Mohamed G. Thabit*, Serry A.A. El Bialy and Magda N.A. Nasr

Synthesis and biological evaluation of new 3-(4-substituted phenyl)aminoquinoxaline derivatives as anticancer agents

Abstract: Quinoxaline derivatives **4–11** were synthesized and evaluated for their *in vitro* growth inhibitory activities against liver carcinoma cell line ($HEPG_2$) using the sulforhodiamine B assay. The synthesis was achieved by reaction of 2,3-dichloroquinoxalines **2a,b** with 4-aminoacetophenone to give the corresponding compounds **3a,b**. Claisen-Schmidt condensation reaction of **3a,b** with furfuraldehyde gave enones **4a,b**, which were transformed into pyridines **6a,b**, **8a,b**, isoxazolines **9a,b**, pyrazolines **10a–d**, and pyrimidines **11a,b** *via* several synthetic routes. Virtual screening was carried out by molecular modeling evaluation of the designed compounds. Biological evaluation of the prepared compounds showed that most of the synthesized compounds exhibit more than 50% growth inhibitory.

Keywords: anticancer; isoxazolines; pyrazolines; pyridines; pyrimidines; quinoxaline.

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Introduction

Cancer is a major health problem in developing and undeveloped countries [1–9]. Accordingly, many diverse strategies have been employed to synthesize new agents or improve existing drugs [10]. Clinically important chemotherapeutics can be classified into three major groups. Alkylating agents react covalently with DNA bases. DNA strand breakers represent the second group. They are reactive radicals that produce cleavage of the double helix and cause a significant change in DNA conformation [11]. Intercalators bind to DNA by non-covalent interactions. The recognized forces that maintain the stability of the DNA-intercalator complex are hydrogen bonding, van der Waals interaction, polarization, and hydrophobic forces [11–14]. The compounds that bear heteroatoms such as nitrogen can increase the strength of the complex by forming hydrogen bonds with DNA [15, 16]. It has been reported that the efficacy of the stacking interaction can be enhanced by the presence of fused polyaromatic nitrogen heterocyclic chromophore substituted with a side chain that penetrates into one of the two DNA grooves [17].

Many quinoxaline derivatives are antiviral [18], antimicrobial [19], antidepressant [20], tuberculostatic [21], anticonvulsant [22], and DNA cleaving agents [23]. There are many quinoxaline derivatives with anticancer activity. For example, 2-[4-(7-chloroquinoxalin-2-yl)oxyphenoxy]propanoic acid (XK469) [24] is a topoisomerase II inhibitor, 4-[3-(4-ethoxycarbonylphenyl)thioureido]-*N*-(quinoxalin-2-yl)benzenesulfonamide (CTBS) shows very potent anticancer activity against a human liver cancer cell line ($HEPG_2$) [25], and *N*-(quinoxalin-2-yl)acetamide (Q22) [26] is a potent kinase (CDK) inhibitor (Figure 1).

These agents are structurally related to 5-(1,3-benzodioxol-5-yl)-3-phenyl-4,5-dihydro-1*H*-pyrazole (**I**), which shows anticancer activity against colon cancer cell line (HCT116) [27], to 2-[4-(3-hydroxyphenyl)isoxazol-5-yl]-4,5-dimethoxyphenol (II), which has antimetastatic activity [28], and to 2,2′:6′,2″-terpyridine (**III**), which has significant cytotoxicity against several human cancer cell lines [29] (Figure 2).

In this work, the output compound of designed feature (Figure 3) is the lead structure for synthesis of new 3-(4-substituted phenyl)aminoquinoxaline derivatives with anticancer activity.

Accordingly, we synthesized a new series of quinoxaline hybrids with different aromatic and heterocyclic moieties including pyridines, isoxazolines, pyrazolines, and pyrimidines **5–11** (Schemes 1–3).

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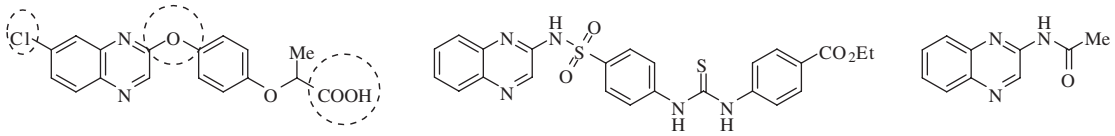


Figure 1 Quinoxaline anticancer agents.

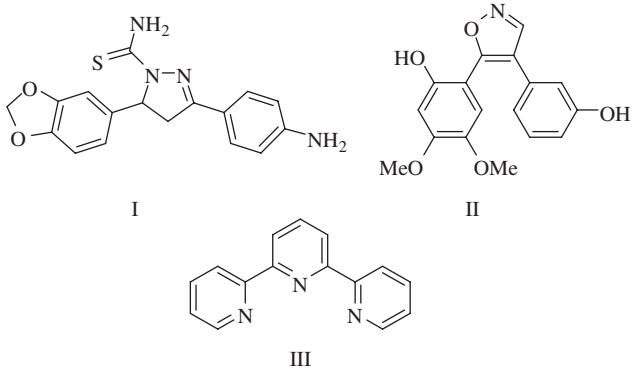


Figure 2 Pyrazole, isoxazole, and pyridine anticancer agents.

Results and discussion

Chemistry

This study was initiated by the synthesis of 2-(4-acetylphenylamino)-3-quinoxaline derivatives (**3a,b**) through the reaction of 2,3-dichloroquioxaline derivatives with an equimolar amount of 4-aminoacetophenone according to the literature method [30].

The synthesis of enones **4a,b** was achieved by condensation of the methyl ketones **3a,b** with furfuraldehyde in the presence of sodium hydroxide [31]. Meanwhile, the hydrazones **5a,b** were synthesized by the reaction of methyl ketones **3a,b** with salicylic acid hydrazide (Scheme 1) [32].

Enones **4a,b** are an important synthon for the construction of variety of heterocycles [33–35]. In this work, the Michael addition reaction of compounds **4a,b** with malononitrile in the presence of sodium alkoxide afforded cyano alkoxypyridines **6a–d**. A similar cyclocondensation of **4a,b** in the presence of ammonium acetate gave aminopyridines **7a,b**. The treatment of enones **4a,b** with ethyl cyanoacetate in the presence of ammonium acetate gave products **8a,b** (Scheme 2). The structures **6a,d**, **7a,b**, and **8a,b** were confirmed by elemental analyses and spectral data.

Reaction of compounds **4a,b** with hydroxylamine in boiling ethanolic solution of potassium hydroxide gave the corresponding oxazole derivatives **9a,b**. Meanwhile, enones **4a,b** were condensed with hydrazine hydrate and phenyl hydrazine to give the corresponding pyrazolines **10a–d**. Also, dihydropyrimidines **11a,b** were synthesized by the reaction of **4a,b** with urea in ethanolic HCl solution (Scheme 3).

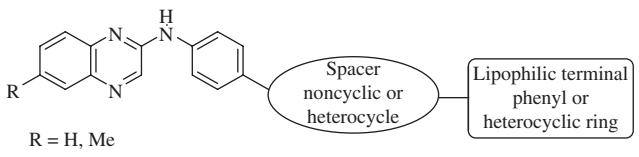
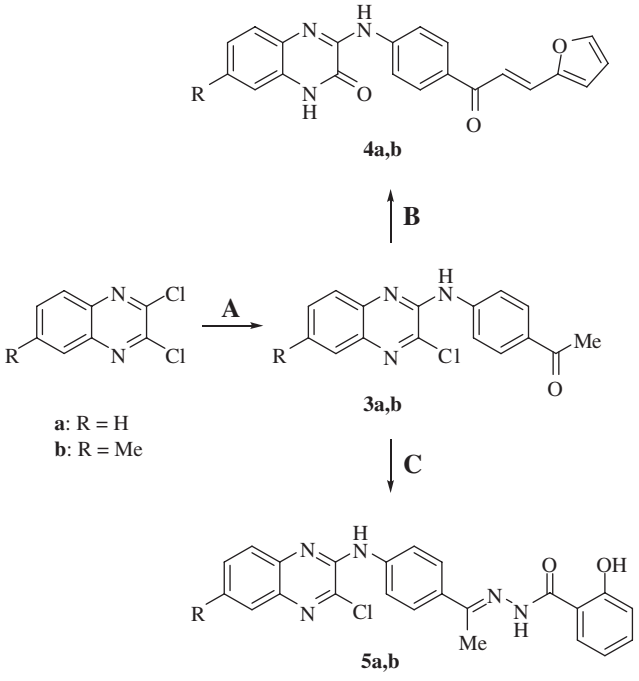


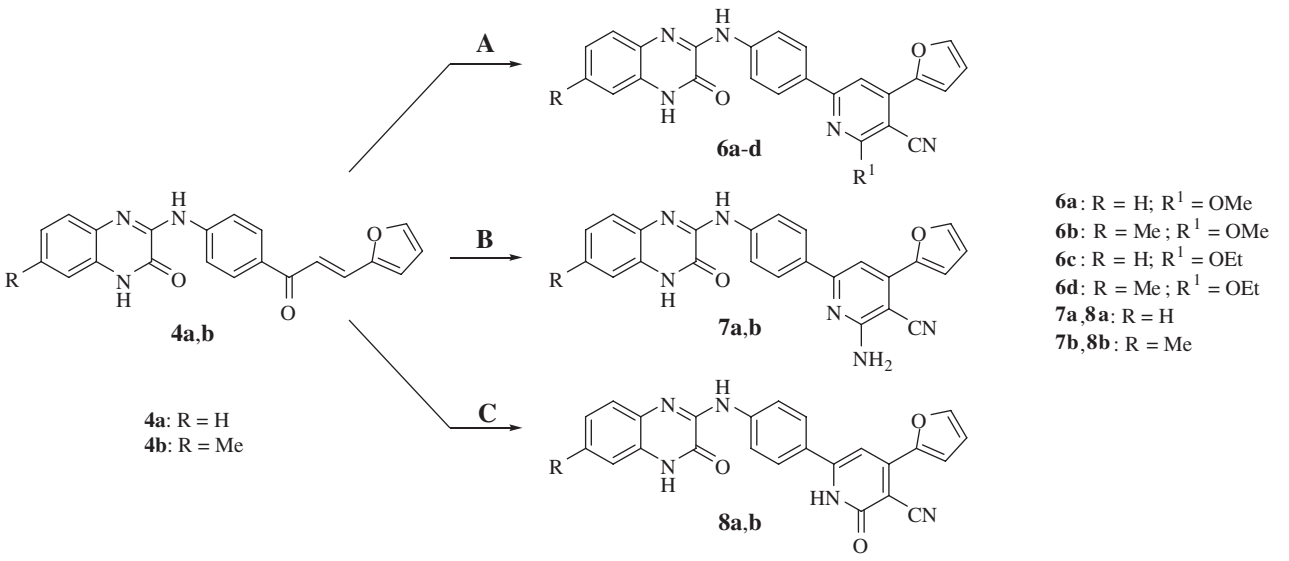
Figure 3 Designed structural features of biological activity.

Molecular modeling

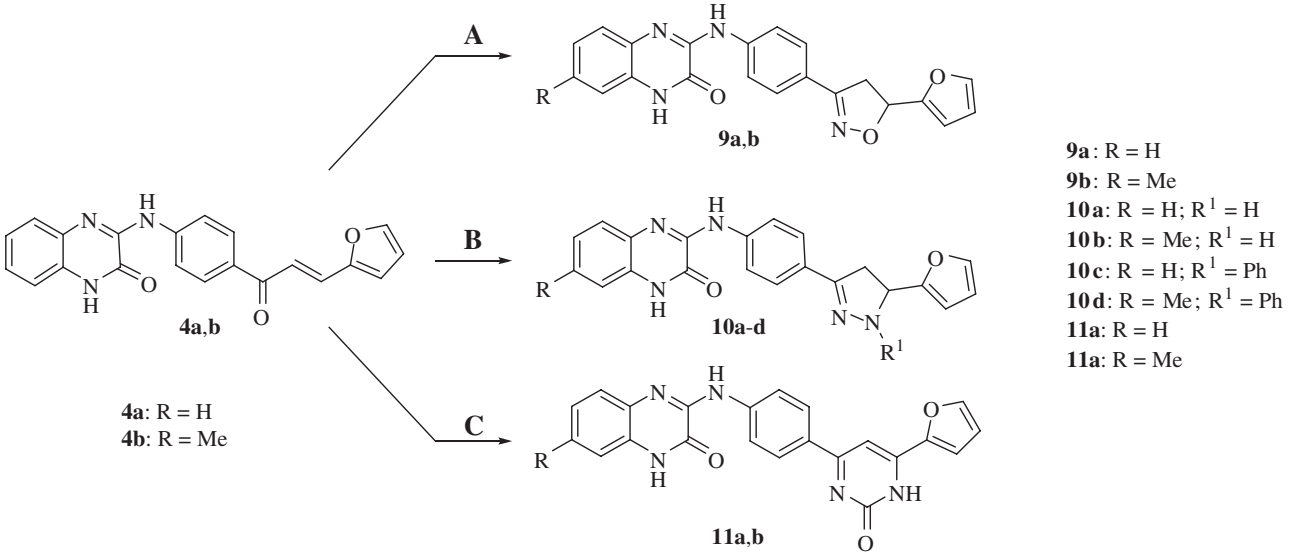
It has been suggested that compound **Q22** is an anticancer agent as CDK inhibitor using docking procedure with the enzyme 1KE8 [26]. The enzyme 1KE8 (Figure 4) was downloaded from the Protein Data Bank (PDB) with the



Scheme 1 The synthesis of **3a,b**, **4a,b**, and **5a,b**: (A) 4-aminoacetophenone, EtOH; (B) ethanolic NaOH, furfuraldehyde; (C) salicylic acid hydrazide, AcOH.



Scheme 2 Synthesis of **6a–d**, **7a,b**, and **8a,b**: (A) Na metal, $CH_2(CN)_2$, EtOH, or MeOH; (B) NH_4OAc, $CH_2(CN)_2$, EtOH; (C) NH_4OAc, $NCCH_2CO_2Et$, EtOH.



Scheme 3 The synthesis of **9a–11b**: (A) NH_2OH, NaOH; (B) NH_2NH_2 or $PhNHNH_2$, EtOH; (C) urea, concentrated HCl, EtOH.

active ligand 4-{[(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene) methyl]amino}-*N*-(1,3-thiazol-2-yl)benzenesulfonamide [36] (Figure 5).

Docking studies have suggested that the most important amino acids residues observed for the complex of this active ligand with 1KE8 are asparagine 132, lysine 129, aspartate 127, glutaminate 12, and threonine 14. This complex is shown in Figure 5. Our docking studies showed that derivatives **4–11** can be docked in the same binding site.

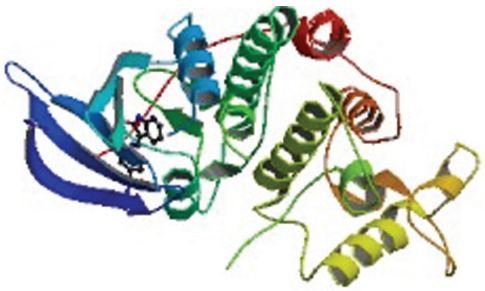


Figure 4 Structure of the enzyme 1KE8.

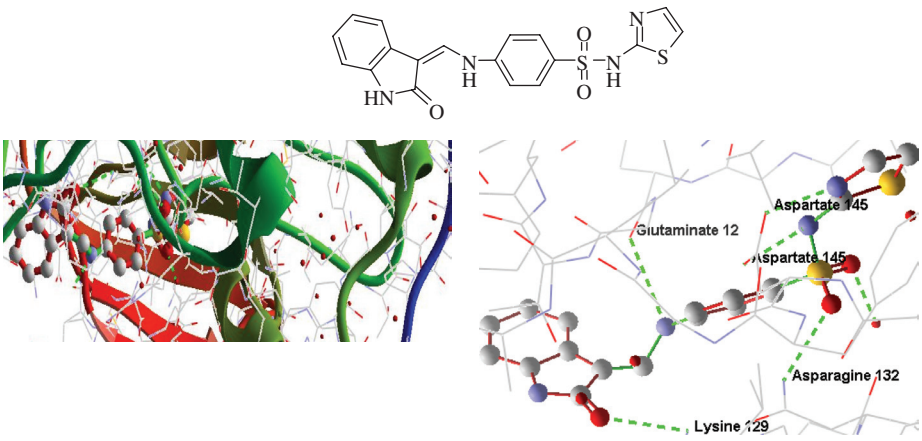


Figure 5 Complex of 1KE8 with the benzenesulfonamide active ligand.

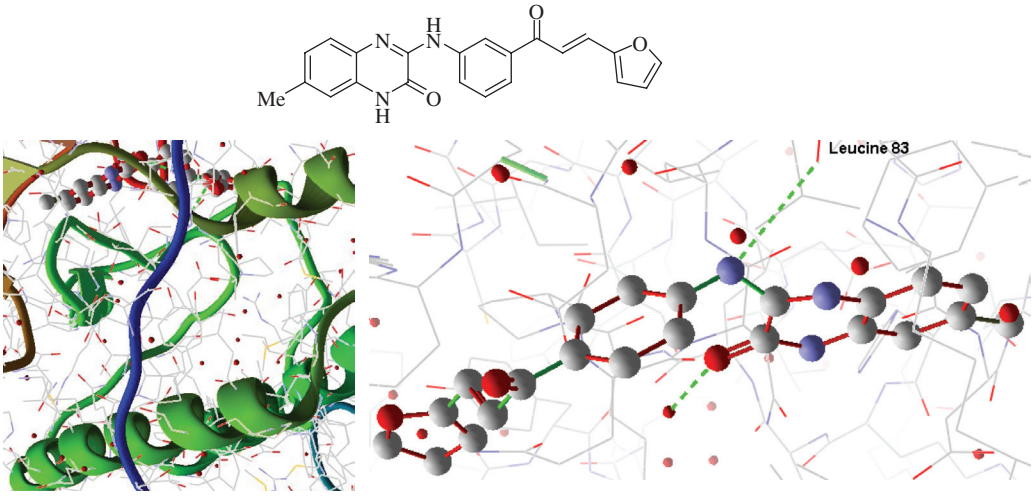


Figure 6 Complex of **4b** with the 1KE8 binding side.

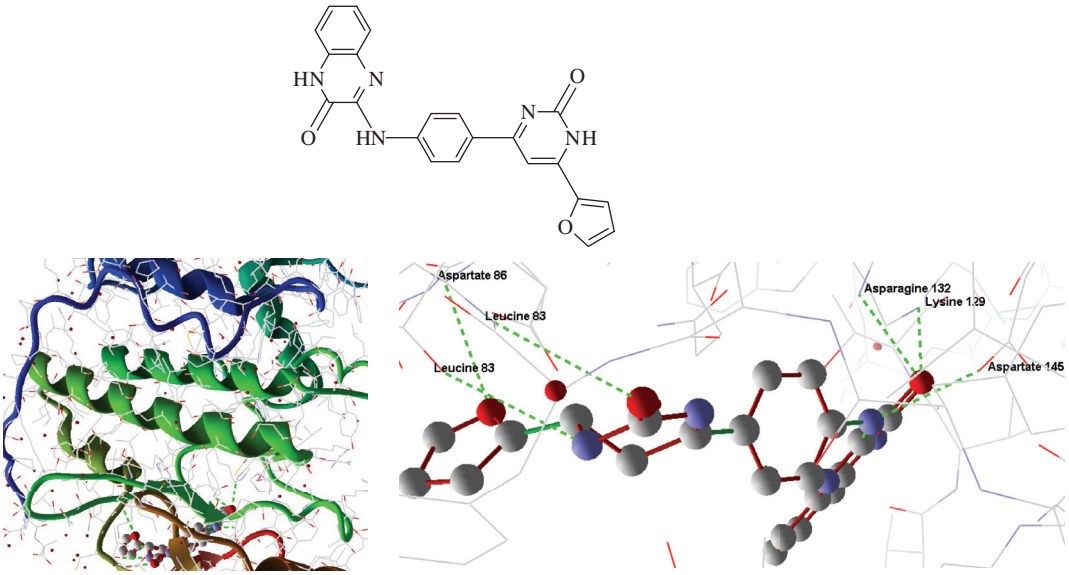
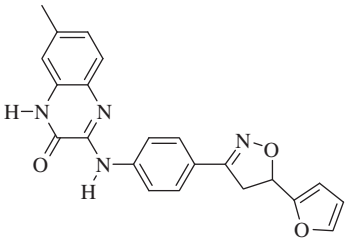


Figure 7 Docking of **11a** in 1KE8 binding side.

Compound **Q22** forms one hydrogen bond with leucine 83 amino acid in the enzyme 1KE8 active side. The enone **4b**, which is a structural analogue of **Q22**, also forms one hydrogen bond with the same amino acid leucine 83, as shown in Figure 6. Importantly, the ligand-enzyme binding energy is decreased to -130.79 kJ/mol from -83.16 kJ/mol from the complex of **Q22**.

Similar complexes were generated for other ligands **6–9** (not shown). For example, compound **11a** forms six hydrogen bonds with the key amino acid residues in the



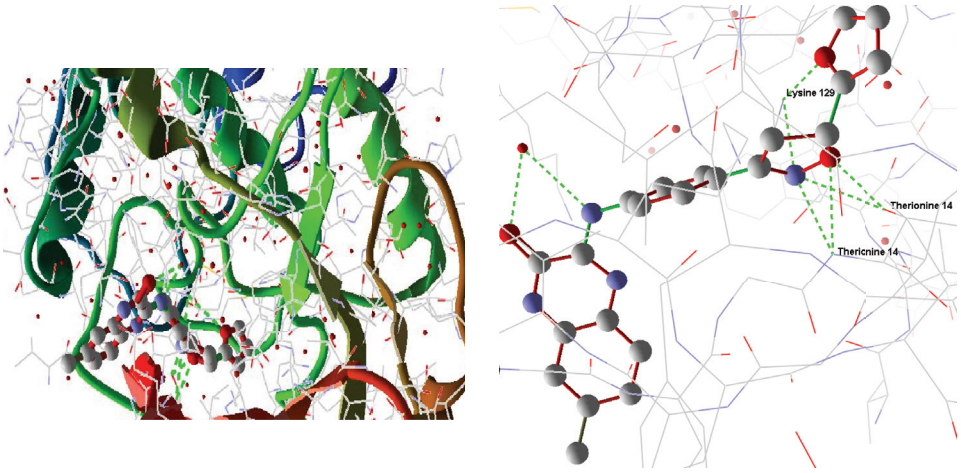
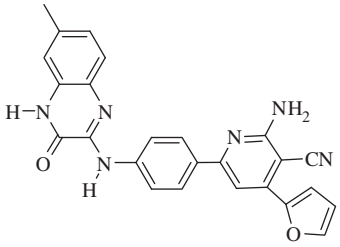


Figure 8 Docking of **9b** in 1KE8 binding side.



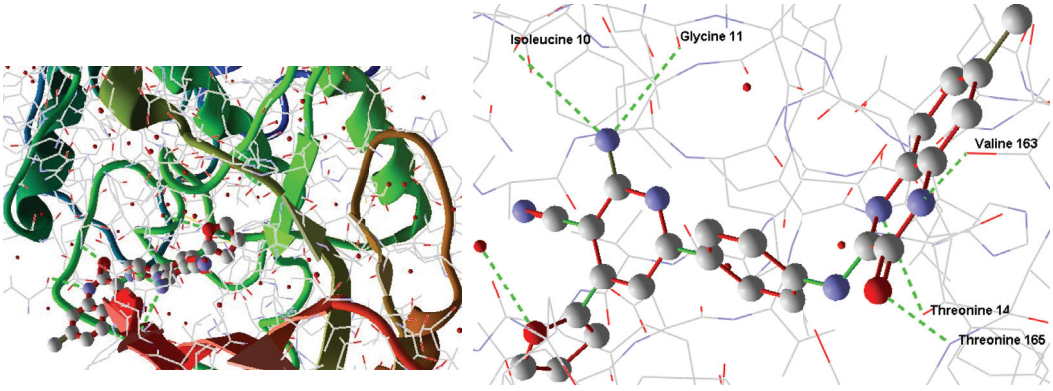


Figure 9 Docking of **7b** in 1KE8 binding side.

Table 1 Molecular modeling results of the interaction of compounds **4–11** with amino acids of the enzyme 1KE8 and biological screening results of these compounds against the $HEPG_2$ human tumor cell line.

Compound no.	Virtual screening				Biological screening % of growth inhibition
	HB no.	E of HB	E of interaction ligand-protein	Amino acids	
11a	6	-7.88	-145.07	Asparagine 132 Lysine 129 Aspartate 14 Aspartate 86 Leucine 83	78
9b	6	-9.39	-136.32	Lysine 129 Threonine 14	77
7b	5	-6.05	-139.42	Isoleucine 10 Glycine 11 Valine 163 Threonine 14 Threonine 165	77
6d	4	-9.11	-163.02	Lysine 129 Leucine 83 Asparagine 132	77
7a	4	-7.79	-152.22	Aspartate 145 Leucine 83 Asparagine 132 Threonine 14	76
10a	4	-6.25	-135.39	Histidine 84 Aspartate 86 Leucine 83 Glutaminate 81	76
10d	2	-3.51	-145.62	Asparagine 132 Leucine 83	76
11b	3	-5.15	-135.51	Threonine 14 Threonine 165	75
6b	2	-3.51	-144.19	Lysine 88 Histidine 84	74
10c	2	-3.54	-144.13	Lysine 89 Leucine 83	71
9a	2	-2.04	-128.13	Aspartate 145	71
5a	1	-2.5	-138.29	Aspartate 145	70
6c	1	-2.47	-148.95	Aspartate 86	70
10b	1	-1.18	-130.39	Leucine 83	70
8b	1	-1.59	-125.24	Glutaminate 12	69.4
4b	1	-1.49	-130.79	Leucine 83	65
6a	3	-0.76	-143.96	Threonine 14 Aspartate 145	59
5b	1	-1.66	-128.54	Glutaminate 12	52
8a	1	-2.22	-146.46	Histidine 84	51
4a	1	-0.88	-116.64	Threonine 14	43
Q22	1	-1.27	-83.16	Leucine 83	

HB, hydrogen bonds; E, energy (kJ/mol).

enzyme active side. One hydrogen bond is between the 3-nitrogen atom of the dihydropyrimidine ring and the oxygen atom of the leucine 83 amino acid. The second bond connects the oxygen atom (2-oxo in the dihydropyrimidine ring) and the nitrogen atom of the leucine 83 amino acid. The third bond is between the furan oxygen atom and the nitrogen atom of the aspartate 86 amino acid. The fourth interaction is formed between the anilino nitrogen atom and the oxygen atom of aspartate 145 amino acid. The last two non-bonding interactions are formed by the bifurcated hydrogen bonds between the oxoquinoxaline oxygen atom and two nitrogen atoms of asparagine 132 lysine 129 amino acids. In comparison to the complex of **4b**, the ligand-enzyme binding

energy is decreased to -145.07 kJ/mol for **11a**. The greater binding affinity of **11a** to the 1KE8-binding side (Figure 7) is nicely paralleled by the greater biological activity of **11a** in comparison to that of **4b**. Thus, cyclization of enone moiety in compound **4a** to the dihydropyrimidine ring of **11a** results in an increased activity. Similar results were obtained for the remaining compounds (Figures 8, 9 and Table 1).

Biological activity

Many natural quinoxaline derivatives are antitumor antibiotics [37–40]. All synthetic quinoxalines **4–11** were screened *in vitro*, using single dose (500 μg/mL), against $HEPG_2$ (human liver carcinoma cell line), using the sulforhodamine-B (SRB) assay [41] (Table 1). Based on the requirement set by NCI that the growth percent of tumor cells (PG%) is 30% or less for active agents lines, it may be concluded that most of these compounds are active because their activities approach this value. The exception is **4a**, which shows a PG% of 43% in $HEPG_2$ cells at a concentration of 500 μg/mL.

Conclusion

The results of molecular modeling and biological screening reveal that the structural modification of the lead structure affects the activity in a predictable manner.

Experimental

Chemistry

Melting points were recorded using Fisher-Johns apparatus and are uncorrected. Elemental analyses were performed at the Microanalytical Center, Cairo University, Egypt. IR spectra were recorded on Mattason 500 FT-IR spectrometer in KBr pellets. The ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were recorded in DMSO-d_6 on a Bruker 400 spectrometer at Georgia State University (Atlanta, GA, USA). Mass spectra were obtained on a JOEL JMS-600H spectrometer at Cairo University.

Synthesis of 1-{4-[3-chloroquinoxalin-2-yl)amino] phenyl}ethan-1-one (3b)

A mixture of 2,3-dichloro-6-methylquinoxaline (**2b**, 2.13 g, 0.01 mol) and 4-aminoacetophenone (2.02 g, 0.015 mol) in absolute ethanol (10 mL) was heated under reflux for 6 h and then cooled. The resultant precipitate was collected by filtration, dried, and crystallized from acetone to give **3b**: yield (2.43 g, 78%); mp 228–230°C; IR: 3311 (NH), 3026 (CH), 1678 cm^{-1} (CO); ^{1}HNMR: δ 2.30 (s, 3H), 2.60 (s, 3H), 7.39–8.01 (m, 7H), 9.00 (s, 1H); ^{13}C NMR: δ 21.5, 28.2, 113.1, 127.5, 130.7, 132.7, 134.4, 137.2, 138.3, 139.9, 140.5, 142.1, 147.1, 165.1, 181.1. Anal. Calcd for $C_{17}H_{14}ClN_3O$ (311.77): C, 65.43; H, 4.49; N, 13.47. Found: C, 65.47; H, 4.46; N, 13.5.

General method for the synthesis of 3-({4[3-(furan-2-yl)-1-oxo-prop-2-en-1-yl]phenyl}amino)-7-substituted quinoxalin-2(1*H*)-one derivatives 4a,b

A mixture of **3a** or **3b** (0.01 mol) and furfuraldehyde (0.96 g, 0.01 mol) in ethanolic sodium hydroxide solution (10%, 25 mL) was stirred at room temperature for 10 h. The precipitated solid was collected by filtration, dried, and crystallized from ethanol.

3-({4[3-(Furan-2-yl)-1-oxo-prop-2-en-1-yl]phenyl}amino)quinoxalin-2(1*H*)-one (4a): Yield (3 g, 84%); mp 160–162°C; IR: 3279 (NH), 3000 (CH), 1657 cm^{-1} (CO); ^{1}H NMR: δ 6.70 (s, 1H), 7.10 (s, 1H), 7.39 (br s, 3H), 7.62 (m, 4H), 7.94 (m, 3H), 8.13 (m, 3H); ^{13}C NMR: δ 113.2, 114.9, 118.8, 121.6, 123.8, 123.9, 130.1, 130.9, 132.5, 137.9, 140.5, 143.5, 145.2, 149.3, 149.5, 154.8, 160.1, 166.2, 182.1; MS: *m*/*z* 357.59 (M$^+$). Anal. Calcd for $C_{21}H_{15}N_3O_3$ (357.36): C, 70.56; H, 4.23; N, 11.74. Found: C, 70.58; H, 4.46; N, 13.5.

3-({4[3-(Furan-2-yl)-1-oxo-prop-2-en-1-yl]phenyl}amino)-7-methyl quinoxalin-2(1*H*)-one (4b): Yield (3.12g, 84%); mp 164–166°C; IR: 3383 (NH), 2900 (CH), 1648 cm^{-1} (CO); ^{1}H NMR: δ 2.47 (s, 3H), 6.65 (s, 1H), 7.05 (s, 1H), 7.51–7.62 (m, 4H), 7.95–8.08 (m, 6H), 9.51 (s, 1H); ^{13}C NMR: δ 22.1, 114.1, 115.1, 118.5, 122.4, 124.8, 128.4, 129.2, 131.2, 133.3, 138.7, 139.5, 142.1, 147.1, 148.1, 148.9, 153.1, 162.1, 165.1, 180.1. MS: *m*/*z* 371.42 (M$^+$). Anal. Calcd for $C_{22}H_{17}N_3O_3$ (371.39): C, 71.08; H, 4.57; N, 11.30. Found: C, 71.10; H, 4.59; N, 11.32.

General procedure for the synthesis of 1-({[4-(3-chloro-6-substituted quinoxalin-2-yl)amino]phenyl}ethylidene)-2-hydroxybenzohydrazide derivatives 5a,b

A mixture of ketone **3a** or **3b** (0.001 mol) and salicylic acid hydrazide (0.152 g, 0.001 mol) in acetic acid (5 mL) was heated under reflux for 10 h. After cooling, the resultant precipitate was collected by filtration, dried, and crystallized from glacial acetic acid.

1-({[4-(3-Chloroquinoxalin-2-yl)amino]phenyl}ethylidene)-2-hydroxy benzohydrazide (5a): Yield (0.217 g, 50%); mp 172–174°C; IR: 3481 (OH), 3324 (NH), 3160 (CH), 1680 cm^{-1} (CO); ^{1}HNMR: δ 2.35 (s, 3H), 4.01 (s, 1H), 7.1–8.01 (m, 11H), 9.75 (s, 1H), 10.62 (s, 1H), 11.32 (s, 1H); ^{13}C NMR: δ 20.1, 113.2, 118.1, 119.5, 122.9, 125.2, 129.1, 129.9, 130.1, 131.5, 134.7, 135.1, 135.9, 137.2, 138.1, 144.7, 145.1, 147.1, 159.1, 162.1, 165.5; MS: *m*/*z* 434.00 (M$^+$). Anal. Calcd for $C_{23}H_{18}ClN_5O_2$ (431.87): C, 63.90; H, 4.16; N, 16.21. Found: C, 63.92; H, 4.18; N, 16.24.

1-({[4-(3-Chloro-6-methylquinoxalin-2-yl)amino]phenyl} ethylidene)-2-hydroxybenzohydrazide (5b): Yield (0.224 g, 50%); mp 178–180°C; IR: 3487 (OH), 3285 (NH), 3186 (CH), 1669 cm^{-1} (CO); ^{1}HNMR: δ 2.41 (s, 3H), 2.65 (s, 3H), 3.92 (s, 1H), 7.1–8.01 (m,

10H), 9.50 (s, 1H), 10.90 (s, 1H), 11.50 (s, 1H); ^{13}C NMR: δ 21.1, 23.1, 112.1, 118.6, 118.9, 123.9, 126.7, 128.9, 129.1, 131.6, 132.6, 133.9, 134.4, 135.1, 136.3, 137.9, 144.9, 145.3, 147.9, 157.7, 162.8, 164.7; MS: *m/z* 448.00 (M^+). Anal. Calcd for $C_{24}H_{20}ClN_5O_2$ (445.90): C, 64.58; H, 4.48; N, 15.69. Found: C, 64.60; H, 4.50; N, 15.71.

General procedure for the synthesis of 2-alkoxy-4-(furan-2-yl)-6-{[4-(3-oxo-6-substituted-3,4dihydroquinoxalin-2-yl) amino]phenyl}-pyridine-3-carbonitrile derivatives 6a–d

A cooled freshly prepared solution of sodium alkoxide (0.001 mol) [0.023 g sodium metal 0.001 mol in 50 mL absolute methanol for compounds (**6a,b**) or absolute ethanol for compounds (**6c,d**)] was treated with malononitrile (0.066 g, 0.001 mol) and then with compound **4a** or **4b** (0.001 mol) at 60°C for 10 h. The precipitated solid was collected by filtration, dried, and crystallized from ethanol.

4-(Furan-2-yl)-2-methoxy-6-{[2-oxo-(1,2dihydroquinoxalin-3-yl) amino] phenyl}pyridine-3-carbonitrile (6a): Yield (0.323 g, 72%); mp 175–177°C; IR: 3384 (NH), 3278 (CH), 2216 (CN), 1648 cm^{-1} (CO); ^{1}HNMR: δ 3.90 (s, 3H), 4.10 (s, 1H), 6.71–8.35 (m, 12H), 9.40 (s, 1H); MS: *m/z* 435.00 (M^+). Anal. Calcd for $C_{25}H_{17}N_5O_3$ (435.43): C, 68.89; H, 3.90; N, 16.07. Found: C, 68.92; H, 3.88; N, 16.08.

4-(Furan-2-yl)-2-methoxy-6-{[4-(6-methyl-3-oxo-3,4dihydroquinoxalin-2-yl)amino]phenyl}pyridine-3-carbonitrile (6b): Yield (0.333 g, 72%); mp 185–187°C; IR: 3385 (NH), 2943 (CH), 2216 (CN), 1614 cm^{-1} (CO); ^{1}HNMR: δ 2.67 (s, 3H), 3.65 (s, 3H), 4.00 (s, 1H), 6.80–8.35 (m, 11H), 9.10 (s, 1H); MS: *m/z* 449.00 (M^+). Anal. Calcd for $C_{26}H_{19}N_5O_3$ (449.46): C, 69.41; H, 4.22; N, 15.57. Found: C, 69.43; H, 4.20; N, 15.60.

2-Ethoxy-4-(furan-2-yl)-6-{[4-(2-oxo-1,2-dihydroquinoxalin-3-yl) amino] phenyl}pyridine-3-carbonitrile (6c): Yield (0.313 g, 72%) mp 195–197°C, IR: 3421 (NH), 2215 (CN), 1656 cm^{-1} (CO); ^{1}HNMR: δ 1.50 (s, 3H), 4.50 (br s, 2H), 7.45 (m, 4H), 7.70 (m, 4H), 8.10 (m, 6H); MS: *m/z* 449.00 (M^+). Anal. Calcd for $C_{26}H_{19}N_5O_3$ (449.46): C, 69.41; H, 4.22; N, 15.57. Found: C, 69.39; H, 4.21; N, 15.59.

2-Ethoxy-4-(furan-2-yl)-6-{[4-(6-methyl-3-oxo-3,4-dihydroquinoxalin-2-yl)amino]phenyl} (6d): Yield (0.323 g, 72%) mp 200–202°C, IR: 3407 (NH), 2922 (CH), 2216 (CN), 1588 cm^{-1} (CO); ^{1}HNMR: δ 1.50 (s, 3H), 2.50 (s, 3H), 4.50 (br s, 2H), 7.45 (m, 4H), 7.70 (m, 4H), 8.10 (m, 5H); MS: *m/z* 463.93 (M^+). Anal. Calcd for $C_{27}H_{21}N_5O_3$ (463.49): C, 69.90; H, 4.53; N, 15.10. Found: C, 69.92; H, 3.51; N, 15.08.

General procedure for the synthesis of 2-amino-4-(furan-2-yl)-6-{[4-(3-oxo-6-substitutd-3,4-dihydroquinoxalin-2-yl)amino]phenyl}pyridine-3-carbonitrile derivatives 7a,b

A mixture of compound **4a** or **4b** (0.001 mol), malononitrile (0.066 g, 0.001 mol), and ammonium acetate (0.616 g, 0.008 mol) in absolute ethanol (25 mL) was heated under reflux for 8 h. The resultant solid was collected by filtration, dried, and crystallized from ethanol.

2-Amino-4-(furan-2-yl)-6-{[4-(2-oxo-1,2-dihydroquinoxalin-3-yl) amino]pyridine-3-carbonitrile (7a): Yield (0.302 g, 72%); mp 160–162°C; IR: 3409 (NH), 2923 (CH), 2220 (CN), 1648 cm^{-1} (CO); ^{1}H NMR: δ 6.70 (s, 1H), 7.10 (s, 1H), 7.45 (m, 2H), 7.60 (m, 2H), 7.72 (m, 2H), 7.95 (m, 2H), 8.00–8.25 (m, 5H), 9.65 (s, 1H); MS: *m/z* 420.00 (M^+). Anal. Calcd for $C_{24}H_{16}N_6O_2$ (420.42): C, 68.50; H, 3.80; N, 19.98. Found: C, 68.52; H, 3.82; N, 19.97.

2-Amino-4-(furan-2-yl)-6-{[4-(6-methyl-3-oxo-3,4-dihydroquinoxalin-2-yl) amino]pyridine-3-carbonitrile (7b): Yield (0.312 g, 72%); mp 178–180°C; IR: 3374 (NH), 2915 (CH), 2203 (CN), 1589 cm^{-1} (CO); ^{1}H NMR: δ 2.53 (s, 3H), 6.70 (s, 1H), 7.10 (s, 1H), 7.45 (m, 2H), 7.60 (m, 3H), 7.72 (m, 2H), 7.95 (m, 2H), 8.00–8.25 (m, 3H), 9.65 (s, 1H); MS: *m/z* 434.53 (M^+). Anal. Calcd for $C_{25}H_{18}N_6O_2$ (434.45): C, 69.05; H, 4.14; N, 19.33. Found: C, 69.07; H, 4.12; N, 19.31.

General method for the synthesis of 4-(furan-2-yl)-2-oxo-6-{4-[(3-oxo-6-substituted-3,4-dihydroquinoxalin-2-yl) amino]phenyl}pyridine-3-carbonitrile derivatives 8a,b

A mixture of compound **4a** or **4b** (0.001 mol), ethyl cyanoacetate (0.112 g, 0.001 mol), and ammonium acetate (0.616 g, 0.008 mol) in absolute ethanol (25 mL) was heated under reflux for 8 h. The resultant solid was collected by filtration, dried, and crystallized from ethanol.

4-(Furan-2-yl)-2-oxo-6-{4-[(2-oxo-1,2-dihydroquinoxalin-3-yl) amino] phenyl}pyridine-3-carbonitrile (8a): Yield (0.253 g, 60%); mp 180–182°C; IR: 3415 (NH), 2977 (CH), 2218 (CN), 1613 cm^{-1} (CO); ^{1}H NMR: δ 6.70 (s, 1H), 7.10 (s, 1H), 7.45 (s, 1H), 7.60(m, 2H), 7.70 (m, 2H), 7.95 (s, 1H), 8.05 (m, 2H), 8.10 (m, 2H), 8.20 (m, 2H), 9.50 (s, 1H); MS: *m/z* 421.33 (M^+). Anal. Calcd for $C_{24}H_{15}N_5O_3$ (421.41): C, 68.34; H, 3.55; N, 16.61. Found: C, 68.32; H, 3.53; N, 16.63.

4-(Furan-2-yl)-2-oxo-6-{4-[(6-methyl-3-oxo-3,4-dihydroquinoxalin-2-yl) amino]pyridine-3-carbonitrile (8b): Yield (0.261 g, 60%); mp 188–190°C; IR: 3287 (NH), 3058 (CH), 2217 (CN), 1668 cm^{-1} (CO); ^{1}HNMR: δ 2.55 (br s, 3H), 7.25 (s, 1H), 7.45 (s, 1H), 7.58 (s, 1H), 7.95(m, 4H), 8.15(m, 5H), 9.90 (br s, 2H); MS: *m/z* 435.33 (M^+). Anal. Calcd for $C_{25}H_{17}N_5O_3$ (435.43): C, 68.89; H, 3.90; N, 16.07. Found: C, 68.91; H, 3.88; N, 16.09.

General procedure for the synthesis of 3-({4-[5-(furan-2-yl)-4,5-dihydroisox azol-3-yl]phenyl}amino)-7-substituted quinoxalin-2(1*H*)-one derivatives 9a,b

A mixture of compound **4a** or **4b** (0.001 mol), hydroxylamine hydrochloride (0.14 g, 0.002 mol), and sodium hydroxide solution (0.5 g in 2 mL water) in absolute ethanol (25 mL) was heated under reflux for 8 h. After addition of ice-cold water, the resultant solid was collected by filtration, dried, and crystallized from ethanol.

3-({4-[5-(Furan-2-yl)dihydroisoxazol-3-yl]phenyl}amino)quinoxalin-2(1*H*)-one (9a): Yield (0.287 g, 72%); mp 150–152°C; IR: 3400 (NH), 2923 (CH), 1644 cm^{-1} (CO); ^{1}H NMR: δ 3.70 (s, 2H), 3.95 (s, 1H), 7.35(m, 2H), 7.57–7.62 (m, 2H), 7.78 (m, 2H), 7.96 (m, 3H), 8.27–8.29 (m, 4H); ^{13}C NMR: δ 41.9, 72.4, 111.4, 111.9, 117.5, 119.1, 122.9, 125.1, 126.9,

130.7, 132.2, 133.4, 143.1, 145.4, 146.9, 154.4, 158.1, 163.5, 167.2; MS: *m/z* 372.10 (M^+). Anal. Calcd for $C_{21}H_{16}N_4O_3$ (372.38): C, 67.67; H, 4.29; N, 15.03. Found: C, 67.69; H, 4.27; N, 15.01.

3-({4-[5-(Furan-2-yl)dihydroisoxazol-3-yl]phenyl}amino)7-methylquinoxalin-2(1*H*)-one (9b): Yield (0.297 g, 72%); mp 155–157°C; IR: 3389 (NH), 2921 (CH), 1643 cm^{-1} (CO); ^1H NMR: δ 3.10 (s, 3H), δ 3.70 (s, 2H), 3.95 (s, 1H), 6.50 (s, 1H), 7.10 (s, 1H) 7.20–7.29 (m, 5H), 7.70–7.80 (m, 5H); ^{13}C NMR: δ 22.1, 41.1, 71.1, 112.6, 113.1, 119.9, 121.6, 123.9, 129.1, 131.5, 137.6, 139.1, 139.9, 142.1, 142.7, 144.9, 154.9, 155.3, 163.5, 167.2; MS: *m/z* 386.40 (M^+). Anal. Calcd for $C_{22}H_{18}N_4O_3$ (386.40): C, 68.32; H, 4.65; N, 14.49. Found: C, 68.34; H, 4.63; N, 14.47.

General method for the synthesis of 3-({4-[5-(furan-2-yl)-4,5-dihydro-1-substituted-pyrazol-3-yl]phenyl} amino)-7-substituted quinoxalin-2(1*H*)-one derivatives 10a–d

A mixture of compound **4a** or **4b** (0.001 mol) and hydrazine hydrate (98%) or phenyl hydrazine (0.001 mol) in ethanol or acetic acid, respectively (5 mL), was heated under reflux for 8 h. The resultant solid was collected by filtration, washed several times with hot ethanol and dried.

3-({4-[5-(Furan-2-yl)-4,5-dihydro-1*H*-pyrazol-3-yl]phenyl}amino) quinoxalin-2(1*H*)-one (10a): Yield (0.223 g, 60%) mp 163–165°C; IR: 3382 (NH), 2921 (CH), 1644 cm^{-1} (CO); ^1H NMR: δ 3.45 (br s, 2H), 4.10 (br s, 2H), 6.55–8.10 (m, 12H), 9.10 (s, 1H); ^{13}C NMR: δ 41.75, 49.10, 111.4, 111.2, 117.5, 118.6, 124.5, 127.2, 128.1, 130.6, 131.1, 132.8, 142.5, 142.9, 144.1, 152.5, 155.1, 163.5, 167.2; MS: *m/z* 371.66 (M^+). Anal. Calcd for $C_{21}H_{17}N_5O_2$ (371.14): C, 67.89; H, 4.58; N, 18.86. Found: C, 67.87; H, 4.56; N, 18.88.

3-({4-[5-(Furan-2-yl)-4,5-dihydro-1*H*-pyrazol-3-yl]phenyl} amino)-7-methyl quinoxalin-2(1*H*)-one (10b): Yield (0.231 g, 60%); mp 166–168°C; IR: 3370 (NH), 2923 (CH), 1600 cm^{-1} (CO).); ^1H NMR: δ 2.45 (s, 3H), 3.69 (br s, 2H), 4.15 (br s, 2H), 6.65–8.00 (m, 11H), 9.50 (s, 1H); ^{13}C NMR: δ 21.7, 43.7, 51.1, 112.7, 115.2, 116.5, 125.6, 128.6, 131.2, 133.3, 137.6, 139.9, 142.2, 144.4, 145.9, 147.1, 153.6, 155.1, 164.5, 166.3; MS: *m/z* 385.00 (M^+). Anal. Calcd for $C_{22}H_{19}N_5O_2$ (385.42): C, 68.54; H, 4.93; N, 18.17. Found: C, 68.56; H, 4.95; N, 18.18.

3-({4-[5-(Furan-2-yl)-1-phenyl-4,5-dihydropyrazol-3-yl]phenyl} amino) quinoxalin-2(1*H*)-one (10c): Yield (0.268 g, 60%); mp 140–142°C; IR: 3378 (NH), 2923 (CH), 1664 cm^{-1} (CO).); ^1H NMR: 3.69 (br s, 2H), 4.00 (s, 1H), 5.00 (s, 1H), 6.65–8.00 (m, 16H), 9.50 (s, 1H); ^{13}C NMR: δ 41.1, 55.2, 110.7, 111.2, 116.9, 118.6, 119.6, 121.2, 124.3, 127.6, 127.9, 132.2, 134.4, 135.9, 137.1, 143.6, 144.1, 145.5, 146.1, 153.9, 154.1, 163.6, 165.0: MS: *m/z* 447.19 (M^+). Anal. Calcd for $C_{27}H_{21}N_5O_2$ (447.49): C, 72.40; H, 4.69; N, 15.64. Found: C, 72.42; H, 4.67; N, 15.62.

3-({4-[5-(Furan-2-yl)-1-phenyl-4,5-dihydropyrazol-3-yl]phenyl} amino)-7-methylquinoxalin-2(1*H*)-one (10d): Yield (0.277 g, 60%); mp 148–150°C; IR: 3376 (NH), 3923 (CH), 1668 cm^{-1} (CO); ^1H NMR: 3.00 (s, 3H), 3.79 (br s, 2H), 4.20 (s, 1H), 5.50 (s, 1H), 6.65–8.00 (m, 15H), 8.90 (s, 1H); ^{13}C NMR: δ 21.9, 40.3, 54.2, 110.2, 111.9, 117.9, 118.1, 121.6, 123.2, 127.3, 129.9, 131.9, 132.8, 137.4, 138.9, 140.1, 143.6, 144.1, 145.5, 146.1, 153.9, 154.1, 163.6, 167.1; MS: *m/z* 461.00 (M^+). Anal. Calcd for $C_{28}H_{23}N_5O_2$ (461.51): C, 72.85; H, 4.98; N, 15.17. Found: C, 72.87; H, 5.00; N, 15.15.

General method for the synthesis of 3-({4-[6-(furan-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl]phenyl}amino)-7-substituted quinoxalin-2(1*H*)-one derivatives 11a,b

A mixture of compound **4a** or **4b** (0.001 mol), urea (0.1 g, 0.001 mol), and concentrated hydrochloric acid (2 mL) in ethanol (25 mL) was heated under reflux for 8 h. After cooling, the precipitated solid was collected by filtration, dried, and crystallized from ethanol.

3-({4-[6-(Furan-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl]phenyl} amino)quinoxalin-2(1*H*)-one (11a): Yield (0.287 g, 72%); mp 153–155°C; ^1H NMR: δ 2.61 (s, 1H), 5.30 (br s, 2H), 6.71 (s, 1H), 7.10 (s, 1H), 7.45 (s, 1H), 7.70 (m, 4H), 7.90(s, 1H), 8.15 (m, 3H), 9.5 (s, 1H); ^{13}C NMR: δ 40.7, 42.4, 110.1, 112.1, 117.8, 118.3, 122.5, 127.5, 131.6, 132.8, 132.9, 134.2, 142.4, 142.9, 143.5, 151.5, 161.9, 164.8, 165.6, 166.7; MS: *m/z* 397.10 (M^+). Anal. Calcd for $C_{22}H_{15}N_5O_3$ (397.39): C, 66.43; H, 3.77; N, 17.61. Found: C, 66.47; H, 3.80; N, 17.65.

3-({4-[6-(Furan-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl]phenyl} amino)-7-methylquinoxalin-2(1*H*)-one (11b): Yield (0.297 g, 72%); mp 158–160°C; ^1H NMR: δ 2.95 (s, 3H), 2.98 (s, 1H), 5.20 (s, 1H), 6.80 (s, 1H), 7.10 (s, 1H), 7.25 (s, 1H), 7.66 (m, 4H), 7.90–8.29 (m, 4H), 9.50 (s, 1H); ^{13}C NMR: δ 21.1, 39.7, 40.4, 113.1, 118.1, 118.8, 128.3, 128.5, 130.5, 130.6, 130.8, 131.1, 134.2, 140.4, 140.9, 145.5, 145.5, 145.9, 159.8, 186.6, 186.7; MS: *m/z* 411.20 (M^+). Anal. Calcd for $C_{23}H_{17}N_5O_3$ (411.41): C, 67.08; H, 4.13; N, 17.01. Found: C, 67.11; H, 4.16; N, 17.04.

Molecular modeling

The Molegro Virtual Docker (MVD) program was used to perform docking. The protein-ligand interaction energies of the examined compounds were calculated using the Pose Organizer option in the MVD program [42] for five different orientations.

The structure of enzyme 1KE8 was downloaded from the PDB. The docking results are shown in Table 1.

Biological activity

SRB assay of cytotoxicity was used to analyze the effect of the synthesized compounds on the $HEPG_2$ human tumor cell line. The tumor cells were obtained frozen in liquid nitrogen (-180°C) from the American type culture collection, RPMI-1640 medium (Sigma Chemicals, St. Louis, MO, USA). Monolayer cells were incubated with the compounds for 48 h before use; the medium was warmed at 37°C in a water bath and supplemented with penicillin/streptomycin and FBS. Cells were planted in 96-multiwell plates (5×10^4–10^5 cells/well) for 24 h before treatment with the compounds to allow attachment of cells to the wall of the plate. Different concentrations of the compounds tested (0, 5, 12.5, 25 and 50 μg/mL) were added to the cell monolayer at 37°C and in an atmosphere of 5% CO_2. Control cells were treated with vehicle alone. Cultures were then fixed with trichloroacetic acid and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and protein-bound dye was extracted with 10 mM unbuffered Tris base [tris(hydroxymethyl)aminomethane] for determination of optical density (OD). The OD of each well was measured spectrophotometrically at 564 nm with an ELIZA microplate reader.

The mean background value of each drug concentration was calculated. The percentage of cell survival was calculated as follows: survival fraction=OD (treated cells)/OD (control cells).

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