ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Convenient Synthesis of *N*-Alkyl-2-(3-phenyl-quinoxalin-2-ylsulfanyl)acetamides and Methyl-2-[2-(3-phenyl-quinoxalin-2-ylsulfanyl)acetylamino]alkanoates

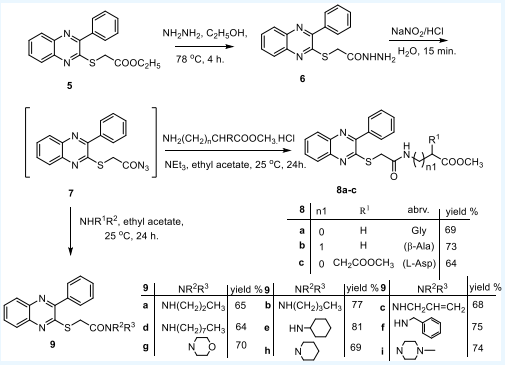
Samir Mohamed El Rayes,* Gaber El-Enany, Mohamed Sayed Gomaa, Ibrahim A. I. Ali, Walid Fathalla, Faheem Hyder Pottoo, and Firdos Alam Khan

Cite This: *ACS Omega* 2022, 7, 34166−34176

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: A series of 27 new quinoxaline derivatives (*N*-alkyl-[2-(3-phenyl-quinoxalin-2-ylsulfanyl)]acetamides, methyl-2-[2-(3-phenylquinoxalin-2-ylsulfanyl)-acetylamino]alkanoates, and their corresponding dipeptides) were prepared from 3-phenylquinoxaline-2(1*H*)-thione based on the chemoselective reaction with soft electrophiles. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to study the efficacy of 27 compounds on cancer cell viability and proliferation. A total of 13 compounds (**4a−c**, **5**, **6**, **8c**, **9c**, **9f**, **10a**, **10b**, **11c**, **12b**, and **12c**) showed inhibitory action on HCT-116 cancer cells and 15 compounds (**4a−c**, **5**, **6**, **8c**, **9a**, **9c**, **9f**, **9h**, **10b**, **11c**, **12a**, **12b**, and **12c**) showed activity on MCF-7 cancer cells, with compound **10b** exhibiting the highest inhibitory action (IC_{50} 1.52 and 2 μg/mL, respectively) on both cell lines. The molecular modeling studies on the human thymidylate synthase (hTS) homodimer interface showed that these compounds are good binders and could selectively inhibit the enzyme by stabilizing its inactive conformation. The study also identified key residues for homodimer binding, which could be used for further optimization and development.



1. INTRODUCTION

Quinoxaline possesses a wide variety of therapeutic properties. Many quinoxaline derivatives have been found with distinct anticancer,[1] antiviral,[2] anthelmintic,[3] antimicrobial,[4] anti-inflammatory,[5] antioxidant,[6] and antiprotozoal activities.[7] Quinoxaline and its derivatives have recently been recognized as effective chemotherapeutic agents against a number of tumors.[8−10] Earlier discussion on the feasibility of quinoxaline anticancer activity revealed a number of pathways including the inhibition of enzymes (tyrosine kinases and c-MET kinase)[11−14] as well as induction of apoptosis and tumor hypoxia.[15−17] Recently, we have studied the structure−activity relationship in methyl-2-[3-(3-phenyl-quinoxalin-2-ylsulfanyl)-propanamido]alkanoates and *N*-alkyl-3-((3-phenylquinoxalin-2-yl)sulfanyl)propanamides by molecular docking via examining the binding affinity to the human thymidylate synthase (hTS) allosteric site.[18] This study proved the significance of the peptidomimetic side chain at position 3 of the quinoxaline ring. These compounds were tested against human HCT-116 and MCF-7 cell lines and showed remarkable results with IC_{50} values in the range of 1.9−7.52 μg/mL compared to the reference drug doxorubicin (IC_{50} 3.23 μg/mL). In continuation to this study, we found it interesting to prepare a series of *N*-alkyl-[2-(3-phenyl-quinoxalin-2-ylsulfanyl)] acetamides, methyl-2-[2-(3-phenylquinoxalin-2-ylsulfanyl)-acetylamino] alkanoates, and their corresponding dipeptides as new anticancer drugs. The newly synthesized derivatives were screened for their antitumor activity against the liver carcinoma cell line (HepG2). The mechanism of the antiproliferative activity of the synthesized compounds was studied through their binding to the human thymidylate synthase (hTS) homodimer interface using molecular docking.

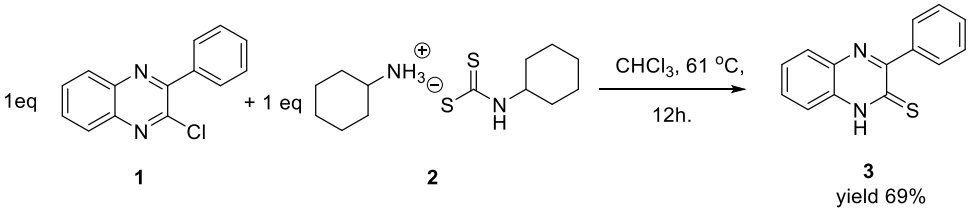
2. RESULTS AND DISCUSSION

2.1. Chemistry. The feasibility of *N*-cyclohexyldithiocarbamatecyclohexylammonium salt **2** as an excellent thiating reagent was earlier discussed in a number of articles.[18−20] 3-Phenylquinoxaline-2(1*H*)-thione (**3**) could be obtained simply by the reaction of chloroquinoxaline **1** with **2** in chloroform for 12 h at 61 °C to afford **3** in excellent yield, Scheme 1.[18,19]

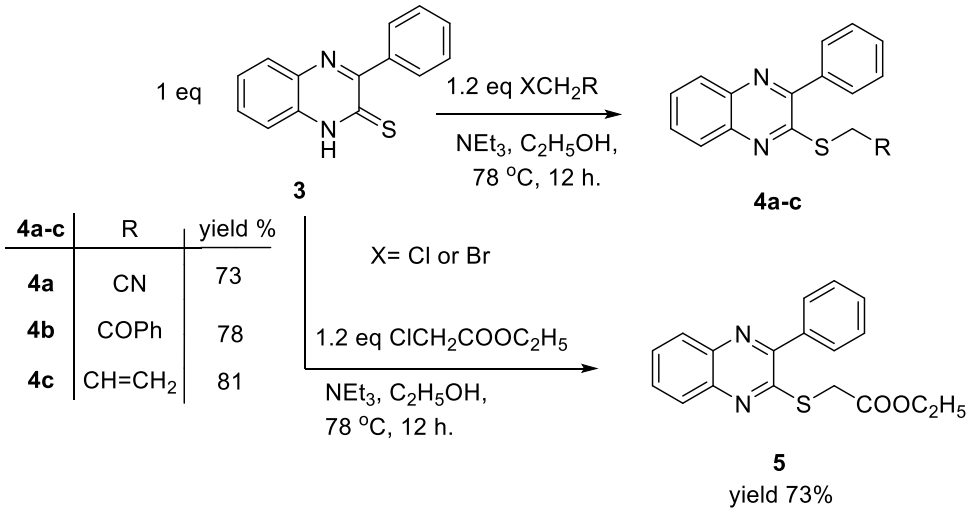
The model compound 3-phenylquinoxaline-2(1*H*)-thione (**3**) as a heterocyclic thioamide is presented in a tautomeric mixture between thiol and thione forms.[21,22] This ambident nucleophile behavior of **3** could be invested to modify the

Received: June 6, 2022
Accepted: September 5, 2022
Published: September 15, 2022

Scheme 1. Preparation of Phenylquinoxaline-2(1*H*)-thione (3)

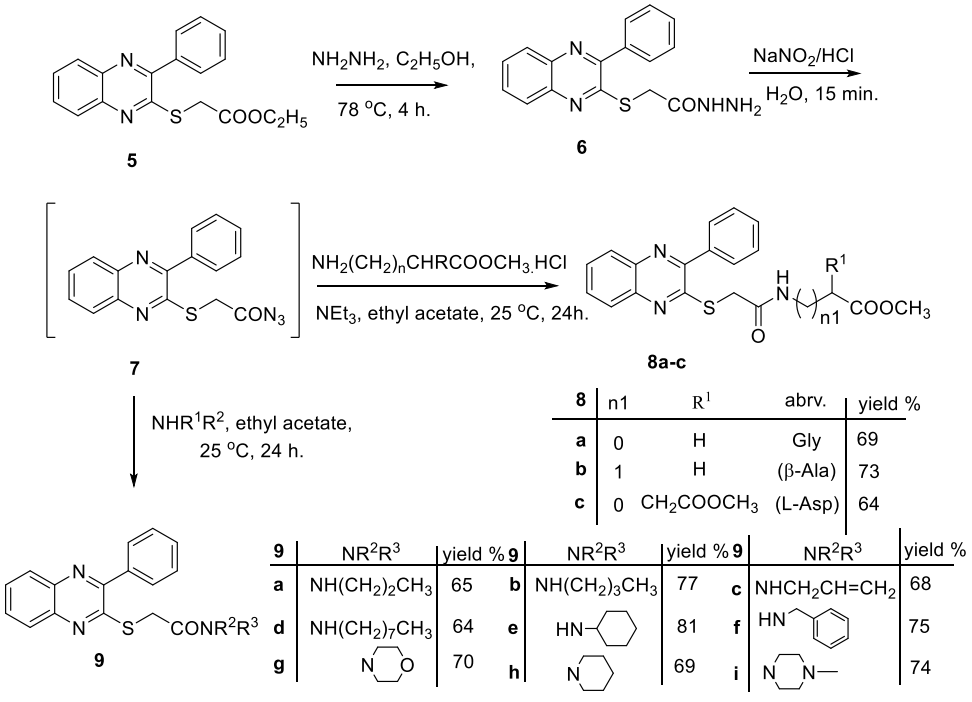


Scheme 2. Reaction of Phenylquinoxaline-2(1*H*)-thione (3) with Soft Electrophiles



4a-c	R	yield %
4a	CN	73
4b	COPh	78
4c	$CH=CH_2$	81

Scheme 3. Preparation of Methyl-2-[2-(3-phenylquinoxalin-2-ylsulfanyl)acetylamino]alkanoates 8a−c and *N*-Alkyl-[2-(3-phenyl-quinoxalin-2-ylsulfanyl)]acetamides 9a−i



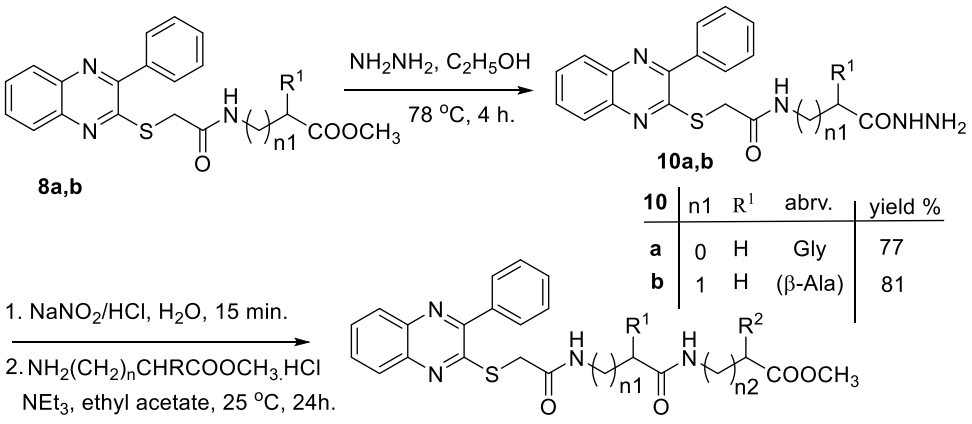
8	n1	R^1	abrv.	yield %
a	0	H	Gly	69
b	1	H	(β-Ala)	73
c	0	CH_2COOCH_3	(L-Asp)	64

9	NR^2R^3	yield %	9	NR^2R^3	yield %	9	NR^2R^3	yield %
a	$NH(CH_2)_2CH_3$	65	b	$NH(CH_2)_3CH_3$	77	c	$NHCH_2CH=CH_2$	68
d	$NH(CH_2)_7CH_3$	64	e	HN-⟨⟩	81	f	HN-⟨⟩	75
g	N⟨⟩O	70	h	N⟨⟩	69	i	N⟨⟩N—	74

quinoxaline ring structure by simple chemoselective alkylation reactions at nitrogen and sulfur atoms. However, the unique structure of **3** bearing a phenyl group contributing to a continuous conjugation in the whole molecule makes the *S*-atom bear both soft and hard characteristics. This was practically proved in our previous research following the reaction of 3-phenylquinoxaline-2(1*H*)-thione (**3**) with hard electrophilic alkylating reagents (activated acrylic acid compounds) to give *S*-substituted derivatives.[18] Herein, we wish to report the reaction of model quinoxaline **3** with soft electrophiles and to invest the products to prepare a number of biologically promising compounds. Thus, **3** reacted with a

https://doi.org/10.1021/acsomega.2c03522
ACS Omega 2022, 7, 34166−34176

Scheme 4. Preparation of Dipeptides 11−12(a−c)



10	n1	R^1	abrv.	yield %
a	0	H	Gly	77
b	1	H	(β-Ala)	81

11	n1	R^1	n2	R^2	abrv.	yield %
a	0	H	0	H	Gly-Gly	57
b	0	H	1	H	Gly-β-Ala	51
c	0	H	0	CH_2COOCH_3	Gly-L-Asp	62

from **8a** and **10a**

12	n1	R^1	n2	R^2	abrv.	yield %
a	1	H	0	H	β-Ala-Gly	65
b	1	H	1	H	β-Ala-β-Ala	62
c	1	H	0	CH_2COOCH_3	β-Ala-L-Asp	51

from **8b** and **10b**

number of soft electrophiles (chloroacetonitrile, phenacyl chloride, allyl bromide, and ethyl chloroacetate) in the presence of triethylamine to give *S*-alkylated derivatives **4a−c** and **5**, respectively, in 73−81 and 73% yields, Scheme 2.

The structure assignment of the prepared *S*-substituted quinoxaline derivatives **4a−c** and **5** is based on ^{1}H and ^{13}C NMR spectral and physicochemical analysis. The ^{1}H NMR spectrum of ethyl(3-phenyl-quinoxalin-2-ylsulfanyl)acetate (**5**) shows an interesting singlet signal at 4.03 ppm corresponding to the SCH_2CO group, which clearly confirms the site of alkylation. The ^{1}H NMR spectrum of **5** also shows two signals at 4.25 and 1.29 ppm corresponding to ester OCH_2CH_3 beside several multiplet signals ranging between 8.08 and 7.51 ppm for nine aromatic protons. The ^{13}C NMR spectrum of **5** displays an interesting signal at 33.4 ppm for SCH_2CO, which once again confirms the site of alkylation. The ^{13}C NMR spectrum of **5** also shows signals at δ 169.2, 61.6, and 14.2 ppm corresponding to C═O, OCH_2, and CH_3 groups, respectively.

The *S*-substituted ester ethyl(3-phenylquinoxalin-2-ylsulfanyl)acetate (**5**) is an excellent precursor for the structural modification of the quinoxaline ring system at the sulfur atom and the introduction of either amino acid or alkyl amine residues via the azide coupling method.[23,24]

Ester **5** was refluxed with hydrazine hydrate in ethyl alcohol to afford the corresponding hydrazides **6** in 82% yield, Scheme 3. Hydrazide **6** was converted to the corresponding carbonyl azide derivative **7** by treatment with a $NaNO_2$ and HCl mixture in an ice bath for 15 min and was extracted with ethyl acetate. The in situ-generated ethyl acetate solution of azide **7** was used without purification and reacted with amino acid methyl ester hydrochlorides (glycine, *β*-alanine, and L-aspartic acid) in the presence of triethylamine to afford a series of *S*-substituted methyl-2-[2-(3-phenylquinoxalin-2-ylsulfanyl)-acetylamino]alkanoates **8a−c** in good yields, Scheme 3. Similarly, the in situ-generated ethyl acetate solution of azide **7** reacted with alkane amines at room temperature for 24 h to afford a series of *N*-alkyl-[2-(3-phenyl-quinoxalin-2-ylsulfanyl)]acetamides **9a−i**, Scheme 3.

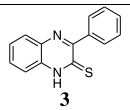
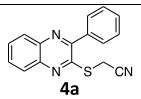
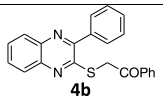
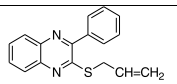
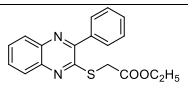
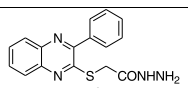
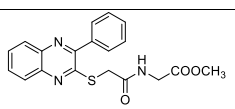
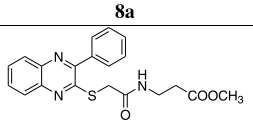
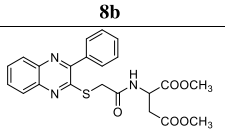
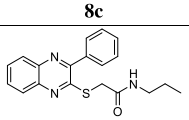
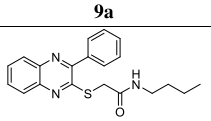
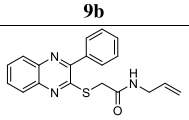
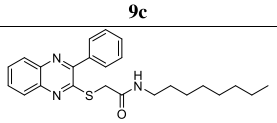
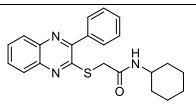
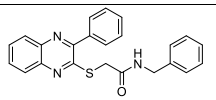
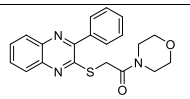
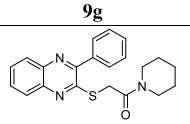
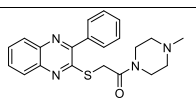
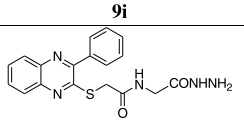
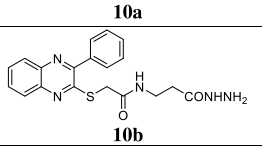
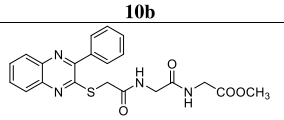
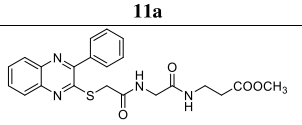
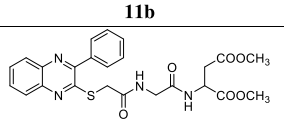
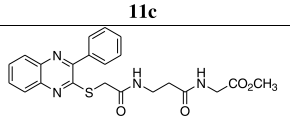
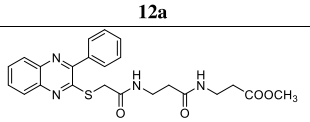
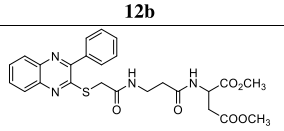
The structure assignment of the prepared methyl-2-[2-(3-phenylquinoxalin-2-ylsulfanyl)acetylamino]alkanoates **8a−c** and *N*-alkyl-[2-(3-phenyl-quinoxalin-2-ylsulfanyl)]acetamides **9a−i** is based on ^{1}H and ^{13}C NMR spectral and physicochemical analysis. The ^{1}H NMR spectrum of methyl-[2-(3-phenyl-quinoxalin-2-ylsulfanyl)acetylamino]acetate (**8a**) showed signals at δ 7.28, 4.22, 3.96, and 3.72 ppm corresponding to NH, SCH_2, NCH_2, and OCH_3, respectively. The ^{13}C NMR spectrum of **8a** showed signals at δ 172.12, 168.3, 52.4, 41.7, and 34.6 ppm corresponding to two C═O, OCH_3, $NHCH_2$, and SCH_2, respectively.

Next, we attempted the modification of the quinoxaline residue chemical structure of our model by the attachment of the dipeptide residue to enhance the biological activity. Thus, the reactions of amino acid derivatives **8a** and **8b** with hydrazine hydrate in ethanol for 4 h afforded hydrazides **10a** and **10b** in good yields. Hydrazides **10a** and **10b** were first reacted with a $NaNO_2$ and HCl mixture in an ice bath for 15 min and then extracted with ethyl acetate and reacted simultaneously with amino acid methyl ester hydrochlorides (glycine, *β*-alanine, and L-aspartic acid) to afford dipeptides **11−12(a−c)** in good yields, Scheme 4.

2.2. Antiproliferative Activities. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out to study the impact of 27 compounds on cancer cell viability and proliferation. The cytotoxic effects of the compounds were observed after 48 h of treatment. Fourteen compounds (**4a−c**, **5**, **6**, **8c**, **9c**, **9f**, **10a−c**, **11c**, **12b**, and **12c**) showed inhibitory action on HCT-116 cancer cells, whereas the remaining 13 compounds did not show any inhibitory action on the cancerous cells. We calculated the IC_{50} values for these compounds, and compound **10b** (*β*-ala hydrazide)

https://doi.org/10.1021/acsomega.2c03522
ACS Omega 2022, 7, 34166−34176

Table 1. Impact of Synthetic Compounds on Cancer Cells Using the MTT Assay[a]

S. No.	Structure of compounds	IC_{50} Value [μg/mL] **HCT-116**	IC_{50} Value [μg/mL] **MCF-7**
1	 **3**	NA	NA
2	 **4a**	3.20	6.62
3	 **4b**	2.19	2.84
4	 **4c**	2.41	2.92
5	 **5**	2.19	2.38
6	 **6**	3.10	7.56
7	 **8a**	NA	NA
8	 **8b**	NA	NA
9	 **8c**	2.74	3.24
10	 **9a**	NA	3.46
11	 **9b**	NA	NA
12	 **9c**	6.74	3.70
13	 **9d**	NA	NA
14	 **9e**	NA	NA
15	 **9f**	1.79	2.24
16	 **9g**	NA	NA
17	 **9h**	NA	7.95
18	 **9i**	NA	NA
19	 **10a**	5.94	NA
20	 **10b**	1.52	2.00
21	 **11a**	NA	NA
22	 **11b**	NA	NA
23	 **11c**	3.20	6.62
24	 **12a**	NA	3.03
25	 **12b**	2.07	2.39
26	 **12c**	1.79	2.24

[a]NA = not active. IC_{50} value [μg/mL] = inhibitory concentration (IC) is expressed in μg/mL.

showed the highest inhibitory action, whereas compound **9c** (allylamine derivative) showed the lowest inhibitory action on HCT-116 (Table 1). We also examined inhibitory action on MCF-7 cells. We found that 15 compounds (**4a−c**, **5**, **6**, **8c**, **9a**,

https://doi.org/10.1021/acsomega.2c03522
ACS Omega 2022, 7, 34166−34176

9c, **9f**, **9h**, **10b**, **11c**, **12a**, **12b**, and **12c**) showed inhibitory action on MCF-7 cancer cells, whereas the remaining 11 compounds did not show any inhibitory action on the cancerous cells. We calculated the IC_{50} values for these compounds and compound **10b** showed the highest inhibitory action, whereas compound **6** showed the lowest inhibitory action on MCF-7 (Table 1).

Next, we wanted to know whether these compounds selectively target the cancerous cells or not. We tested these compounds on normal cells (HEK-293) at the same concentrations and duration of treatments. The cell viability assay using MTT revealed null cytotoxic effects on the normal cells.

We do not know the molecular mechanism of cancer cell death, so it would be interesting to study the role of apoptotic pathways in synthetic compound-mediated cancer cell death. There are reports of nanoparticle-induced nuclear fragmentation and disintegration in cancer cells.[25−29] We suggest that these synthetic compounds possess selective targeting capability to cancerous cells and could be potential candidates for cancer treatments.

2.3. Molecular Modeling. The preliminary structure−activity relationship of the synthesized peptidomimetics showed that substitution of the thiol group with a simple methyl-bearing group capable of HB formation showed good activity (**4a−c**, and **5**). Substitution of the thiol at position 3 with a peptidomimetic side chain bearing a single peptide or amide bond gave variable results with some compounds showing as good activity as simple alkyl substitution (**8c**, **10b**, and **9f**), while others were inactive (**8a**, **8b**, and **9d**).

Further extension of the molecules through formation of dipeptides that are connected to the quinazoline scaffold through a glycine structure gave almost inactive compounds (**11a**, **11b**, and **12a**). However, when the dipeptide is connected through a *β*-alanine structure, the activity was regained (**12b** and **12c**).

Molecular docking was carried out to explain the results of the antiproliferative assay further and obtain better insights into the binding requirements of these quinazoline peptidomimetics at the hTS interface.

Key interactions at protein−protein interfaces represent important targets for small molecule inhibition. This kind of inhibition, unlike targeting the active site, inhibits intracellular hTS and cell growth without leading to overexpression of the protein, thereby conferring more selectivity and specificity.[30]

Peptide and nonpeptide[31] inhibitors were demonstrated by X-ray crystallographic studies to bind hTS at the homodimer interface and showed allosteric inhibition of the enzyme through stabilizing its inactive form. Our peptidomimetics were shown to bind the hTS dimer interface and potentially stabilize its di-inactive form. The designed peptidomimetics use their peptide-like structure for optimal binding without being peptides in nature, which makes them more suitable for pharmaceutical manipulations and development.

Upon computational docking, the inhibitors were found predominantly at the dimer interface in poses that align with the cocrystallized peptide and maintained the key interactions with the target protein (hTS, 3N5E). This was mainly through conserving H-bonding with key residues: Gln172, Arg175, Ile 190, Met191, and Cys192 from chain A and Leu204 and Pro205 from chain B (Figure 1).

The docking results showed that the most active compounds (**5**, **10b**, **9f**, **12b**, and **12c**) lie at the interface of the homodimer and established interactions with both chains of the homodimer and with the mentioned key residues (Figure 1).

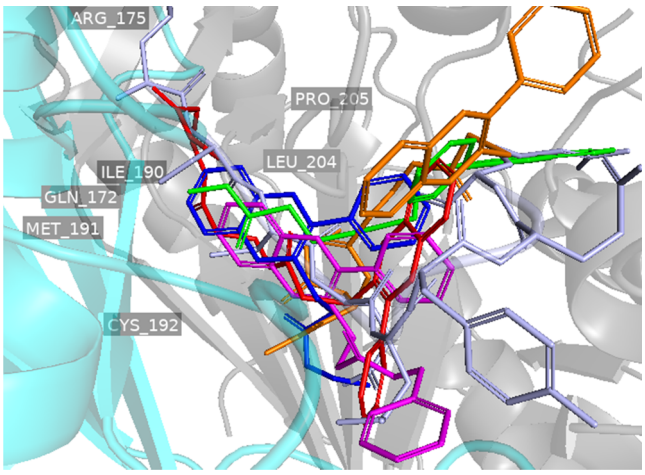


Figure 1. Most active compounds **10b** green, **12b** red, **12c** brown, **5** blue, and **9f** magenta and the crystallized inhibitor light blue docked at the hTS homodimer interface and showing key binding residues. The backbone is represented as cartoons: gray for chain A and cyan for chain B.

The most active compounds showed good stability and affinity to the active site by holding very close conformations and key interactions for most of the provided docked conformations with a relatively low root-mean-square deviation (RMSD) value (Figure 2).

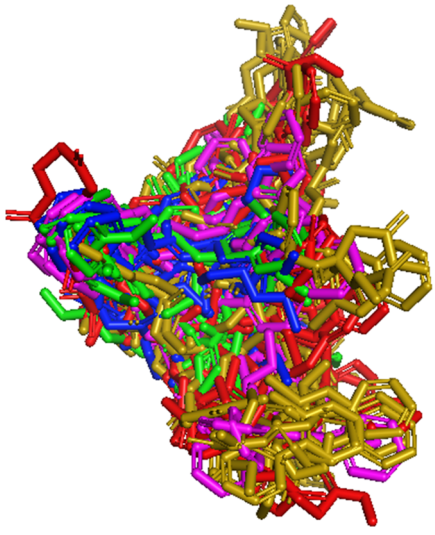


Figure 2. Docking conformations of most active compounds. **10b** green, **12b** red, **12c** brown, **5** blue, and **9f** magenta.

In these compounds, the binding affinity also correlates well with the experimental IC_{50} value (Table 2).

Inactive compound **9d** was not able to maintain the interaction with key residues in most of the docking poses and showed variable poses at the interface that could reflect the low stability and affinity of this compound, as shown in Figure 3. The long hydrophobic alkyl chain was mainly found solvent exposed, and this could have affected the stability of the compound in the binding pocket.

https://doi.org/10.1021/acsomega.2c03522
ACS Omega 2022, 7, 34166−34176

Table 2. Binding Affinities and IC_{50} for Most Active Compounds

	binding affinity (kcal/mol)	IC_{50} values (μg/mL) HCT-116	IC_{50} values (μg/mL) MCF-7
10b	−10.3	1.52	2.00
12b	−8.9	2.07	2.39
12c	−10.7	1.79	2.24
5	−8.8	2.19	2.38
9f	−9.2	1.79	2.24
crystallized inhibitor	−7.6		

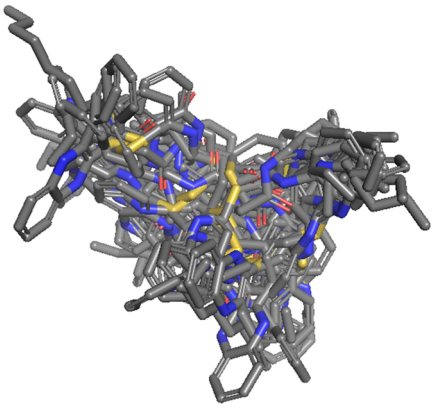


Figure 3. Compound **9d** docking conformations.

3. EXPERIMENTAL SECTION

3.1. Chemistry. *3.1.1. General Procedures.* The solvent was purified and dried in the usual way. The boiling range of petroleum ether used was 40−60 °C. Thin-layer chromatography (TLC) silica gel 60 F_{254} plastic plates (E. Merck, layer thickness 0.2 mm) were detected by UV absorption. Elemental analyses were performed on a Flash EA-1112 instrument at the Microanalytical Laboratory, Faculty of Science, Suez Canal University, Ismailia, Egypt. Melting points were determined on a Buchi 510 melting point apparatus, and the values are uncorrected. NMR spectra were measured with a Bruker 400 MHz, and tetramethylsilane (TMS) (0.00 ppm) was used as an internal standard. 2-Chloro-3-phenylquinoxaline (**1**) was prepared according to the method described.[32]

*3.1.1.1. Preparation of Phenylquinoxaline-2(1H)-thione (**3**).* To a solution of 2-chloro-3-phenylquinoxaline (**1**, 2.5 mmol) in $CHCl_3$ (25 mL) was added *N*-cyclohexyldithiocarbamatecyclohexylammonium salt **2** (0.69 g, 2.5 mmol). The reaction mixture was refluxed at 61 °C for 12 h. The reaction mixture was evaporated under reduced pressure, and 25 mL of ethanol was added to the solid residue. A yellowish precipitate was filtered to give the desired product and crystallized from ethanol. Yield 69%, yellow powder, mp 224−225 °C. ^{1}H NMR spectrum (300 MHz, dimethyl sulfoxide (DMSO)), δ, ppm (*J*, Hz): 14.56 (1H, bs, NH), 8.48−8.37 (1H, m, ArH), 8.18−8.01 (2H, m, ArH), 7.85−7.78 (1H, m, ArH), 7.41−7.33 (5H, m, ArH). Found, %: C, 70.13; H, 3.84; N, 11.29. For $C_{14}H_{10}N_2S$ (236.1). Calcd, %: C, 70.56; H, 4.23; N, 11.76.

3.1.2. General Procedure for Alkylation. To a mixture of quinoxaline **3** (0.24 g, 1.0 mmol) and triethylamine (0.2 mL, 2.0 mmol) in ethyl alcohol (30 mL, 95%), alkylating agents (chloroacetonitrile, 2-bromo-1-phenyl-ethanone, allyl bromide, and/or ethyl chloroacetate) (1.0 mmol) were added. The reaction mixture was heated under reflux for 12 h and concentrated under reduced pressure. The solid obtained was filtered and crystallized from ethyl alcohol.

*3.1.2.1. (3-Phenyl-quinoxalin-2-ylsulfanyl)acetonitrile (**4a**).* From chloroacetonitrile. Yield 73%, yellow powder, mp 137−139 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 8.07 (1H, d, *J* = 8 Hz, ArH), 8.04 (1H, d, *J* = 8 Hz, ArH), 7.81−7.79 (2H, m, ArH), 7.71 (2H, t, *J* = 8.0 Hz, ArH), 7.53−7.50 (3H, m, ArH), 4.43 (2H, s, SCH_2). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 154.7, 153.4, 141.3, 139.7, 136.3, 129.8, 129.4 129.1, 129.0, 128.5, 128.3, 127.6 (C Ar), 115.6 (CN), 25.3 (CH_2). Mass spectrometry (MS) (matrix-assisted laser desorption ionization (MALDI), positive mode, matrix DHB) *m*/*z*: 300 $(M + Na)^+$. Found, % C, 69.78; H, 3.96; N, 15.02. For $C_{16}H_{11}N_3S$ (277.3). Calcd % C, 69.29; H, 4.00; N, 15.15; S, 11.56.

*3.1.2.2. 1-Phenyl-2-(3-phenyl-quinoxalin-2-ylsulfanyl)-ethanone (**4b**).* From phenacyl bromide. Yield 78%, yellow powder, mp 120−122 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 8.09 (1H, d, *J* = 8 Hz, ArH), 8.07−8.03 (3H, m, ArH), 7.84−7.82 (2H, m, ArH), 7.80 (2H, d, *J* = 8 Hz, ArH), 7.64−7.61 (3H, m, ArH), 7.51−7.48 (3H, m, ArH), 4.98 (2H, s, SCH_2). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 189.3 (C═O), 153.4, 153.3, 141.3, 139.7, 136.3, 135.4, 131.9, 129.8, 129.1, 129.0, 128.6, 128.4, 127.4, 118.3, 43.3 (SCH_2). MS (MALDI, positive mode, matrix DHB) *m*/*z*: 379 $(M + Na)^+$. Found, % C, 74.51; H, 4.21; N, 7.77. For $C_{22}H_{16}N_2OS$ (356.4). Calcd % C, 74.13; H, 4.52; N, 7.86; S, 9.00.

*3.1.2.3. 2-Allylsulfanyl-3-phenyl-quinoxaline (**4c**).* From allyl bromide. Yield 81%, yellow powder, mp 79−81 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 8.10 (1H, d, *J* = 8 Hz, ArH), 7.91 (1H, d, *J* = 8 Hz, ArH), 7.80−7.78 (2H, m, ArH), 7.74−7.72 (2H, m, ArH), 7.56−7.53 (3H, m, ArH), 5.89−5.86 (1H, m, CH); 5.15 (1H, d, CH_2, *J* = 17.2 Hz), 5.03 (1H, d, CH_2, *J* = 9.2 Hz), 4.02 (2H, d, SCH_2, *J* = 5.6 Hz). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 154.5, 153.2, 141.8, 139.7, 136.9, 132.4 (CH), 13.1, 129.7, 129.3, 129.0, 128.6, 128.3 127.0, 117.9 (CH_2), 34.8 (SCH_2). MS (MALDI, positive mode, matrix DHB) *m*/*z*: 302 $(M + Na)^+$. Found, % C, 73.21; H, 5.12; N, 10.47. For $C_{17}H_{14}N_2S$ (278.4). Calcd % C, 73.35; H, 5.07; N, 10.06; S, 11.52.

*3.1.2.4. Ethyl-(3-phenyl-quinoxalin-2-ylsulfanyl)acetate (**5**).* From ethyl chloroacetate. Yield 73%, yellow powder, mp 93−95 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 8.08 (1H, d, *J* = 8 Hz, ArH), 8.05 (1H, d, *J* = 8 Hz, ArH), 7.83−7.80 (2H, m, ArH), 7.69 (1H, *t*, *J* = 8.0 Hz, ArH), 7.64 (1H, *t*, *J* = 8.0 Hz, ArH), 7.54−7.51 (3H, m, ArH), 4.25 (2H, *q*, *J* = 7.2 Hz, OCH_2), 4.03 (2H, s, SCH_2), 1.29 (3H, *t*, *J* = 7.2 Hz, CH_3). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 169.2 (C═O), 153.6, 153.0, 141.2, 139.6, 136.9, 129.9, 129.1, 129.0, 128.5, 128.3, 127.4, 61.6 (OCH_2), 33.4 (SCH_2), 14.2 (CH_3). MS (MALDI, positive mode, matrix DHB) *m*/*z*: 347 $(M + Na)^+$. Found, % C, 66.55; H, 5.05; N, 8.72. For $C_{18}H_{16}N_2O_2S$ (324.4). Calcd % C, 66.64; H, 4.97; N, 8.64; S, 9.88.

*3.1.2.5. (3-Phenyl-quinoxalin-2-ylsulfanyl)acetic Acid Hydrazide (**6**).* Hydrazine hydrate (80%, 2.4 mL, 5 mmol) was added to a solution of ester **5** (0.33 g, 1.0 mmol) in absolute ethanol (30 mL). The reaction mixture was refluxed for 4 h and cooled. The resultant precipitate was filtered off, washed

https://doi.org/10.1021/acsomega.2c03522
ACS Omega 2022, 7, 34166−34176

with ethanol and diethyl ether, and then crystallized from aqueous ethanol to yield the corresponding hydrazide.

Yield 82%, yellow powder, mp 166−168 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (J, Hz): 9.36 (1H, bs, NH), 8.05−8.02 (2H, m, ArH), 7.85−7.80 (4H, m, ArH), 7.18−7.16 (3H, m, ArH), 4.39 (2H, bs, NH_2), 3.98 (2H, s, SCH_2). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 171.2 (C═O). 153.3, 153.2, 141.7, 139.5, 136.7, 129.8, 129.3, 129.1, 128.7, 127.8, 34.5 (SCH_2). MS (MALDI, positive mode, matrix DHB) m/z: 333 $(M + Na)^+$. Found, % C, 61.87; H, 4.63; N, 18.11. For $C_{16}H_{14}N_4OS$ (310.4). Calcd % C, 61.92; H, 4.55; N, 18.05; S, 10.33.

*3.1.2.6. Preparation of Methyl-2-[2-(3-phenylquinoxalin-2-ylsulfanyl)acetylamino]alkanoates **8a−c** and N-Alkyl-[2-(3-phenyl-quinoxalin-2- ylsulfanyl)]acetamides **9a−i**.* General method: A solution of $NaNO_2$ (0.34 g, 5.0 mmol) in cold water (3 mL) was added to a cold solution (−5 °C) of hydrazide **6** (0.31 g, 1.0 mmol) in AcOH (6 mL), 1 N HCl (3 mL), and water (25 mL). After stirring at −5 °C for 15 min, a yellowish syrup started to form. The reaction mixture was stirred in an ice bath for another 1 h. The reaction mixture was extracted twice with ethyl acetate (30 mL). The combined organic layer was washed with 0.5 N HCl (30 mL), 3% $NaHCO_3$ (30 mL), and H_2O (30 mL) and finally dried over Na_2SO_4 (10 g) to give an ethyl acetate solution of azide **7**. A solution of an appropriate amino acid ester hydrochloride (1.0 mmol) in ethyl acetate (20 mL) containing triethylamine (0.2 mL, 2 mmol) or the appropriate alkane amine (1.0 mmol) in ethyl acetate (20 mL) was added to the solution of azide **7**. The mixture was kept at −5 °C for 24 h and then at 25 °C for another 24 h, followed by washing with 0.5 N HCl (30 mL), 3% $NaHCO_3$ (30 mL), and H_2O (30 mL), and finally dried over Na_2SO_4 (10 g). The solution was evaporated to dryness, and the residue was recrystallized from petroleum ether−ethyl acetate, 1:3, to give desired *S*-coupled products **8a−c** and **9a−i**.

*3.1.2.7. Methyl-2-[2-(3-phenyl-quinoxalin-2-ylsulfanyl)-acetylamino]acetate (**8a**).* From GlyOMe. Yield 69%, yellow powder, mp 84−85 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (J, Hz): 8.11 (1H, d, J = 8 Hz, ArH), 8.07 (1H, d, J = 8 Hz, ArH), 7.76−7.72 (2H, m, ArH), 7.69 (1H, t, J = 8.0 Hz, ArH), 7.57 (1H, t, J = 8.0 Hz, ArH), 7.48−7.45 (3H, m, ArH), 7.28 (1H, bs, NH), 4.22 (2H, s, CH_2), 3.96 (2H, s, $\underline{SCH_2}$); 3.72 (3H, s, OCH_3). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 172.1 (C═O), 168.3 (C═O), 153.5, 153.1, 141.2, 139.5, 137.0, 129.7, 129.3, 129.1, 128.7, 127.5, 52.4 (OCH_3), 41.7 (NCH_2), 34.6 (SCH_2). MS (MALDI, positive mode, matrix DHB) m/z: 390 $(M + Na)^+$. Found, % C, 62.02; H, 4.43; N, 11.21. For $C_{19}H_{17}N_3O_3S$ (367.4). Calcd % C, 62.11; H, 4.66; N, 11.44; S, 8.73.

*3.1.2.8. Methyl-3-[2-(3-phenyl-quinoxalin-2-ylsulfanyl)-acetylamino]propanoate (**8b**).* From β-AlaOMe. Yield 73%, yellow powder, mp 77−78 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (J, Hz): 8.14 (1H, d, J = 8 Hz, ArH), 8.03 (1H, d, J = 8 Hz, ArH), 7.77−7.75 (2H, m, ArH), 7.74−7.73 (2H, m, ArH), 7.55−7.53 (3H, m, ArH), 7.36−7.34 (1H, m, NH), 3.89 (2H, s, SCH_2), 3.49−3.47 (2H, m, $NHCH_2$), 3.35 (3H, s, OMe), 2.45−2.43 (2H, m, CH_2CO). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 172.8 (C═O), 168.9 (C═O), 153.4, 153.2, 141.4, 139.6, 137.4, 129.5, 129.4, 129.2, 128.6, 127.7, 51.6 (OCH_3), 36.9 ($NHCH_2$), 34.9 (SCH_2), 33.8 (CH_2CO). MS (MALDI, positive mode, matrix DHB) m/z: 404 $(M + Na)^+$. Found, % C, 62.86; H, 4.96; N, 11.16. For $C_{20}H_{19}N_3O_3S$ (381.5). Calcd % C, 62.97; H, 5.02; N, 11.02; S, 8.41.

*3.1.2.9. Dimethyl-2-[2-(3-phenyl-quinoxalin-2-ylsulfanyl)-acetylamino]succinate (**8c**).* From L-AspOMe. Yield 64%, yellow powder, mp 132−134 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (J, Hz): 8.10 (1H, d, J = 8 Hz, ArH), 7.98 (1H, d, J = 8 Hz, ArH), 7.78−7.76 (2H, m, ArH), 7.71−7.69 (2H, m, ArH), 7.55−7.52 (3H, m, ArH), 6.86−6.84 (1H, m, NH), 4.84−4.82 (1H, m, NHCH), 3.93 (2H, s, SCH_2), 3.60 (3H, s, OMe), 3.32 (3H, s, OMe), 2.88−2.86 (2H, m, CH_2CO). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 172.7 (C═O), 172.6 (C═O), 168.3 (C═O), 153.5, 153.3, 141.4, 139.4, 137.7, 129.8, 129.6, 129.3, 128.7, 127.7, 55.4 (CH), 52.4 (OCH_3), 51.9 (OCH_3), 34.6 (SCH_2), 34.1 (CH_2CO). MS (MALDI, positive mode, matrix DHB) m/z: 462 $(M + Na)^+$. Found, % C, 60.07; H, 4.95; N, 9.62. For $C_{22}H_{21}N_3O_5S$ (439.5). Calcd % C, 60.12; H, 4.82; N, 9.56; S, 7.30.

*3.1.2.10. N-Propyl 2-(3-phenyl-quinoxalin-2-ylsulfanyl)-acetamide (**9a**).* From n-propylamine. Yield 65%, yellow powder, mp 71−73 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (J, Hz): 8.14 (1H, d, J = 8 Hz, ArH); 7.98 (1H, d, J = 8 Hz, ArH); 7.80−7.78 (2H, m, ArH); 7.76 (1H, t, J = 8.0 Hz, ArH); 7.73 (1H, t, J = 8.0 Hz, Ar H); 7.56−7.54 (3H, m, ArH); 7.10 (1H, bs, NH); 3.95 (2H, s, SCH_2); 3.23−3.21 (2H, m, $HN\underline{CH_2}$); 1.50−1.47 (2H, m, CH_2); 0.83 (3H, t, J = 7.2 Hz, CH_3). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 168.6 (C═O), 153.4, 153.2, 141.3, 139.5, 137.6, 129.7, 129.5, 129.2, 128.6, 127.5, 41.8 ($NHCH_2$), 34.9 (SCH_2), 22.9 (CH_2), 12.1 (CH_3). MS (MALDI, positive mode, matrix DHB) m/z: 360 $(M + Na)^+$. Found, % C, 67.55; H, 5.72; N, 12.52. For $C_{19}H_{19}N_3OS$ (337.4). Calcd % C, 67.63; H, 5.68; N, 12.45; S, 9.50.

*3.1.2.11. N-Butyl-2-(3-phenylquinoxalin-2-ylsulfanyl)-acetamide (**9b**).* From n-butylamine. Yield 77%, yellow powder, mp 86−87 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (J, Hz): 8.11 (1H, d, J = 8 Hz, ArH), 7.96 (1H, d, J = 8 Hz, ArH), 7.79−7.77 (2H, m, ArH), 7.74 (1H, t, J = 8.0 Hz, ArH), 7.69 (1H, t, J = 8.0 Hz, Ar H), 7.55−7.53 (3H, m, ArH), 7.05 (1H, bs, NH), 3.93 (2H, s, SCH_2), 3.27−3.25 (2H, m, $HNCH_2$), 1.42−1.39 (2H, m, CH_2), 1.24−1.21 (2H, m, CH_2), 0.79 (3H, t, J = 7.2 Hz, CH_3). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 168.5 (C═O), 153.6, 153.4, 141.4, 139.6, 137.7, 129.8, 129.6, 129.3, 128.5, 127.4, 42.1 (NCH_2), 34.2 (SCH_2), 31.2 (CH_2), 22.3 (CH_2), 17.1 (CH_3). MS (MALDI, positive mode, matrix DHB) m/z: 374 $(M + Na)^+$. Found, % C, 68.42; H, 6.11; N, 12.04. For $C_{20}H_{21}N_3OS$ (351.5). Calcd % C, 68.35; H, 6.02; N, 11.96; S, 9.12.

*3.1.2.12. N-Allyl 2-(3-Phenyl-quinoxalin-2-ylsulfanyl)-acetamide (**9c**).* From allylamine. Yield 68%, yellow powder, mp 67−68 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (J, Hz): 8.12 (1H, d, J = 8 Hz, ArH), 7.95 (1H, d, J = 8 Hz, ArH), 7.80−7.78 (2H, m, ArH), 7.73−7.70 (2H, m, ArH), 7.55−7.52 (3H, m, ArH), 7.00 (1H, bs, NH), 5.89−5.86 (1H, m, CH), 5.12 (1H, d, J = 16.0 Hz, CH_2), 5.05 (1H, d, J = 8.4 Hz, CH═CH_2), 3.95 (2H, s, SCH_2), 3.90−3.88 (2H, d, J = 6.4 Hz, CH_2). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 168.5 (C═O), 153.5. 153.3, 140.9, 139.8, 136.6, 133.8 (CH), 130.1, 129.3, 129.0, 128.6, 127.0, 116.2 (CH_2), 41.9 (NCH_2), 34.4 (SCH_2). MS (MALDI, positive mode, matrix DHB) m/z: 358 $(M + Na)^+$. Found, % C, 68.27; H, 5.10; N,

https://doi.org/10.1021/acsomega.2c03522
ACS Omega 2022, 7, 34166−34176

12.61. For $C_{19}H_{17}N_3OS$ (335.4). Calcd % C, 68.03; H, 5.11; N, 12.53; S, 9.56.

*3.1.2.13. N-Octyl-2-(3-phenyl-quinoxalin-2-ylsulfanyl)-acetamide (**9d**).* From *n*-octylamine. Yield 64%, yellow powder, mp 90−91 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), *δ*, ppm (*J*, Hz): 8.09 (1H, d, *J* = 8.0 Hz, ArH), 8.02 (1H, d, *J* = 8.0 Hz, ArH), 7.73−7.71 (2H, m, ArH), 7.68 (1H, *t*, *J* = 8.0 Hz, ArH), 7.54 (1H, *t*, *J* = 8.0 Hz, ArH), 7.50−7.48 (3H, m, ArH), 7.14−7.13 (1H, m, NH), 4.11 (2H, s, S$\underline{CH_2}$), 3.20−3.18 (2H, m, HN$\underline{CH_2}$), 1.63−1.61 (2H, m, $\underline{CH_2}$), 1.31−1.29 (2H, m, $\underline{CH_2}$), 1.28−1.20 (10H, m, $5CH_2$), 0.88 (3H, *t*, *J* = 7.2 Hz, CH_3). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), *δ*, ppm: 168.3 (C═O), 153.5, 153.6, 141.5, 139.7, 137.8, 129.7, 129.5, 129.2, 128.6, 127.5, 42.6 (NCH_2), 34.9 (SCH_2), 33.2, 32.0, 31.2, 30.8, 28.1, 24.1, 18.2 (CH_3). MS (MALDI, positive mode, matrix DHB) *m*/*z*: 444 $(M + Na)^+$. Found, % C, 71.17; H, 7.32; N, 10.04. For $C_{25}H_{31}N_3OS$ (421.6). Calcd % C, 71.22; H, 7.41; N, 9.97; S, 7.61.

*3.1.2.14. N-Cyclohexyl-2-(3-phenyl-quinoxalin-2-ylsulfanyl)acetamide (**9e**).* From cyclohexylamine. Yield 81%, yellow powder, mp 111−113 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), *δ*, ppm (*J*, H): 8.10 (1H, d, *J* = 8 Hz, ArH), 7.95 (1H, d, *J* = 8 Hz, ArH), 7.79−7.77 (2H, m, ArH), 7.74 (1H, *t*, *J* = 8.0 Hz, ArH), 7.69 (1H, *t*, *J* = 8.0 Hz, ArH), 7.54−7.52 (3H, m, ArH), 7.03 (1H, s, NH), 3.87 (2H, s, SCH_2), 3.76−3.74 (1H, m, CH), 1.83−1.81 (2H, m, CH_2), 1.56−1.54 (2H, m, CH_2), 1.42−1.21 (4H, m, $2CH_2$), 1.08−1.06 (2H, m, CH_2). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), *δ*, ppm: 168.7, 153.6, 153.5, 141.4, 139.6, 137.9, 129.8, 129.6, 129.3, 128.4, 127.6, 45.7 (CH), 34.9 (SCH_2), 33.4, 26.4, 23.1. MS (MALDI, positive mode, matrix DHB) *m*/*z*: 400 $(M + Na)^+$. Found, % C, 70.12; H, 6.19; N, 11.07. For $C_{22}H_{23}N_3OS$ (377.5). Calcd % C, 70.00; H, 6.14; N, 11.13; S, 8.49.

*3.1.2.15. N-Benzyl-2-(3-phenyl-quinoxalin-2-ylsulfanyl)-acetamide (**9f**).* From benzylamine. Yield 75%, yellow powder, mp 124−125 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), *δ*, ppm (*J*, Hz): 8.11−8.09 (2H, m, ArH), 7.76−7.74 (3H, m, ArH), 7.73−7.71 (1H, m, ArH), 7.54−7.52 (3H, m, ArH), 7.41 (1H, bs, NH), 7.17−7.15 (5H, m, ArH), 4.44 (2H, d, *J* = 7.2 Hz, CH_2), 3.97 (2H, s, SCH_2). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), *δ*, ppm: 168.9 (C═O), 153.7, 153.6, 144.1, 141.5, 139.7, 137.8, 129.7, 129.6, 129.2, 128.5, 128.3, 127.9, 127.7, 127.4, 48.6 (CH_2), 34.7 (SCH_2). MS (MALDI, positive mode, matrix DHB) *m*/*z*: 408 $(M + Na)^+$. Found, % C, 71.76; H, 5.06; N, 10.84. For $C_{23}H_{19}N_3OS$ (385.5). Calcd % C, 71.66; H, 4.97; N, 10.90; S, 8.32.

*3.1.2.16. 1-Morpholin-4-yl-2-(3-phenyl-quinoxalin-2-ylsulfanyl)ethanone (**9g**).* From morpholine. Yield 70%, yellow powder, mp 138−139 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), *δ*, ppm (*J*, Hz): 8.09 (1H, d, *J* = 8 Hz, ArH), 7.92 (1H, d, *J* = 8 Hz, ArH), 7.80−7.78 (2H, m, ArH), 7.71 (1H, *t*, *J* = 8.0 Hz, ArH), 7.67 (1H, *t*, *J* = 8.0 Hz, ArH), 7.53−7.51 (3H, m, ArH), 4.23 (2H, s, SCH_2), 3.72−3.68 (8H, m, $4CH_2$). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), *δ*, ppm: 168.8 (C═O), 153.8, 153.7, 142.0, 139.8, 137.7, 129.6, 129.5, 129.4, 128.4, 127.8, 127.6, 64.5 (OCH_2), 65.6 (OCH_2), 64.2 (OCH_2), 46.2 (NCH_2), 45.7 (NCH_2), 34.3 (SCH_2). MS (MALDI, positive mode, matrix DHB) *m*/*z*: 388 $(M + Na)^+$. Found, % C, 65.81; H, 5.33; N, 11.62. For $C_{20}H_{19}N_3O_2S$ (365.5). Calcd % C, 65.73; H, 5.24; N, 11.50; S, 8.77.

*3.1.2.17. 2-(3-Phenyl-quinoxalin-2-ylsulfanyl)-1-piperidin-1-yl-ethanone (**9h**).* From piperidine. Yield 69%, yellow powder, mp 106−107 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), *δ*, ppm (*J*, Hz): 8.09 (1H, d, *J* = 8 Hz, ArH), 7.94 (1H, d, *J* = 8 Hz, ArH), 7.82−7.80 (2H, m, ArH), 7.70 (1H, *t*, *J* = 8.0 Hz, ArH), 7.64 (1H, t, *J* = 8.0 Hz, ArH), 7.52−7.49 (3H, m, ArH), 4.26 (2H, s, SCH_2), 3.62−3.58 (4H, m, $2CH_2$); 1.68−1.64 (6H, m, $3CH_2$); ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), *δ*, ppm: 168.7 (C═O), 153.6, 153.4, 142.2, 139.7, 137.6, 129.6, 129.4, 129.4, 128.3, 127.7, 127.5, 46.2 (NCH_2), 45.2 (NCH_2), 34.9 (SCH_2), 26.0 (CH_2), 25.3 (CH_2), 25.1 (CH_2). MS (MALDI, positive mode, matrix DHB) *m*/*z*: 386 $(M + Na)^+$. Found, % C, 69.42; H, 5.96; N, 11.51. For $C_{21}H_{21}N_3OS$ (363.5). Calcd % C, 69.39; H, 5.82; N, 11.56; S, 8.82.

*3.1.2.18. N-(4-Methyl-piperazin-1-yl)-2-(3-phenyl-quinoxalin-2-ylsulfanyl)acetamide (**9i**).* From *N*-methylpiperazine. Yield 74%, yellow powder, mp 142−143 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), *δ*, ppm (*J*, Hz): 8.12 (1H, d, *J* = 8 Hz, ArH), 8.01 (1H, d, *J* = 8 Hz, ArH), 7.84−7.82 (2H, m, ArH), 7.77 (1H, *t*, *J* = 8.0 Hz, ArH), 7.69 (1H, *t*, *J* = 8.0 Hz, Ar H),7.60−7.58 (3H, m, ArH), 4.07 (2H, s, SCH_2), 2.74−2.70 (4H, m, $2CH_2$), 2.44−2.40 (4H, m, $2CH_2$), 2.22 (3H, s, $\underline{CH_3}$). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), *δ*, ppm: 167.9 (C═O), 153.7, 153.6, 142.1, 139.7, 137.6, 129.4, 129.3, 129.3, 128.55 127.7, 127.5, 54.8 (CH_2), 54.3 (CH_2), 44.8 (CH_2), 44.3 (CH_2), 42.4 (NCH_3), 34.8 (SCH_2). MS (MALDI, positive mode, matrix DHB) *m*/*z*: 416 $(M + Na)^+$. Found, % C, 64.22; H, 5.91; N, 17.87. For $C_{21}H_{23}N_5OS$ (393.5). Calcd % C, 64.10; H, 5.89; N, 17.80; S, 8.15.

*3.1.2.19. General Procedure for the Synthesis of Hydrazides **10a** and **10b**.* Hydrazine hydrate (80%, 5 mmol) was added to a solution of esters **8a** and **b** (1.0 mmol) in absolute ethanol (30 mL). The reaction mixture was refluxed for 4 h and cooled. The resultant precipitate was filtered off, washed with ethanol and diethyl ether, and then crystallized from aqueous ethanol to yield the corresponding hydrazide.

*3.1.2.20. N-Hydrazinocarbonylmethyl-2-(3-phenyl-quinoxalin-2-ylsulfanyl)acetamide (**10a**).* Yield 77%, yellow powder, mp 170−171 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), *δ*, ppm (*J*, Hz): 9.87 (1H, bs, NH), 8.08 (1H, d, *J* = 8 Hz, ArH), 8.01 (1H, d, *J* = 8 Hz, ArH), 7.88−7.90 (2H, m, ArH), 7.71 (1H, *t*, *J* = 8.0 Hz, ArH), 7.62 (1H, *t*, *J* = 8.0 Hz, ArH), 7.44−7.41 (3H, m, ArH), 7.11−7.07 (1H, m, NH), 4.22 (2H, bs, NH_2), 4.05 (2H, s, SCH_2), 3.87 (2H, s, NCH_2). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), *δ*, ppm: 170.8 (C═O), 168.2 (C═O), 153.4, 153.1, 141.3, 139.4, 137.1, 129.4, 129.2, 129.0, 128.8, 127.6, 45.6 (NCH_2), 34.6 (SCH_2). MS (MALDI, positive mode, matrix DHB) *m*/*z*: 390 $(M + Na)^+$. Found, % C, 58.76; H, 4.39; N, 19.15. For $C_{18}H_{17}N_5O_2S$ (367.4). Calcd % C, 58.84; H, 4.66; N, 19.06; S, 8.73.

*3.1.2.21. N-(2-Hydrazinocarbonyl-ethyl)-2-(3-phenyl-quinoxalin-2-ylsulfanyl)acetamide (**10b**).* Yield 81%, yellow powder, mp 135−136 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), *δ*, ppm (*J*, Hz): 9.93 (1H, bs, NH), 8.11 (1H, d, *J* = 8 Hz, ArH), 8.07 (1H, d, *J* = 8 Hz, ArH), 7.89−7.87 (2H, m, ArH), 7.80−7.78 (2H, m, ArH), 7.59−7.57 (3H, m, ArH), 7.44−7.36 (1H, m, NH), 4.03 (2H, bs, NH_2), 3.87 (2H, s, SCH_2), 3.57−3.55 (2H, m, $NHCH_2$), 2.31−2.29 (2H, m, CH_2CO), ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), *δ*, ppm: 171.9, 168.2, 153.3, 153.1, 141.3, 139.6, 137.2, 129.4, 129.3, 129.2, 128.5, 127.6, 42.4 (NCH_2), 37.5 (SCH_2), 34.8 (CH_2CO). MS (MALDI, positive mode, matrix DHB) *m*/*z*: 404 $(M + Na)^+$. Found, % C, 59.88; H, 4.94; N, 18.42. For

https://doi.org/10.1021/acsomega.2c03522
ACS Omega 2022, 7, 34166−34176

$C_{19}H_{19}N_5O_2S$ (381.5). Calcd % C, 59.82; H, 5.02; N, 18.36; S, 8.41.

*3.1.2.22. {2-[2-(3-Phenyl-quinoxalin-2-ylsulfanyl)-acetylamino]-acetylamino}-acetic Acid Methyl Ester (**11a**).* Abbreviated: Gly-Gly. Yield 57%, yellow powder, mp 76−78 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (J, Hz): 8.12 (1H, d, J = 8 Hz, ArH), 8.06 (1H, d, J = 8 Hz, ArH), 7.93−7.91 (2H, m, ArH), 7.67 (1H, *t*, J = 8.0 Hz, ArH), 7.62 (1H, *t*, J = 8.0 Hz, ArH), 7.36−7.34 (3H, m, ArH), 7.14−7.12 (1H, m, NH), 6.17−6.14 (1H, m, NH), 4.13−4.11 (2H, m, CH_2), 4.01−3.99 (2H, m, CH_2), 3.87 (2H, s, S$\underline{CH_2}$), 3.54 (3H, s, OMe). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 171.8 (C═O), 168.2 (C═O), 167.8 (C═O), 153.4, 153.3, 141.4, 139.5, 137.2, 129.5, 129.3, 129.1, 128.7, 127.5, 126.9, 52.9 (OCH_3), 45.7 (NCH_2), 42.6 (NCH_2), 34.6 (SCH_2). MS (MALDI, positive mode, matrix DHB) m/z: 447 $(M + Na)^+$. Found, % C, 59.38; H, 4.80; N, 13.26; S, 7.59. For $C_{21}H_{20}N_4O_4S$ (424.5). Calcd % C, 59.42; H, 4.75; N, 13.20; S, 7.55

*3.1.2.23. 3-{2-[2-(3-Phenyl-quinoxalin-2-ylsulfanyl)-acetylamino]acetylamino}-propionic Acid Methyl Ester (**11b**).* Abbreviated: Gly-β-Ala. Yield 51%, yellow powder, mp 77−79 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (J, Hz): 8.09 (1H, d, J = 8 Hz, ArH), 8.04 (1H, d, J = 8.0 Hz, ArH), 7.90−7.89 (2H, m, ArH), 7.61 (1H, *t*, J = 8.0 Hz, ArH), 7.58 (1H, *t*, J = 8.0 Hz, ArH), 7.32−7.30 (3H, m, ArH), 7.09−7.06 (1H, m, NH), 6.12−6.10 (1H, m, NH), 4.07−3.94 (4H, m, $2NCH_2$), 3.87 (2H, s, SCH_2), 3.54 (3H, s, OMe), 2.89−2.87 (2H, m, CH_2). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 171.9, 168.4, 167.8, 153.5, 153.3, 141.5, 139.4, 137.3, 129.6, 129.4, 129.1, 128.7, 127.4, 127.0, 52.9 (OCH_3), 45.6 (NCH_2), 42.5 (NCH_2), 34.2 (SCH_2), 32.9 (CH_2CO). MS (MALDI, positive mode, matrix DHB) m/z: 461 $(M + Na)^+$. Found, % C, 60.29; H, 5.11; N, 12.81. For $C_{22}H_{22}N_4O_4S$ (438.5). Calcd % C, 60.26; H, 5.06; N, 12.78; S, 7.31.

*3.1.2.24. 2-{2-[2-(3-Phenyl-quinoxalin-2-ylsulfanyl)-acetylamino]-acetyl}-malonic Acid Dimethyl Ester (**11c**).* Abbreviated: Gly-L-Asp. Yield 62%, yellow powder, mp 98−99 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (J, Hz): 8.16 (1H, d, J = 8 Hz, ArH), 8.09 (1H, d, J = 8 Hz, ArH), 7.97−7.95 (2H, m, ArH), 7.74 (1H, *t*, J = 8.0 Hz, ArH), 7.61 (1H, *t*, J = 8.0 Hz, ArH), 7.39−7.36 (3H, m, ArH), 7.13−7.11 (1H, m, NH), 6.15−6.13 (1H, m, NH), 5.14−5.12 (1H, m, CH), 4.23−4.21 (2H, m, NCH_2), 3.73 (2H, s, SCH_2), 3.66 (3H, s, OMe), 3.59 (3H, s, OMe), 2.76−2.63 (2H, m, CH_2). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 171.9 (C═O), 171.3 (C═O), 168.4 (C═O), 168.2 (C═O), 153.4, 153.1, 141.4, 139.4, 137.2, 129.5, 129.4, 129.1, 128.8, 127.5, 127.1, 56.1 (CH), 52.8 (OCH_3), 52.3 (OCH_3), 45.7 (NCH_2), 34.4 (SCH_2), 33.8 (CH_2). MS (MALDI, positive mode, matrix DHB) m/z: 505 $(M + Na)^+$. Found, % C, 57.29; H, 4.66; N, 11.67. For $C_{23}H_{22}N_4O_6S$ (482.5). Calcd % C, 57.25; H, 4.60; N, 11.61; S, 6.65.

*3.1.2.25. {3-[2-(3-Phenyl-quinoxalin-2-ylsulfanyl)-acetylamino]propionyl-amino}-acetic Acid Methyl Ester (**12a**).* Abbreviated: β-Ala-Gly. Yield 65%, yellow powder, mp 101−102 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (J, Hz): 8.12 (1H, d, J = 8 Hz, ArH), 8.06 (1H, d, J = 8 Hz, ArH), 7.88−7.86 (2H, m, ArH), 7.59 (1H, *t*, J = 8.0 Hz, ArH), 7.54 (1H, *t*, J = 8.0 Hz, ArH), 7.29−7.27 (3H, m, ArH), 7.02−7.00 (1H, m, NH), 6.88−6.86 (1H, m, NH), 4.12−4.10 (4H, m, $2NCH_2$), 3.85 (2H, s, SCH_2), 3.68 (3H, s, OMe), 2.27−2.25 (2H, m, CH_2). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 171.8 (C═O), 168.4 (C═O), 168.1 (C═O), 153.5, 153.3, 141.5, 139.4, 137.3, 129.5, 129.4, 129.0, 128.6, 127.4, 127.0, 52.8 (OCH_3), 45.5 (NCH_2), 42.6 (NCH_2), 34.3 (SCH_2), 31.3 (CH_2CO). MS (MALDI, positive mode, matrix DHB) m/z: 461 $(M + Na)^+$. Found, % C, 60.31; H, 5.09; N, 12.84. For $C_{22}H_{22}N_4O_4S$ (438.5). Calcd % C, 60.26; H, 5.06; N, 12.78; S, 7.31.

*3.1.2.26. 3-{3-[2-(3-Phenyl-quinoxalin-2-ylsulfanyl)-acetylamino]propionyl-amino}-propionic Acid Methyl Ester (**12b**).* Abbreviated: β-Ala-β-Ala. Yield 62%, yellow powder, mp 84−86 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (J, Hz): 8.04 (1H, d, J = 8 Hz, ArH), 8.00 (1H, d, J = 8 Hz, ArH), 7.84−7.82 (2H, m, ArH), 7.61 (1H, *t*, J = 8.0 Hz, ArH), 7.52 (1H, *t*, J = 8.0 Hz, ArH), 7.13−7.11 (3H, m, ArH), 7.04−7.02 (1H, m, NH), 6.24−6.22 (1H, m, NH), 3.91−3.72 (6H, s, SCH_2, $2NCH_2$), 3.63 (3H, s, OMe), 2.11−2.08 (4H, m, $2CH_2$). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 172.7 (C═O), 168.9 (C═O), 168.2 (C═O), 153.6, 153.4, 141.6, 139.5, 137.4, 129.6, 129.51 129.1, 128.7, 127.3, 127.0, 52.9 (OCH_3), 45.6 (NCH_2), 42.4 (NCH_2), 34.3 (SCH_2), 31.7 (CH_2CO), 31.3 (CH_2CO). MS (MALDI, positive mode, matrix DHB) m/z: 475 $(M + Na)^+$. Found, % C, 61.12; H, 5.39; N, 12.27. For $C_{23}H_{24}N_4O_4S$ (452.5). Calcd % C, 61.05; H, 5.35; N, 12.38; S, 7.09.

*3.1.2.27. 2-{3-[2-(3-Phenyl-quinoxalin-2-ylsulfanyl)-acetylamino]propionyl-amino}-malonic Acid Dimethyl Ester (**12c**).* Abbreviated: β-Ala-L-Asp. Yield 51%, yellow powder, mp 96−97 °C. ^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm (J, Hz): 8.11 (1H, d, J = 8 Hz, ArH), 8.05 (1H, d, J = 8 Hz, ArH), 7.84−7.82 (2H, m, ArH), 7.56 (1H, *t*, J = 8.0 Hz, ArH), 7.51 (1H, *t*, J = 8.0 Hz, ArH), 7.27−7.25 (3H, m, ArH), 7.07−7.05 (1H, m, NH), 6.97−6.95 (1H, m, NH), 4.78−4.76 (1H, m, CH), 3.93−3.82 (4H, m, SCH_2, NCH_2), 3.72 (3H, s, OMe), 3.68 (3H, s, OMe), 2.96−2.94 (2H, m, CH_2), 2.44−2.42 (2H, m, CH_2). ^{13}C NMR spectrum (75.0 MHz, $CDCl_3$), δ, ppm: 172.1 (C═O), 171.9 (C═O), 168.2 (C═O), 167.0 (C═O), 153.4, 153.2, 141.5, 139.4, 137.3, 129.5, 129.2, 129.0, 128.7, 127.5, 127.0, 55.8 (CH), 52.8 (OCH_3), 51.3 (OCH_3), 45.5 (NCH_2), 34.4 (SCH_2), 33.7 (CH_2), 31.3 (CH_2CO). MS (MALDI, positive mode, matrix DHB) m/z: 519 $(M + Na)^+$. Found, % C, 58.09; H, 4.91; N, 11.32. For $C_{24}H_{24}N_4O_4S$ (496.5). Calcd % C, 58.05; H, 4.87; N, 11.28; S, 6.46.

3.2. Pharmacological Studies. *3.2.1. MTT Assay.* Human embryonic kidney cells (HEK-293), human colorectal (HCT-116) carcinoma cells, and human adenocarcinoma (MCF-7) cells were cultured in the media containing Dulbecco's modified Eagle's medium (DMEM), (10%) L-glutamine, (10%) fetal bovine serum (FBS), (10%) selenium chloride, and (120 unit/mL) penicillin/streptomycin. The cultures were placed in a CO_2 (5%) incubator (Thermo Scientific Heracell-150) at 37 °C to achieve 70−80% confluence and thereafter exposed to different concentrations (2−40 μg/mL) of 27 synthetic compounds for 48 h. After this, cell cultures were incubated with MTT (5.0 mg/mL) for 4 h. DMSO was added to each well, plates were read at 570 nm using an ELISA plate reader (Biotek Instruments, Winooski), and % cell viability was calculated.

3.2.2. Microscopic Analysis. All cells (HCT-116, MCF-7, and HEK-293) were observed under different magnifications of an inverted microscope (TS-100F-Eclipse, Nikon, Japan). The structural morphology of both treated and untreated cells was observed, and the structural morphological difference between

https://doi.org/10.1021/acsomega.2c03522
ACS Omega 2022, 7, 34166−34176

cancerous cells (HCT-116 and MCF-7) and healthy normal cells (HEK-293) was also examined.

3.2.3. Statistical Evaluation. The mean ± standard deviation (SD) from the control and treated groups was calculated. All statistical analyses were completed with GraphPad Prism 6 (GraphPad Software). The difference between control and compound **1**, **2**, and **3** treated groups was calculated by one-way analysis of variance (ANOVA), and *p*-values were calculated by Student's *t*-test (*$p < 0.05$, **$p < 0.01$).

3.2.4. Molecular Modeling. All molecular modeling studies were performed on a Hewlett−Packard Pentium Dual-Core T4300 2.10 GHz running Windows 10 using Molecular Operating Environment (AUTODOCK) 2008.10 molecular modeling software for molecular docking simulations and ligand binding energy calculations and Pymol for output data visualization and figure generation. The crystal structure of the human TS dimer bound to a short peptide LSCQLYQR (PDB code: 3N5E) was chosen as a receptor. This structure was a homodimer in its closed conformation and represented the inactive conformation of the enzyme. The putative ligand binding site was assigned based on the positions of the heavy atoms of the peptide reported.[33] The selected targets were used after deleting the cocrystallized inhibitors, all hydrogens were added to the ligand PDB file, and partial charges were computed. Docking was performed using an AUTODOCK dock tool in AUTODOCK and performed with default values. Amino acid residues involved in binding the cocrystallized ligand were used to define the active site for ligand binding.

The docking results were evaluated using binding energy calculation in AUTODOCK and checking the ligand binding position through interaction with key residues and were further validated through comparative docking with the crystallized ligand position in Pymol.

4. CONCLUSIONS

The results of the biological testing and molecular docking studies showed that the designed and synthesized quinoxaline peptidomimetics possess good antiproliferative activity, particularly against breast cancer hepatic carcinoma, and apparent selectivity that is potentially mediated through binding the hTS homodimer interface and stabilizing its inactive conformation. The compounds are also peptidomimetic in nature and therefore are suitable for further pharmaceutical and preclinical development.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c03522.

NMR spectra for the synthesized compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Samir Mohamed El Rayes − *Department of Chemistry, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt;* orcid.org/0000-0003-2667-3855; Email: samir_elrayes@yahoo.com

Authors

Gaber El-Enany − *Department of Physics, College of Science and Arts in Uglat Asugour, Qassim University, Buraydah 52571, Kingdom of Suadi Arabia; Science & Math Department, Faculty of Engineering, Port Said University, Port Said 42526, Egypt*

Mohamed Sayed Gomaa − *Department of Pharmaceutical Chemistry, College of Clinical Pharmacy, Imam Abdulrahman Bin Faisal University, Dammam 31441, Kingdom of Saudi Arabia*

Ibrahim A. I. Ali − *Department of Chemistry, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt*

Walid Fathalla − *Science & Math Department, Faculty of Engineering, Port Said University, Port Said 42526, Egypt*

Faheem Hyder Pottoo − *Department of Pharmacology, College of Clinical Pharmacy, Imam Abdulrahman Bin Faisal University, Dammam 31441, Kingdom of Saudi Arabia*

Firdos Alam Khan − *Department of Stem Cell Research, Institute of Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, Dammam 31441, Kingdom of Saudi Arabia;* orcid.org/0000-0002-6892-1530

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c03522

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Harmenberg, J.; Akesson-Johansson, A.; Graslund, A.; Malmfors, T.; Bergman, J.; Wahren, B.; Akerfeldt, S.; Lundblad, L.; Cox, S. The mechanism of action of the anti-herpes virus compound 2,3-dimethyl-6(2-dimethylaminoethyl)-6*H*-indolo-(2,3-*b*)quinoxaline. *Antiviral Res.* **1991**, *15*, 193−204.

(2) Naylor, M. A.; Stephen, M. A.; Nolan, J.; Sutton, B.; Tocher, J. H.; Fielden, E. M.; Adams, J. E.; Strafford, I. Heterocyclic mono-N-oxides with potential applications as bioreductive antitumour drugs: Part 1. 8-Alkylamino-substituted phenylimidazo [1,2-*a*] quinoxalines. *Anticancer Drug Des.* **1993**, *8*, 439−461.

(3) Hui, X.; Desrivot, J.; Bories, C.; Loiseau, C. P. M.; Franck, X.; Hocquemiller, R.; Figadere, B. Synthesis and antiprotozoal activity of some new synthetic substituted quinoxalines. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 815−820.

(4) Kim, Y. B.; Kim, Y. H.; Park, J. Y.; Kim, S. K. Synthesis and biological activity of new quinoxaline antibiotics of echinomycin analogueS. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 541−544.

(5) El-Sabbagh, O. I.; El-Sadek, M. E.; Lashine, S. M.; Yassin, S. H.; El-Nabtity, S. M. Synthesis of new 2 (1H)-quinoxalinone derivatives for antimicrobial and antiinflammatory evaluation. *Med. Chem. Res.* **2009**, *18*, 782−797.

(6) Settypalli, T.; Chunduri, V. R.; Maddineni, A. K.; Begari, N.; Allagadda, R.; Kotha, P.; Chippada, A. R. Design, synthesis, in silico docking studies and biological evaluation of novel quinoxaline-hydrazide hydrazone-1,2,3-triazole hybrids as _-glucosidase inhibitors and antioxidants. *New J. Chem.* **2019**, *43*, 15435−15452.

(7) Hui, X.; Desrivot, J.; Bories, C.; Loiseau, P. M.; Franck, X.; Hocquemiller, R.; Figadere, B. Synthesis and antiprotozoal activity of some new synthetic substituted quinoxalines. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 815−820.

(8) Kotb, E. R.; Anwar, A. M.; Soliman, S. M.; Salama, A. M. Synthesis and reactions of some novel quinoxalines foranticancer evaluation. *Phosphorus, Sulfur Silicon Relat. Elem.* **2007**, *182*, 1119−1130.

(9) Carta, A.; Loriga, M.; Piras, S.; Paglietti, G.; La Colla, P.; Busonera, B.; Collu, G.; Loddo, R. Synthesis of variously substituted 3-phenoxymethyl quinoxalin-2-ones and quinoxalines capable to potentiate in vitro the antiproliferative activity of anticancer drugs in multi-drug resistant cell lines. *Med. Chem.* **2006**, *2*, 113−122.

(10) Piras, S.; Loriga, M.; Paglietti, G. Quinoxaline chemistry. Part XVII. Methyl [4-(substituted 2-quinoxalinyloxy) phenyl] acetates and ethyl N-{[4-(substituted 2-quinoxalinyloxy) phenyl] acetyl} glutamates analogues of methotrexate: Synthesis and evaluation of in vitro anticancer activity. *Farmaco* **2004**, *59*, 185−194.
(11) El Newahie, A. M. S.; Ismail, N. S. M.; Abou El Ella, D. A.; Abouzid, K. A. M. Quinoxaline-Based Scaffolds Targeting Tyrosine Kinases and Their Potential Anticancer Activity. *Arch. Pharm.* **2016**, *349*, 309−326.
(12) Ingle, R.; Marathe, R.; Magar, D.; Patel, H. M.; Surana, S. J. Sulphonamido-quinoxalines: Search for anticancer agent. *Eur. J. Med. Chem.* **2013**, *65*, 168−186.
(13) Noolvi, M. N.; Patel, H. M.; Bhardwaj, V.; Chauhan, A. Synthesis and in vitro antitumor activity of substituted quinazoline and quinoxaline derivatives: Search for anticancer agent. *Eur. J. Med. Chem.* **2011**, *46*, 2327−2346.
(14) Abbas, H.-A. S.; Al-Marhabi, A. R.; Ammar, Y. A.; Ammar, Y. A. Molecular modeling studies and synthesis of novel quinoxaline derivatives with potential anticancer activity as inhibitors of c-Met kinase. *Bioorg. Med. Chem.* **2015**, *23*, 6560−6572.
(15) El Newahie, A.; Nissan, Y.; Ismail, N.; Abou El Ella, D.; Khojah, S.; Abouzid, K. Design and Synthesis of New Quinoxaline Derivatives as Anticancer Agents and Apoptotic Inducers. *Molecules* **2019**, *24*, No. 1175.
(16) Tseng, C.-H.; Chen, Y.-R.; Tzeng, C.-C.; Liu, W.; Chou, C.-K.; Chiu, C.-C.; Chen, Y.-L. Discovery of indeno[1,2-b]quinoxaline derivatives as potential anticancer agents. *Eur. J. Med. Chem.* **2016**, *108*, 258−273.
(17) Ismail, M. M. F.; Amin, K. M.; Noaman, E.; Soliman, D. H.; Ammar, Y. A. New quinoxaline 1, 4-di-N-oxides: Anticancer and hypoxia-selective therapeutic agents. *Eur. J. Med. Chem.* **2010**, *45*, 2733−2738.
(18) El Rayes, S. M.; Aboelmagd, A.; Gomaa, M. S.; Ali, A. I. A.; Fathalla, W.; Pottoo, F. H.; Khan, F. A. Convenient synthesis and antiproliferative activity of methyl 2-[3-(3-phenyl-quinoxalin-2-ylsulfanyl)propanamido]alkanoates and N-Alkyl 3-((3-phenylquinoxalin-2-yl)sulfanyl) propanamides. *ACS Omega* **2019**, *4*, 18555−18566.
(19) Fathalla, W.; Ali, I. A. I.; Pazdera, P. A novel method for heterocyclic amide−thioamide transformations. *Beilstein J. Org. Chem.* **2017**, *13*, 174−181.
(20) Megahed, M. I.; Fathalla, W. Synthesis and antimicrobial activity of methyl (2-(2-(2-arylquinazolin-4-yl)sulfanyl)acetyl)amino alkanoates. *J. Heterocycl. Chem.* **2018**, *55*, 2809.
(21) El Rayes, S. M.; Ali, I. A. I.; Fathalla, W. Convenient Synthesis of Some Novel Pyridazinone Bearing Triazole Moieties. *J. Heterocycl. Chem.* **2019**, *56*, 51.
(22) Fathalla, W.; El Rayes, S. M.; Ali, I. A. I. Convenient synthesis of 1-substituted-4-methyl-5-oxo[1,2,4]triazolo[4,3-a]quinazolines. *Arkivoc* **2007**, 173.
(23) Han, S.-Y.; Kim, Y.-A. Recent Development of Peptide Coupling Reagents in Organic Synthesis. *Tetrahedron* **2004**, *60*, 2447.
(24) Ali, I. A. I.; Al-Masoudi, I. A.; Saeed, B.; Al-Masoudi, N. A.; La Colla, P. Amino Acid Derivatives, Part 2: Synthesis, Antiviral, and Antitumor Activity of Simple Protected Amino Acids Functionalized at *N*-terminus with Naphthalene Side Chain. *Heteroatom Chem.* **2005**, *16* (2), 148−155.
(25) Baharara, J.; Ramezani, T.; Divsalar, A.; Mousavi, M.; Seyedarabi, A. Induction of Apoptosis by Green Synthesized Gold Nanoparticles Through Activation of Caspase-3 and 9 in Human Cervical Cancer Cells. *Avicenna J. Med. Biotechnol.* **2016**, *8*, 75−83.
(26) Mytych, J.; Lewinska, A.; Zebrowski, J.; Wnuk, M. Gold nanoparticles promote oxidant-mediated activation of NF-κB and 53BP1 recruitment-based adaptive response in human astrocytes. *BioMed Res. Int.* **2015**, *2015*, No. 304575.
(27) Wang, L.-W.; Ai-Ping, Q.; Wen-Lou, L.; Jia-Mei, C.; Jing-Ping, Y.; Han, W.; Yan, L.; Juan, L. Quantum dots-based double imaging combined with organic dye imaging to establish an automatic computerized method for cancer Ki67 measurement. *Sci. Rep.* **2016**, *6*, No. 20564.
(28) Irani, S.; Shahmirani, Z.; Atyabi, S. M.; Mirpoor, S. Induction of growth arrest in colorectal cancer cells by cold plasma and gold nanoparticles. *Arch. Med. Sci.* **2015**, *11*, 1286−1295.
(29) Khan, F. A.; Akhtar, S.; Almofty, S. A.; Almohazey, D.; Alomari, M. FMSP-Nanoparticles Induced Cell Death on Human Breast Adenocarcinoma Cell Line (MCF-7 Cells): Morphometric Analysis. *Biomolecules* **2018**, *8*, No. 32.
(30) Fletcher, S.; Hamilton, A. D. Targeting protein−protein interactions by rational design: mimicry of protein surfaces. *J. R. Soc. Interface* **2006**, *3*, 215−233.
(31) Carosati, E.; Tochowicz, A.; Marverti, G.; Guaitoli, G.; Benedetti, P.; Ferrari, S.; Stroud, R. M.; Finer-Moore, J.; Luciani, R.; Farina, D.; Cruciani, G.; Costi, M. P. Costi, Inhibitor of ovarian cancer cells growth by virtual screening: a new thiazole derivative targeting human thymidylate synthase. *J. Med. Chem.* **2012**, *55*, 10272−10276.
(32) Aggarwal, R.; Eakta, M.; Garima, S. Facile and Efficient Regioselective Synthesis of 1-(3′-Substituted Quinoxalin-2′-yl)-3-aryl/heteroaryl-5-methylpyrazoles. *Synth. Commun.* **2013**, *43*, 1842−1848.
(33) Cardinale, D.; Guaitoli, G.; Tondi, D.; Luciani, R.; Henrich, S.; Frassineti, O.; Salo-Ahen, M.; Ferrari, S.; Marverti, G.; Guerrieri, D.; A Ligabue, C. C.; Pozzi, S.; Mangani, D.; Fessas, R.; Guerrini, G.; Ponterini, R. C.; Wade, M. P.; Costi. Protein−protein interface-binding peptides inhibit the cancer therapy target human thymidylate synthase. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, E542−E549.

https://doi.org/10.1021/acsomega.2c03522
ACS Omega 2022, 7, 34166−34176