine nucleoside into thymine and 2-deoxy-$_D$-ribose-1-phosphate by cleaving glycoside bond [4, 5]. The intermediate obtained through dephosphorylation is 2-deoxy-$_D$-ribose that plays a significant role in prompting the tumor angiogenesis and hence favors cancer metastasis [6–8]. With respect to tumor angiogenesis, TP play a major role, it helps in the proliferation process of endothelial cells throughout the body in cancer metastasis [9-10]. TP performs the same function as platelet endothelial cell growth factor (PD-ECGF) [11, 12]. TP belonging to mammalians shares 39% sequence similarity with TP of E. coli, while the enzyme of mammalians also shares 65% resemblance with the active sites of residues of E. coli enzyme [13]. The production of 2′-deoxy-D-ribose can be limited through TP inhibitors which in turn suffocate the growth of tumor cells [14, 15]. Therefore, medicinal chemists have tried to synthesize novel inhibitors of thymidine phosphorylase which have the potential to overcome the formation of new blood vessels and decline the tumor cells growth. Various attempts have been reported to developed TP inhibitor [16-23]. The most potent inhibitor belong to human TP known up-to-now is 5-chloro-6-[1-(2-iminopyrrolidinyl)methyl] uracil hydrochloride (TPI), while 7-deazaxanthine (7DX) is the first purine analog labeled as TP inhibitor [24-26].

Nitrogen-containing heterocycles have attracted considerable attention due to their wide range of pharmacological importance. Quinoxaline has a six-membered cyclic ring with two nitrogen atoms inside the cyclic ring. Quinoxaline and their analogs have attracted the medicinal chemists over the decades. Quinoxalines are used as antimicrobial [27], antibacterial [28], antifungal [29, 30], anti-protozoan [31], anti-inflammatory, antianalgesic [32], anti-cancer [33, 34], antidiabetic and as anti-proliferative agents [35, 36]. Our research group has been working on the design and synthesis of heterocyclic compounds in search of potential lead compounds for many years and had found promising results [37-42]. Thus, we decided to screen a library of quinoxaline for thymidine phosphorylase inhibitory activity. Here in this study, we are reporting the synthesis of quinoxaline derivatives as a novel class of thymidine phosphorylase inhibitors.

2. Results and Discussion

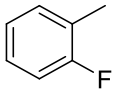
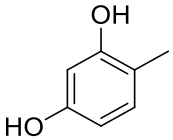
2.1. Chemistry

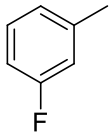
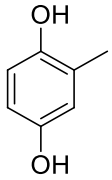
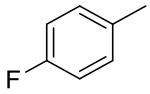
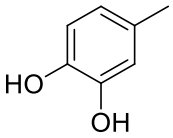
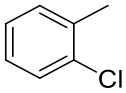
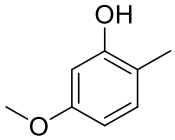
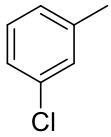
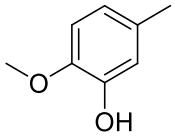
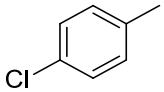
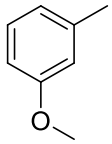
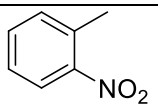
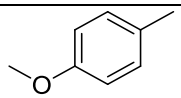
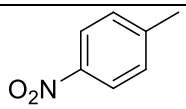
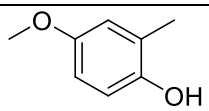
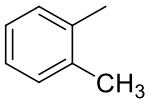
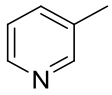
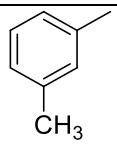
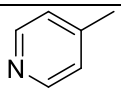
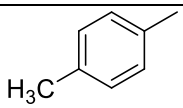
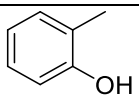
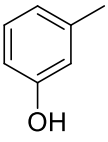
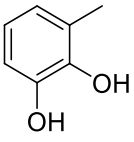
Synthesis of quinoxaline derivatives (**1-25**) started with treating quinoxaline-2-carbohydrazide (**I**) with potassium thiocyanate in the presence of acid to form quinoxaline thiosemicarbazone (**II**) which was treated with basic solution to cyclize to form 5-(quinoxalin-3-yl)-4H-1,2,4-triazole-3-thiol (**III**) which was treated with different substituted phenacyl bromide to afford (**1-25**) target compounds. The crude product was washed with water and recrystallized in methanol to afford pure product in 80-75%. All synthesized compounds were characterized by different spectroscopic methods given in scheme 1.

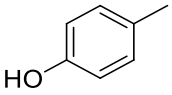


Scheme. 1: synthesis of 2-(5-arylthiazolo[2,3-c][1,2,4]triazol-3-yl)quinoxaline 1-25 derivatives

Table-1: Different substituent of Quinoxaline Derivatives (**1-25**) and their Thymidine Phosphorylase inhibitory activity.

S.No.	R	**IC_{50} (mM ± SEM_a)**	**S.No.**	R	**IC_{50} (mM ± SEM_a)**
1		13.60 ± 0.4	**14**		13.20 ± 0.40

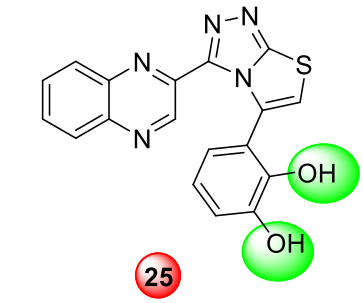
2		26.10 ± 0.70	**15**		15.20 ± 0.50
3		18.10 ± 0.50	**16**		3.50 ± 0.20
4		27.40 ± 0.60	**17**		24.20 ± 0.70
5		33.40 ± 0.80	**18**		16.90 ± 0.60
6		24.40 ± 0.60	**19**		N. A.
7		34.70 ± 0.80	**20**		N. A.
8		47.50 ± 0.90	**21**		26.20 ± 0.50
9		56.40 ± 1.20	**22**		N. A.
10		N. A.	**23**		N. A.
11		N. A.	**24**		13.10 ± 0.30
12		33.20 ± 0.75	**25**		3.20 ± 0.10

13		18.30 ± 0.55	-	-	-
7-Deazaxanthine (7DX)			**38.68 ± 4.42 *m*M**		

2.2. ***In vitro*** **Thymidine Phosphorylase Inhibitory Activity:**

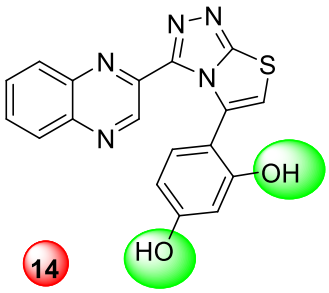
We have synthesized twenty-five analogs of 5-phenyl-3-quinoxalin (**1-25**) and screened for inhibitory potential against thymidine phosphorylase enzyme. With respect to inhibitory potential, many analogs of the series showed a variable degree of inhibition with IC_{50} values ranging between 3.50 ± 0.20 to 56.40 ± 1.20 μM when compared with standard 7-Deazaxanthine (IC_{50} = 38.68 ± 1.12 μM). The analogs **1, 2, 3, 4, 5, 6,** 7**, 12, 13, 14, 15, 16, 17, 18, 21, 24** and **25** showed excellent inhibitory potential with IC_{50} values 13.60 ± 0.4, 26.10 ± 0.70, 18.10 ± 0.50, 27.40 ± 0.60, 33.40 ± 0.80, 24.40 ± 0.60, 34.70 ± 0.80, 33.20 ± 0.75, 18.30 ± 0.55, 13.20 ± 0.40, 15.20 ± 0.50, 3.50 ± 0.20, 24.20 ± 0.70, 16.90 ± 0.60, 26.20 ± 0.50, 13.10 ± 0.30 and 3.20 ± 0.10 μM respectively by comparing with standard 7-Deazaxanthine. Two analogs **8** and **9** showed moderate inhibitory activity with IC_{50} values 47.50 ± 0.90 and 56.40 ± 1.20 μM respectively, while six analogs **10, 11, 19, 20, 22** and **23** were found inactive. Structure activity relationship has been established for all compounds, mainly based on substituents pattern of phenyl ring.

Compound **25**, a 2,3-dihydroxy analog was found the most active analog among the series with IC_{50} value 3.20 ± 0.10 μM. If we compare analog **25** with other dihydroxy analogs like **14**, a 2,4-dihydroxy analog **(**IC_{50} = 13.20 ± 0.40 μM) **15,** a 2,5-dihydroxy analog (IC_{50} = 15.20 ± 0.50 μM) and **16,** a 2,4-dihydroxy analog **(**IC_{50} = 3.50 ± 0.20 μM**),** analog **25** was found superior. Although all the four analogs have two hydroxyl groups at the phenyl ring, the position of attachment on phenyl ring are different. The difference in inhibitory activity of these four analogs seems due to the different position of the hydroxyl group on the phenyl ring.



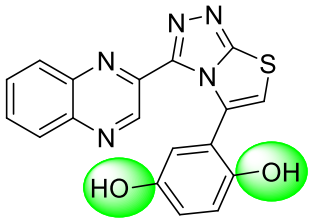
25

IC50= 3.20 ± 0.10 µM



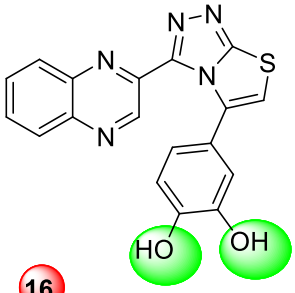
14

IC50= 13.20 ± 0.40 µM



15

IC50= 15.20 ± 0.50 µM

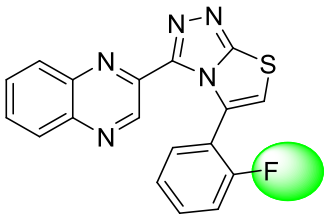


16

IC50= 3.50 ± 0.20 µM

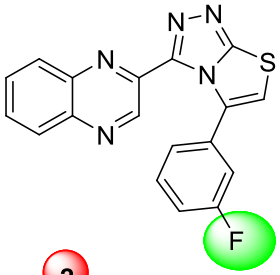
By comparing dihydroxy analogs with monohydroxy analog like **12, 13, 17, 18, 21** and **24** the dihydroxy analogs were found to be more potent. This greater potential seems due to the greater number of hydroxy groups on the phenyl ring.

Similarly, a pattern was also observed in flouro substituted analogs like **1, 2** and **3** IC_{50} value 13.60 ± 0.4, 26.10 ± 0.70 and 18.10 ± 0.50 μM respectively**. A**ll three analogs possess flouro group at the phenyl ring, but the analog **1** shows greater potential than analogs **2** and **3**. The difference in the inhibitory potential in analog **1, 2** and **3** seems due to attachment of flouro group at various positions on the phenyl part.



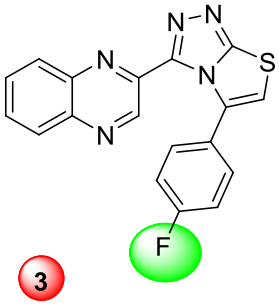
1

IC50= 13.60 ± 0.4µM



2

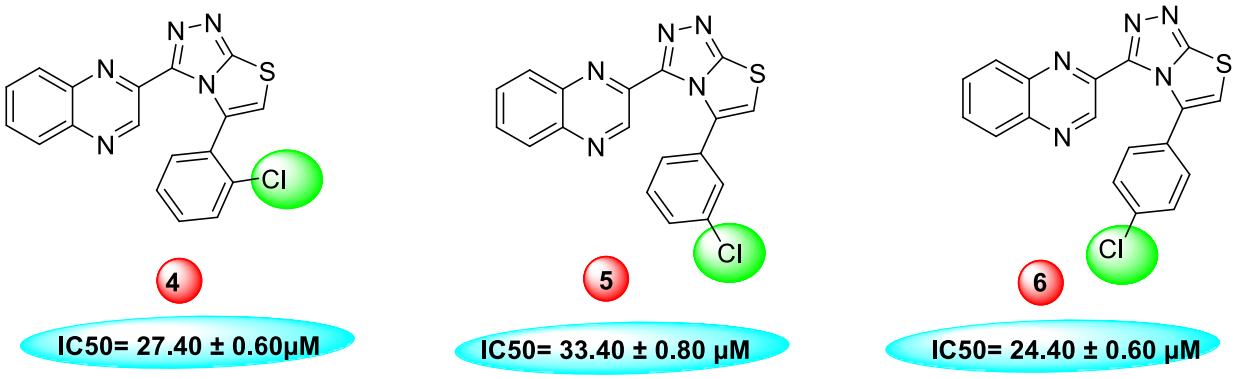
IC50= 26.10 ± 0.70 µM



3

IC50= 18.10 ± 0.50 µM

The same trend of difference in inhibitory activity was found in chloro substituted analogs **4, 5** and **6** with IC_{50} value 27.40 ± 0.60, 33.40 ± 0.80 and 24.40 ± 0.60 μM respectively. All three analogs have chloro group but their attachment on phenyl ring differs from each other, and the difference in inhibitory activity it seems due to attachment of chloro group at variable position on the phenyl part.



So, it was concluded that in our designed molecules the position, nature, and number of substituent play critical role in thymidine phosphorylase inhibition.

Molecular docking

The IC_{50} values of quinoxaline derivatives as thymidine phosphorylase inhibitors are showed in Table 1. The thymidine phosphorylase inhibition by the synthesized derivatives is mainly due to the type, number and positions of the functional group in the substitute group R of the basic skeleton (Table 1). For a better understanding of the enzyme inhibition by the synthesized compounds, molecular docking study has been carried out to shed light on the established binding modes of the five selected synthesized compounds **14**, **15**, **16** and **25**. The selected compounds differ by the substitution position of the hydroxyl group in the aromatic ring (Scheme 1). Compounds **16** and **25** with OH groups in *ortho* position to each other show higher activity than **14** and **15** where OH groups are in *meta* position to each other (Table 1). Table 2 summarized the calculated binding energies of the stable complex's ligand- thymidine phosphorylase, number of established intermolecular hydrogen bonding between the synthesized compounds (**14**, **15**, **16** and **25**) and active site residues of thymidine phosphorylase.

Table 2 IC_{50}, docking binding energies, hydrogen bonding and the number of closest residues to the docked ligands in the active site of the selected quinoxaline derivatives **14**, **15**, **16** and **25** within the active binding site of thymidine phosphorylase.

No. of Compound	Free binding energy (kcal/mol)	H-Bonds (HBs)	Number of closest residues to the docked ligand in the active site	IC_{50} ± SEM
14	-7.71	5	10	13.20 ± 0.40
15	-7.61	3	8	15.20 ± 050
16	-8.05	3	8	3.50 ± 0.20
25	-8.25	4	8	3.20 ± 0.10

The complexes formed between the docked selected compounds and amino acids of the binding active site of thymidine phosphorylase exhibited negative bending energies, which is a signpost that the inhibition of thymidine phosphorylase by the selected compounds is thermodynamically favorable (Table 2). As can be seen from the docking results in Table 2 and Figure 1, the highest activity of **16** and **25** compared with **14** and **15** mainly refers to the stability of the formed complexes between the docked compounds (**16** and **25**) and thymidine phosphorylase compared with the formed complexes between the docked compounds (**14** and **15**) and thymidine phosphorylase ones. The higher activity of **25** compared with **16** may refer to the number intermolecular hydrogen bonding formed with substituted OH groups in the complex **25**-receptor compared to **16**-receptor one. Indeed, three hydrogen bonds are formed between OH groups of **25** and SER 86 and HIS 85 of the active site of thymidine phosphorylase of 1.71, 2.24 and 3.38 Å, respectively. However, two hydrogen bonds are formed between OH groups of **16** and THR 120 of the active site of thymidine phosphorylase of 1.26 and 1.78 Å, respectively.

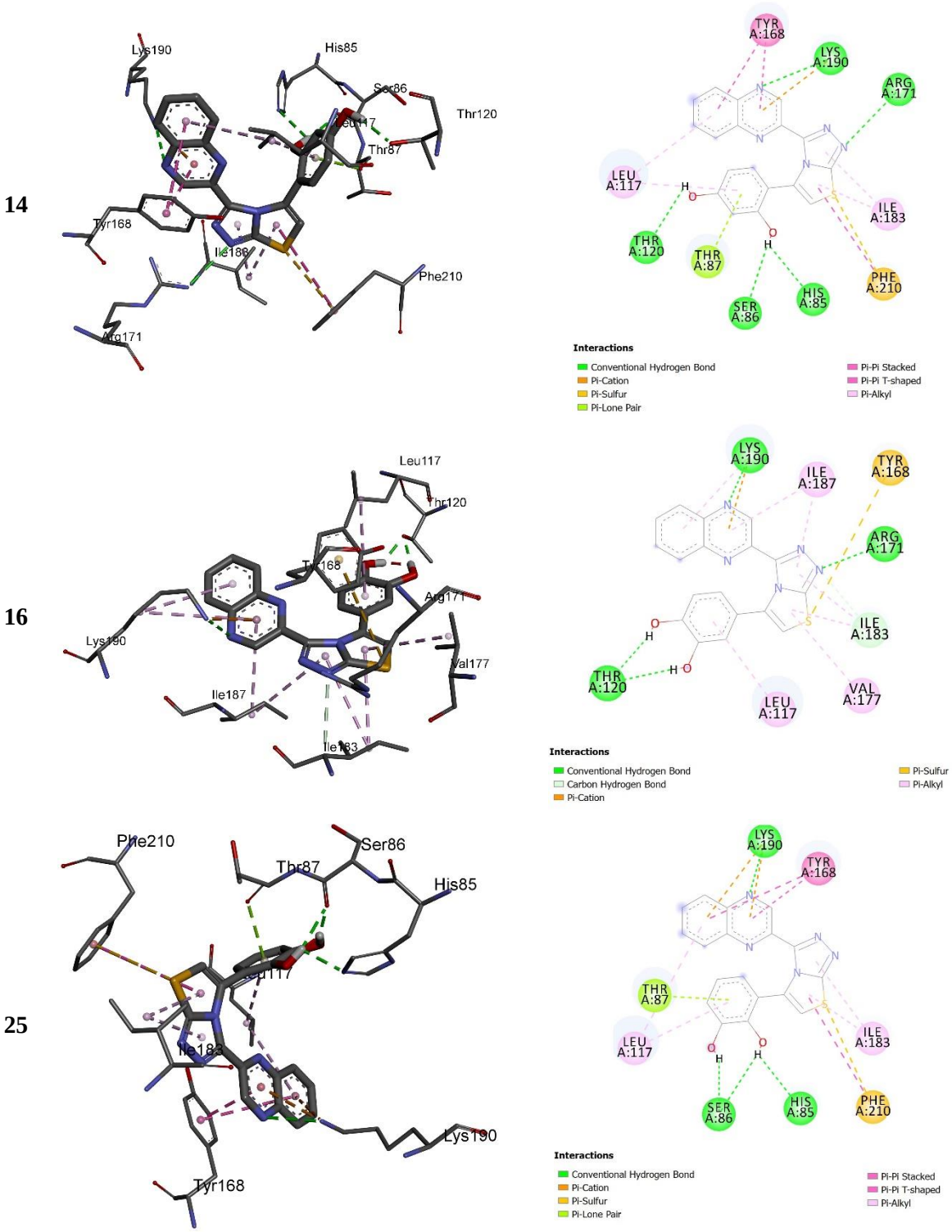


Figure 1: 3D (right) and 2D (left) closest interactions between active site residues of thymidine phosphorylase and synthesized compounds **14**, **16**, and **25**.

3.0. Experimental Section

3.1. *General Methods*

All nuclear magnetic resonance experiments had been carried out using on Avance Bruker 500 MHz. Electron impact mass spectra (EI-MS) were recorded on a Finnigan MAT-311A, Germany. Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm.

3.1.1 Thymidine phosphorylase assay

Since human TP is not easy to get, we used commercially available recombinant *E. coli* TP. The primary sequence of TP is frequently preserved throughout evolution as mammalian TP is reported to share 39% sequence resemblance with the TP of *E. coli*. The mammalian enzyme as well shared up to 70% resemblance with the active site residues, and three-dimensional structure of *E. coli* TP enzyme [43]. The Thymidine phosphorylase/PD-ECGF (E. coli) activity was determined by measuring the absorbance at 290 nm spectrophotometrically. The original method was described by Krenitsky and Bushby [44]. In brief, the total reaction mixture of 200 µL contained 145 µL of potassium phosphate buffer (pH 7.4), 30 µL of enzyme (human and E. coli) at concentration 0.05 and 0.002 U, respectively, were incubated with 5 µL of test materials for 10 min at 25 °C in a microplate reader. After incubation, pre-read at 290 nm was taken to deduce the absorbance of substrate particles. The substrate (20 µL, 1.5 mM), dissolved in potassium phosphate buffer, was immediately added to the plate and continuously read after 10, 20, and 30 min in a microplate reader (spectra max, molecular devices, CA, USA). All assays were performed in triplicate.

3.1.2. Calculations

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. The percent inhibition for above mentioned biological activities was calculated by following formula:

Percent Inhibition=100-(ODtest compound/ODcontrol)×100

3.1.3 Synthesis of quinoxaline thiosemicarbazone (II)

quinoxaline-2-carbohydrazide (5 g, 26.60 mmol), potassium thiocyanate (2.61 g, 26.90 mmol) and 4 ml of conc. HCl in 40 ml of water were refluxed for 4 h. The reaction progress was monitored by TLC. After completion of the reaction, reaction mixture was left for cooling and white solid ppts appeared then the solid was filtered and dried. Yield 5.62 g (85.6%); mp 289-290 °C.

3.1.4. Synthesis of 5-(quinoxalin-3-yl)-4H-1,2,4-triazole-3-thiol (III)

The 5-(quinoxalin-3-yl)-4H-1,2,4-triazole-3-thiol (**III**) was synthesized as reported in [45].

3.5. General procedure for synthesis of quinoxaline derivatives (1-25)

5-(quinoxalin-3-yl)-4H-1,2,4-triazole-3-thiol (**III**) (1 mmol) was refluxed with appropriate arylacyl bromide (1 mmol) in 15 ml ethanol for 12 h. The reaction was monitored by TLC. On completion of the reaction, the product was left for cooling. The solid was filtered, and the crude products were recrystallized from methanol.

4.2.1. 5-(2-flourophenyl)-3(Quinoxalin-2yl)thiazolo[2,3-c][1,2,4]triazole

Yield: 81%. m.p.: 299-300°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.32 (s, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.88 (d, J = 7.0 Hz, 1H), 7.70-7.67 (m, 3H), 7.52-7.48 (m, 2H), 7.30-7.26 (m, 2H). ^{13}C-NMR (150 MHz, DMSO -d_6): δ 158.5, 155.5, 145.9, 145.4, 144.5, 142.4, 142.3, 141.11, 130.5, 129.8, 129.7, 129.5, 129.4, 129.3, 124.10, , 123.7, 115.7, 114.9. HR-ESI-MS: m/z calcd for $C_{18}H_{10}FN_5S$, $[M]^+$ 347.0641; Found 347.0623.

4.2.2. 5-(3-flourophenyl)-3(Quinoxalin-2yl)thiazolo[2,3-c][1,2,4]triazole

Yield: 80%. m.p.: 304-305 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.10 (s, 1H), 7.90 (d, J = 7.5 Hz, 1H), 7.67 (d, J = 7.5 Hz, 2H), 7.61 (d, J = 8.0 Hz, 1H), 7.56 (d, J = 7.5 Hz, 1H), 7.50–7.46 (m, 2H), 7.22 (t, J = 8.0 Hz, 1H), 7.18-7.16 (m, 1H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 162.2, 155.5, 145.9, 145.4, 144.5, 142.4, 142.3, 141.11, 134.8, 129.8, 129.7, 129.5, 129.4, 127.7, 115.7, 123.3, 115.10, 115.7. HR-ESI-MS: m/z calcd for $C_{18}H_{10}FN_5S$, $[M]^+$ 347.0641; Found 347.0625.

4.2.3. 5-(4-flourophenyl)-3(Quinoxalin-2yl)thiazolo[2,3-c][1,2,4]triazole

Yield: 77%. m.p.: 308-309 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.11(s, 1H), 7.90 (d, J = 7.5 Hz, 1H), 7.80 (d, J = 8.0 Hz, 2H), 7.72–7.68 (m, 2H), 7.50–7.45 (m, 2H), 7.24 (d, J = 7.5 Hz, 2H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 162.9, 155.5, 145.9, 145.5, 144.5, 142.4, 142.3, 141.11, 130.8, 130.7, 129.8, 129.7, 129.5, 129.4, 128.8, 116.3, 116.2, 115.7. HR-ESI-MS: m/z calcd for $C_{18}H_{10}FN_5S$, $[M]^+$ 347.0641; Found 347.0617.

4.2.4. 5-(2-chlorophenyl)-3(Quinoxalin-2yl)thiazolo[2,3-c][1,2,4]triazole

Yield: 82%. m.p.: 280-281°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.50 (s, 1H), 8.12 (d, J = 7.0 Hz, 1H), 7.90 (d, J = 7.0 Hz, 1H), 7.72-7.68 (m, 3H), 7.54–7.49 (m, 2H), 7.42–7.38 (m, 2H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 155.5, 145.9, 145.4, 144.5, 142.4, 142.3, 141.11, 132.7, 132.4, 130.8, 130.3, 129.9, 129.8, 129.7, 129.5, 129.4, 128.9, 115.7. HR-ESI-MS: m/z calcd for $C_{18}H_{10}ClN_5S$, $[M]^+$ 363.0345; Found 363.0319.

4.2.5. 5-(3-chlorophenyl)-3(Quinoxalin-2yl)thiazolo[2,3-c][1,2,4]triazole

Yield: 80%. m.p.: 285-286 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.90 (s, 1H), 8.57 (d, J = 6.5 Hz, 1H), 8.16 (d, J = 7.5 Hz, 1H), 8.05 (s, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.70 (d, J = 7.5 Hz, 2H), 7.50-7.47 (m, 2H), 7.42 (d, J = 8.0 Hz, 1H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 155.5, 145.9, 145.4, 144.5, 142.4, 142.3, 141.11, 134.9, 134.6, 129.9, 129.8, 129.7, 129.6, 129.5, 129.4, 128.9, 125.8, 115.7. HR-ESI-MS: m/z calcd for $C_{18}H_{10}ClN_5S$, $[M]^+$ 363.0345; Found 363.0323.

4.2.6. 5-(4-chlorophenyl)-3(Quinoxalin-2yl)thiazolo[2,3-c][1,2,4]triazole

Yield: 89%. m.p.: 249-250 °C. 1H NMR (500 MHz, DMSO-d6): δ 8.08 (s, 1H), 7.88 (d, J = 7.5 Hz, 1H), 7.70 (d, J = 8.5 Hz, 2H), 7.69-7.64 (m, 2H), 7.52-7.45 (m, 4H). ^{13}C NMR (150 MHz, DMSO -d_6): δ 155.5, 145.9, 145.4, 144.5, 142.4, 142.3, 141.11, 134.5, 131.3, 129.9, 129.8, 129.7, 129.6, 129.5, 129.4, 128.9, 127.9, 115.7. HR-ESI-MS: m/z calcd for $C_{18}H_{10}ClN_5S$, $[M]^+$ 363.0345; Found 363.0327.

4.2.7. 5-(2-nitrophenyl)-3(Quinoxalin-2yl)thiazolo[2,3-c][1,2,4]triazole

Yield: 83%. m.p.: 310-311°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.26 (d, J = 7.0 Hz, 1H), 7.98 (d, J = 7.5 Hz, 1H), 7.90 (d, J = 7.5 Hz, 1H), 7.74 (d, J = 7.0 Hz, 1H), 7.70 (d, J = 7.0 Hz, 1H), 7.63 (d, J = 7.0 Hz, 1H), 7.61 (d, J = 7.0 Hz, 1H), 7.50-7.46 (m, 2H), 7.43 (s, 1H); ^{13}C NMR (150 MHz, DMSO -d_6): δ155.5, 148.9, 145.9, 145.5, 144.5, 142.4, 142.3, 141.11, 135.5, 132.8, 129.9,

129.8, 129.7, 129.5, 129.4, 125.4, 124.6,115.7. HR-ESI-MS: m/z calcd for $C_{18}H_{10}N_6O_2S$, $[M]^+$ 374.0586; Found 374.0568.

4.2.8. 5-(4-nitrophenyl)-3(Quinoxalin-2yl)thiazolo[2,3-c][1,2,4]triazole

Yield: 81%. m.p.: 315-316°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.22 (d, J = 8.3 Hz, 2H), 8.18 (s, 1H), 7.98 (d, J = 8.0 Hz, 2H), 7.90 (d, J = 7.5 Hz, 1H), 7.70-7.65 (m, 2H), 7.54 (d, J = 7.0 Hz, 1H), 7.44 (s, 1H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 155.5, 147.9, 145.9, 145.4, 144.5, 142.4, 142.3, 142.1, 141.9, 141.8, 139.3, 129.8, 129.7, 129.5, 129.4, 126.4, 126.3, 124.6,124.5,115.7. HR-ESI-MS: m/z calcd for $C_{18}H_{10}N_6O_2S$, $[M]^+$ 374.0586; Found 374.0559.

4.2.9. 3-(quinoxalin-2-yl)-5-o-tolylthiazolo[2,3-c][1,2,4]triazole

^{13}C NMR (150 MHz, DMSO -d_6): δ 155.5, 145.9, 145.4, 144.5, 142.4, 142.3, 141.11, 136.9, Yield: 88%. m.p.: 265-266 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.40 (s, 1H), 7.89 (d, J = 8.0 Hz, 2H), 7.68 (d, J = 7.0 Hz, 2H), 7.48-7.42 (m, 2H), 7.35-7.22 (m, 3H), 2.49 (s, 3H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 130.1, 129.9, 129.8, 129.7, 129.5, 129.4, 128.8, 126.4, 122.9, 115.7, 18.9. HR-ESI-MS: m/z calcd for $C_{19}H_{13}N_5S$, $[M]^+$ 343.0892; Found 343.0864.

4.2.10. 3-(quinoxalin-2-yl)-5-m-tolylthiazolo[2,3-c][1,2,4]triazole

Yield: 84%. M.P.: 270-271 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.08 (s, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.72-7.68 (m, 2H), 7.57 (s, 1H), 7.52 (d, J = 7.0 Hz, 1H), 7.43 (s, 1H), 2.46 (s, 3H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 155.5, 145.9, 145.4, 144.5, 142.4, 142.3, 141.11, , 139.1, 133.1, 130.6, 129.9, 129.8, 129.7, 129.5, 129.4, 128.9, 124.6, 115.7, 21.8. HR-ESI-MS: m/z calcd for $C_{19}H_{13}N_5S$, $[M]^+$ 343.0892; Found 343.0871.

4.2.11. 3-(quinoxalin-2-yl)-5-p-tolylthiazolo[2,3-c][1,2,4]triazole

Yield: 81%. m.p.: 280-281 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.10 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.71-7.65 (m, 4H), 7.47-7.42 (m, 2H), 7.27 (d, J = 7.9 Hz, 2H), 2.49 (s, 3H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 155.5, 145.9, 145.4, 145.3, 144.5, 142.4, 142.3, 141.11, 130.2, 131.9, 129.9, 129.8, 129.7, 129.5, 129.4, 129.3, 125.9, 125.7, 115.7, 21.5. HR-ESI-MS: m/z calcd for $C_{19}H_{13}N_5S$, $[M]^+$ 343.0892; Found 343.0873.

4.2.12. 3-(3-(quinoxalin-2-yl)thiazolo[2,3-c][1,2,4]triazol-5-yl)phenol

Yield: 85%. m.p.: 289-290 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.05 (s, 1H, OH), 8.04 (s, 1H), 7.90 (d, J = 7.5 Hz, 1H), 7.70-7.65 (m, 2H), 7.49-7.43 (m, 2H), 7.25 (d, J = 7.5 Hz, 1H), 7.19-7.15 (m, 1H), 7.15 (d, J = 7.0 Hz, 1H), 6.81 (d, J = 7.0 Hz, 1H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 157.7, 155.5, 145.9, 145.4, 144.5, 142.4, 142.3, 141.11, 134.6, 130.8, 129.8, 129.7, 129.5, 129.4, 120.3, 115.10, 115.9, 115.7. HR-ESI-MS: m/z calcd for $C_{18}H_{11}N_5OS$, $[M]^+$ 345.0684; Found 345.0666.

4.2.13. 4-(3-(quinoxalin-2-yl)thiazolo[2,3-c][1,2,4]triazol-5-yl)phenol

Yield: 82%. m.p.: 297-298 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.82 (s, 1H, OH), 8.03 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.65-7.62 (m, 2H), 7.58 (d, J = 7.5 Hz, 2H), 7.46-7.40 (m, 2H), 6.80 (d, J = 8.0 Hz, 2H). ^{13}C NMR (150 MHz, DMSO -d_6): δ 158.7, 155.5, 145.9, 145.5, 144.5, 142.4, 142.3, 141.11, 129.8, 129.7, 129.5, 129.4, 128.9, 128.7, 125.7, 116.6, 116.4,115.7. HR-ESI-MS: m/z calcd for $C_{18}H_{11}N_5OS$, $[M]^+$ 345.0684; Found 345.0661.

4.2.14. 4-(3-(quinoxalin-2-yl)thiazolo[2,3-c][1,2,4]triazol-5-yl)benzene-1,3-diol

Yield: 79%. m.p.: 299-300 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.70 (s, 1H, OH), 9.80 (s, 1H, OH), 8.18 (s, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.71-7.65 (m, 3H), 7.50-7.43 (m, 2H), 6.32 (d, J = 7.0, 6.0 Hz, 2H). ^{13}C NMR (150 MHz, DMSO -d_6): δ 160.1, 156.2, 155.5, 145.9, 145.5, 144.5, 142.4, 142.3, 141.11, 133.4, 129.8, 129.7, 129.5, 129.4, 113.3, 109.0, 105.8, 115.7. HR-ESI-MS: m/z calcd for $C_{18}H_{11}N_5O_2S$, $[M]^+$ 361.0633; Found 361.0615.

4.2.15. 2-(3-(quinoxalin-2-yl)thiazolo[2,3-c][1,2,4]triazol-5-yl)benzene-1,4-diol

Yield: 87%. m.p.: 301-302 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.72 (s, 2H, OH), 8.30 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.74-7.65 (m, 2H), 7.52-7.40 (m, 2H), 7.17 (d, J = 6.0 Hz, 1H), 6.75 (d, J = 8.0 Hz, 1H), 6.68 (d, J = 7.0 Hz, 1H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 155.5, 150.3, 147.9, 145.9, 145.5, 144.5, 142.4, 142.3, 141.11, 129.8, 129.7, 129.5, 129.4, 122.1, 117.9, 117.5, 115.7, 114.5. HR-ESI-MS: m/z calcd for $C_{18}H_{11}N_5O_2S$, $[M]^+$ 361.0633; Found 361.0617.

4.2.16. 4-(3-(quinoxalin-2-yl)thiazolo[2,3-c][1,2,4]triazol-5-yl)benzene-1,2-diol

Yield: 83%. m.p.: 293-294 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.30 (s, 1H, OH), 9.10 (s, 1H, OH), 7.96 (s, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.68(d, J = 6.0 Hz, 2H), 7.53-7.40 (m, 2H), 7.20 (s, 1H), 6.95 (d, J = 7.0 Hz, 1H), 6.70 (d, J = 8.0 Hz, 1H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 155.5,

147.5, 145.9, 145.7, 145.5, 144.5, 142.4, 142.3, 141.11, 129.8, 129.7, 129.5, 129.4, 127.2, 121.7, 116.4, 115.7, 114.5. HR-ESI-MS: m/z calcd for $C_{18}H_{11}N_5O_2S$, $[M]^+$ 361.0633; Found 361.0612.

4.2.17. 5-methoxy-2-(3-(quinoxalin-2-yl)thiazolo[2,3-c][1,2,4]triazol-5-yl)phenol

Yield: 86%. m.p.: 305-306 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.87 (s, 1H, OH), 8.30 (s, 1H), 7.82 (d, J = 7.5 Hz, 1H), 7.62 (s, 2H), 7.55 (d, J = 8.0 Hz, 1H), 7.46-7.40 (s, 2H), 6.51 (d, J = 8.0 Hz, 1H), 6.42 (d, J = 6.0 Hz, 1H), 3.75 (s, 3H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 162.2, 156.2, 155.5, 145.9, 145.5, 144.5, 142.4, 142.3, 141.11, 132.9, 129.8, 129.7, 129.5, 129.4, 115.7, 112.9, 107.6, 104.4, 55.9. HR-ESI-MS: m/z calcd for $C_{19}H_{13}N_5O_2S$, $[M]^+$ 375.0790; Found 375.0772.

4.2.18. 2-methoxy-5-(3-(quinoxalin-2-yl)thiazolo[2,3-c][1,2,4]triazol-5-yl)phenol

Yield: 83%. m.p.: 293-294 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.22 (s, 1H, OH), 8.04 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.76-7.69 (m, 2H), 7.46-7.41 (m, 2H), 7.28 (d, J = 6.0Hz, 1H), 7.04 (d, J = 8.0 Hz, 1H), 6.94 (d, J = 8.0 Hz, 1H), 3.77 (s, 3H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 155.5, 145.7, 147.5, 147.4, 145.5, 144.5, 142.4, 142.3, 141.11, 129.8, 129.7, 129.5, 129.4, 126.9, 121.7, 114.1, 115.7, 111.6, 56.3. . HR-ESI-MS: m/z calcd for $C_{19}H_{13}N_5O_2S$, $[M]^+$ 375.0790; Found 375.0768.

4.2.19. 5-(3-methoxyphenyl)-3-(quinoxalin-2-yl)thiazolo[2,3-c][1,2,4]triazole

Yield: 75%. m.p.: 179-180 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (s, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.70-7.59 (m, 3H), 7.46-7.40 (m, 2H), 7.30 (d, J = 6.5 Hz, 2H), 6.96- 6.89 (m, 1H), 3.80 (s, 3H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 161.2, 155.5, 145.7, 145.5, 144.5, 142.4, 142.3, 141.11, 134.2, 130.3, 129.8, 129.7, 129.5, 129.4, 119.9, 115.7, 114.1, 113.8, 55.9. HR-ESI-MS: m/z calcd for $C_{19}H_{13}N_5OS$, $[M]^+$ 359.0841; Found 359.0843.

4.2.20. 5-(4-methoxyphenyl)-3-(quinoxalin-2-yl)thiazolo[2,3-c][1,2,4]triazole

Yield: 87%. m.p.: 301-302 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.07 (s, 1H), 7.88 (d, J = 8.0 Hz, 2H), 7.70 (d, J = 8.0 Hz, 2H), 7.62 (d, J = 7.0 Hz, 1H), 7.47-7.42 (s, 2H), 7.02 (d, J = 8.0 Hz, 2H), 3.81 (s, 3H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 160.8, 155.5, 145.7, 145.5, 144.5, 142.4, 142.3, 141.11, 129.8, 129.7, 129.5, 129.4, 128.7, 128.5, 125.5, 115.7, 114.9, 114.7, 55.9. HR-ESI-MS: m/z calcd for $C_{19}H_{13}N_5OS$, $[M]^+$ 359.0841; Found 359.0819.

4.2.21. 4-methoxy-2-(3-(quinoxalin-2-yl)thiazolo[2,3-c][1,2,4]triazol-5-yl)phenol

Yield: 83%. m.p.: 308-309 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.98 (s, 1H, OH), 8.40 (s, 1H), 7.88 (d, J = 8.2 Hz, 1H), 7.72-7.67 (m, 2H), 7.47-7.42 (m, 2H), 7.30 (s, 1H), 6.82 (d, J = 2.0 Hz, 2H), 3.79 (s, 3H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 155.5, 153.9, 145.7, 147.5, 145.4, 144.5, 142.4, 142.3, 141.11, 129.8, 129.7, 129.5, 129.4, 121.7, 117.6, 115.7, 115.5, 112.9, 55.9. HR-ESI-MS: m/z calcd for $C_{19}H_{13}N_5O_2S$, $[M]^+$ 375.0790; Found 375.0772.

4.2.22. 5-(pyridin-3-yl)-3-(quinoxalin-2-yl)thiazolo[2,3-c][1,2,4]triazole

Yield: 82%. m.p.: 276-277 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.42 (s, 1H), 8.12 (d, J = 8.0 Hz, 1H), 7.90 (d, J = 7.0 Hz, 1H), 7.70-7.65 (m, 4H), 7.51 (d, J = 7.0 Hz, 1H), 7.47-7.42 (m, 2H). ^{13}C NMR (150 MHz, DMSO -d_6): δ147.9, 145.7, 147.5, 145.4, 144.5, 143.4, 142.4, 142.3, 141.11, 134.2, 133.2, 129.8, 129.7, 129.5, 129.4, 124.2,120.3. HR-ESI-MS: m/z calcd for $C_{17}H_{10}N_6S$, $[M]^+$ 330.0688; Found 330.0667.

4.2.23. 5-(pyridin-4-yl)-3-(quinoxalin-2-yl)thiazolo[2,3-c][1,2,4]triazole

Yield: 86%. m.p.: 276-277 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.60 (d, J = 7.0 Hz, 2H), 8.13 (s, 1H), 7.92 (d, J = 7.9 Hz, 1H), 7.74-7.66 (m, 4H), 7.50-7.42 (m, 2H). ^{13}C NMR (150 MHz, DMSO -d_6): δ149.9, 149.8, 145.7, 145.5, 144.5, 143.4, 142.4, 142.3, 141.11, 140.5, 129.8, 129.7, 129.5, 129.4, 121.5, 121.5, 120.3. HR-ESI-MS: m/z calcd for $C_{17}H_{10}N_6S$, $[M]^+$ 330.0688; Found 330.0670.

4.2.24. 2-(3-(quinoxalin-2-yl)thiazolo[2,3-c][1,2,4]triazol-5-yl)phenol

Yield: 82%. m.p.: 286-287 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.54 (s, 1H, OH), 8.20 (s, 1H), 7.90 (d, J = 7.5 Hz, 1H), 7.72 (d, J = 7.0 Hz, 1H), 7.65 (d, J = 8.0 Hz, 2H), 7.52-7.44 (m, 2H), 7.25-7.20 (m, 2H), 6.90 (d, J = 6.5 Hz, 1H). ^{13}C NMR (150 MHz, DMSO -d_6): δ 155.5, 155.3, 145.7, 145.5, 144.5, 142.4, 142.3, 141.11, 131.7, 130.3, 129.8, 129.7, 129.5, 129.4, 120.7, 117.9, 121.7, 115.7. HR-ESI-MS: m/z calcd for $C_{18}H_{11}N_5OS$, $[M]^+$ 345.0684; Found 345.0668.

4.2.25. 3-(3-(quinoxalin-2-yl)thiazolo[2,3-c][1,2,4]triazol-5-yl)benzene-1,2-diol

Yield: 82%. m.p.: 309-310 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.08 (s, 1H, OH), 9.35 (s, 1H, OH), 8.40 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.64 (d, J = 6.0 Hz, 2H), 7.45-7.40 (m, 2H), 7.14 (d, J = 7.0 Hz, 1H), 6.80 (d, J = 7.0 Hz, 1H), 6.70 (t, J = 7.5 Hz, 1H); ^{13}C NMR (150 MHz, DMSO -d_6): δ 155.5, 145.7, 145.5, 145.4, 144.5, 143.7, 142.4, 142.3, 141.11, 129.8, 129.7, 129.5, 129.4, 124.3, 123.4, 122.1, 117.5, 115.7. HR-ESI-MS: m/z calcd for $C_{18}H_{11}N_5O_2S$, $[M]^+$ 361.0633; Found 361.0605.

3.3. Molecular docking details

The intermolecular binding modes between the docked selected synthesized quinoxaline derivatives and the active residues of thymidine phosphorylase have been explored using Autodock package [46]. The geometries of thymidine phosphorylase and the original docked ligand 3'-azido-2'-fluoro-dideoxyuridine were obtained from the RCSB data bank website (PDB code 4EAD) [47]. Water molecules were removed; polar hydrogen atoms and Kollman charge were added to the extracted receptor using the automated tool in AutoDock Tools 4.2. The active site is identified based on the co-crystallized receptor-ligand complex structure of thymidine phosphorylase. The re-docking of the original ligand 3'-azido-2'-fluoro-dideoxyuridine into the active site is well reproduced with an RMSD value less than 1.14 Å and a binding energy of -6.63 kcal/mol. The molecular structures geometries of quinoxaline derivatives were minimized at Merck molecular force field 94 (MMFF94) level44. The optimized structures were saved as PDB files. Nonpolar hydrogens were merged, and rotatable bonds were defined for each docked ligand. Docking studies were performed by the Lamarckian genetic algorithm, with 500 as a total number of the run for binding site for original ligand the synthesized derivatives. In each particularrun, a population of 150 individuals with 27000 generations and 250000 energy evaluations were employed. Operator weights for crossover, mutation, and elitism were set to 0.8, 0.02, and 1, respectively. The binding site was defined using a grid of 35 × 35 × 35 points each with a grid spacing of 0.375 Å. The docking calculation has been carried out using an Intel (R) Core (TM) i5-3770 CPU @ 3.40 GHz workstation.

4.0. Conclusion:

Twenty-five quinoxaline analogs (**1-25**) have been synthesized, characterized through different spectroscopic techniques such as ^{1}H NMR and HREI-MS and were screened against thymidine phosphorylase. Result profile obtained through *in vitro* activity showed that hydroxyl and halogen groups on the phenyl part showed a significant role in inhibitory potential against thymidine phosphorylase much folds better than when compared with standard 7-Deazaxanthine. The binding interactions of the most active analogs were determined by molecular docking study.

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