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

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ABSTRACT: A series of 27 new quinoxaline derivatives (N-alkyl-2-(3-phenyl-quinoxalin-2-ylsulfanyl)acetamides, methyl-2-[2-(3-phenylquinaxaln-2-ylsulfan-yl)-acetylamino]alkanoates, and their corresponding dipeptides) were prepared from 3-phenylquinaxaline-2(1H)-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, 9f, 10a, 10b, 11c, 12b, and 12c) showed inhibitory action on HCT-116 cancer cells and 15 compounds (4a−c, 5, 6, 8c, 9a, 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,1−3 antiviral,4−6 anthelmintic,7−9 antimicrobial,9−11 anti-inflammatory,12 antioxidant,12−14 and antiprotozoal activities.15 Quinoxaline and its derivatives have recently been recognized as effective chemotherapeutic agents against a number of tumors.3,8−10 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-phenylquinaxaln-2-ylsulfanyl)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-[2-(3-phenyl-quinoxalin-2-ylsulfanyl)] acetamides, methyl-2-[2-(3-phenylquinaxaln-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-cyclohexylthiocarbamatecyclohexylammonium salt 2 as an excellent thiating reagent was earlier discussed in a number of articles.18−20 3-Phenylquinaxaline-2(1H)-thione (3) could be obtained simply by the reaction of chloroquinaxaline 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-phenylquinaxaline-2(1H)-thione (3) as a heterocyclic thiaoamide 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
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(1H)-thione (3) with hard electrophilic alkylating reagents (activated acrylic acid compounds) to give S-substituted derivatives. 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

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

![Scheme 1](image)

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

| 4a-c | R   | yield % |
|------|------|---------|
| 4a   | CN   | 73      |
| 4b   | COPh | 78      |
| 4c   | CH=CH₂ | 81    |

**Scheme 3. Preparation of Methyl-2-[(3-phenylquinoxalin-2-y)sulfanyl]acetamino]alkanoates 8a–c and N-Alkyl-[(3-phenylquinoxalin-2-y)sulfanyl]acetamides 9a–i**

![Scheme 3](image)
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 1H and 13C NMR spectral and physicochemical analysis. The 1H NMR spectrum of ethyl(3-phenyl-quinoxalin-2-ylsulfanyl)acetate (5) shows an interesting singlet signal at 4.03 ppm corresponding to the SCH2CO group, which clearly confirms the site of alkylation. The 13C NMR spectrum of 5 also shows two signals at 4.25 and 1.29 ppm corresponding to ester OCH2, beside several multiplet signals ranging between 8.08 and 7.51 ppm for nine aromatic protons. The 13C NMR spectrum of ethyl(3-phenyl-quinoxalin-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 NaN3O 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-phenyl-quinoxalin-2-ylsulfanyl)]acylamino]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-phenyl-quinoxalin-2-ylsulfanyl)]acylamino]alkanoates 8a−c and N-alkyl-[2-(3-phenyl-quinoxalin-2-ylsulfanyl)]acetamides 9a−i is based on 1H and 13C NMR spectral and physicochemical analysis. The 1H NMR spectrum of methyl-2-[2-(3-phenyl-quinoxalin-2-ylsulfanyl)]acylamino]acetate (8a) showed signals at δ 7.28, 4.22, 3.96, and 3.72 ppm corresponding to NH, SCH2, and OCH3, respectively. The 13C NMR spectrum of 8a showed signals at δ 172.12, 168.3, 52.4, 41.7, and 34.6 ppm corresponding to C==O, OCH3, NHCH2, and SCH2, 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 hydrazide hydrate in ethanol for 4 h afforded hydrazides 10a and 10b in good yields. Hydrazides 10a and 10b were first reacted with a NaN3O 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 IC50 values for these compounds, and compound 10b (β-alanine hydrazide)
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,

| S. No. | Structure of compounds | IC$_{50}$ Value [µg/mL] HCT-116 | IC$_{50}$ Value [µg/mL] MCF-7 |
|-------|------------------------|-------------------------------|-------------------------------|
| 1     | ![Structure](image1)   | NA                            | NA                            |
| 2     | ![Structure](image2)   | 3.20                          | 6.62                          |
| 3     | ![Structure](image3)   | 2.19                          | 2.84                          |
| 4     | ![Structure](image4)   | 2.41                          | 2.92                          |
| 5     | ![Structure](image5)   | 2.19                          | 2.38                          |
| 6     | ![Structure](image6)   | 3.10                          | 7.56                          |
| 7     | ![Structure](image7)   | NA                            | NA                            |
| 8     | ![Structure](image8)   | NA                            | NA                            |
| 9     | ![Structure](image9)   | 2.74                          | 3.24                          |
| 10    | ![Structure](image10)  | NA                            | 3.46                          |
| 11    | ![Structure](image11)  | NA                            | NA                            |
| 12    | ![Structure](image12)  | 6.74                          | 3.70                          |
| 13    | ![Structure](image13)  | NA                            | NA                            |

*NA = not active. IC$_{50}$ value [µg/mL] = inhibitory concentration (IC) is expressed in µg/mL.*
9c, 9f, 9b, 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 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).

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).

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 alky chain was mainly found solvent exposed, and this could have affected the stability of the compound in the binding pocket.
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 F254 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 tetramethysilane (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 CHCl3 (25 mL) was added N-cyclohexylthiocarbamoylchexylammonium 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. 1H 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 C12H9N2O2S (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. 1H NMR spectrum (300 MHz, CDCl3), 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, SCH2). 13C NMR spectrum (75.0 MHz, CDCl3), 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 (CH3). Mass spectrometry (MS) (matrix-assisted laser desorption ionization (MALDI), positive mode, matrix DHB) m/z: 300 (M + Na+)2. Found, % C, 69.78; H, 3.96; N, 15.02. For C14H11N2S (277.4). 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. 1H NMR spectrum (300 MHz, CDCl3), 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, SCH2). 13C NMR spectrum (75.0 MHz, CDCl3), 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 (SCH2). MS (MALDI, positive mode, matrix DHB) m/z: 379 (M + Na+)2. Found, % C, 74.51; H, 4.21; N, 7.77. For C12H16N2S (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. 1H NMR spectrum (300 MHz, CDCl3), 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, CH2, J = 17.2 Hz), 5.03 (1H, d, CH2, J = 9.2 Hz), 4.02 (2H, d, SCH2, J = 5.6 Hz). 13C NMR spectrum (75.0 MHz, CDCl3), δ ppm: 154.5, 153.2, 141.8, 139.7, 136.2, 132.4 (CH), 13.1, 129.7, 129.3, 129.0, 128.6, 128.3, 127.0, 117.9 (CH3), 34.8 (SCH2). MS (MALDI, positive mode, matrix DHB) m/z: 302 (M + Na+)2. Found, % C, 73.21; H, 5.12; N, 10.47. For C12H14N2S2 (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. 1H NMR spectrum (300 MHz, CDCl3), 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, OCH2), 4.03 (2H, s, SCH2), 1.29 (3H, t, J = 7.2 Hz, CH3), 1.42 (2H, CH2). MS (MALDI, positive mode, matrix DHB) m/z: 347 (M + Na+)2. Found, % C, 66.55; H, 5.05; N, 8.72. For C14H16N2O2S (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 resulting precipitate was filtered off, washed.
with ethanol and diethyl ether, and then crystallized from aqueous ethanol to yield the corresponding hydrazide.

Yield 82%, yellow powder, mp 166–168 °C. 1H NMR spectrum (300 MHz, CDCl3), 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, NH2), 3.98 (2H, s, SCH2). 13C NMR spectrum (75.0 MHz, CDCl3), δ ppm: 171.2 (C=O), 153.3, 152.3, 141.7, 139.5, 136.7, 129.8, 129.3, 129.1, 128.7, 127.8, 34.5 (SCH2). MS (MALDI, positive mode, matrix DHB) m/z: 333 (M + Na+) 3. Found, % C, 61.87; H, 4.63; N, 18.11. For C27H19N3O3S (381.5). Calculd % C, 62.97; H, 5.02; N, 11.02; S, 8.41.

3.1.2.9. Dimethyl-2-[2-(3-phenyl-quinoxalin-2-ylsulfonyl)-acetylaminoluccinate (8C). From L-AspOMe. Yield 64%, yellow powder, mp 132–134 °C. 1H NMR spectrum (300 MHz, CDCl3), δ ppm (J, Hz): 8.10 (1H, d, J = 8 Hz, ArH), 7.98 (1H, d, J = 8 Hz, ArH), 7.78–7.78 (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, SCH2), 3.60 (3H, s, OMe), 3.28–3.28 (2H, m, CH2O). 13C NMR spectrum (75.0 MHz, CDCl3), δ ppm: 172.7 (C=O), 172.6 (C=O), 168.3 (C=O), 153.5, 153.3, 141.4, 139.7, 127.9, 129.6, 129.3, 128.7, 55.4 (CH), 52.4 (OCH3), 51.9 (OCH3), 34.6 (SCH2), 34.1 (CH2O). MS (MALDI, positive mode, matrix DHB) m/z: 462 (M + Na+) 4. Found, % C, 60.07; H, 4.95; N, 9.62. For C27H19N3O3S (439.5). Calculd % C, 60.12; H, 4.82; N, 9.56; S, 7.30.

3.1.2.10. N-Propyl-2-(3-phenyl-quinoxalin-2-ylsulfonyl)acetamide (9A). From n-propylamine. Yield 65%, yellow powder, mp 71–73 °C. 1H NMR spectrum (300 MHz, CDCl3), 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 Hz, ArH), 7.73 (1H, t, J = 8 Hz, ArH), 7.56–7.54 (3H, m, ArH), 7.10 (1H, bs, NH), 3.95 (2H, s, SCH2), 3.23–3.21 (2H, m, HNCH2), 1.50–1.47 (2H, m, CH2O), 0.83 (3H, t, J = 7.2 Hz, CH3). 13C NMR spectrum (75.0 MHz, CDCl3), δ ppm: 116.8 (C=O), 153.4, 153.2, 141.3, 139.5, 137.6, 129.7, 129.5, 129.2, 128.6, 127.5, 41.8 (NHCH3), 34.9 (SCH2), 22.9 (CH2), 12.1 (CH3). MS (MALDI, positive mode, matrix DHB) m/z: 360 (M + Na)+. Found, % C, 67.55; H, 5.72; N, 12.52. For C27H19N3O3S (373.4). Calculd % C, 67.63; H, 5.68; N, 12.45; S, 9.50.

3.1.2.11. N-Butyl-2-(3-phenyl-quinoxalin-2-ylsulfonyl)acetamide (9B). From n-butylamine. Yield 77%, yellow powder, mp 86–87 °C. 1H NMR spectrum (300 MHz, CDCl3), 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 Hz, ArH), 7.69 (1H, t, J = 8 Hz, ArH), 7.55–7.53 (3H, m, ArH), 7.05 (1H, bs, NH), 3.93 (2H, s, SCH2), 3.27–3.25 (2H, m, HNCH2), 1.42–1.39 (2H, m, CH2), 1.24–1.21 (2H, m, CH2), 0.79 (3H, t, J = 7.2 Hz, CH3). 13C NMR spectrum (75.0 MHz, CDCl3), δ ppm: 168.5 (C=O), 153.6, 153.4, 141.4, 139.6, 137.7, 129.8, 129.6, 129.3, 128.7, 127.4, 42.1 (NCH3), 34.2 (SCH2), 31.2 (CH2), 22.3 (CH2), 17.1 (CH3). MS (MALDI, positive mode, matrix DHB) m/z: 374 (M + Na)+. Found, % C, 68.42; H, 6.11; N, 12.04. For C27H19N3O3S (351.5). Calculd % C, 68.35; H, 6.02; N, 11.96; S, 9.12.

3.1.2.12. N-Allyl-2-(3-Phenyl-quinoxalin-2-yl-sulfonyl)acetamide (9C). From allylamine. Yield 68%, yellow powder, mp 67–68 °C. 1H NMR spectrum (300 MHz, CDCl3), 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, CH2), 5.05 (1H, d, J = 8.4 Hz, CH2), 3.95 (2H, s, SCH2), 3.90–3.88 (2H, m, J = 6.4 Hz, CH2). 13C NMR spectrum (75.0 MHz, CDCl3), 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 (CH2), 41.9 (NCH2), 34.4 (SCH2). MS (MALDI, positive mode, matrix DHB) m/z: 358 (M + Na)+. Found, % C, 68.27; H, 5.10; N,
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. 1H NMR spectrum (300 MHz, CDCl3), δ ppm (J, Hz): 8.09 (1H, d, J = 8 Hz, ArH), 8.02 (1H, d, J = 8 Hz, ArH), 7.73–7.77 (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, SCH2), 3.20–3.18 (2H, m, HNCH2), 1.63–1.61 (2H, m, CH2), 1.31–1.29 (2H, m, CH2), 1.28–1.20 (10H, m, 5CH2). MS (MALDI, positive mode, matrix DHB) m/z: 386 (M + Na)+. Found, % C, 69.42; H, 5.96; N, 11.51. For C23H25N2O3S (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. 1H NMR spectrum (300 MHz, CDCl3), 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, ArH), 7.60–7.58 (3H, m, ArH), 4.07 (2H, s, SCH2), 2.74–2.70 (4H, m, 2CH2), 2.44–2.40 (4H, m, 2CH2), 2.22 (3H, s, CH3). 13C NMR spectrum (75.0 MHz, CDCl3), δ ppm: 167.9 (C=O), 153.7, 153.6, 142.1, 139.7, 137.6, 129.4, 129.3, 128.6, 127.7, 127.4, 127.3, 127.2, 126.8 (CH), 134.9 (SCH2), 33.4, 26.4, 23.1. MS (MALDI, positive mode, matrix DHB) m/z: 416 (M + Na)+. Found, % C, 64.22; H, 5.91; N, 17.87. For C23H25N2O3S (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. 1H NMR spectrum (300 MHz, CDCl3), 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, NH2), 4.05 (2H, s, SCH2), 3.87 (2H, s, SCH2). 13C NMR spectrum (75.0 MHz, CDCl3), δ ppm: 170.8 (C=O), 168.2 (C=O), 153.4, 153.1, 143.1, 139.4, 137.1, 129.4, 129.2, 128.9, 128.4, 127.6, 45.6 (NCH), 34.6 (SCH2). MS (MALDI, positive mode, matrix DHB) m/z: 390 (M + Na)+. Found, % C, 58.76; H, 4.39; N, 19.15. For C22H22N4O3S (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. 1H NMR spectrum (300 MHz, CDCl3), 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, NH2), 3.87 (2H, s, SCH2), 3.57–3.55 (2H, m, NCH), 2.31–2.29 (2H, m, CH2CO), 13C NMR spectrum (75.0 MHz, CDCl3), δ ppm: 171.9, 168.2, 153.3, 153.1, 143.1, 139.6, 137.2, 129.4, 129.3, 129.2, 128.5, 127.6, 42.4 (NCH2), 37.5 (SCH2), 34.8 (CH2CO). MS (MALDI, positive mode, matrix DHB) m/z: 404 (M + Na)+. Found, % C, 59.88; H, 4.94; N, 18.42. For
C8H14N2O,S (381.5). Calcd % C, 59.82; H, 5.02; N, 18.36; S, 8.41.

3.1.2.22. 2-[2-[3-Phenyl-quinazalin-2-ylsulfanyl]-acetylamino]-acetylamino)-acetic Acid Methyl Ester (11a). Abbreviated: Gly-Gly. Yield 57%, yellow powder, mp 76–78 °C. 1H NMR spectrum (300 MHz, CDCl3), 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, CH2), 4.01–3.99 (2H, m, CH2), 3.87 (2H, s, SCH2), 3.54 (3H, s, OMe). 13C NMR spectrum (75.0 MHz, CDCl3), δ ppm: 171.8 (C=O), 168.2 (C=O), 153.5, 153.3, 151.9, 139.4, 137.3, 129.5, 129.0, 128.6, 127.4, 127.0, 52.8 (OCH3), 45.5 (NCH), 42.6 (NCH3), 34.3 (SCH2), 31.3 (CH2CO). MS (MALDI, positive mode, matrix DHB) m/z: 447 (M + Na)+. Found, % C, 60.31; H, 5.09; N, 12.84. For C18H16N6O12S (438.5). Calcd % C, 60.26; H, 5.06; N, 12.78; S, 7.31.

3.1.2.26. 3-[2-[3-Phenyl-quinazolin-2-ylsulfanyl]-acetylamino)propionyl-amino)-propionic Acid Methyl Ester (12b). Abbreviated: β-Ala-β-Ala. Yield 62%, yellow powder, mp 84–86 °C. 1H NMR spectrum (300 MHz, CDCl3), 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, SCH2, 2NCH3), 3.63 (3H, s, OMe), 2.11–2.08 (4H, m, 2CH2). 13C NMR spectrum (75.0 MHz, CDCl3), δ ppm: 172.7 (C=O), 168.9 (C=O), 153.6, 153.4, 141.6, 139.5, 137.4, 137.4, 129.61, 129.1, 128.7, 127.3, 127.0, 52.9 (OCH3), 45.6 (NCH3), 42.4 (NCH3), 34.3 (SCH2), 31.7 (CH2CO), 31.3 (CH3CO). MS (MALDI, positive mode, matrix DHB) m/z: 475 (M + Na)+. Found, % C, 61.12; H, 5.39; N, 12.27. For C18H16N6O12S (452.5). Calcd % C, 61.05; H, 5.35; N, 12.38; S, 7.09.

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%) fetal bovine serum (FBS), (10%) selenium chloride, (10%) glutamine, and (10%) penicillin/streptomycin. The cultures were placed in a CO2 (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

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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 performed 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. 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)

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Notes

The authors declare no competing financial interest.

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