Scientific paper

Uses of anthranilic acid for the synthesis of dihydroquinazolin derivatives with antitumor, anti-proliferative and Pim-1kinase activities

Rafat M. Mohareb*[a], Peter A. Halim[b]

[a]*Department of Chemistry, Faculty of Science, Cairo University, Giza, A. R. Egypt*

[b] *Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt*

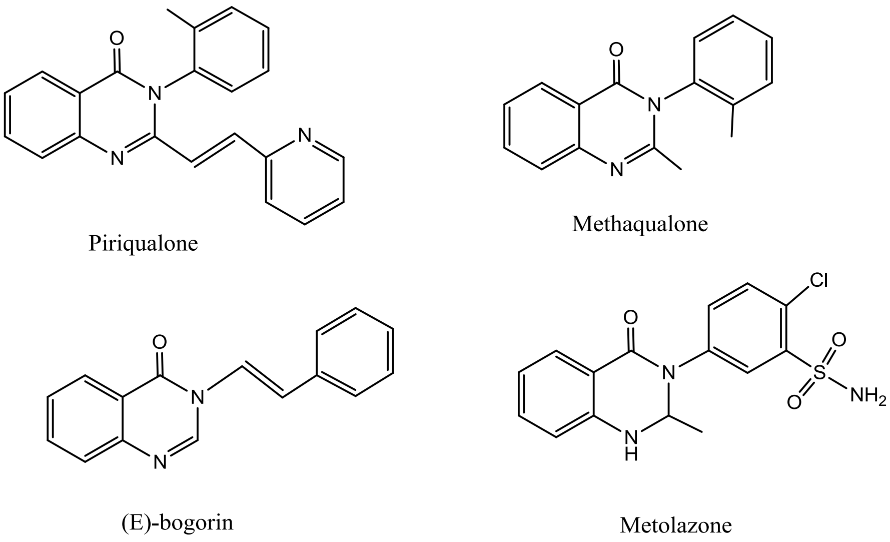
Abstract

The reaction of anthranilic acid with ethoxycarbonylisothiocyanate gave the ethyl 4-oxo-2-thioxo-1,2-dihydroquinazoline-3(4H)-carboxylate (**4**). The reaction of compound 4 with hydrazine hydrate and α-halocarbnyl derivatives was studied to give either hydrazono or S-alkylated products. Heterocyclization reactions of some of the S-alkylated derivatives **8** and **12** were carried to afford thiophene, 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 **8**, **10**, **16a**, **19d-f**, **21c**, **21e**, **21f**, **24a and 24b** showed the highest cytotoxicity. The c-Met kinase inhibition for some selected compounds showed that compounds **8**, **13**, **19d**, **21e**, **21f** and **24a** with the highest inhibitory effect. Activities towards tyrosine kinases revealed that compounds **13**, **21e** and **24a** with the highest potency their molecular modeling was described. 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 widely present in natural products as well as medicinal, and pharmacological compounds.[1] In addition, several thioxoquinazolin analogues have been developed as antitumor, antibiotic, antidefibrillator and antipyretic agent (Fig. 1). Further- more, they display a broad range of applications for diabetes,[2] cancer,[3] and selective plant grow regulators.[4,5] Given the importance of these nitrogen heterocyclic compounds, the devel- opment of mild, high yielding and clean synthesis of these important compounds is daunting challenge and has been extensively investigated in literatures.[6-12] The classical methods for the synthesis of quinazolinedione ring system are the reaction of anthranilic acid and their derivatives with isothiocyanates or their equivalents.[13-27] In this context, we aimed to develop an efficient and facile approach to synthesize a series of thioxoquinazolin-4(1*H*)-ones from anthranilic acid and ethoxycarbonylisothiocyanate.



Piriqualone

Methaqualone

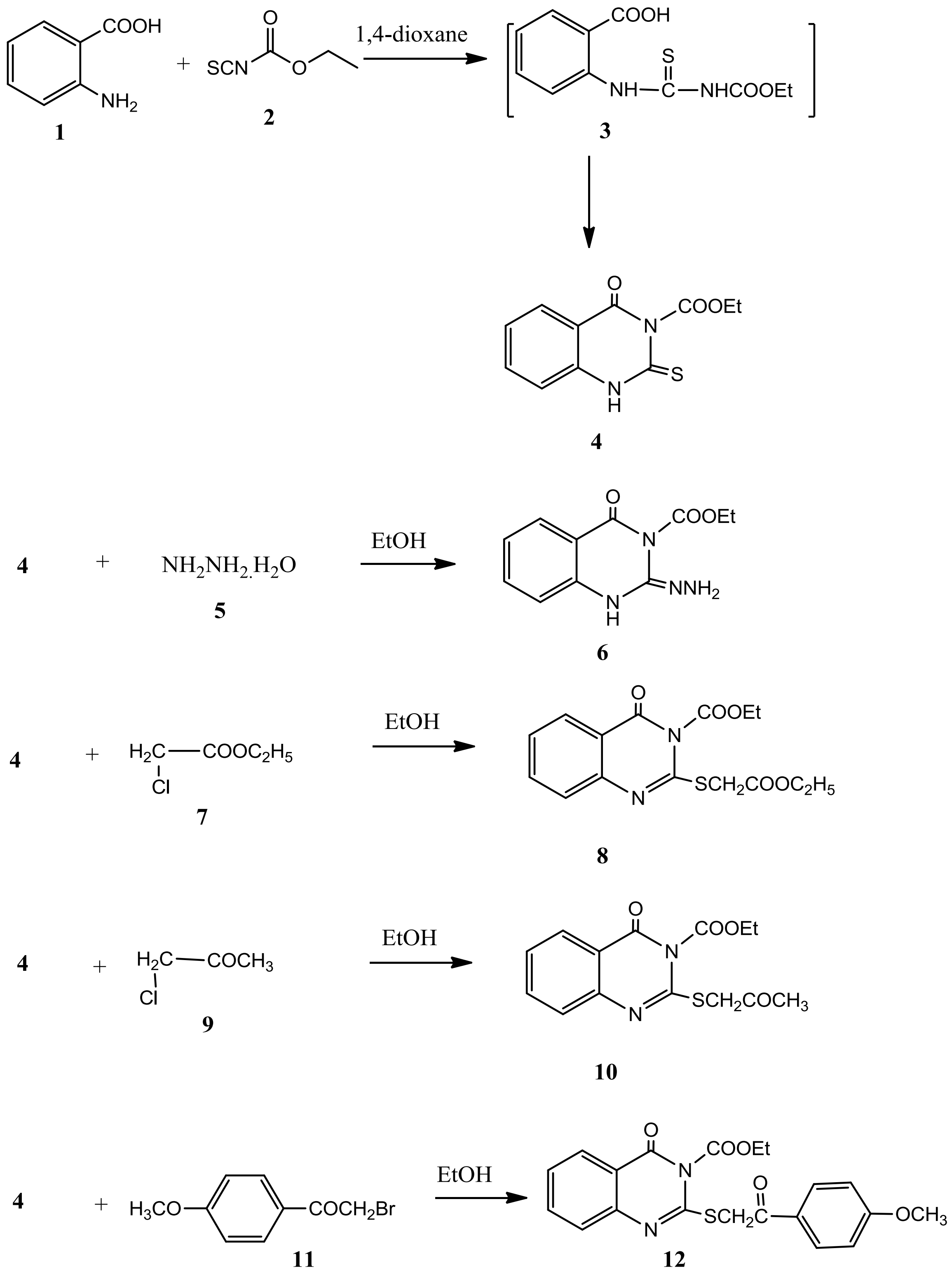
(E)-bogorin

Metolazone

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

2. Results and discussion

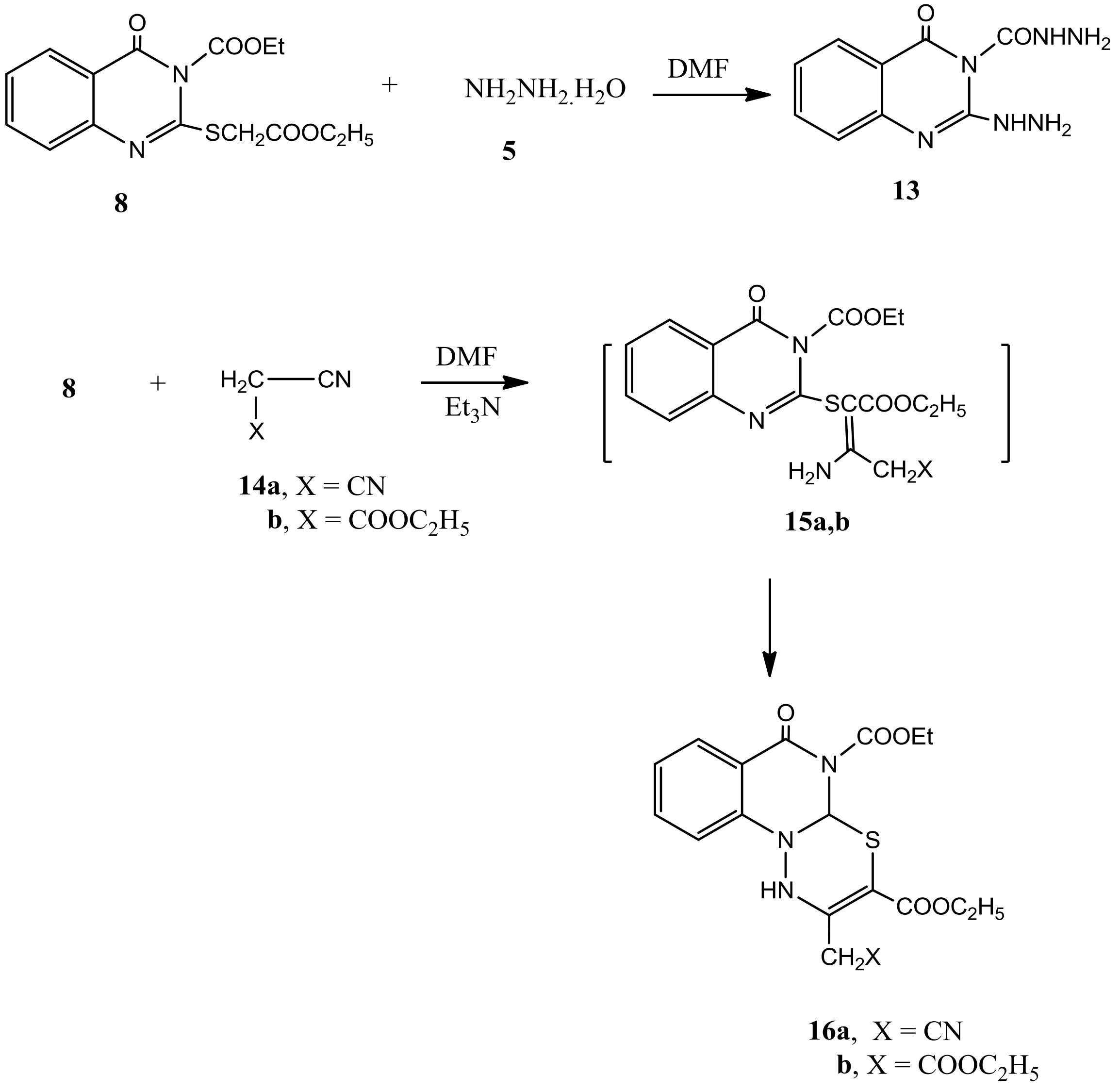
In the present work we aimed to synthesis a series of heterocyclic compounds derived from dihydroquinazoline derivative. The reaction of anthranilic acid (**1**) with ethoxycarbonylisothiocyanate (**2**) in 1,4-dioxane gave the ethyl 2-thioxo-2,3-dihydroquinazolin-4(1*H*)-one-3-carboxylate (**4**). The 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 different chemical 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 α-halocarbonyl compounds. Thus, the reaction of compound **4** with any of the ethyl chloroacetate (**7**), chloroacetone (**9**) or 2-bromo-1-(4-chlorophenyl)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 the analytical and spectral data. Thus, the ^{1}H NMR spectrum of compound **12** (as an example) showed the presence of a triplet at δ 1.13 and a quartet at δ 4.22 ppm equivalent for the ester CH_3 and CH_2, respectively, a singlet at δ 3.73 ppm equivalent for the OCH_3 group, a singlet at δ 5.49 for the CH_2 group and a multiplet at δ 7.23-7.42 ppm for the two C_6H_4 groups. In addition the ^{13}C NMR spectrum showed δ 16.4 (ester CH_3), 37.5 (CH_2), 53.4 (ester CH_2), 55.2 (OCH_3), 119.2, 120.8, 122.3, 124.4, 124.9, 126.2, 126.8, 127.8, 128.4, 129.1 indicating the C_6H_5 and C_6H_4 groups and three signals at δ 163.2, 164.4, 165.8 confirming the three CO group.



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

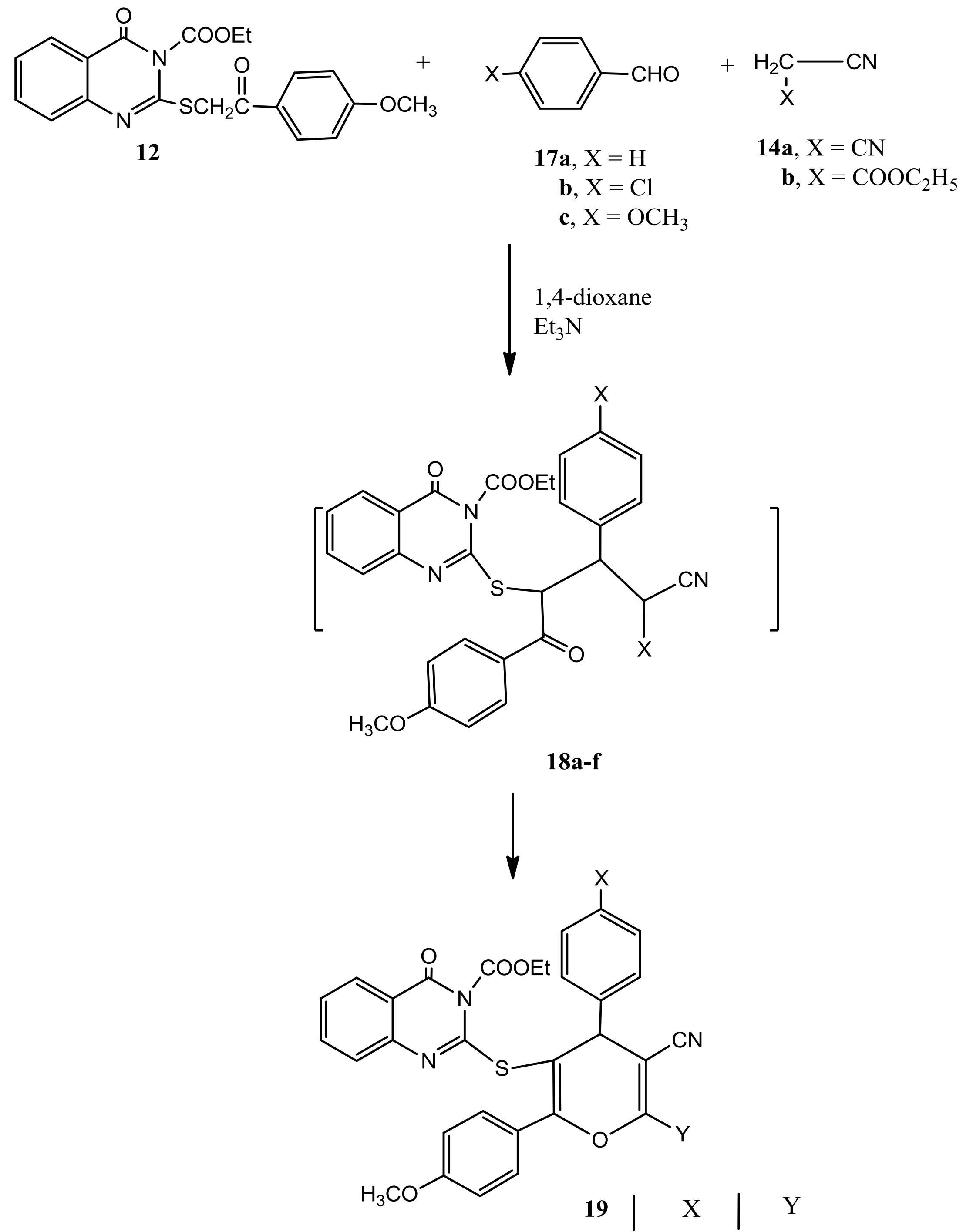
On the other hand, the reaction of compound **4** with chloroacetylchloride (**13**) in a boiling dimethylformamide containing potassium carbonate gave the 1-(2-chloroacetyl)-3-phenyl-2-thioxo-2,3-dihydroquinazolin-4(1*H*)-one (**14**).

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}H NMR spectrum of **16a** showed, beside the expected signals, two triplets at δ 1.12, 1.15 and two quartets at δ 4.16, 4.20 ppm indicating the presence of two esters CH_3 and CH_2 groups, respectively, a singlet at δ 6.01 ppm equivalent to the pyrimidine H-2 and a singlet at δ 8.28 ppm for the NH group. In addition its ^{13}C NMR spectrum showed two signals at δ 16.3, 16.5 for the two ester CH_3 groups and two quartets at δ 52.1, 53.8 for the two ester CH_2 groups, three signats at δ 163.8, 164.4, 165.2 equivalent to the three CO groups.



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

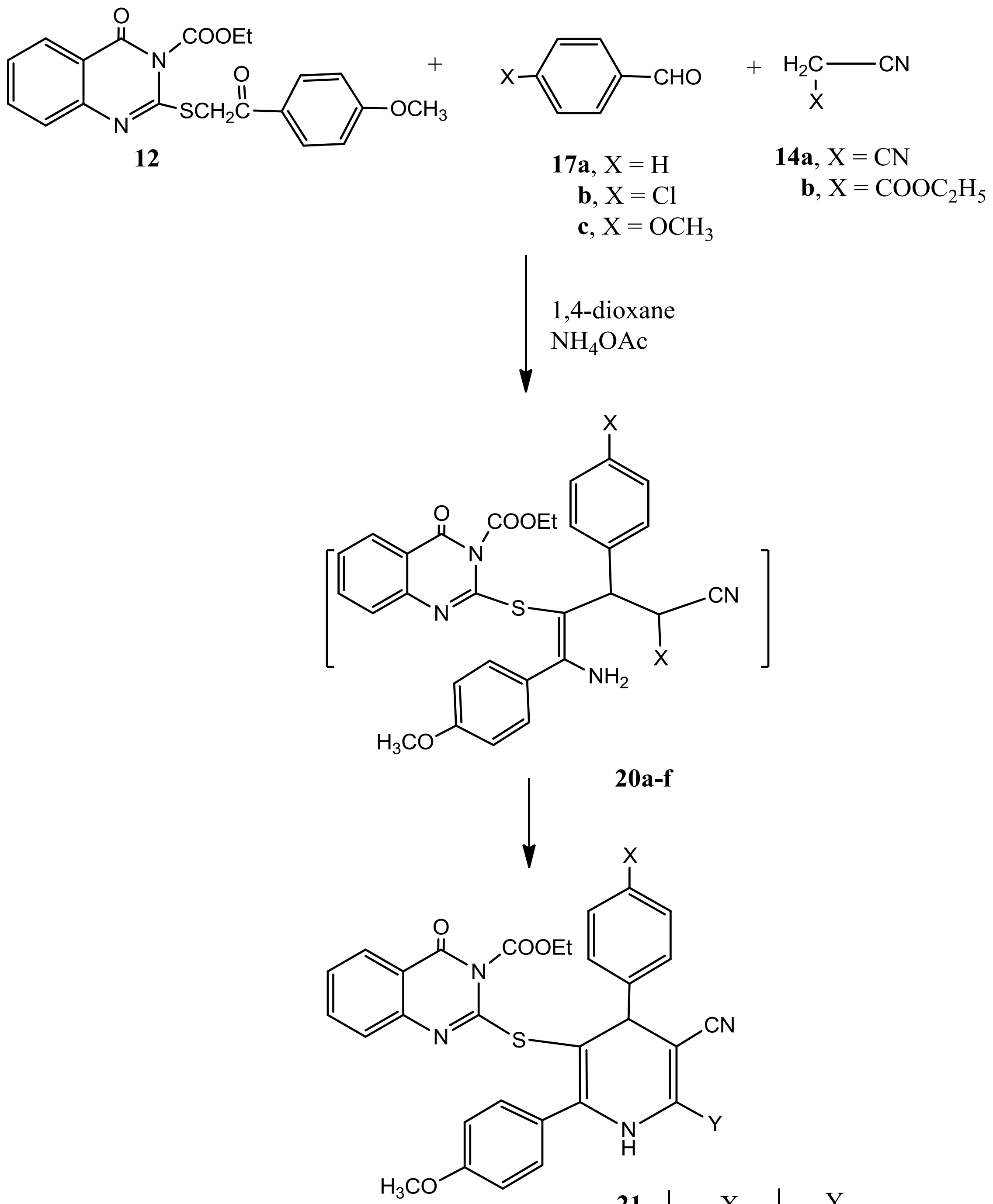
In continuation of our recent interest to conduct multi-component reactions using aromatic aldehydes and cyanomethylene reagents[28-30] we studied the multi-component reactions of compound **12**. Thus, the multi-component reaction of compound **12** with any of the aromatic aldehydes namely, benzaldehyde (**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 pyran derivatives **19a-f**, respectively through the intermediate formation of **18a-f**. The analytical and spectral data of the compounds **19a-f** (Scheme 3) were based on their respective analytical and spectral data (see experimental section).



19	X	Y
a	H	NH_2
b	H	OH
c	Cl	NH_2
d	Cl	OH
e	OCH_3	NH_2
f	OCH_3	OH

Scheme 3. Synthesis of compounds **19a-f**.

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**.



12

17a, X = H
b, X = Cl
c, X = OCH$_3$

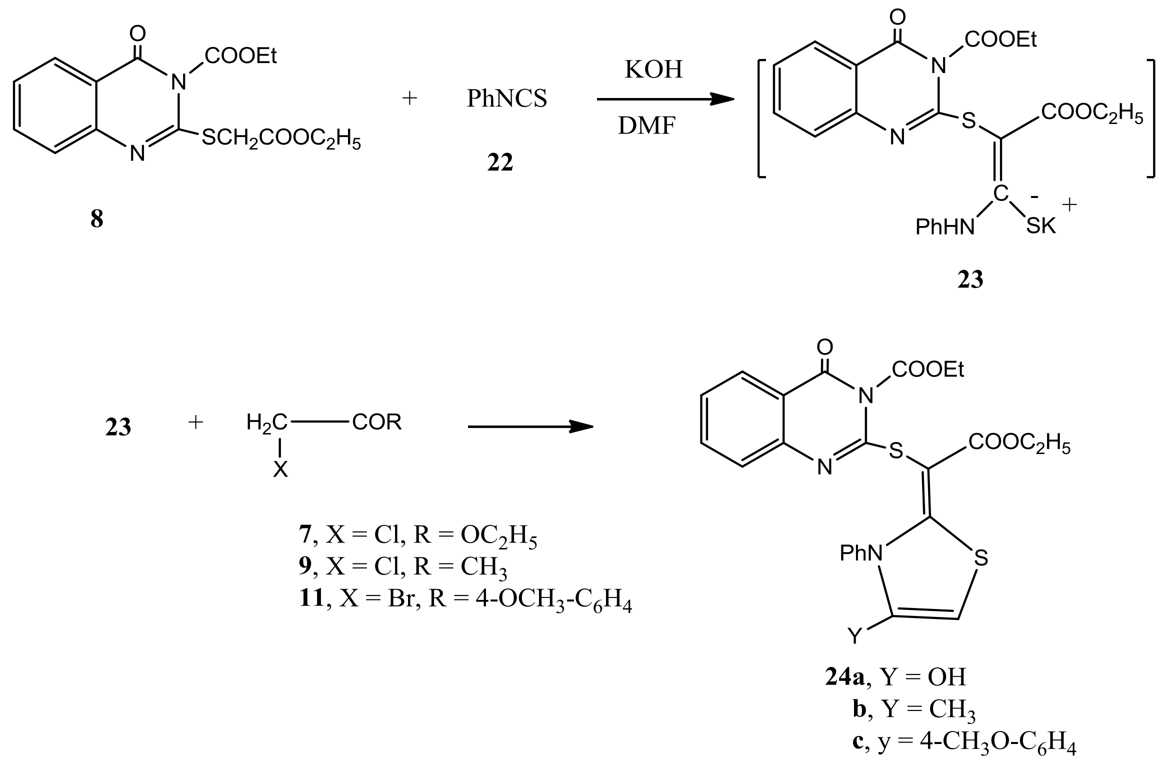
14a, X = CN
b, X = COOC$_2$H$_5$

20a-f

21	X	Y
a	H	NH$_2$
b	H	OH
c	Cl	NH$_2$
d	Cl	OH
e	OCH$_3$	NH$_2$
f	OCH$_3$	OH

Scheme 4. Synthesis of compounds **21a-f**.

Recently, our research group was involved through a comprehensive program involving the reaction of active methylene reagents with phenylisothiocyanate in basic dimethylformamde solution followed by heterocyclization with α-halocarbonyl compounds.[31-33] Products of the reaction were either thiophene or thiazole derivatives or a mixture of both depending on the reaction conditions and the nature of the α-halocarbonyl compound. In continuation of this program we conducted the reaction of compound **8** with phenylisothiocyanate (**23**) in dimethylformamide containing potassium hydroxide gave the intermediate potassium salt **22**. The reaction of the intermediate **22** with any of ethyl chloroacetate (**7**) chloroacetone (**9**) or 2-bromo-1-(4-chlorophenyl)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), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (Saint Louis, USA).

Cell cultures

Was obtained from the European Collection of cell Cultures (ECACC, Salisbury, UK) and human gastric cancer (NUGC and HR), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and normal fibroblast cells (WI38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 μM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 lg/mL), at 37 ^{o}C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5 x 10^5 cells/mL for the seven human cancer cell lines including cells derived from 0.75 x 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), nasopharyngeal carcinoma (HONE1) and a normal fibroblast cells (WI38). All of IC_{50} values were listed in Table 1. Some heterocyclic compounds was observed with significant cytotoxicity against most of the cancer cell lines tested (IC_{50}=10–1000 nM). Normal fibroblasts cells (WI38) were affected to a much lesser extent (IC50>10,000 nM). The reference compound used is 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_{50}'s 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_{50}'s 69 and 125 nM, respectively.

On the other hand, the reaction of compound **4** with ethyl α-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$_{50}$'s 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 the high nitrogen content, showed relatively high potency against the six cancer cell lines. On the other hand, the dihydro-[1,3,4]thiadiazino[3,2-*a*]quinazoline derivatives **16a,b** showed low potency. The multi-component reactions of compound **12** with any of the artomatic aldehydes **17a-c** and either of malononitrile or ethyl cyanoacetate gave the pyran derivatives **19a-f**. Compounds **19c** (X = Cl, Y = NH$_2$), **19d** (X = Cl, Y = OH) and **19e** (X = OCH$_3$ Y = NH$_2$) showed the highest cytotoxicity among such series of compounds. On the other hand, for the pyridine derivatives **21a-f**, the four compounds **21b** (X = H, Y = OH), **21d** (X = Cl, Y = OH), **21e** (X = OCH$_3$, Y = NH$_2$) and **21f** (X = OCH$_3$, Y = OH) showed the highest potency. Compound **21f** showed cyctotoxicity higher than foretinib against the DLD-1 cell line with IC$_{50}$ 219 nM Considering the thiazole derivatives **24a-c**, it is obvious that compounds **23a (**Y = OH) and **24c** (Y = 4-OCH$_3$-phenyl) showed higher potency than **24b** (Y = CH$_3$). It is clear that throughout the synthesized compounds the presence of the electronegative groups like the Cl and OH groups or the electron reach NH$_2$ groups enhance the potency of the compound.

Table 1: Cytotoxicity of the newly synthesized products against a variety of cancer cell lines [IC$_{50}$^{a} (nM)]

Compound No.	Cytotoxocity (IC$_{50}$ in nM)						
	NUGC^{b}	DLD-1^{b}	HA22T^{b}	HEPG-2^{b}	HONE-1^{b}	MCF-7^{b}	WI-38^{b}
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

[a] Drug concentration required to inhibit tumor cell proliferation by 50% after continuous exposure of 48 h.

[b] 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.

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** were 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.[34,35] The cancer cell lines were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). Approximate 4 x 10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 ^oC 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 μg/mL, and incubated with cells at 37 ^oC for 4 h. The formazan crystals were dissolved in 100 μL of DMSO each well, and the absorbency 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_{50} (inhibitory concentration 50%) were the averages of three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

2.1.4. In vitro cell assays

The antitumor evaluations[36] of the synthesized compounds were measured against A549 (non-small cell lung cancer), H460 (human lung cancer), HT-29 (human colon cancer) and MKN-45 (human gastric cancer cancer), U87MG (human glioblastoma) and SMMC-7721 (human liver cancer) cell lines. cancer cell lines. Foretinib was used as the positive control by a MTT assay. The results expressed as IC_{50} after three different experiments were summarized in (Table 2). The data listed in (Table 2) revealed that the compounds possessed moderate to strong cytotoxicity against the five tested cell lines in the single-digit lM range, and high selectivity for inhibition A549, H460 and MKN-45 cells. The promising compounds were **13**, **19c**, **19e**, **21d**, **21e** and **24c** were the most active IC_{50} values of 0.09 and 0.83 μM, respectively. Moreover, compound 13 showed potency higher that foretinib towards H460 with IC_{50} 0.06 μM. Compounds **6**, **13**, **19e**, **21d**, **21e** showed activities toward U87MG cell line higher that foretinib with IC'_{50} 0.39, 0.69, 0.26, 0.70 and 0.42 μM, respectively. It is of great value to note that compound **6** showed higher potency than foretinib with IC_{50} 0.29 μM.

Table **2**. In vitro growth inhibitory effects IC_{50} ± SEM (μM) of the most potent compounds

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

2.1.5. HTRF kinase assay

The c-Met kinase activity (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.[37,38] In addition, the most potent compounds toward c-Met kinase were further evaluated against other five tyrosine kinase (c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR) using the same method [Table 4]. Briefly, 20 μg/mL poly (Glu, Tyr) 4:1 (Sigma) was used as a substrate in 384-well plates. Then 50 μL of 10 mMATP (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 μL of 1% DMSO (v/v) were used as the negative control. The kinase reaction was started by the addition of purified tyrosine kinase proteins diluted in 39 μL of kinase reaction buffer solution. The incubation time for the reactions was 30 min at 25 ^oC and ceased by the addition of 5 μL of Streptavidin-XL665 and 5 μL Tk Antibody Cryptate working solution to all of wells. The plates were read using Envision (PerkinElmer) 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)] x 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
6	-	-	10.22 ± 2.38
8	-	-	1.34 ± 0.81
13	-	-	0.06 ± 0.001
19c	Cl	NH_2	2.26 ± 0.80
19d	Cl	OH	0.83 ± 0.19
21b	H	OH	4.30±1.89
21d	Cl	OH	12.28 ± 2.69
21e	OCH_3	NH_2	1.27 ±0.98
21f	OCH_3	OH	1.40±0.51

24a	-	OH	0.79 ± 0.09
24c	-	4-OCH_3-C_6H_4	8.50 ±2.19
Foretinib	-	-	1.16 ± 0.17

2.1.6. Inhibitory of the most active compounds towards Tyrosine kinases

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

Table 4. inhibitory effect of compounds **8**, **19d**, **21e**, **21f** and **24a** towards tyrosine kinases [Enzyme IC_{50} (nM)]

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

2.1.7. Pim-1 kinase inhibition: 13, 21e and 24a

Compounds **13**, **21e** and **24a** were selected to examine their Pim-1 kinase inhibition activity[39] at a range of 10 concentrations and the IC_{50} values were calculated. Our slection 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 **13** and **24a** with IC_{50} value of 0.36 and 0.28 μM, while **21e** was less effective (IC_{50} > 10 μM). SGI-1776 was used as positive control with IC_{50} 0.048 μM in the assay. These profiles in combination with cell growth inhibition data of compounds **13**, **21e** and **24a** was listed in Table 5 indicated that Pim-1 was 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_{50} (μM)
13	86	0.36
21e	34	> 10
24a	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 2.02A° resolution. Deleting all water of crystallization away from the active site except the one involved in interaction 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 retrieve the co-crystallized pose with RMSD value of 0.55A°. The three-dimensional structures of the most active compounds **13**, **21e** & **24a** were built using MOE molecular builder, then they were energy minimized by Merk Molecular Force Field (MMFF94x). Hydrogens and partial charges were added to the system using protonate 3D application.

Docking results

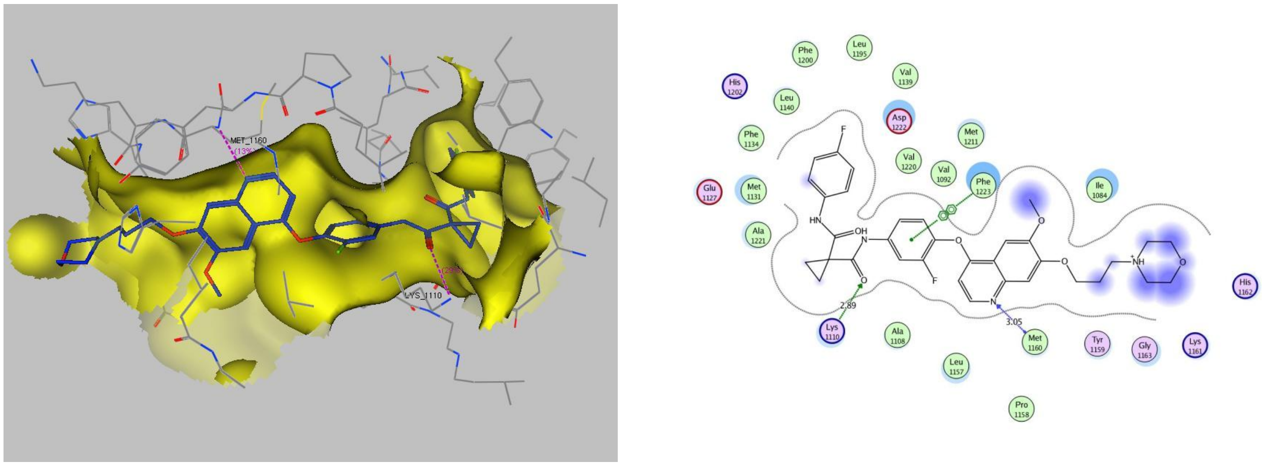
For each docked compound, only one pose was selected based on number of binding interactions, superposition with the original ligand, docking score and the formed H-bonds were measured. The docking results obtained from the docking study were summarized in table 6.

Table 6. Docking study data showing amino acid interactions and the hydrogren 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^{1223}**	**Atoms of compound forming H-bond**	**Amino acid residues forming H-bonds (H-bond length in A°)**	**Binding energy score (Kcal/mol)**
Ligand (Foretinib)	2	1	Quinoline N (H-acceptor) CO (H-acceptor)	Met^{1160} (3.05) Lys^{1110} (2.89)	-16.37
13	3	-	Quinolone N1 (H-acceptor) NH$\underline{NH_2}$ (H-acceptor) CONH$\underline{NH_2}$ (H-acceptor)	Asp^{1222} (3.03) Asp^{1222} (2.98) Lys^{1110} (2.75)	-11.45
21e	3	-	NH_2 (H-donor) OCH_3 (H-acceptor) OCH_3 (H-acceptor)	Asp^{1164} (1.46) Asn^{1171} (2.94) His^{1094} (2.86)	-7.83
24a	2	-	OH (H-donor) OH (H-acceptor)	Asp^{1164} (1.23) Asn^{1171} (2.66)	-11.58

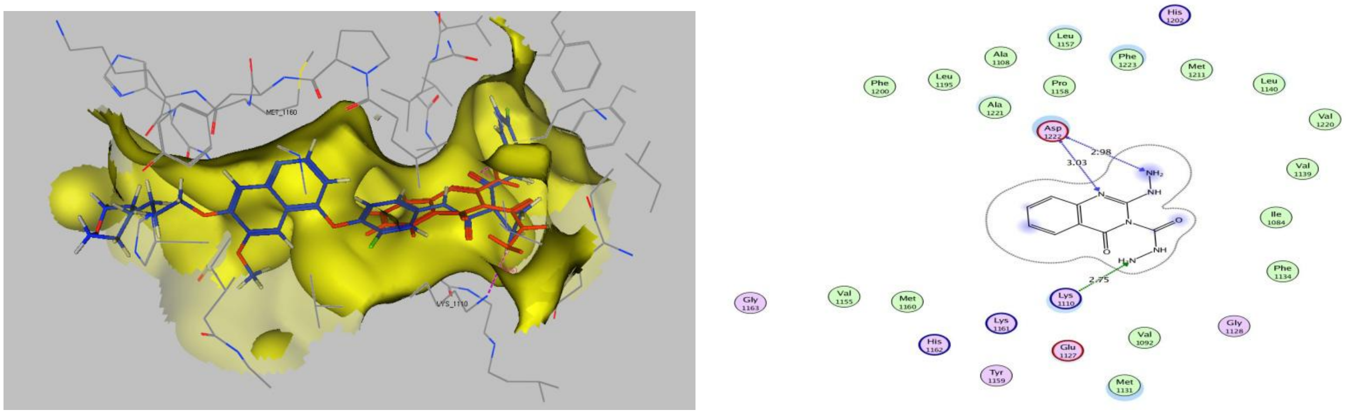
Discussion of molecular modeling

The X-ray crystallographic structure of XL880 (Foretinib) in complex with c-Met kinase showed that the inhibitor forms two hydrogen bonds between quinoline N and Met^{1160}, CO of malonamide moiety and Lys^{1110}. Phe^{1223} of the activation loop has relocated from the position in the active conformation to stack underneath the fluorophenyl ring (π–π interaction), placing the kinase in a pseudo-unactivated conformation[40] (Fig. 2). Compounds **13**, **21e** and **24a** showed good fitting to the active binding site of c-Met kinase by interaction with Asp^{1222}, Lys^{1110}, Asp^{1164}, Asn^{1171} & His^{1094} amino acid residues (Fig. 2-5).



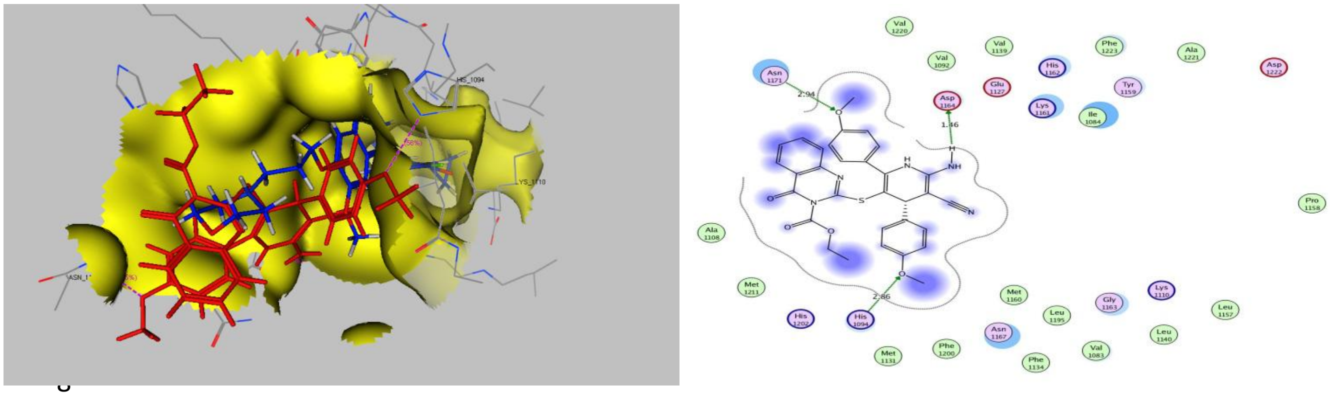
(a) (b)

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



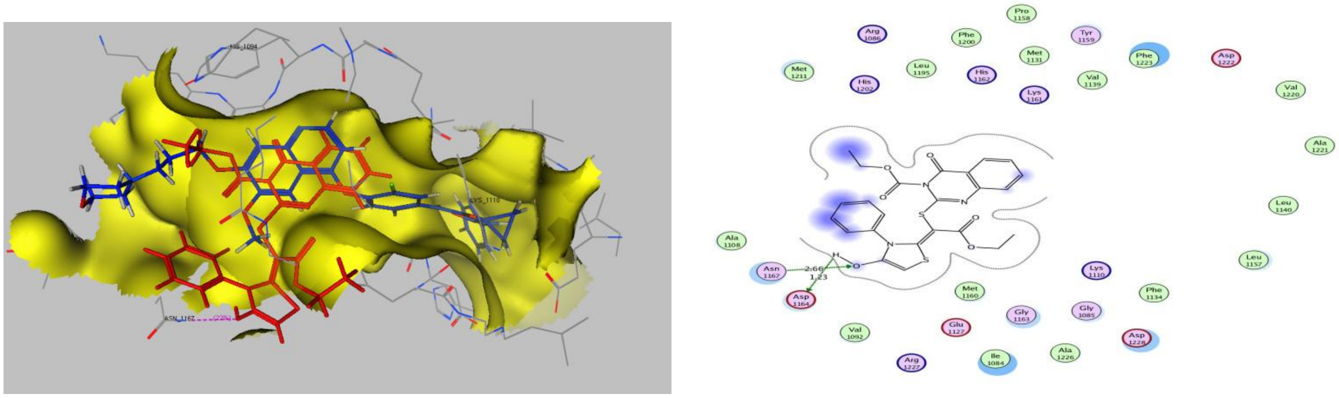
(a) (b)

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.



(a) (b)

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.



(a) (b)

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.

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. ^{13}C-NMR and ^{1}H-NMR spectra were recorded on Bruker DPX200 instrument in DMSO with TMS as internal standard for protons and solvent signals as internal standard for carbon spectra. Chemical shift values are mentioned in δ (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 x 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(4*H*)-carboxylate (4)

To a solution of anthranilic acid (1.37 g, 0.01 mol) in 1,4-dioxane (40 mL) a solution of ethyl carbonisothiocyanatidate (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 ^{o}C; IR (KBr) νmax 3480-3345 (NH), 3054 (CH aromatic), 2987, 2880 (CH_3, CH_2), 1688, 1682 (2CO), 1631 (C=C), 1205 (C=S) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.13 (t, 3H, J = 5.82 Hz, CH_3), 4.19 (q, 2H, CH_2), 7.29-7.38 (m, 4H, C_6H_4), 8.34 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.2 ($OCH_2\underline{CH_3}$), 53.8 ($O\underline{CH_2}CH_3$), 119.4, 122.3, 125.4, 126.3, 128.4, 130.3 (C_6H_4), 164.2, 164.8 (2CO), 179.4 (C=S); Anal. Calcd. for $C_{11}H_{10}N_2O_3S$ (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; EIMS (m/z, %): 250 [M^{+}, 42].

3.1.2. Ethyl 2-hydrazono-4-oxo-1,2-dihydroquinazoline-3(4*H*)-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 few drops of hydrochloric acid and the formed solid product was collected by filtration.

Yellow crystals from ethanol; m.p. 266-268 ^{o}C; IR (KBr) νmax 3469-3339 (NH_2, NH), 3056 (CH aromatic), 2989, 2883 (CH_3, CH_2), 1689, 1684 (2CO), 1655 (C=N), 1630 (C=C) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.14 (t, 3H, J = 7.04 Hz, CH_3), 4.19 (q, 2H, J = 7.04 Hz, CH_2), 4.76 (s, 2H, D_2O exchangeable, NH_2), 7.26-7.37 (m, 4H, C_6H_4), 8.31 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.1 ($OCH_2\underline{CH_3}$), 53.6 ($O\underline{CH_2}CH_3$), 120.5, 121.8, 124.8, 125.1, 127.8, 129.1 (C_6H_4), 164.3, 164.7 (2CO), 174.6 (C=N); Anal. Calcd. for $C_{11}H_{12}N_4O_3$ (248.24): C, 53.22; H, 4.87; N, 22.57. Found: C, 53.41; H, 4.69; N, 22.69; EIMS (m/z, %): 248 [M^{+}, 26].

3.1.3. Synthesis of the thioether derivatives 8, 10 and 12

To a solution of compound **4** in ethanol (40 mL) any of compound **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 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(4*H*)-carboxylate (8)

Yellow crystals from ethanol; m.p. 168-171 ^{o}C; IR (KBr) νmax 3055 (CH aromatic), 2989, 2883 (CH_3, CH_2), 1691, 1686, 1884 (3CO), 1654 (C=N), 1630 (C=C) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.12, 1.14 (2t, 6H, J = 5.93, 6.73 Hz, $2CH_3$), 4.16, 4.20 (2q, 4H, J = 5.93, 6.73 Hz, $2CH_2$), 5.21 (s, 2H, CH_2), 7.28-7.38 (m, 4H, C_6H_4); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.2, 16.4 (2 $OCH_2\underline{CH_3}$), 37.5 (CH_2), 53.3, 53.5 (2 $O\underline{CH_2}CH_3$), 120.8, 122.5, 125.3, 127.5, 127.8, 130.2 (C_6H_4), 164.3, 164.5, 165.0 (3CO), 174.6 (C=N); Anal. Calcd. for $C_{15}H_{16}N_2O_5S$ (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; EIMS (m/z, %): 336 [M^{+}, 36].

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

Orange crystals from ethanol; m.p. 210-213 ^{o}C; IR (KBr) νmax 3055 (CH aromatic), 2986, 2887 (CH_3, CH_2), 1694, 1686, 1882 (3CO), 1655 (C=N), 1631 (C=C) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.13 (t, 3H, J = 7.04 Hz, CH_3), 2.68 (s, 3H, CH_3), 4.23 (q, 2H, J = 7.04 Hz, CH_2), 5.38 (s, 2H, CH_2), 7.25-7.35 (m, 4H, C_6H_4); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.3 ($OCH_2\underline{CH_3}$), 22.8 (CH_3), 37.8 (CH_2), 53.2 ($O\underline{CH_2}CH_3$), 120.4, 121.6, 123.4, 125.0, 127.8, 129.1 (C_6H_4), 163.8, 164.2, 165.2 (3CO), 174.8 (C=N); Anal. Calcd. for $C_{14}H_{14}N_2O_4S$ (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; EIMS (m/z, %): 306 [M^{+}, 28].

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

Orange crystals from ethanol; m.p. 148-151 ^{o}C; IR (KBr) νmax 3056 (CH aromatic), 2983, 2889 (CH_3, CH_2), 1690, 1689, 1882 (3CO), 1653 (C=N), 1630 (C=C) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.13 (t, 3H, J = 6.99 Hz, CH_3), 3.73 (s, 3H, OCH_3), 4.22 (q, 2H, J = 6.99 Hz, CH_2), 5.49 (s, 2H, CH_2), 7.23-7.42 (m, 8H, $2C_6H_4$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.4 (OCH_2CH_3), 37.5 (CH_2), 53.4 (OCH_2CH_3), 55.2 (OCH_3), 119.2, 120.8, 122.3, 124.4, 124.9, 126.2, 126.8, 127.8, 128.4, 129.1 ($2C_6H_4$), 163.2, 164.4, 165.8 (3CO), 174.6 (C=N); Anal. Calcd. for $C_{20}H_{18}N_2O_5S$ (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; EIMS (m/z, %): 306 [M^{+}, 28].

3.1.4. 2-Hydrazinyl-4-oxoquinazoline-3(4*H*)-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 few drops of hydrochloric acid and the formed solid product was collected by filtration.

White crystals from ethanol; m.p. 233-236 ^{o}C; IR (KBr) νmax 3480-3320 ($2NH_2$, 2NH), 3053 (CH aromatic), 1687, 1883 (2CO), 1656 (C=N), 1632 (C=C) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 4.83, 4.91 (2s, 4H, D_2O exchangeable, $2NH_2$), 7.26-7.36 (m, 4H, C_6H_4), 8.21, 8.27 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 120.3, 124.4, 127.5, 127.8, 128.0, 129.1 (C_6H_4), 163.0, 164.2 (2CO), 174.4 (C=N); Anal. Calcd.

for $C_9H_{10}N_6O_2$ (234.21): C, 46.15; H, 4.30; N, 35.88. Found: C, 46.28; H, 4.46; N, 35.93; EIMS (m/z, %): 234 [M^+, 36].

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

To a solution of compound **8** (3.36 g, 0.01 mol) in dimethylformamide (30 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 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(1*H*)-dicarboxylate (16a)

Pale yellow crystals from 1,4-dioxane; m.p. 184-186 ^oC; IR (KBr) νmax 3468-3341 (NH), 3056 (CH aromatic), 1689, 1885-1883 (3CO), 2220 (CN), 1653 (C=N), 1630 (C=C) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 1.12, 1.15 (2t, 6H, J = 6.16, 6.90 Hz, 2 $OCH_2\underline{CH_3}$), 4.16, 4.20 (2q, 4H, J = 6.16, 6.90 Hz, 2 $O\underline{CH_2}CH3$), 5.29 (s, 2H, CH_2), 6.01 (s, 1H, pyrimidine H-2), 7.27-7.38 (m, 4H, C_6H_4), 8.28 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.3, 16.5 (2 $OCH_2\underline{CH_3}$), 28.1 (CH_2), 52.1, 53.8 (2 $O\underline{CH_2}CH3$), 92.6 (pyrimidine C-2), 117.8 (CN), 122.4, 124.8, 125.6, 126.3, 128.6, 143.8, (C_6H_4, thiadiazine C-5, C-6), 163.8, 164.4, 165.2 (3CO); Anal. Calcd. for $C_{18}H_{18}N_4O_5S$ (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; EIMS (m/z, %): 402 [M^+, 18].

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

Pale yellow crystals from 1,4-dioxane; m.p. 132-135 ^oC; IR (KBr) νmax 3468-3341 (NH), 3056 (CH aromatic), 1689, 1885, 1883 (3CO), 2220 (CN), 1653 (C=N), 1630 (C=C) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ Pale yellow crystals from 1,4-dioxane; m.p. 184-186 ^oC; IR (KBr) νmax 3468-3341 (NH), 3056 (CH aromatic), 1691- 1883 (4CO), 1656 (C=N), 1630 (C=C) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 1.12-1.15 (3t, 9H, 3 $OCH_2\underline{CH_3}$), 4.16-

4.20 (3q, 6H, J = 6.16, 6.90 Hz, 3 O$\underline{CH_2}$$CH_3$), 5.29 (s, 2H, CH_2), 6.01 (s, 1H, pyrimidine H-2), 7.27-7.38 (m, 4H, C_6H_4), 8.28 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.3, 16.4, 16.9 (3 $OCH_2\underline{CH_3}$), 28.1 (CH_2), 52.6, 52.9, 53.8 (3 O$\underline{CH_2}$$CH_3$), 92.6 (pyrimidine C-2), 120.8, 122.4, 123.9, 125.3, 125.8, 129.3, 141.3, 144.2 (C_6H_4, thiadiazine C-5, C-6), 163.3, 163.8, 164.4, 165.8 (4CO); Anal. Calcd. for $C_{20}H_{23}N_3O_7S$: C, 53.44; H, 5.16; N, 9.35; S, 7.13. Found: C, 53.28; H, 5.28; N, 4.93; S, 7.32; EIMS (m/z, %): 449 [M^+, 48].

3.1.6. General procedure for the synthesis of the pyran derivatives 19a-f

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-4*H*-pyran-3-yl)thio)-4-oxoquinazoline-3(4*H*)-carboxylate (19a)

Yellow crystals from ethanol; m.p.: 188-190 °C; IR (KBr) νmax 3469-3316 (NH_2), 2223 (CN), 3056 (CH aromatic), 2984, 2870 (CH_3, CH_2), 1688, 1686 (2CO), 1652 (C=N), 1630 (C=C) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.14 (t, 3H, J = 7.18 Hz, $OCH_2\underline{CH_3}$), 3.65 (s, 3H, OCH_3), 4.22 (q, 2H, J = 7.18 Hz, O$\underline{CH_2}$$CH_3$), 4.58 (s, 2H, D_2O exchangeable, NH_2), 6.92 (s, 1H, pyran H-4), 7.27-7.36 (m, 13H, C_6H_5, 2C_6H_4); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.2 ($OCH_2\underline{CH_3}$), 52.8 (OCH_3), 55.3 (O$\underline{CH_2}$$CH_3$), 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_6H_5, 2C_6H_4, pyran C), 164.3, 164.8 (2CO), 174.8 (C=N); Anal. Calcd. for $C_{30}H_{24}N_4O5_3S$ (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; EIMS (m/z, %): 552 [M^+, 25].

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

Yellow crystals from 1,4-dioxane; m.p.: 203-205 ^{o}C; IR (KBr) νmax 3583-3327 (OH), 2222 (CN), 3058 (CH aromatic), 2986 (CH_3), 1687. 1865 (2CO), 1650 (C=N), 1632 (C=C) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.14 (t, 3H, J = 6.80 Hz, $OCH_2\underline{CH_3}$), 3.67 (s, 3H, OCH_3), 4.26 (q, 2H, J = 6.80 Hz, $O\underline{CH_2}CH_3$), 6.94 (s, 1H, pyran H-4), 7.24-7.43 (m, 13H, C_6H_5, $2C_6H_4$), 10.33 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.1 ($OCH_2\underline{CH_3}$), 52.6 (CH_3), 55.1 ($O\underline{CH_2}CH_3$), 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_6H_5, $2C_6H_4$, pyran C), 164.5, 164.7 (2CO), 174.3 (C=N); Anal. Calcd. for $C_{30}H_{23}N_3O_6S$ (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; EIMS (m/z, %): 553 [M^{+}, 32].

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

Yellow crystals from ethanol; m.p.: 166-169 ^{o}C; IR (KBr) νmax 3472, 3329 (NH_2), 2220 (CN), 3052 (CH aromatic), 2986 (CH_3), 1688, 1686 (2CO), 1653 (C=N), 1633 (C=C) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.13 (t, 3H, J = 6.29 Hz, $OCH_2\underline{CH_3}$), 3.69 (s, 3H, OCH_3), 4.19 (q, 2H, J = 6.29 Hz, $O\underline{CH_2}CH_3$), 4.28 (s, 2H, D_2O exchangeable, NH_2), 6.91 (s, 1H, pyran H-4), 7.25-7.48 (m, 12H, $3C_6H_4$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.1 ($OCH_2\underline{CH_3}$), 52.6 (CH_3), 54.3 ($O\underline{CH_2}CH_3$), 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 ($3C_6H_4$, pyrimidine C-2, pyran C), 164.3, 165.0 (2CO), 174.6 (C=N); Anal. Calcd. for $C_{30}H_{23}ClN_4O_5S$ (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; EIMS (m/z, %): 587 [M^{+}, 28].

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

Pale yellow crystals from 1,4-dioxane; m.p.: 193-195 ^{o}C; IR (KBr) νmax 3563-3336 (OH), 2221 (CN), 3054 (CH aromatic), 2987 (CH_3), 1689, 1687 (2CO), 1650 (C=N), 1632 (C=C) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.15 (t, 3H, J = 7.37 Hz, $OCH_2\underline{CH_3}$), 3.69 (s, 3H, CH_3), 4.19 (q, 2H, J = 7.37 Hz, $O\underline{CH_2}CH_3$), 6.91 (s, 1H, pyran H-4), 7.25-7.46 (m, 12H, $3C_6H_4$), 10.31 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.4 (ester

CH_3), 52.4 (CH_3), 54.8 (ester CH_2), 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 ($3C_6H_4$, pyrimidine C-2, pyran C), 164.2, 164.5 (2CO), 174.6 (C=N); Anal. Calcd. for $C_{30}H_{22}ClN_3O_6S$ (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; EIMS (m/z, %): 588 [M^+, 25].

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

Yellow crystals from ethanol; m.p.: 148-150 °C; IR (KBr) νmax 3468-3315 (NH_2), 2221 (CN), 3056 (CH aromatic), 2987 (CH_3), 2220 (CN), 1689, 1687 (2CO), 1633 (C=C) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 1.13 (t, 3H, J = 7.26 Hz, $OCH_2\underline{CH_3}$), 3.67, 3.72 (2s, 6H, 2 OCH_3), 4.19 (q, 2H, J = 7.26 Hz, $O\underline{CH_2}CH_3$), 4.28 (s, 2H, D_2O exchangeable, NH_2), 6.93 (s, 1H, pyran H-4), 7.24-7.49 (m, 12H, $3C_6H_4$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.0 ($OCH_2\underline{CH_3}$), 52.6, 53.2 (2 OCH_3), 54.3 ($O\underline{CH_2}CH_3$), 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 ($3C_6H_4$, pyrimidine C-2, pyran C), 164.3, 164.8 (2CO), 174.4 (C=N); Anal. Calcd. for $C_{31}H_{26}N_4O_6S$ (582.63): C, 63.91; H, 4.50; N, 9.62; S, 5.50. Found: C, 61.73; H, 4.53; N, 9.63; S, 5.72; EIMS (m/z, %): 582 [M^+, 18].

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

Yellow crystals from ethanol; m.p.: 242-146 °C; IR (KBr) νmax 3530-3336 (OH), 2220 (CN), 3056 (CH aromatic), 2986 (CH_3), 2221 (CN), 1689, 1687 (2CO), 1634 (C=C) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 1.14 (t, 3H, J = 6.52 Hz, $OCH_2\underline{CH_3}$), 3.66, 3.73 (2s, 6H, 2 OCH_3), 4.20 (q, 2H, J = 6.52 Hz, $O\underline{CH_2}CH_3$), 6.90 (s, 1H, pyran H-4), 7.23-7.48 (m, 12H, $3C_6H_4$), 10.37 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.4 ($OCH_2\underline{CH_3}$), 52.8, 53.4 (2 OCH_3), 54.6 ($O\underline{CH_2}CH_3$), 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 ($3C_6H_4$, pyrimidine C-2, pyran C), 164.3, 164.6 (2CO), 174.8 (C=N); Anal. Calcd. for $C_{34}H_{25}N_3O_5S$ (583.61): C, 63.80; H, 4.32; N, 7.20; S, 5.49. Found: C, 63.69; H, 4.38; N, 7.29; S, 5.53; EIMS (m/z, %): 583 [M^+, 18].

3.1.8. 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(4*H*)-carboxylate (21a)

Yellow crystals from ethanol; m.p.: 231-235 ^{o}C; IR (KBr) νmax 3483-3342 (NH, NH_2), 2220 (CN), 3054 (CH aromatic), 2989 (CH_3), 1689, 1687 (2CO), 1656 (C=N), 1630 (C=C) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.15 (t, 3H, J = 7.28 Hz, $OCH_2\underline{CH_3}$), 3.66 (s, 3H, CH_3), 4.23 (q, 2H, J = 7.28 Hz, $O\underline{CH_2}CH_3$), 4.59 (s, 2H, D_2O exchangeable, NH_2), 6.89 (s, 1H, pyridine H-4), 7.27-7.38 (m, 13H, C_6H_5, $2C_6H_4$), 8.29 (s, 1H, D_2O exchangeable NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.1 ($OCH_2\underline{CH_3}$), 52.5 (CH_3), 54.1 ($O\underline{CH_2}CH_3$), 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_6H_5, C_6H_4, pyridine C), 164.2, 164.7 (2CO), 174.3 (C=N); Anal. Calcd. for $C_{30}H_{25}N_5O_4S$ (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; EIMS (m/z, %): 551 [M^{+}, 25].

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

Yellow crystals from 1,4-dioxane; m.p.: 230-233 ^{o}C; IR (KBr) νmax 3571-3320 (OH, NH), 2220 (CN), 3055 (CH aromatic), 2989 (CH_3), 2220 (CN), 1689, 1684 (2CO), 1653 (C=N), 1630 (C=C) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.14 (t, 3H, J = 6.89 Hz, $OCH_2\underline{CH_3}$), 3.68 (s, 3H, CH_3), 4.22 (q, 2H, J = 6.89 Hz, $O\underline{CH_2}CH_3$), 6.93 (s, 1H, pyridine H-4), 7.26-7.46 (m, 13H, C_6H_5, $2C_6H_4$), 8.23 (s, 1H, D_2O exchangeable, NH); 10.33 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.4 ($OCH_2\underline{CH_3}$), 52.8 (CH_3), 54.6

(O$\underline{CH_2}$CH$_3$), 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_6H_5, $2C_6H_4$, pyridine C), 164.2, 164.8 (2CO), 174.6 (C=N); Anal. Calcd. for $C_{30}H_{24}N_4O_5S$ (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; EIMS (m/z, %): 552 [M$^+$, 32].

Ethyl 2-((6-amino-4-(4-chlorophenyl)-5-cyano-2-(4-methoxyphenyl)-1,4-dihydropyridin-3-yl)thio)-4-oxoquinazoline-3(4*H*)-carboxylate (21c)

Yellow crystals from ethanol; m.p.: 184-187 ^{o}C; IR (KBr) νmax 3480, 3323 (NH$_2$, NH), 3054 (CH aromatic), 2988 (CH$_3$), 2220 (CN), 1656 (C=N), 1633 (C=C) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d$_6$) δ 1.13 (t, 3H, J = 7.01 Hz, OCH$_2\underline{CH_3}$), 3.66 (s, 3H, OCH$_3$), 4.19 (q, 2H, J = 7.01 Hz, O$\underline{CH_2}$CH$_3$), 4.68 (s, 2H, D$_2$O exchangeable, NH$_2$), 6.93 (s, 1H, pyridine H-4), 7.22-7.45 (m, 12H, $3C_6H_4$), 8.28 (s, 1H, D$_2$O exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 16.3 (OCH$_2\underline{CH_3}$), 52.8 (OCH$_3$), 54.3 (O$\underline{CH_2}$CH$_3$), 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 ($3C_6H_4$, pyridine C), 164.2, 164.6 (2CO), 174.8 (C=N); Anal. Calcd. for $C_{30}H_{24}ClN_5O_4S$ (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; EIMS (m/z, %): 586 [M$^+$, 36].

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

Yellow crystals from 1,4-dioxane; m.p.: 166-168 ^{o}C; IR (KBr) νmax 3572-3333 (OH, NH), 2221 (CN), 3054 (CH aromatic), 2987 (CH$_3$), 2220 (CN), 1689, 1684 (2CO), 1650 (C=N), 1632 (C=C) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d$_6$) δ 1.13 (t, 3H, J = 6.47 Hz, OCH$_2\underline{CH_3}$), 3.69 (s, 3H, OCH$_3$), 4.18 (q, 2H, J = 6.47 Hz, O$\underline{CH_2}$CH$_3$), 6.90 (s, 1H, pyran H-4), 7.22-7.46 (m, 12H, $3C_6H_4$), 8.28 (s, 1H, D$_2$O exchangeable, NH), 10.31 (s, 1H, D$_2$O exchangeable, OH); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 16.6 (OCH$_2\underline{CH_3}$), 52.6 (OCH$_3$), 54.6 (O$\underline{CH_2}$CH$_3$), 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 (C_6H_5, $3C_6H_4$, pyran C), 164.2, 164.8 (2CO), 174.6 (C=N); Anal. Calcd. for $C_{30}H_{23}ClN_4O_5S$

(587.05): C, 61.38; H, 3.95; N, 9.54; S, 5.46. Found: C, 61.42; H, 3.49; N, 9.70; S, 5.59; EIMS (m/z, %): 587 [M^+, 48].

2-Amino-4,6-bis(4-methoxyphenyl)-5-((4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)thio)-4*H*-pyran-3-carbonitrile (11e)

Yellow crystals from ethanol; m.p.: 148-152 ^oC; IR (KBr) νmax 3468-3315 (NH_2, NH), 3056 (CH aromatic), 2987 (CH_3), 2220 (CN), 1689, 1682 (2CO), 1633 (C=C) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 1.14 (t, 3H, J = 7.19 Hz, $OCH_2\underline{CH_3}$), 3.65, 3.74 (2s, 6H, 2 OCH_3), 4.20 (q, 2H, J = 7.19 Hz, $O\underline{CH_2}CH_3$), 4.67 (s, 2H, D_2O exchangeable, NH_2), 6.91 (s, 1H, pyran H-4), 7.25-7.47 (m, 12H, $3C_6H_4$), 8.30 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.3 ($OCH_2\underline{CH_3}$), 52.5, 53.8 ($2CH_3$), 54.6 ($O\underline{CH_2}CH_3$), 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, 135.6, 136.3, 137.1, 140.9, 142.7, 143.5, 143.5 ($3C_6H_4$, pyridine C), 163.9, 164.5 (2CO), 174.6 (C=N); Anal. Calcd. for $C_{31}H_{27}N_5O_5S$ (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; EIMS (m/z, %): 581 [M^+, 22].

Ethyl 2-((5-cyano-6-hydroxy-2,4-bis(4-methoxyphenyl)-1,4-dihydropyridin-3-yl)thio)-4-oxoquinazoline-3(4H)-carboxylate (11f)

Yellow crystals from ethanol; m.p.: 263-265^oC; IR (KBr) νmax 3548-3322 (OH NH), 3054 (CH aromatic), 2987 (CH_3), 2221 (CN), 1688, 1684 (2CO), 1635 (C=C) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 1.13 (t, 3H, J = 7.08 Hz, $OCH_2\underline{CH_3}$), 3.62, 3.73 (2s, 6H, 2 OCH_3), 4.21 (q, 2H, J = 7.08 Hz, $O\underline{CH_2}CH_3$), 6.91 (s, 1H, pyran H-4), 7.25-7.48 (m, 12H, $3C_6H_4$), 8.26 (s, 1H, D_2O exchangeable, NH), 10.31 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.1 ($OCH_2\underline{CH_3}$), 52.4, 53.8 ($2CH_3$), 54.4 ($O\underline{CH_2}CH_3$), 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 ($3C_6H_4$, pyridine C), 164.1, 164.3 (2CO), 174.3 (C=N); Anal. Calcd. for $C_{31}H_{26}N_4O_6S$ (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; EIMS (m/z, %): 582 [M^+, 31].

3.1.9. 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 chloroaceate (1.22 g, 0.01 mol), α-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 another 24 hours then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

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

Orange crystals from ethanol; m.p.: 193-196 °C; IR (KBr) νmax 3562-3345 (OH), 3055 (CH aromatic), 2985, 2889 (CH_3, CH_2), 1689, 1685, 1682 (3CO), 1632 (C=C) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 1.13, 1.15 (2t, 6H, J = 6.59, 6.18 Hz, 2 $OCH_2\underline{CH_3}$), 4.18, 4.26 (2q, 4H, J = 6.59, 6.18 Hz, 2 $O\underline{CH_2}CH_3$), 6.06 (s, 1H, thiazole H-5), 7.28-7.41 (m, 9H, C_6H_5, C_6H_4), 9.42 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.1, 16.8 (2 $OCH_2\underline{CH_3}$), 54.2, 54.6 (2 $O\underline{CH_2}CH_3$), 69.2 (thiazole C-5), 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_6H_5, C_6H_4, thiazole C-3, C-4), 163.4, 164.3, 164.6 (3CO), 174.4 (C=N); Anal. Calcd. for $C_{24}H_{21}N_5O_6S_2$ (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; EIMS (m/z, %): 511 [M^+, 38].

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

Orange crystals from acetic acid; m.p.: 205-208 °C; IR (KBr) νmax 3053 (CH aromatic), 2989, 2884 (CH_3, CH_2), 1688, 1686, 1680 (3CO), 1634 (C=C) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 1.14, 1.15 (2t, 6H, J = 7.26, 6.37 Hz, 2 $OCH_2\underline{CH_3}$), 2.80 (s, 3H, CH_3), 4.18, 4.23 (2q, 4H, J = J = 7.26, 6.37 Hz, 2 $O\underline{CH_2}CH_3$), 6.07 (s, 1H, thiazole H-5), 7.26-7.44 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.6, 16.9 (2 $OCH_2\underline{CH_3}$), 54.6, 54.8 (2 $O\underline{CH_2}CH_3$), 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_6H_5, C_6H_4, thiazole C-3, C-4), 163.8, 164.8, 164.9 (3CO), 174.2 (C=N); Anal. Calcd. for $C_{25}H_{23}N_3O_5S_2$ (509.60): C, 58.92; H, 4.55; N, 8.25; S, 12.58. Found: C, 56.79; H, 4.70; N, 8.39; S, 12.39; EIMS (m/z, %): 509 [M$^+$, 19].

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

Orange crystals from acetic acid; m.p.: 177-179 ^oC; IR (KBr) νmax 3055 (CH aromatic), 2987, 2888 (CH_3, CH_2), 1689, 1686, 1683 (3CO), 1632 (C=C) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d$_6$) δ 1.16, 1.18 (2t, 6H, J = 7.42, 7.07 Hz, 2 $OCH_2\underline{CH_3}$), 3.69 (s, 3H, OCH_3), 4.18, 4.23 (2q, 4H, J = J = 7.42, 713 Hz, 2 $O\underline{CH_2}CH_3$), 6.06 (s, 1H, thiazole H-5), 7.22-7.48 (m, 13H, C_6H_5, $2C_6H_4$); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 16.6, 16.8 (2 $OCH_2\underline{CH_3}$), 52.6 (OCH_3), 54.3, 54.6 (2 $O\underline{CH_2}CH_3$), 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_6H_5, C_6H_4, thiazole C-3, C-4), 163.3, 164.6, 164.5 (3CO), 174.5 (C=N); Anal. Calcd. for $C_{31}H_{27}N_3O_6S_2$ (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; EIMS (m/z, %): 601 [M$^+$, 22].

4. Conclusions

In the presented work The reaction of anthranilic acid with ethoxycarbonylisothiocyanate gave the ethyl 4-oxo-2-thioxo-1,2-dihydroquinazoline-3(4H)-carboxylate (**4**). The reaction of compound **4** with hydrazine hydrate and α-halocarbnyl derivatives was studied to give either hydrazono or S-alkylated products. Heterocyclization reactions of some of the S-alkylated derivatives **8** and **12** were carried to afford thiophene, 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 **8**, **10**, **16a**, **19d-f**, **21c**, **21e**, **21f**, **24a and 24b** showed the highest cytotoxicity. The c-Met kinase inhibition for some selected compounds showed that compounds **8**, **13**, **19d**, **21e**, **21f** and **24a** with the highest inhibitory effect. Activities towards tyrosine kinases revealed that compounds **13**, **21e** and **24a** with the highest potency. Compounds **13** and **24a** showed the highest activities towards Pim-1 kinase.

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