

Scientific paper

# Uses of Anthranilic Acid for the Synthesis of Dihydroquinazolin Derivatives with Antitumor, Antiproliferative and Pim-1 kinase Activities

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Received: 26-12-2017

## Abstract

The reaction of anthranilic acid with ethoxycarbonylthiocyanate gave the ethyl 4-oxo-2-thioxo-1,2-dihydroquinazoline-3(4*H*)-carboxylate (**4**). The reaction of compound **4** with hydrazine hydrate and  $\alpha$ -halocarbonyl derivatives was found to give either hydrazono or *S*-alkylated products. Heterocyclization reactions of some of the *S*-alkylated derivatives **8** and **12** were carried out to afford thiazole, pyran and pyridine derivatives. The cytotoxicity of the newly synthesized compounds towards the six cancer cell lines NUGC, DLD-1, HA22T, HEPG-2, HONE-1 and MCF-7 showed that compounds **6**, **8**, **13**, **19c–f**, **21b–f**, **24a** and **24c** with the highest cytotoxicity. The *c*-Met kinase inhibition for some of the selected compounds showed that compounds **8**, **13**, **19d**, **21e**, **21f** and **24a** were the most active compounds. Screening toward tyrosine kinases revealed that compounds **13**, **21e** and **24a** exhibit the highest inhibitions and therefore their molecular modeling was described. In addition, compounds **13** and **24a** showed the highest activities towards Pim-1 kinase.

**Keywords:** Dihydroquinazolin; thiophene; thiazole; pyran; pyridine; cytotoxicity

## 1. Introduction

2-Thioxoquinazolin-4(1*H*)-ones are important heterocyclic compounds that are widely present in natural products as well as medicinal, and pharmacological compounds.<sup>1</sup> In addition, several thioxoquinazolin analogues have been developed as antitumor, antibiotic, antifibrilator and antipyretic agents (Fig. 1). Furthermore, they display a broad range of applications against diabetes,<sup>2</sup> cancer,<sup>3</sup> and as selective plant grow regulators.<sup>4,5</sup> Given the importance of these nitrogen heterocyclic compounds, the development of mild, high-yielding and clean synthesis of these important compounds is a daunting challenge and has been extensively investigated and described in the literature.<sup>6–12</sup> The classical methods for the synthesis of quinazolinone ring system are the reaction of anthranilic acid and its derivatives with isothiocyanates or their equivalents.<sup>13–27</sup> Recently, our research group was involved in the synthesis and determination of the anti-proliferative and anti-inflammatory activity of a large number of heterocyclic compounds.<sup>28,29</sup> In the continuation of this pro-

gram, in this context, we aimed to develop an efficient and facile approach to synthesize and measure the cytotoxicity of a series of thioxoquinazolin-4(1*H*)-ones from anthranilic acid and ethoxycarbonylthiocyanate.

## 2. Results and Discussion

Quinazoline derivatives showed a wide range spectrum of biological applications, especially in the field of anti-cancer therapy<sup>30</sup> which encouraged us to direct our work towards their synthesis. Thus, in the present work we aimed to synthesis a series of heterocyclic compounds derived from dihydroquinazolin derivative. The reaction of anthranilic acid (**1**) with ethoxycarbonylthiocyanate (**2**) in 1,4-dioxane gave the ethyl 4-oxo-2-thioxo-1,2-dihydroquinazoline-3(4*H*)-carboxylate (**4**). The formation of the latter product took place through intermediate formation of the thiourea derivative followed by cyclization. Compound **4** was used for the synthesis of different heterocyclic compounds through its reaction with various chemi-

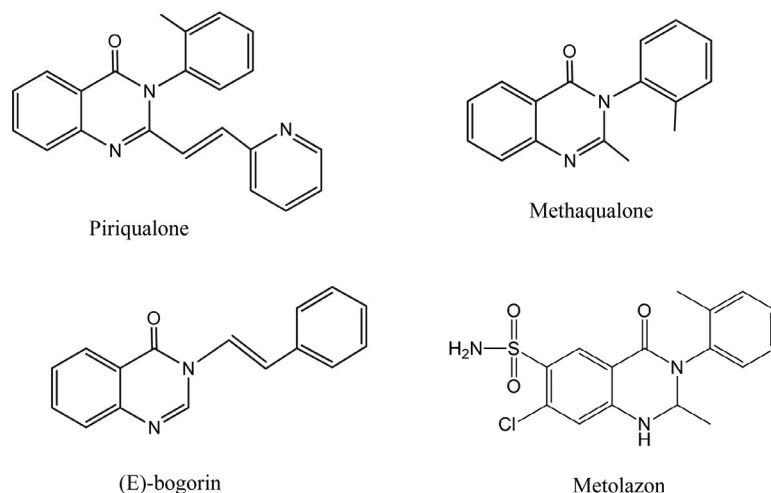
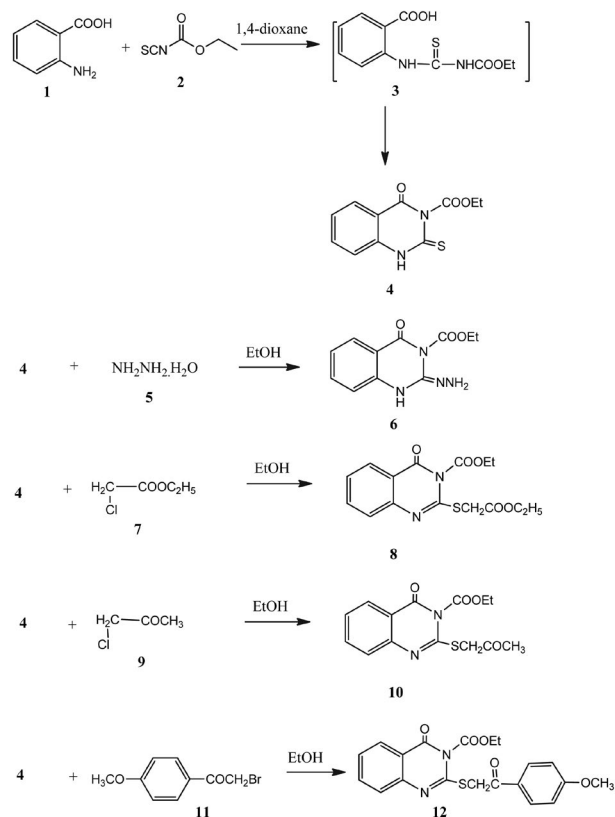


Fig. 1. Selected examples of quinazolin analogues with pharmacological activities

cal reagents. Thus, the reaction of compound **4** with hydrazine hydrate (**5**) gave the hydrazone derivative **6**. Compound **4** was capable to form thioether derivatives through its reaction with  $\alpha$ -halocarbonyl compounds. Thus, the reaction of compound **4** with any of the following: ethyl chloroacetate (**7**), chloroacetone (**9**) or 2-bromo-1-(4-methoxyphenyl)ethanone (**11**), gave the thioether derivatives **8**, **10** and **12**, respectively (Scheme 1). The structures of compounds **8**, **10** and **12** were established on the basis of their analytical and spectral data. Thus, the  $^1\text{H}$  NMR spectrum of compound **12** (as an example) showed the presence of a triplet at  $\delta$  1.13 and a quartet at  $\delta$  4.22 ppm showing the presence of an ester  $\text{CH}_3$  and  $\text{CH}_2$ , respectively, a singlet at  $\delta$  3.73 ppm showing the  $\text{OCH}_3$  group, a singlet at  $\delta$  5.49 ppm for the  $\text{CH}_2$  group and a multiplet at  $\delta$  7.23–7.42 ppm for the two  $\text{C}_6\text{H}_4$  groups. In addition, the  $^{13}\text{C}$  NMR spectrum showed signals at  $\delta$  16.4 (ester  $\text{CH}_3$ ), 37.5 ( $\text{CH}_2$ ), 53.4 (ester  $\text{CH}_2$ ), 55.2 ( $\text{OCH}_3$ ), 119.2, 120.8, 122.3, 124.4, 124.9, 126.2, 126.8, 127.8, 128.4, 129.1 indicating the presence of two  $\text{C}_6\text{H}_4$  groups and three signals at  $\delta$  163.2, 164.4, 165.8 confirming the three CO groups.

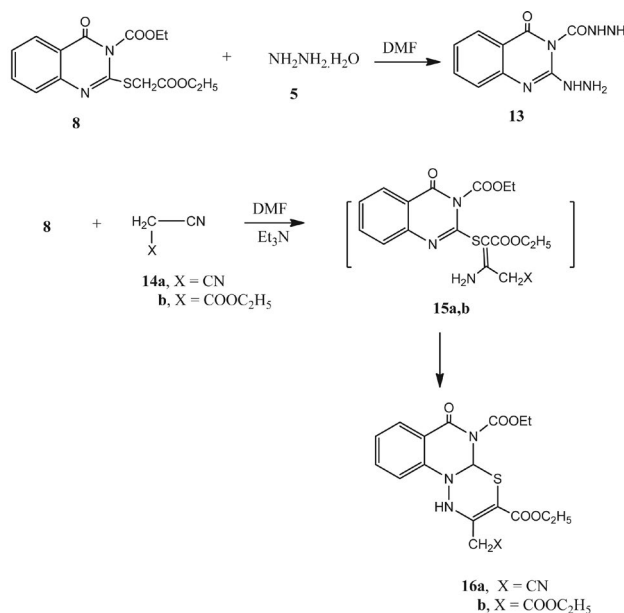
The reaction of compound **8** with hydrazine hydrate (**5**) gave the hydrazino derivative **13**. On the other hand, the reaction of compound **8** with either of malononitrile (**14a**) or ethyl cyanoacetate (**14b**) gave the dihydro[1,3,4]thiadiazino[3,2-*a*]quinazolin-6(1*H*)-one derivatives **16a** and **16b**, respectively (Scheme 2). Formation of compounds **16a** and **16b** took place through the intermediate formation of **15a,b** followed by cyclization. The structures of compounds **16a** and **16b** were confirmed on the basis of analytical and spectral data. Thus, the  $^1\text{H}$  NMR spectrum of **16a** showed, besides the expected signals, two triplets at  $\delta$  1.12, 1.15 and two signals at  $\delta$  4.16, 4.20 ppm indicating the presence of two ester  $\text{CH}_3$  and  $\text{CH}_2$  groups, respectively, a singlet at  $\delta$  6.01 ppm belonging to the pyrimidine H-2 and a singlet at  $\delta$  8.28 ppm for the NH group. In addition its  $^{13}\text{C}$  NMR spectrum showed two signals at  $\delta$  16.3, 16.5



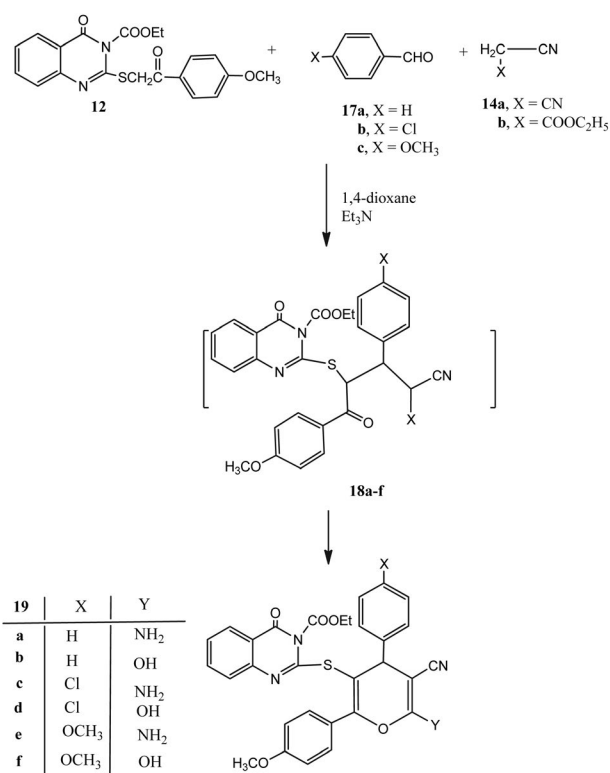
Scheme 1: Synthesis of compounds **4**, **6**, **8**, **10** and **12**.

for the two ester  $\text{CH}_3$  groups and two quartets at  $\delta$  52.1, 53.8 for the two ester  $\text{CH}_2$  groups as well as three signals at  $\delta$  163.8, 164.4, 165.2 belonging to the three CO groups.

In continuation of our recent interest to conduct multi-component reactions using aromatic aldehydes and cyanomethylene reagents<sup>31–33</sup> we studied the multi-component reactions of compound **12**. Thus, the multi-component reaction of compound **12** with any of the aromatic



Scheme 2: Synthesis of compounds 13 and 16a,b.



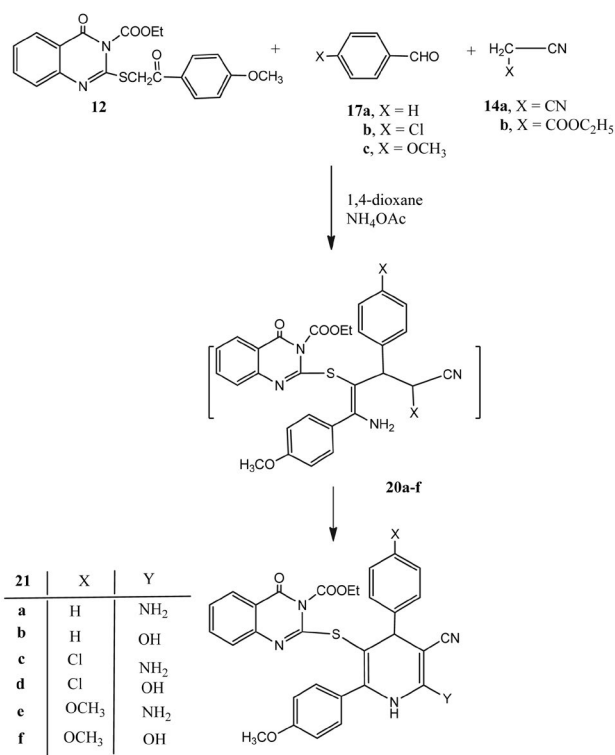
Scheme 3: Synthesis of compounds 19a-f.

aldehydes namely, benzaldehyde (17a), 4-chlorobenzaldehyde (17b) or 4-methoxybenzaldehyde (17c) and either of malononitrile (14a) or ethyl cyanoacetate (14b) in 1,4-dioxane containing triethylamine gave the pyridine derivatives 19a–f, respectively through the intermediate formation of 18a–f. The characterization of the compounds 19a–f

(Scheme 3) is based on their respective analytical and spectral data (see Experimental section).

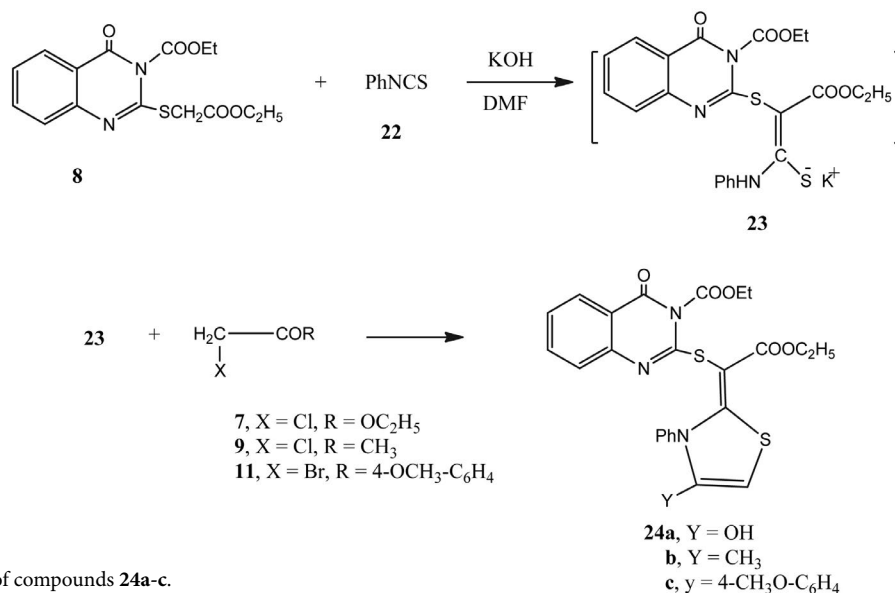
On the other hand, the multi-component reaction of compound 12 with any of benzaldehyde (17a), 4-chlorobenzaldehyde (17b) or 4-methoxybenzaldehyde (17c) and either of malononitrile (14a) or ethyl cyanoacetate (14b) in 1,4-dioxane containing ammonium acetate gave the pyridine derivatives 21a–f, respectively (Scheme 4). Formation of the latter products took place through the intermediate formation of 20a–f.

Recently, our research group was involved in a comprehensive program involving the reactions of active methylene reagents with phenylisothiocyanate in basic dimethylformamide solutions, followed by heterocyclization with  $\alpha$ -halocarbonyl compounds.<sup>34–36</sup> Products of the re-



Scheme 4: Synthesis of compounds 21a-f.

actions were either thiophene or thiazole derivatives or a mixture of both depending on the reaction conditions and the nature of the  $\alpha$ -halocarbonyl compound. In continuation of this program we carried out the reaction of compound 8 with phenylisothiocyanate (22) in dimethylformamide containing potassium hydroxide to give the intermediate potassium salt 23. The reaction of the intermediate 23 with any of ethyl chloroacetate (7), chloroacetone (9) or 2-bromo-1-(4-methoxyphenyl)ethanone (11) afforded the thiazole derivatives 24a–c, respectively (Scheme 5). All synthesized compounds were obtained in good yields and their cytotoxicity against cancer cell lines was measured.



Scheme 5: Synthesis of compounds 24a-c.

## 2. 1. Biological Evaluation

### 2. 1. 1. *In vitro* Cytotoxic Assay

#### Chemicals

Fetal bovine serum (FBS) and L-glutamine were purchased from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was purchased from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), foretinib, penicillin, streptomycin and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (Saint Louis, USA).

#### Cell Cultures

Were obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK) and human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF-7), nasopharyngeal carcinoma (HONE1) and normal fibroblast cells (WI38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as a monolayer and were routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2  $\mu\text{M}$  glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100  $\mu\text{g}/\text{mL}$ ), at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>. Exponentially growing cells were obtained by plating  $1.5 \times 10^5$  cells/mL for the six human cancer cell lines including cells derived from  $0.75 \times 10^4$  cells/mL followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

The heterocyclic compounds, prepared in this study, were evaluated according to standard protocols for their *in vitro* cytotoxicity against six human cancer cell lines, including cells derived from human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharynge-

al carcinoma (HONE1) and a normal fibroblast cells (WI38). All of IC<sub>50</sub> values are listed in Table 1. Some heterocyclic compounds were observed to display significant cytotoxicity against most of the cancer cell lines tested (IC<sub>50</sub> = 10–1000 nM). Normal fibroblasts cells (WI38) were affected to a much lesser extent (IC<sub>50</sub> >10,000 nM). The reference compound used was the foretinib anti-tumor agent.

### 2. 1. 2. Structure Activity Relationship

It is clear from Table 1 that most of the tested compounds showed cytotoxicity against the selected cancer cell lines. Compound 4 showed high cytotoxicity against HEPG-2 and MCF-7 cell lines with IC<sub>50</sub> values of 683 and 460 nM, respectively. The reaction of compound 4 with hydrazine hydrate gave the hydrazone derivative 6 which showed high potency against the six cancer cell lines, this was attributed to the high nitrogen content in this compound. It is worthy to note that compound 6 showed higher cytotoxicity than foretinib against DLD-1 and HEPG-2 cell lines with IC<sub>50</sub> values of 69 and 125 nM, respectively. On the other hand, the reaction of compound 4 with ethyl  $\alpha$ -chloroacetate gave the thioether derivative 8 with high cytotoxicity against the six cancer cell lines but its potency is a little bit less than that of compound 6. It is obvious that compound 8 showed higher cytotoxicity than foretinib against DLD-1 and HEPG-2 cell lines with IC<sub>50</sub> values of 230 and 64 nM, respectively. On the other hand, the thioether 10 showed high potency against HONE-1 and MCF-7 cell lines but compound 12 showed low potency against the six cancer cell lines. The reaction of compound 8 with hydrazine hydrate gave the hydrazino derivative 13 with a high nitrogen content, showing relatively high potency against the six cancer cell lines. On the other hand, the dihydro-[1,3,4]thiadiazino[3,2-*a*]quinazoline deriva-

**Table 1:** Cytotoxicity of the newly synthesized products against a variety of cancer cell lines [IC<sub>50</sub><sup>a</sup> (nM)]

Compound No.	Cytotoxicity (IC <sub>50</sub> in nM)						
	NUGC <sup>b</sup>	DLD-1 <sup>b</sup>	HA22T <sup>b</sup>	HEPG-2 <sup>b</sup>	HONE-1 <sup>b</sup>	MCF-7 <sup>b</sup>	WI-38 <sup>b</sup>
4	1250	1280	1088	683	1159	460	NA
6	75	69	59	125	312	189	NA
8	137	230	219	64	312	109	NA
10	1089	1694	1741	2493	253	290	NA
12	1224	1476	2251	1122	1373	1229	NA
13	430	784	352	280	1879	128	NA
16a	1466	2369	1763	2461	2749	1863	NA
16b	2557	2590	2430	1461	2893	1279	NA
19a	2539	3167	2577	2690	1993	3289	NA
19b	1368	1273	1549	1366	2165	2540	NA
19c	65	530	250	149	259	426	NA
19d	159	250	59	114	189	550	NA
19e	30	62	74	39	1330	88	NA
19f	1089	1146	89	122	320	422	320
21a	2166	2253	1259	2769	2429	2760	NA
21b	88	79	194	520	287	1221	NA
21c	1243	138	229	1821	128	220	NA
21d	480	679	1293	580	744	124	NA
21e	129	226	183	480	136	229	NA
21f	55	79	134	109	85	93	NA
24a	380	219	179	229	312	59	NA
24b	2848	2218	2214	2373	1072	1238	NA
24c	93	68	169	421	629	229	NA
Foretinib	23	258	48	240	35	35	NA

<sup>a</sup> Drug concentration required to inhibit tumor cell proliferation by 50% after continuous exposure of 48 h. <sup>b</sup> NUGC, gastric cancer; DLD-1, colon cancer; HA22T, liver cancer; HEPG-2, liver cancer; HONE-1, nasopharyngeal carcinoma; MCF-7, breast cancer; WI-38, normal fibroblast cells. NA: Not Active.

tives **16a,b** showed low potency. The multi-component reactions of compound **12** with any of the aromatic aldehydes **17a–c** and either of malononitrile or ethyl cyanoacetate gave the pyran derivatives **19a–f**. Compounds **19c** (X = Cl, Y = NH<sub>2</sub>), **19d** (X = Cl, Y = OH) and **19e** (X = OCH<sub>3</sub>, Y = NH<sub>2</sub>) showed the highest cytotoxicity among this series of compounds. On the other hand, the pyridine derivatives **21a–f** where the four compounds **21b** (X = H, Y = OH), **21d** (X = Cl, Y = OH), **21e** (X = OCH<sub>3</sub>, Y = NH<sub>2</sub>) and **21f** (X = OCH<sub>3</sub>, Y = OH) showed the highest potency. Compound **21f** showed cytotoxicity higher than foretinib against the DLD-1 cell line with IC<sub>50</sub> 79 nM. Considering the thiazole derivatives **24a–c**, it is obvious that compounds **24a** (Y = OH) and **24c** (Y = 4-OCH<sub>3</sub>-phenyl) showed higher potency than **24b** (Y = CH<sub>3</sub>). It is clear that throughout the synthesized compounds the presence of the electronegative groups, like the Cl and OH groups, or the electron-rich NH<sub>2</sub> groups enhance the potency of the compound.

### 2. 1. 3. Cell Proliferation Assay

The anti-proliferative activity of compounds **6**, **8**, **13**, **19c**, **19d**, **19e**, **21b**, **21d**, **21e**, **21f**, **24a** and **24c** was evaluated (Table 2) against the five c-Met-dependent cancer cell lines (A549, HT-29, MKN-45, U87MG, and SMMC-7721)

and one c-Met-independent cancer cell line (H460) using the standard MTT assay *in vitro*, with foretinib as the positive control.<sup>37,38</sup> The cancer cell lines were cultured in the minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). Approximate 4 × 10<sup>3</sup> cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO<sub>2</sub> at 37 °C for 24 h. The compounds tested at the indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 mg/mL, and incubated with cells at 37 °C for 4 h. The formazan crystals were dissolved in 100 μL of DMSO for each well, and the absorbance at 492 nM (for absorbance of MTT formazan) and 630 nM (for the reference wavelength) was measured with an ELISA reader. All of the compounds were tested three times in each cell line. The results expressed as IC<sub>50</sub> (inhibitory concentration 50%) are the averages of three determinations and were calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

### 2. 1. 4. In vitro Cell Assays

The antitumor evaluations<sup>39</sup> of the synthesized compounds were measured against A549 (non-small cell lung

Table 2. In vitro growth inhibitory effects  $IC_{50} \pm SEM$  ( $\mu M$ ) of the most potent compounds

Compound No	$IC_{50} \pm SEM$ ( $\mu M$ )					
	A549	H460	HT29	MKN-45	U87MG	SMMC-7721
<b>6</b>	1.28 ± 0.67	1.58 ± 0.65	0.65 ± 0.32	1.58 ± 0.49	0.39 ± 0.18	0.29 ± 0.19
<b>8</b>	1.02 ± 0.80	1.27 ± 0.69	1.28 ± 0.79	1.04 ± 0.48	1.49 ± 0.72	1.26 ± 0.73
<b>13</b>	0.09 ± 0.06	0.06 ± 0.01	0.04 ± 0.06	0.83 ± 0.26	0.69 ± 0.32	0.73 ± 0.25
<b>19c</b>	0.77 ± 0.34	0.28 ± 0.06	0.93 ± 0.24	0.72 ± 0.14	0.48 ± 0.13	0.93 ± 0.18
<b>19d</b>	1.02 ± 0.36	1.18 ± 0.42	0.93 ± 0.26	0.63 ± 0.14	1.63 ± 0.87	0.32 ± 0.16
<b>19e</b>	0.63 ± 0.28	0.36 ± 0.25	0.03 ± 0.53	0.28 ± 0.11	0.26 ± 0.07	0.80 ± 0.24
<b>21b</b>	3.26 ± 1.72	3.59 ± 1.30	1.42 ± 0.80	2.83 ± 0.46	1.74 ± 0.79	1.40 ± 0.73
<b>21d</b>	0.87 ± 0.22	0.69 ± 0.21	0.59 ± 0.11	0.69 ± 0.21	0.70 ± 0.12	0.83 ± 0.24
<b>21e</b>	0.18 ± 0.02	0.25 ± 0.09	0.36 ± 0.09	0.16 ± 0.07	0.42 ± 0.16	0.55 ± 0.19
<b>21f</b>	2.31 ± 0.69	2.49 ± 0.80	1.43 ± 0.79	1.08 ± 0.75	2.39 ± 0.93	1.38 ± 0.91
<b>24a</b>	1.02 ± 0.53	1.08 ± 0.55	2.39 ± 0.88	1.48 ± 0.93	0.95 ± 0.29	1.63 ± 0.82
<b>24c</b>	0.19 ± 0.01	0.28 ± 0.07	0.80 ± 0.09	0.57 ± 0.08	0.93 ± 0.27	0.75 ± 0.16
<b>Foretinib</b>	0.08 ± 0.01	0.18 ± 0.03	0.15 ± 0.023	0.03 ± 0.0055	0.90 ± 0.13	0.44 ± 0.062

cancer), H460 (human lung cancer), HT-29 (human colon cancer) and MKN-45 (human gastric cancer), U87MG (human glioblastoma) and SMMC-7721 (human liver cancer) cancer cell lines. Foretinib was used as the positive control by a MTT assay. The results are expressed as  $IC_{50}$  after three different experiments were summarized and are shown in Table 2. The data listed in Table 2 reveal that the compounds possess moderate to strong cytotoxicity against the six tested cell lines in the single-digit  $\mu M$  range, and high selectivity for inhibition of A549, H460 and MKN-45 cells. The promising compounds were **13**, **19c**, **19e**, **21d**, **21e** and **24c**, these were the most active with  $IC_{50}$  values of 0.09 and 0.93  $\mu M$ , respectively. Moreover, compound **13** showed potency higher than foretinib towards H460 with  $IC_{50}$  0.06  $\mu M$ . Compounds **6**, **13**, **19e**, **21d**, **21e** showed activities toward U87MG cell line higher than foretinib with  $IC_{50}$  values of 0.39, 0.69, 0.26, 0.70 and 0.42  $\mu M$ , respectively. It is of great value to note that compound **6** showed higher potency than foretinib with  $IC_{50}$  0.29  $\mu M$  against SMMC-7721.

### 2. 1. 5. HTRF Kinase Assay

The c-Met kinase activities (Table 3) of the most potent compounds **6**, **8**, **13**, **19c**, **19d**, **19e**, **21b**, **21d**, **21e**, **21f**, **24a** and **24c** were measured using homogeneous time-resolved fluorescence (HTRF) assay as previously reported.<sup>40,41</sup> In addition, the most potent compounds toward c-Met kinase were further evaluated against other five tyrosine kinases (c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR) using the same method (Table 4). Briefly, 20 mg/mL poly (Glu, Tyr) 4:1 (Sigma) was used as a substrate in 384-well plates. Then, 50  $\mu L$  of 10 mM ATP (Invitrogen) solution diluted in kinase reaction buffer (50 mM HEPES, Ph 7.0, 1 M DTT, 1 M  $MgCl_2$ , 1 M  $MnCl_2$ , and 0.1%  $NaN_3$ ) was added to each well. Various concentrations of the tested compounds diluted in 10  $\mu L$  of 1% DMSO (v/v) were used as the negative control. The kinase reaction was start-

ed by the addition of the purified tyrosine kinase proteins diluted in 39  $\mu L$  of kinase reaction buffer solution. The incubation times for the reactions were 30 min at 25 °C and were ceased by the addition of 5  $\mu L$  of Streptavidin-XL665 and 5  $\mu L$  Tk Antibody Cryptate working solution added to all of wells. The plates were read using Envision (Perkin-Elmer) at 320 and 615 nm. The inhibition rate (%) was calculated using the mathematical equation: % inhibition =  $100 - [(Activity\ of\ enzyme\ with\ tested\ compounds - Min)/(Max - Min)] \times 100$  (Max: the observed enzyme activity measured in the presence of enzyme, substrates, and cofactors; Min: the observed enzyme activity in the presence of substrates, cofactors and in the absence of enzyme).  $IC_{50}$  values were calculated using the inhibition curves.

According to Table 3 it is clear that compounds **8**, **13**, **19d**, **21e**, **21f** and **24a** were the most active compounds towards c-Met kinase. Moreover, compounds **13**, **19d** and **24a** showed activity towards c-Met enzymatic activity higher than that of foretinib.

Table 3. c-Met enzymatic activity and line of the most potent compounds

Compound No	X	Y	$IC_{50}$ (nM) c-Met
<b>6</b>	–	–	10.22 ± 2.38
<b>8</b>	–	–	1.34 ± 0.81
<b>13</b>	–	–	0.06 ± 0.001
<b>19c</b>	Cl	NH <sub>2</sub>	2.26 ± 0.80
<b>19d</b>	Cl	OH	0.83 ± 0.19
<b>21b</b>	H	OH	4.30 ± 1.89
<b>21d</b>	Cl	OH	12.28 ± 2.69
<b>21e</b>	OCH <sub>3</sub>	NH <sub>2</sub>	1.27 ± 0.98
<b>21f</b>	OCH <sub>3</sub>	OH	1.40 ± 0.51
<b>24a</b>	–	OH	0.79 ± 0.09
<b>24c</b>	–	4-OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	8.50 ± 2.19
<b>Foretinib</b>	–	–	1.16 ± 0.17

### 2. 1. 6. Inhibitory Effects of the Most Active Compounds Towards Tyrosine Kinases

The most potent compounds **8**, **13**, **19d**, **21e**, **21f** and **24a** towards c-Met enzymatic activity of the five tyrosine kinases (c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR) were determined using the same method described for c-Met enzymatic activity and the data are shown in Table 4. Here foretinib was also used as the reference drug. It is clear from Table 4 that compounds **13**, **21e** and **24a** showed the highest inhibitory effect towards the five tyrosine kinases.

Table 4. inhibitory effect of compounds **8**, **13**, **19d**, **21e**, **21f** and **24a** towards tyrosine kinases [Enzyme IC<sub>50</sub> (nM)]

Compound	c-Kit	Flt-3	VEGFR-2	EGFR	PDGFR
<b>8</b>	9.26	8.18	7.66	4.50	6.85
<b>13</b>	1.32	2.59	1.08	1.26	0.99
<b>19d</b>	10.39	8.68	9.09	6.49	7.30
<b>21e</b>	0.42	0.76	0.69	0.74	0.25
<b>21f</b>	8.57	8.55	10.39	8.48	10.99
<b>24a</b>	0.82	2.80	0.79	1.59	1.33
<b>Foretinib</b>	0.19	0.17	0.20	0.13	0.26

### 2. 1. 7. Pim-1 Kinase Inhibition of Compounds **13**, **21e** and **24a**

Compounds **13**, **21e** and **24a** were selected to examine their Pim-1 kinase inhibition activity<sup>42</sup> at a range of 10 concentrations and the IC<sub>50</sub> values were calculated. Our selection for **13**, **21e** and **24a** was based on their relative activity towards c-Met kinase, together with their inhibition towards tyrosine kinases; the more potent to inhibit Pim-1 activity were **13** and **24a** with IC<sub>50</sub> value of 0.36 and 0.28

μM, while **21e** was less effective (IC<sub>50</sub> > 10 μM). SGI-1776 was used as the positive control with IC<sub>50</sub> 0.048 μM in the assay. These profiles in combination with cell growth inhibition data of compounds **13**, **21e** and **24a** are listed in Table 5 and indicate that Pim-1 is a potential target of these compounds.

Table 5. The inhibitor activity of compounds **13**, **21e** and **24a** on Pim-1 Kinase.

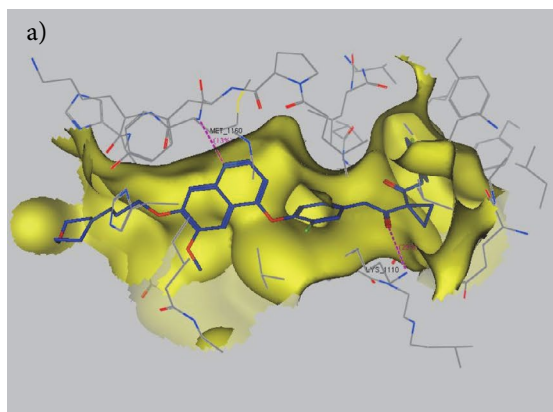
Compound	Inhibition ratio At 10 μM	IC <sub>50</sub> (μM)
<b>13</b>	86	0.36
<b>21e</b>	34	> 10
<b>24a</b>	95	0.28
SGI-1776	–	0.048

### Experimental Protocol of Docking Study

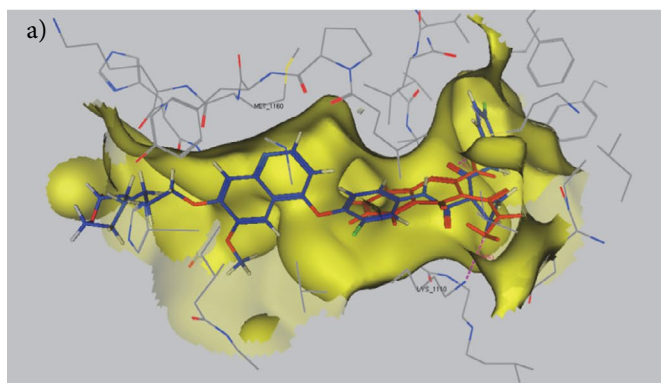
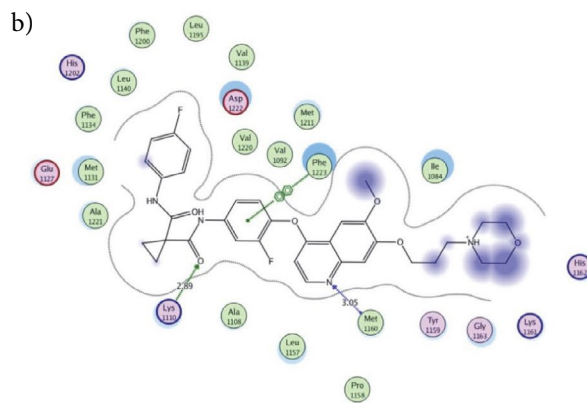
All the molecular modeling studies were carried out on an Intel Pentium 2.3 GHz processor, 8 GB memory with Windows 7 operating system using Molecular Operating Environment (MOE, 10.2008; Chemical Computing Group, Canada) software. The X-ray crystallographic structure of c-Met kinase enzyme with its co-crystallized ligand XL880 (Foretinib) in the file (PDB ID: 3LQ8) was obtained from RCSB Protein Data Bank with a 2.02 Å resolution. All water of crystallization was deleted from the active site except the one involved in interactions with the ligand. Hydrogens and partial charges were added to the system using protonate 3D application. Isolation of the active site, recognition of the amino acids and the backbone was hidden. The docking algorithm was validated via docking of the native ligand (Foretinib) into its c-Met kinase active site where the docking procedure was able to

Table 6. Docking study data showing amino acid interactions and the hydrogen bond lengths of target compounds and foretinib on c-Met kinase enzyme.

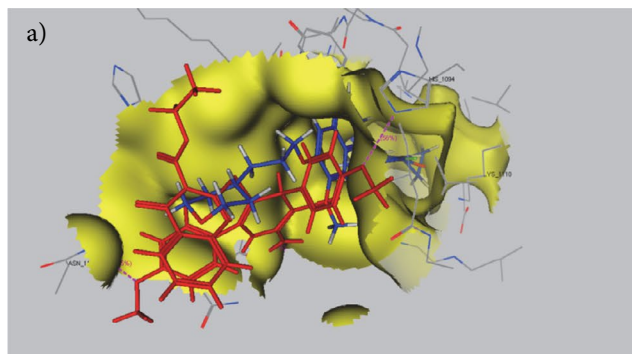
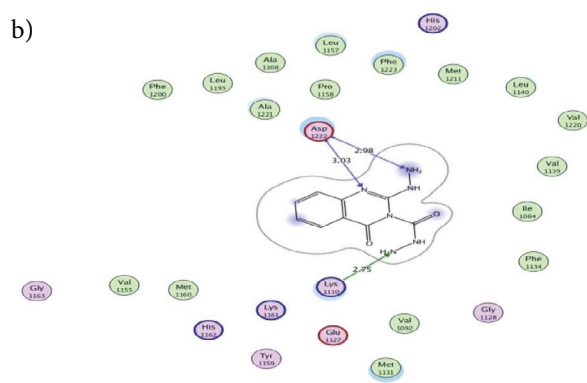
Compound number	Number of H-bonds	Number of π-π interactions with Phe <sup>1223</sup>	Atoms of compound forming H-bond	Amino acid residues forming H-bonds (H-bond length in Å)	Binding energy score (kcal/mol)
Ligand (Foretinib)	2	1	Quinazoline N (H-acceptor) CO (H-acceptor)	Met <sup>1160</sup> (3.05) Lys <sup>1110</sup> (2.89)	-16.37
<b>13</b>	3	-	Quinazoline N (H-acceptor) NHNH <sub>2</sub> (H-acceptor) CONHNH <sub>2</sub> (H-acceptor)	Asp <sup>1222</sup> (3.03) Asp <sup>1222</sup> (2.98) Lys <sup>1110</sup> (2.75)	-11.45
<b>21e</b>	3	-	NH <sub>2</sub> (H-donor) OCH <sub>3</sub> (H-acceptor) OCH <sub>3</sub> (H-acceptor)	Asp <sup>1164</sup> (1.46) Asn <sup>1171</sup> (2.94) His <sup>1094</sup> (2.86)	-7.83
<b>24a</b>	2	-	OH (H-donor) OH (H-acceptor)	Asp <sup>1164</sup> (1.23) Asn <sup>1171</sup> (2.66)	-11.58



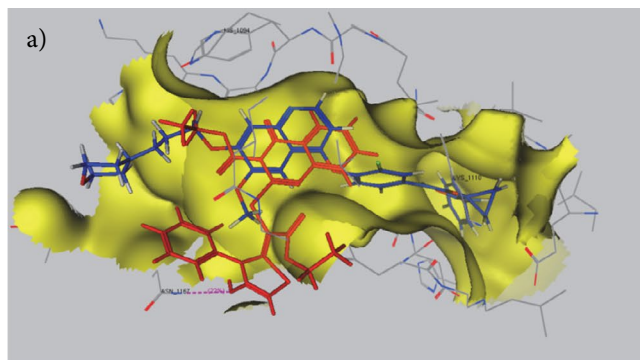
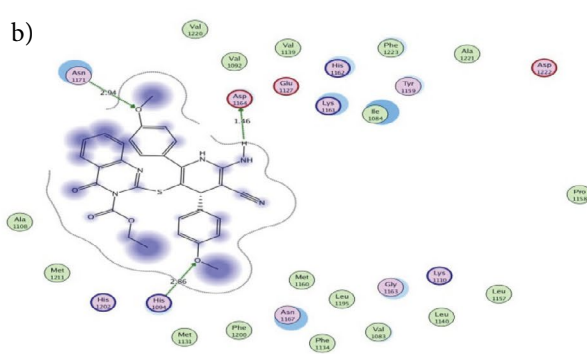
**Figure 2.** Interactions of XL880 (foretinib) with the amino acid residues of the active site of c-Met 3D(a) and 2D(b)



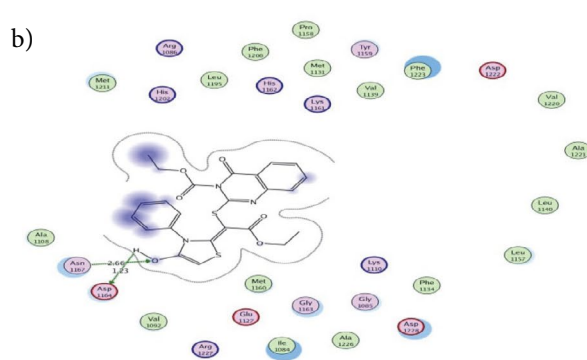
**Figure 3.** (a) The superposition of foretinib (blue) and compound **13** (red) docked in the binding site of c-Met, the dotted lines represent H-bonding interactions; (b) 2D ligand interaction of **13** in binding site of c-Met.



**Figure 4.** (a) The superposition of foretinib (blue) and compound **21e** (red) docked in the binding site of c-Met, the dotted lines represent H-bonding interactions; (b) 2D ligand interaction of **21e** in binding site of c-Met.



**Figure 5.** (a) The superposition of foretinib (blue) and compound **24a** (red) docked in the binding site of c-Met, the dotted lines represent H-bonding interactions; (b) 2D ligand interaction of **24a** in binding site of c-Met.





retrieve the co-crystallized pose with RMSD value of 0.55 Å. The three-dimensional structures of the most active compounds **13**, **21e** & **24a** were built using MOE molecular builder, then their energy was minimized by Merck Molecular Force Field (MMFF94x). Hydrogens and partial charges were added to the system using protonate 3D application.

### Docking Results

For each docked compound, only one pose was selected based on number of binding interactions, superposition with the original ligand, docking score and the formed H-bonds were measured. The docking results obtained from the docking study are summarized in Table 6.

### Discussion of Molecular Modeling

The X-ray crystallographic structure of XL880 (foretinib) in complex with c-Met kinase shows that the inhibitor forms two hydrogen bonds between quinoline N and Met<sup>1160</sup>, CO of malonamide moiety and Lys<sup>1110</sup>. Phe<sup>1223</sup> of the activation loop has relocated from the position in the active conformation to stack underneath the fluorophenyl ring ( $\pi$ - $\pi$  interaction), placing the kinase in a pseudo-unactivated conformation<sup>43</sup> (Fig. 2). Compounds **13**, **21e** and **24a** showed good fitting to the active binding site of c-Met kinase by interaction with Asp<sup>1222</sup>, Lys<sup>1110</sup>, Asp<sup>1164</sup>, Asn<sup>1171</sup> and His<sup>1094</sup> amino acid residues (Fig. 3–5).

## 3. Experimental

### 3.1. General

All melting points were determined on an electrothermal apparatus (Büchi 535, Switzerland) in an open capillary tube and are uncorrected. <sup>13</sup>C NMR and <sup>1</sup>H NMR spectra were recorded on Bruker DPX200 instrument in DMSO with TMS as internal standard for proton spectra and solvent signals as internal standard for carbon spectra. Chemical shift values are given in  $\delta$  (ppm). Mass spectra were recorded on EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. Elemental analyses were carried out by the Microanalytical Data Unit at Cairo University. The progress of all reactions was monitored by TLC on 2 × 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck).

#### 3.1.1. Ethyl 4-oxo-2-thioxo-1,2-dihydroquinazoline-3(4H)-carboxylate (4)

To a solution of anthranilic acid (1.37 g, 0.01 mol) in 1,4-dioxane (40 mL) a solution of ethyl carbonisothiocyanatide (1.31 g, 0.01 mol) in 1,4-dioxane [prepared by the addition of ammonium thiocyanate (0.76 g, 0.01 mol) to ethyl chloroformate (1.08 g, 0.01 mol) in 1,4-dioxane (20 mL) and heating under reflux for 10 min] was added. The reaction mixture was heated under reflux for 1 h, then

poured onto ice/water and the formed solid product was collected by filtration.

Yellow crystals from ethanol; m.p. 210–212 °C; IR (KBr)  $\nu_{\text{max}}$  3480–3345 (NH), 3054 (CH aromatic), 2987, 2880 (CH<sub>3</sub>, CH<sub>2</sub>), 1688, 1682 (2CO), 1631 (C=C), 1205 (C=S) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.13 (t, 3H, *J* = 5.82 Hz, CH<sub>3</sub>), 4.19 (q, 2H, *J* = 5.85, CH<sub>2</sub>), 7.29–7.38 (m, 4H, C<sub>6</sub>H<sub>4</sub>), 8.34 (s, 1H, D<sub>2</sub>O exchangeable, NH); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  16.2 (OCH<sub>2</sub>CH<sub>3</sub>), 53.8 (OCH<sub>2</sub>CH<sub>3</sub>), 119.4, 122.3, 125.4, 126.3, 128.4, 130.3 (C<sub>6</sub>H<sub>4</sub>), 164.2, 164.8 (2CO), 179.4 (C=S); Anal. Calcd. for C<sub>11</sub>H<sub>10</sub>N<sub>2</sub>O<sub>3</sub>S (250.27): C, 52.79; H, 4.03; N, 11.19; S, 12.81. Found: C, 52.83; H, 3.86; N, 11.37; S, 13.05; EI-MS (*m/z*, %): 250 [M<sup>+</sup>, 42].

#### 3.1.2. Ethyl 2-hydrazono-4-oxo-1,2-dihydroquinazoline-3(4H)-carboxylate (6)

To a solution of compound **4** (2.50 g, 0.01 mol) in ethanol (50 mL) hydrazine hydrate (0.50 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h then poured onto ice/water containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

Yellow crystals from ethanol; m.p. 266–268 °C; IR (KBr)  $\nu_{\text{max}}$  3469–3339 (NH<sub>2</sub>, NH), 3056 (CH aromatic), 2989, 2883 (CH<sub>3</sub>, CH<sub>2</sub>), 1689, 1684 (2CO), 1655 (C=N), 1630 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.14 (t, 3H, *J* = 7.04 Hz, CH<sub>3</sub>), 4.19 (q, 2H, *J* = 7.04 Hz, CH<sub>2</sub>), 4.76 (s, 2H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 7.26–7.37 (m, 4H, C<sub>6</sub>H<sub>4</sub>), 8.31 (s, 1H, D<sub>2</sub>O exchangeable, NH); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  16.1 (OCH<sub>2</sub>CH<sub>3</sub>), 53.6 (OCH<sub>2</sub>CH<sub>3</sub>), 120.5, 121.8, 124.8, 125.1, 127.8, 129.1 (C<sub>6</sub>H<sub>4</sub>), 164.3, 164.7 (2CO), 174.6 (C=N); Anal. Calcd. for C<sub>11</sub>H<sub>12</sub>N<sub>4</sub>O<sub>3</sub> (248.24): C, 53.22; H, 4.87; N, 22.57. Found: C, 53.41; H, 4.69; N, 22.69; EI-MS (*m/z*, %): 248 [M<sup>+</sup>, 26].

#### 3.1.3. Synthesis of the thioether derivatives **8**, **10**

To a solution of compound **4** in ethanol (40 mL) any of compounds **7** (1.22 g, 0.01 mol), **9** (0.92 g, 0.01 mol) or **11** (2.29 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 3 h then poured into ice/water mixture containing a few drops of sodium hydroxide solution and the formed solid product was collected by filtration.

#### Ethyl 2-((2-ethoxy-2-oxoethyl)thio)-4-oxoquinazoline-3(4H)-carboxylate (8)

Yellow crystals from ethanol; m.p. 168–171 °C; IR (KBr)  $\nu_{\text{max}}$  3055 (CH aromatic), 2989, 2883 (CH<sub>3</sub>, CH<sub>2</sub>), 1691, 1686, 1684 (3CO), 1654 (C=N), 1630 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.12, 1.14 (2t, 6H, *J* = 5.93, 6.73 Hz, 2CH<sub>3</sub>), 4.16, 4.20 (2q, 4H, *J* = 5.93, 6.73 Hz, 2CH<sub>2</sub>), 5.21 (s, 2H, CH<sub>2</sub>), 7.28–7.38 (m, 4H, C<sub>6</sub>H<sub>4</sub>); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  16.2, 16.4 (two OCH<sub>2</sub>CH<sub>3</sub>), 37.5 (CH<sub>2</sub>),

53.3, 53.5 (two  $\text{OCH}_2\text{CH}_3$ ), 120.8, 122.5, 125.3, 127.5, 127.8, 130.2 ( $\text{C}_6\text{H}_4$ ), 164.3, 164.5, 165.0 (3CO), 174.6 (C=N); Anal. Calcd. for  $\text{C}_{15}\text{H}_{16}\text{N}_2\text{O}_5\text{S}$  (336.36): C, 53.56; H, 4.79; N, 8.33; S, 9.53. Found: C, 53.63; H, 4.49; N, 8.40; S, 9.70; EI-MS ( $m/z$ , %): 336 [ $\text{M}^+$ , 36].

#### Ethyl 4-oxo-2-((2-oxopropyl)thio)quinazoline-3(4H)-carboxylate (10)

Orange crystals from ethanol; m.p. 210–213 °C; IR (KBr)  $\nu_{\text{max}}$  3055 (CH aromatic), 2986, 2887 ( $\text{CH}_3$ ,  $\text{CH}_2$ ), 1694, 1686, 1682 (3CO), 1655 (C=N), 1631 (C=C)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{DMSO}-d_6$ )  $\delta$  1.13 (t, 3H,  $J = 7.04$  Hz,  $\text{CH}_3$ ), 2.68 (s, 3H,  $\text{CH}_3$ ), 4.23 (q, 2H,  $J = 7.04$  Hz,  $\text{CH}_2$ ), 5.38 (s, 2H,  $\text{CH}_2$ ), 7.25–7.35 (m, 4H,  $\text{C}_6\text{H}_4$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  16.3 ( $\text{OCH}_2\text{CH}_3$ ), 24.8 ( $\text{CH}_3$ ), 37.8 ( $\text{CH}_2$ ), 53.2 ( $\text{OCH}_2\text{CH}_3$ ), 120.4, 121.6, 123.4, 125.0, 127.8, 129.1 ( $\text{C}_6\text{H}_4$ ), 163.8, 164.2, 165.2 (3CO), 174.8 (C=N); Anal. Calcd. for  $\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}_4\text{S}$  (306.34): C, 54.89; H, 4.61; N, 9.14; S, 10.47. Found: C, 55.17; H, 4.53; N, 9.05; S, 10.66; EI-MS ( $m/z$ , %): 306 [ $\text{M}^+$ , 28].

#### Ethyl 2-((2-(4-methoxyphenyl)-2-oxoethyl)thio)-4-oxoquinazoline-3(4H)-carboxylate (12)

Orange crystals from ethanol; m.p. 148–151 °C; IR (KBr)  $\nu_{\text{max}}$  3056 (CH aromatic), 2983, 2889 ( $\text{CH}_3$ ,  $\text{CH}_2$ ), 1690, 1689, 1682 (3CO), 1653 (C=N), 1630 (C=C)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{DMSO}-d_6$ )  $\delta$  1.13 (t, 3H,  $J = 6.99$  Hz,  $\text{CH}_3$ ), 3.73 (s, 3H,  $\text{OCH}_3$ ), 4.22 (q, 2H,  $J = 6.99$  Hz,  $\text{CH}_2$ ), 5.49 (s, 2H,  $\text{CH}_2$ ), 7.23–7.42 (m, 8H,  $2\text{C}_6\text{H}_4$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  16.4 ( $\text{OCH}_2\text{CH}_3$ ), 37.5 ( $\text{CH}_2$ ), 53.4 ( $\text{OCH}_2\text{CH}_3$ ), 55.2 ( $\text{OCH}_3$ ), 119.2, 120.8, 122.3, 124.4, 124.9, 126.2, 126.8, 127.8, 128.4, 129.1 ( $2\text{C}_6\text{H}_4$ ), 163.2, 164.4, 165.8 (3CO), 174.6 (C=N); Anal. Calcd. for  $\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_5\text{S}$  (398.43): C, 60.29; H, 4.55; N, 7.03; S, 8.05. Found: C, 60.46; H, 4.69; N, 7.27; S, 7.86; EI-MS ( $m/z$ , %): 398 [ $\text{M}^+$ , 24].

#### 3. 1. 4. 2-Hydrazinyl-4-oxoquinazoline-3(4H)-carbohydrazide (13)

To a solution of compound **8** (3.36 g, 0.01 mol) in dimethylformamide (30 mL) hydrazine hydrate (1.0 mL, 0.02 mol) was added. The reaction mixture was heated under reflux for 1 h then poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

White crystals from ethanol; m.p. 233–236 °C; IR (KBr)  $\nu_{\text{max}}$  3480–3320 ( $2\text{NH}_2$ ,  $2\text{NH}$ ), 3053 (CH aromatic), 1687, 1683 (2CO), 1656 (C=N), 1632 (C=C)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{DMSO}-d_6$ )  $\delta$  4.83, 4.91 (2s, 4H,  $\text{D}_2\text{O}$  exchangeable,  $2\text{NH}_2$ ), 7.26–7.36 (m, 4H,  $\text{C}_6\text{H}_4$ ), 8.21, 8.27 (2s, 2H,  $\text{D}_2\text{O}$  exchangeable,  $2\text{NH}$ );  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  120.3, 124.4, 127.5, 127.8, 128.0, 129.1 ( $\text{C}_6\text{H}_4$ ), 163.0, 164.2 (2CO), 174.4 (C=N); Anal. Calcd. for  $\text{C}_9\text{H}_{10}\text{N}_6\text{O}_2$  (234.21): C, 46.15; H, 4.30; N, 35.88. Found: C, 46.28; H, 4.46; N, 35.93; EI-MS ( $m/z$ , %): 234 [ $\text{M}^+$ , 36].

#### 3. 1. 5. Synthesis of the 4a,5-dihydro-[1,3,4]thiadiazino[3,2-a]quinazolin-6(1H)-one derivatives 16a,b

To a solution of compound **8** (3.36 g, 0.01 mol) in dimethylformamide (30 mL) containing triethylamine (1.0 mL) either of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

#### Diethyl 2-(cyanomethyl)-6-oxo-4a,6-dihydro-[1,3,4]thiadiazino[3,2-a]quinazoline-3,5(1H)-dicarboxylate (16a)

Pale yellow crystals from 1,4-dioxane; m.p. 184–186 °C; IR (KBr)  $\nu_{\text{max}}$  3468–3341 (NH), 3056 (CH aromatic), 1689, 1685–1683 (3CO), 2220 (CN), 1653 (C=N), 1630 (C=C)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{DMSO}-d_6$ )  $\delta$  1.12, 1.15 (2t, 6H,  $J = 6.16$ , 6.90 Hz,  $2\text{OCH}_2\text{CH}_3$ ), 4.16, 4.20 (2q, 4H,  $J = 6.16$ , 6.90 Hz,  $2\text{OCH}_2\text{CH}_3$ ), 5.29 (s, 2H,  $\text{CH}_2$ ), 6.01 (s, 1H, pyrimidine H-2), 7.27–7.38 (m, 4H,  $\text{C}_6\text{H}_4$ ), 8.28 (s, 1H,  $\text{D}_2\text{O}$  exchangeable, NH);  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  16.3, 16.5 ( $2\text{OCH}_2\text{CH}_3$ ), 28.1 ( $\text{CH}_2$ ), 52.1, 53.8 ( $2\text{OCH}_2\text{CH}_3$ ), 92.6 (pyrimidine C-2), 117.8 (CN), 122.4, 124.8, 125.6, 126.3, 128.6, 143.8, ( $\text{C}_6\text{H}_4$ , thiadiazine C-5, C-6), 163.8, 164.4, 165.2 (3CO); Anal. Calcd. for  $\text{C}_{18}\text{H}_{18}\text{N}_4\text{O}_5\text{S}$  (402.42): C, 53.72; H, 4.51; N, 13.92; S, 7.97. Found: C, 53.92; H, 4.61; N, 14.05; S, 8.29; EI-MS ( $m/z$ , %): 402 [ $\text{M}^+$ , 18].

#### Diethyl 2-(2-ethoxy-2-oxoethyl)-6-oxo-4a,6-dihydro-[1,3,4]thiadiazino[3,2-a]quinazoline-3,5(1H)-dicarboxylate (16b)

Pale yellow crystals from 1,4-dioxane; m.p. 132–135 °C; IR (KBr)  $\nu_{\text{max}}$  3468–3341 (NH), 3056 (CH aromatic), 1689–1683 (4CO), 1630 (C=C)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{DMSO}-d_6$ )  $\delta$  1.12–1.15 (3t, 9H,  $3\text{OCH}_2\text{CH}_3$ ), 4.16–4.20 (3q, 6H,  $3\text{OCH}_2\text{CH}_3$ ), 5.29 (s, 2H,  $\text{CH}_2$ ), 6.01 (s, 1H, pyrimidine H-2), 7.27–7.38 (m, 4H,  $\text{C}_6\text{H}_4$ ), 8.28 (s, 1H,  $\text{D}_2\text{O}$  exchangeable, NH);  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ )  $\delta$  16.3, 16.4, 16.9 (three  $\text{OCH}_2\text{CH}_3$ ), 28.1 ( $\text{CH}_2$ ), 52.6, 52.9, 53.8 (three  $\text{OCH}_2\text{CH}_3$ ), 92.6 (pyrimidine C-2), 120.8, 122.4, 123.9, 125.3, 125.8, 129.3, 141.3, 144.2 ( $\text{C}_6\text{H}_4$ , thiadiazine C-5, C-6), 163.3, 163.8, 164.4, 165.8 (4CO); Anal. Calcd. for  $\text{C}_{20}\text{H}_{23}\text{N}_3\text{O}_7\text{S}$  (449.48): C, 53.44; H, 5.16; N, 9.35; S, 7.13. Found: C, 53.28; H, 5.28; N, 9.53; S, 7.32; EI-MS ( $m/z$ , %): 449 [ $\text{M}^+$ , 48].

#### 3. 1. 6. General procedure for the synthesis of the pyran derivatives 19a–4

To a solution of compound **12** (3.06 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL) any of benzaldehyde (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (1.40 g, 0.01 mol) or 4-methoxybenzaldehyde (1.36 g, 0.01 mol) and either of malononitrile (0.66 g, 0.01 mol) or

ethyl cyanoacetate (1.13 g, 0.01 mol) were added. The whole reaction mixture was heated under reflux for 3 h then left to cool and the formed solid product, in each case, was collected by filtration.

**Ethyl 2-((6-amino-5-cyano-2-(4-methoxyphenyl)-4-phenyl-4H-pyran-3-yl)thio)-4-oxoquinazoline-3(4H)-carboxylate (19a)**

Yellow crystals from ethanol; m.p.: 188–190 °C; IR (KBr)  $\nu_{\text{max}}$  3469–3316 (NH<sub>2</sub>), 2223 (CN), 3056 (CH aromatic), 2984, 2870 (CH<sub>3</sub>, CH<sub>2</sub>), 1688, 1686 (2CO), 1652 (C=N), 1630 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.14 (t, 3H, *J* = 7.18 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 3.65 (s, 3H, OCH<sub>3</sub>), 4.22 (q, 2H, *J* = 7.18 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 4.58 (s, 2H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 6.92 (s, 1H, pyran H-4), 7.27–7.36 (m, 13H, C<sub>6</sub>H<sub>5</sub>, 2C<sub>6</sub>H<sub>4</sub>); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  16.2 (OCH<sub>2</sub>CH<sub>3</sub>), 52.8 (OCH<sub>3</sub>), 55.3 (OCH<sub>2</sub>CH<sub>3</sub>), 59.6 (pyran C-4), 116.6 (CN), 119.6, 120.8, 122.5, 125.5, 127.4, 127.8, 130.9, 134.4, 136.8, 137.9, 138.8, 139.2, 140.6, 141.3, 142.6, 144.6 (C<sub>6</sub>H<sub>5</sub>, 2C<sub>6</sub>H<sub>4</sub>, pyran C), 164.3, 164.8 (2CO), 174.8 (C=N); Anal. Calcd. for C<sub>30</sub>H<sub>24</sub>N<sub>4</sub>O<sub>5</sub>S (552.60): C, 65.20; H, 4.38; N, 10.14; S, 5.80. Found: C, 65.42; H, 4.63; N, 9.87; S, 5.83; EI-MS (*m/z*, %): 552 [M<sup>+</sup>, 25].

**Ethyl 2-((5-cyano-6-hydroxy-2-(4-methoxyphenyl)-4-phenyl-4H-pyran-3-yl)thio)-4-oxoquinazoline-3(4H)-carboxylate (19b)**

Yellow crystals from 1,4-dioxane; m.p.: 203–205 °C; IR (KBr)  $\nu_{\text{max}}$  3583–3327 (OH), 2222 (CN), 3058 (CH aromatic), 2986 (CH<sub>3</sub>), 1687, 1665 (2CO), 1650 (C=N), 1632 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.14 (t, 3H, *J* = 6.80 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 3.67 (s, 3H, OCH<sub>3</sub>), 4.26 (q, 2H, *J* = 6.80 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 6.94 (s, 1H, pyran H-4), 7.24–7.43 (m, 13H, C<sub>6</sub>H<sub>5</sub>, 2C<sub>6</sub>H<sub>4</sub>), 10.33 (s, 1H, D<sub>2</sub>O exchangeable, OH); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  16.1 (OCH<sub>2</sub>CH<sub>3</sub>), 52.6 (CH<sub>3</sub>), 55.1 (OCH<sub>2</sub>CH<sub>3</sub>), 59.2 (pyran C-4), 116.7 (CN), 120.2, 120.6, 121.9, 124.9, 126.2, 127.1, 129.1, 131.4, 132.3, 133.9, 134.2, 136.3, 138.5, 139.0, 141.2, 141.8, 142.9, 143.7 (C<sub>6</sub>H<sub>5</sub>, 2C<sub>6</sub>H<sub>4</sub>, pyran C), 164.5, 164.7 (2CO), 174.3 (C=N); Anal. Calcd. for C<sub>30</sub>H<sub>23</sub>N<sub>3</sub>O<sub>6</sub>S (553.59): C, 65.09; H, 4.19; N, 7.59; S, 5.79. Found: C, 65.28; H, 4.38; N, 7.62; S, 5.68; EI-MS (*m/z*, %): 553 [M<sup>+</sup>, 32].

**Ethyl 2-((6-amino-4-(4-chlorophenyl)-5-cyano-2-(4-methoxyphenyl)-4H-pyran-3-yl)thio)-4-oxoquinazoline-3(4H)-carboxylate (19c)**

Yellow crystals from ethanol; m.p.: 166–169 °C; IR (KBr)  $\nu_{\text{max}}$  3472, 3329 (NH<sub>2</sub>), 2220 (CN), 3052 (CH aromatic), 2986 (CH<sub>3</sub>), 1688, 1686 (2CO), 1653 (C=N), 1633 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.13 (t, 3H, *J* = 6.29 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 3.69 (s, 3H, OCH<sub>3</sub>), 4.19 (q, 2H, *J* = 6.29 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 4.28 (s, 2H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 6.91 (s, 1H, pyran H-4), 7.25–7.48 (m, 12H, 3C<sub>6</sub>H<sub>4</sub>); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  16.1 (OCH<sub>2</sub>CH<sub>3</sub>), 52.6 (CH<sub>3</sub>), 54.3 (OCH<sub>2</sub>CH<sub>3</sub>), 59.3 (pyran C-4), 116.4 (CN),

120.3, 121.3, 122.4, 123.5, 126.8, 127.8, 129.1, 130.2, 132.3, 132.7, 134.0, 135.2, 138.9, 139.3, 140.0, 141.4, 142.5, 143.9 (3C<sub>6</sub>H<sub>4</sub>, pyran C), 164.3, 165.0 (2CO), 174.6 (C=N); Anal. Calcd. for C<sub>30</sub>H<sub>23</sub>ClN<sub>4</sub>O<sub>5</sub>S (587.05): C, 61.38; H, 3.95; N, 9.54; S, 5.46. Found: C, 61.27; H, 4.04; N, 9.59; S, 5.53; EI-MS (*m/z*, %): 587 [M<sup>+</sup>, 28].

**Ethyl 2-((4-(4-chlorophenyl)-5-cyano-6-hydroxy-2-(4-methoxyphenyl)-4H-pyran-3-yl)thio)-4-oxoquinazoline-3(4H)-carboxylate (19d)**

Pale yellow crystals from 1,4-dioxane; m.p.: 193–195 °C; IR (KBr)  $\nu_{\text{max}}$  3563–3336 (OH), 2221 (CN), 3054 (CH aromatic), 2987 (CH<sub>3</sub>), 1689, 1687 (2CO), 1650 (C=N), 1632 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.15 (t, 3H, *J* = 7.37 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 3.69 (s, 3H, CH<sub>3</sub>), 4.19 (q, 2H, *J* = 7.37 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 6.91 (s, 1H, pyran H-4), 7.25–7.46 (m, 12H, 3C<sub>6</sub>H<sub>4</sub>), 10.31 (s, 1H, D<sub>2</sub>O exchangeable, OH); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  16.4 (OCH<sub>2</sub>CH<sub>3</sub>), 52.4 (CH<sub>3</sub>), 54.8 (OCH<sub>2</sub>CH<sub>3</sub>), 59.4 (pyran C-4), 116.4 (CN), 120.3, 121.4, 123.6, 126.0, 127.3, 129.6, 130.9, 131.6, 132.8, 133.4, 134.5, 135.1, 136.8, 138.3, 141.1, 142.3, 142.9, 143.4 (3C<sub>6</sub>H<sub>4</sub>, pyran C), 164.2, 164.5 (2CO), 174.6 (C=N); Anal. Calcd. for C<sub>30</sub>H<sub>22</sub>ClN<sub>3</sub>O<sub>6</sub>S (588.03): C, 61.28; H, 3.77; N, 7.15; S, 5.45. Found: C, 61.18; H, 3.80; N, 7.29; S, 5.64; EI-MS (*m/z*, %): 588 [M<sup>+</sup>, 25].

**Ethyl 2-((6-amino-5-cyano-2,4-bis(4-methoxyphenyl)-4H-pyran-3-yl)thio)-4-oxoquinazoline-3(4H)-carboxylate (19e)**

Yellow crystals from ethanol; m.p.: 148–150 °C; IR (KBr)  $\nu_{\text{max}}$  3468–3315 (NH<sub>2</sub>), 3056 (CH aromatic), 2987 (CH<sub>3</sub>), 2220 (CN), 1689, 1687 (2CO), 1633 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.13 (t, 3H, *J* = 7.26 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 3.67, 3.72 (2s, 6H, 2 OCH<sub>3</sub>), 4.19 (q, 2H, *J* = 7.26 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 4.28 (s, 2H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 6.93 (s, 1H, pyran H-4), 7.24–7.49 (m, 12H, 3C<sub>6</sub>H<sub>4</sub>); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  16.0 (OCH<sub>2</sub>CH<sub>3</sub>), 52.6, 53.2 (2 OCH<sub>3</sub>), 54.3 (OCH<sub>2</sub>CH<sub>3</sub>), 59.1 (pyran C-4), 116.4 (CN), 120.6, 121.8, 123.9, 125.8, 127.9, 128.8, 131.6, 132.6, 133.8, 134.8, 135.3, 135.6, 136.3, 137.1, 140.9, 142.1, 143.0, 143.5 (3C<sub>6</sub>H<sub>4</sub>, pyran C), 164.3, 164.8 (2CO), 174.4 (C=N); Anal. Calcd. for C<sub>31</sub>H<sub>26</sub>N<sub>4</sub>O<sub>6</sub>S (582.63): C, 63.91; H, 4.50; N, 9.62; S, 5.50. Found: C, 63.73; H, 4.53; N, 9.63; S, 5.72; EI-MS (*m/z*, %): 582 [M<sup>+</sup>, 18].

**Ethyl 2-((5-cyano-6-hydroxy-2,4-bis(4-methoxyphenyl)-4H-pyran-3-yl)thio)-4-oxoquinazoline-3(4H)-carboxylate (19f)**

Yellow crystals from ethanol; m.p.: 242–246 °C; IR (KBr)  $\nu_{\text{max}}$  3530–3336 (OH), 3056 (CH aromatic), 2986 (CH<sub>3</sub>), 2221 (CN), 1689, 1687 (2CO), 1634 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.14 (t, 3H, *J* = 6.52 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 3.66, 3.73 (2s, 6H, 2 OCH<sub>3</sub>), 4.20 (q, 2H, *J* = 6.52 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 6.90 (s, 1H, pyran H-4), 7.23–7.48 (m, 12H, 3C<sub>6</sub>H<sub>4</sub>), 10.37 (s, 1H, D<sub>2</sub>O exchangeable, OH); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  16.4 (OCH<sub>2</sub>CH<sub>3</sub>), 52.8,

53.4 (2 OCH<sub>3</sub>), 54.6 (OCH<sub>2</sub>CH<sub>3</sub>), 59.0 (pyran C-4), 116.7 (CN), 120.2, 123.1, 124.2, 126.8, 127.9, 129.4, 131.8, 133.3, 133.8, 134.3, 135.3, 135.6, 136.3, 137.1, 141.3, 142.6, 142.50, 143.9 (3C<sub>6</sub>H<sub>4</sub>, pyran C), 164.3, 164.6 (2CO), 174.8 (C=N); Anal. Calcd. for C<sub>34</sub>H<sub>25</sub>N<sub>3</sub>O<sub>7</sub>S (583.61): C, 63.80; H, 4.32; N, 7.20; S, 5.49. Found: C, 63.69; H, 4.48; N, 7.29; S, 5.53; EI-MS (*m/z*, %): 583 [M<sup>+</sup>, 18].

### 3. 1. 7. General procedure for the synthesis of the pyridine derivatives 21a–f

To a solution of compound **12** (3.06 g, 0.01 mol) in 1,4-dioxane (40 mL) containing ammonium acetate (0.50 g) any of benzaldehyde (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (1.40 g, 0.01 mol) or 4-methoxybenzaldehyde (1.36 g, 0.01 mol) and either of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) were added. The whole reaction mixture was heated under reflux for 3 h then left to cool and the formed solid product, in each case, was collected by filtration.

#### Ethyl 2-((6-amino-5-cyano-2-(4-methoxyphenyl)-4-phenyl-1,4-dihydropyridin-3-yl)thio)-4-oxoquinazoline-3(4H)-carboxylate (21a)

Yellow crystals from ethanol; m.p.: 231–235 °C; IR (KBr)  $\nu_{\text{max}}$  3483–3342 (NH, NH<sub>2</sub>), 2220 (CN), 3054 (CH aromatic), 2989 (CH<sub>3</sub>), 1689, 1687 (2CO), 1656 (C=N), 1630 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.15 (t, 3H, *J* = 7.28 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 3.66 (s, 3H, CH<sub>3</sub>), 4.23 (q, 2H, *J* = 7.28 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 4.59 (s, 2H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 6.89 (s, 1H, pyridine H-4), 7.27–7.38 (m, 13H, C<sub>6</sub>H<sub>5</sub>, 2C<sub>6</sub>H<sub>4</sub>), 8.29 (s, 1H, D<sub>2</sub>O exchangeable NH); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  16.1 (OCH<sub>2</sub>CH<sub>3</sub>), 52.5 (CH<sub>3</sub>), 54.1 (OCH<sub>2</sub>CH<sub>3</sub>), 59.5 (pyridine C-4), 116.5 (CN), 120.8, 121.6, 123.1, 125.8, 126.9, 128.3, 128.9, 129.6, 132.3, 131.6, 135.2, 136.5, 138.3, 139.7, 140.3, 142.6, 143.3, 144.3 (C<sub>6</sub>H<sub>5</sub>, C<sub>6</sub>H<sub>4</sub>, pyridine C), 164.2, 164.7 (2CO), 174.3 (C=N); Anal. Calcd. for C<sub>30</sub>H<sub>25</sub>N<sub>5</sub>O<sub>4</sub>S (551.62): C, 65.32; H, 4.57; N, 12.70; S, 5.81. Found: C, 65.48; H, 4.61; N, 12.87; S, 5.92; EI-MS (*m/z*, %): 551 [M<sup>+</sup>, 25].

#### Ethyl 2-((5-cyano-6-hydroxy-2-(4-methoxyphenyl)-4-phenyl-1,4-dihydropyridin-3-yl)thio)-4-oxoquinazoline-3(4H)-carboxylate (21b)

Yellow crystals from 1,4-dioxane; m.p.: 230–233 °C; IR (KBr)  $\nu_{\text{max}}$  3571–3320 (OH, NH), 3055 (CH aromatic), 2989 (CH<sub>3</sub>), 2220 (CN), 1689, 1684 (2CO), 1653 (C=N), 1630 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.14 (t, 3H, *J* = 6.89 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 3.68 (s, 3H, CH<sub>3</sub>), 4.22 (q, 2H, *J* = 6.89 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 6.93 (s, 1H, pyridine H-4), 7.26–7.46 (m, 13H, C<sub>6</sub>H<sub>5</sub>, 2C<sub>6</sub>H<sub>4</sub>), 8.23 (s, 1H, D<sub>2</sub>O exchangeable, NH), 10.33 (s, 1H, D<sub>2</sub>O exchangeable, OH); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  16.4 (OCH<sub>2</sub>CH<sub>3</sub>), 52.8 (CH<sub>3</sub>), 54.6 (OCH<sub>2</sub>CH<sub>3</sub>), 59.9 (pyridine C-4), 116.7 (CN), 120.4, 121.2, 121.9, 122.6, 125.9, 126.2, 128.2, 129.2, 131.7, 132.3, 134.2, 136.3, 138.5, 139.0, 139.6, 141.8, 142.9, 143.8

(C<sub>6</sub>H<sub>5</sub>, 2C<sub>6</sub>H<sub>4</sub>, pyridine C), 164.2, 164.8 (2CO), 174.6 (C=N); Anal. Calcd. for C<sub>30</sub>H<sub>24</sub>N<sub>4</sub>O<sub>5</sub>S (552.60): C, 65.20; H, 4.38; N, 10.14; S, 5.80. Found: C, 65.14; H, 4.42; N, 10.08; S, 5.72; EI-MS (*m/z*, %): 552 [M<sup>+</sup>, 32].

#### Ethyl 2-((5-cyano-6-hydroxy-2-(4-methoxyphenyl)-4-phenyl-1,4-dihydropyridin-3-yl)thio)-4-oxoquinazoline-3(4H)-carboxylate (21c)

Yellow crystals from ethanol; m.p.: 184–187 °C; IR (KBr)  $\nu_{\text{max}}$  3480, 3323 (NH<sub>2</sub>, NH), 3054 (CH aromatic), 2988 (CH<sub>3</sub>), 2220 (CN), 1687, 1683 (2CO), 1656 (C=N), 1633 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.13 (t, 3H, *J* = 7.01 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 3.66 (s, 3H, OCH<sub>3</sub>), 4.19 (q, 2H, *J* = 7.01 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 4.68 (s, 2H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 6.93 (s, 1H, pyridine H-4), 7.22–7.45 (m, 12H, 3C<sub>6</sub>H<sub>4</sub>), 8.28 (s, 1H, D<sub>2</sub>O exchangeable, NH); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  16.3 (OCH<sub>2</sub>CH<sub>3</sub>), 52.8 (OCH<sub>3</sub>), 54.3 (OCH<sub>2</sub>CH<sub>3</sub>), 59.6 (pyridine C-4), 116.7 (CN), 120.3, 122.0, 123.5, 124.6, 126.8, 127.4, 129.0, 130.2, 132.3, 132.7, 134.0, 135.2, 138.9, 139.3, 140.0, 141.4, 142.5, 143.9 (3C<sub>6</sub>H<sub>4</sub>, pyridine C), 164.2, 164.6 (2CO), 174.8 (C=N); Anal. Calcd. for C<sub>30</sub>H<sub>24</sub>ClN<sub>5</sub>O<sub>4</sub>S (586.06): C, 61.48; H, 4.13; N, 11.95; S, 5.47. Found: C, 61.53; H, 4.24; N, 12.28; S, 5.60; EI-MS (*m/z*, %): 586 [M<sup>+</sup>, 36].

#### Ethyl 2-((4-(4-chlorophenyl)-5-cyano-6-hydroxy-2-(4-methoxyphenyl)-1,4-dihydropyridin-3-yl)thio)-4-oxoquinazoline-3(4H)-carboxylate (21d)

Yellow crystals from 1,4-dioxane; m.p.: 166–168 °C; IR (KBr)  $\nu_{\text{max}}$  3572–3333 (OH, NH), 2221 (CN), 3054 (CH aromatic), 2987 (CH<sub>3</sub>), 1689, 1684 (2CO), 1650 (C=N), 1632 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.13 (t, 3H, *J* = 6.47 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 3.69 (s, 3H, OCH<sub>3</sub>), 4.18 (q, 2H, *J* = 6.47 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 6.90 (s, 1H, pyridine H-4), 7.22–7.46 (m, 12H, 3C<sub>6</sub>H<sub>4</sub>), 8.28 (s, 1H, D<sub>2</sub>O exchangeable, NH), 10.31 (s, 1H, D<sub>2</sub>O exchangeable, OH); <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  16.6 (OCH<sub>2</sub>CH<sub>3</sub>), 52.6 (OCH<sub>3</sub>), 54.6 (OCH<sub>2</sub>CH<sub>3</sub>), 59.4 (pyridine C-4), 116.6 (CN), 120.6, 122.8, 123.4, 125.2, 126.8, 127.3, 130.9, 131.3, 132.5, 133.4, 134.5, 135.5, 136.8, 138.3, 141.0, 142.3, 142.9, 143.6 (3C<sub>6</sub>H<sub>4</sub>, pyran C), 164.2, 164.8 (2CO), 174.6 (C=N); Anal. Calcd. for C<sub>30</sub>H<sub>23</sub>ClN<sub>4</sub>O<sub>5</sub>S (587.05): C, 61.38; H, 3.95; N, 9.54; S, 5.46. Found: C, 61.42; H, 3.69; N, 9.70; S, 5.59; EI-MS (*m/z*, %): 587 [M<sup>+</sup>, 48].

#### Ethyl 2-((6-amino-5-cyano-2,4-bis(4-methoxyphenyl)-1,4-dihydropyridin-3-yl)thio)-4-oxoquinazoline-3(4H)-carboxylate (21e)

Yellow crystals from ethanol; m.p.: 148–152 °C; IR (KBr)  $\nu_{\text{max}}$  3468–3315 (NH<sub>2</sub>, NH), 3056 (CH aromatic), 2987 (CH<sub>3</sub>), 2220 (CN), 1689, 1682 (2CO), 1633 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.14 (t, 3H, *J* = 7.19 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 3.65, 3.74 (2s, 6H, 2 OCH<sub>3</sub>), 4.20 (q, 2H, *J* = 7.19 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 4.67 (s, 2H, D<sub>2</sub>O exchangeable, NH<sub>2</sub>), 6.91 (s, 1H, pyridine H-4), 7.25–7.47 (m, 12H, 3C<sub>6</sub>H<sub>4</sub>), 8.30 (s, 1H, D<sub>2</sub>O exchangeable, NH); <sup>13</sup>C NMR

(75 MHz, DMSO- $d_6$ )  $\delta$  16.3 (OCH<sub>2</sub>CH<sub>3</sub>), 52.5, 53.8 (2CH<sub>3</sub>), 54.6 (OCH<sub>2</sub>CH<sub>3</sub>), 59.5 (pyridine C-4), 116.8 (CN), 119.3, 123.4, 124.1, 124.5, 128.3, 129.6, 130.3, 132.8, 134.3, 134.8, 135.6, 136.3, 137.1, 140.9, 142.7, 143.5, 143.5 (3C<sub>6</sub>H<sub>4</sub>, pyridine C), 163.9, 164.5 (2CO), 174.6 (C=N); Anal. Calcd. for C<sub>31</sub>H<sub>27</sub>N<sub>5</sub>O<sub>5</sub>S (581.64): C, 64.01; H, 4.68; N, 12.04; S, 5.51. Found: C, 63.93; H, 4.56; N, 11.84; S, 5.69; EI-MS ( $m/z$ , %): 581 [M<sup>+</sup>, 22].

**Ethyl 2-((5-cyano-6-hydroxy-2,4-bis(4-methoxyphenyl)-1,4-dihydropyridin-3-yl)thio)-4-oxoquinazoline-3(4H)-carboxylate (21f)**

Yellow crystals from ethanol; m.p.: 263–265 °C; IR (KBr)  $\nu_{\max}$  3548–3322 (OH NH), 3054 (CH aromatic), 2987 (CH<sub>3</sub>), 2221 (CN), 1688, 1684 (2CO), 1635 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO- $d_6$ )  $\delta$  1.13 (t, 3H,  $J$  = 7.08 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 3.62, 3.73 (2s, 6H, 2 OCH<sub>3</sub>), 4.21 (q, 2H,  $J$  = 7.08 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 6.91 (s, 1H, pyridine H-4), 7.25–7.48 (m, 12H, 3C<sub>6</sub>H<sub>4</sub>), 8.26 (s, 1H, D<sub>2</sub>O exchangeable, NH), 10.31 (s, 1H, D<sub>2</sub>O exchangeable, OH); <sup>13</sup>C NMR (75 MHz, DMSO- $d_6$ )  $\delta$  16.1 (OCH<sub>2</sub>CH<sub>3</sub>), 52.4, 53.8 (2CH<sub>3</sub>), 54.4 (OCH<sub>2</sub>CH<sub>3</sub>), 59.6 (pyridine C-4), 116.6 (CN), 119.6, 121.8, 128.3, 130.3, 133.6, 133.8, 135.8, 136.1, 136.3, 137.1, 140.2, 142.3, 143.5, 143.8 (3C<sub>6</sub>H<sub>4</sub>, pyridine C), 164.1, 164.3 (2CO), 174.3 (C=N); Anal. Calcd. for C<sub>31</sub>H<sub>26</sub>N<sub>4</sub>O<sub>6</sub>S (582.63): C, 63.91; H, 4.50; N, 9.62; S, 5.50. Found: C, 63.88; H, 4.53; N, 9.71; S, 5.73; EI-MS ( $m/z$ , %): 582 [M<sup>+</sup>, 31].

**Synthesis of the thiazole synthesis of the thiazole derivatives 24a–c**

To a solution of compound **8** (3.36 g, 0.01 mol) in dimethylformamide (40 mL) containing potassium hydroxide (0.40 g, 0.01 mol), phenylisothiocyanate (1.30 g, 0.01 mol) was added. The reaction mixture was stirred at room temperature for 24 h. On the second day any of ethyl chloroacetate (1.22 g, 0.01 mol),  $\alpha$ -chloroacetone (0.92 g, 0.01 mol) or 2-bromo-1-(4-bromophenyl)ethanone (2.75 g, 0.01 mol) was added. The whole reaction mixture was stirred at room temperature for an additional 24 h then poured onto ice/water containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

**Ethyl 2-((2-ethoxy-1-(4-hydroxy-3-phenylthiazol-2(3H)-ylidene)-2-oxoethyl)thio)-4-oxo-quinazoline-3(4H)-carboxylate (24a)**

Orange crystals from ethanol; m.p.: 193–196 °C; IR (KBr)  $\nu_{\max}$  3562–3345 (OH), 3055 (CH aromatic), 2985, 2889 (CH<sub>3</sub>, CH<sub>2</sub>), 1689, 1685, 1682 (3CO), 1632 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO- $d_6$ )  $\delta$  1.13, 1.15 (2t, 6H,  $J$  = 6.59, 6.18 Hz, 2 OCH<sub>2</sub>CH<sub>3</sub>), 4.18, 4.26 (2q, 4H,  $J$  = 6.59, 6.18 Hz, 2 OCH<sub>2</sub>CH<sub>3</sub>), 6.06 (s, 1H, thiazole H-5), 7.28–7.41 (m, 9H, C<sub>6</sub>H<sub>5</sub>, C<sub>6</sub>H<sub>4</sub>), 9.42 (s, 1H, D<sub>2</sub>O exchangeable, OH); <sup>13</sup>C NMR (75 MHz, DMSO- $d_6$ )  $\delta$  16.1, 16.8 (2 OCH<sub>2</sub>CH<sub>3</sub>), 54.2, 54.6 (2 OCH<sub>2</sub>CH<sub>3</sub>), 84.5, 148.2 (C=C),

119.3, 124.1, 124.5, 128.3, 130.3, 132.8, 134.3, 135.6, 135.6, 137.1, 142.7, 143.5 (C<sub>6</sub>H<sub>5</sub>, C<sub>6</sub>H<sub>4</sub>, thiazole C-4, C-5), 163.4, 164.3, 164.6 (3CO), 174.4 (C=N); Anal. Calcd. for C<sub>24</sub>H<sub>21</sub>N<sub>3</sub>O<sub>6</sub>S<sub>2</sub> (511.57): C, 56.35; H, 4.14; N, 8.21; S, 12.54. Found: C, 56.48; H, 4.32; N, 8.40; S, 12.70; EI-MS ( $m/z$ , %): 511 [M<sup>+</sup>, 38].

**Ethyl 2-((2-ethoxy-1-(4-methyl-3-phenylthiazol-2(3H)-ylidene)-2-oxoethyl)thio)-4-oxoquinazoline-3(4H)-carboxylate (24b)**

Orange crystals from acetic acid; m.p.: 205–208 °C; IR (KBr)  $\nu_{\max}$  3053 (CH aromatic), 2989, 2884 (CH<sub>3</sub>, CH<sub>2</sub>), 1688, 1686, 1680 (3CO), 1634 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO- $d_6$ )  $\delta$  1.14, 1.15 (2t, 6H,  $J$  = 7.26, 6.37 Hz, 2 OCH<sub>2</sub>CH<sub>3</sub>), 2.80 (s, 3H, CH<sub>3</sub>), 4.18, 4.23 (2q, 4H,  $J$  = 7.26, 6.37 Hz, 2 OCH<sub>2</sub>CH<sub>3</sub>), 6.07 (s, 1H, thiazole H-5), 7.26–7.44 (m, 9H, C<sub>6</sub>H<sub>5</sub>, C<sub>6</sub>H<sub>4</sub>); <sup>13</sup>C NMR (75 MHz, DMSO- $d_6$ )  $\delta$  16.6, 16.9 (two OCH<sub>2</sub>CH<sub>3</sub>), 54.6, 54.8 (two OCH<sub>2</sub>CH<sub>3</sub>), 69.1 (thiazole C-5), 88.6, 148.0 (C=C), 119.8, 124.3, 124.8, 129.1, 130.6, 133.2, 134.6, 135.4, 136.3, 137.5, 140.8, 143.7 (C<sub>6</sub>H<sub>5</sub>, C<sub>6</sub>H<sub>4</sub>, thiazole C-4, C-5), 163.8, 164.8, 164.9 (3CO), 174.2 (C=N); Anal. Calcd. for C<sub>25</sub>H<sub>23</sub>N<sub>3</sub>O<sub>6</sub>S<sub>2</sub> (509.60): C, 58.92; H, 4.55; N, 8.25; S, 12.58. Found: C, 58.79; H, 4.70; N, 8.39; S, 12.39; EI-MS ( $m/z$ , %): 509 [M<sup>+</sup>, 19].

**Ethyl 2-((2-ethoxy-1-(4-(4-methoxyphenyl)-3-phenylthiazol-2(3H)-ylidene)-2-oxoethyl)thio)-4-oxoquinazoline-3(4H)-carboxylate (24c)**

Orange crystals from acetic acid; m.p.: 177–179 °C; IR (KBr)  $\nu_{\max}$  3055 (CH aromatic), 2987, 2888 (CH<sub>3</sub>, CH<sub>2</sub>), 1689, 1686, 1683 (3CO), 1632 (C=C) cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO- $d_6$ )  $\delta$  1.16, 1.18 (2t, 6H,  $J$  = 7.42, 7.07 Hz, 2 OCH<sub>2</sub>CH<sub>3</sub>), 3.69 (s, 3H, OCH<sub>3</sub>), 4.18, 4.23 (2q, 4H,  $J$  = 7.42, 7.07 Hz, 2 OCH<sub>2</sub>CH<sub>3</sub>), 6.06 (s, 1H, thiazole H-5), 7.22–7.48 (m, 13H, C<sub>6</sub>H<sub>5</sub>, 2C<sub>6</sub>H<sub>4</sub>); <sup>13</sup>C NMR (75 MHz, DMSO- $d_6$ )  $\delta$  16.6, 16.8 (two OCH<sub>2</sub>CH<sub>3</sub>), 52.6 (OCH<sub>3</sub>), 54.3, 54.6 (two OCH<sub>2</sub>CH<sub>3</sub>), 69.3 (thiazole C-5), 88.7, 148.0 (C=C), 120.3, 122.4, 123.8, 124.1, 124.8, 125.6, 126.9, 129.2, 130.8, 133.2, 134.6, 135.4, 136.3, 137.6, 142.4, 143.5 (C<sub>6</sub>H<sub>5</sub>, C<sub>6</sub>H<sub>4</sub>, thiazole C-4, C-5), 163.3, 164.6, 164.5 (3CO), 174.5 (C=N); Anal. Calcd. for C<sub>31</sub>H<sub>27</sub>N<sub>3</sub>O<sub>6</sub>S<sub>2</sub> (601.69): C, 61.88; H, 4.52; N, 6.98; S, 10.66. Found: C, 61.79; H, 4.66; N, 7.05; S, 10.81; EI-MS ( $m/z$ , %): 601 [M<sup>+</sup>, 22].

## 4. Conclusions

The work deals with the synthesis of a series of 1,2-dihydroquinazoline derivatives. The cytotoxicity of the newly synthesized compounds towards the six cancer cell lines NUGC, DLD-1, HA22T, HEPG-2, HONE-1 and MCF-7 showed that compounds **6**, **8**, **13**, **19c–f**, **21b–f**, **24a** and **24c** displayed the highest cytotoxicity. The c-Met kinase inhibition for some selected compounds showed that compounds **8**, **13**, **19d**, **21e**, **21f** and **24a** possess the high-

est inhibitory effect. Activities towards tyrosine kinases revealed that compounds **13**, **21e** and **24a** have the highest potency. Compounds **13** and **24a** showed the highest activities towards Pim-1 kinase.

## 5. Acknowledgments

R. M. Mohareb would like to thank the Alexander von Humboldt for affording him regular fellowships in Germany for doing research and completing this work.

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## Povzetek

Pri reakciji antranilne kisline z etoksikarbonilizotiocianatom nastane etil 4-okso-2-tioakso-1,2-dihidrokinazolin-3(4*H*)-karboksilat (**4**). Ugotovili smo, da reakcija spojine **4** s hidrazin hidratom in  $\alpha$ -halokarbonilnimi derivati daje ali hidrazono ali *S*-alkilirane produkte. Izvedli smo tudi heterociklizacijske reakcije nekaterih *S*-alkiliranih derivatov **8** in **12** in na ta način pripravili tiofenske, tiazolne, piranske in piridinske derivate. Raziskali smo citotoksičnost novih sintetiziranih spojin na šest rakavih celičnih linij: NUGC, DLD-1, HA22T, HEPG-2, HONE-1 in MCF-7. Ugotovili smo, da spojine **8**, **10**, **16a**, **19d–f**, **21c**, **21e**, **21f**, **24a** in **24b** izkazujejo največjo citotoksičnost. Test inhibicije *c*-Met kinaze za nekatere izbrane spojine je pokazal, da derivati **8**, **13**, **19d**, **21e**, **21f** in **24a** predstavljajo najbolj aktivne spojine. Test s tirozin kinazo pa je razkril, da spojine **13**, **21e** in **24a** kažejo največjo inhibitorno aktivnost in zato smo zanje izvedli študijo molekulskega modeliranja. Dodatno se je pokazalo, da spojini **13** in **24a** izkazujeta največjo aktivnost na Pim-1 kinazo.