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Original article

Quinazoline–tyrphostin as a new class of antitumor agents, molecular properties prediction, synthesis and biological testing

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ABSTRACT

A new series of substituted quinazolin-4-(3*H*)-one-tyrphostin derivatives was prepared and screened for their cytotoxic activity against three tumor cell lines, namely human breast cancer cell line (MCF-7), human cervical cancer cell line (HeLa) and human hepatocellular liver carcinoma cell line (HepG2) using the colorimetric MTT assay. Among the current series, **10** compounds exhibited remarkable *in vitro* antiproliferative activity against the three tested cell lines with the IC_{50} values ranging from 0.009 to 0.015 mM. All the compounds showed suitable drug like characteristics according to Lipinski's rule.

1. Introduction

Cancer is one of the most challenging disorders in the world and chemotherapy regimens led to unsatisfactory results. Future improvements are likely to come from novel agents targeting molecular pathways that promote tumor cell growth and survival [1]. Protein tyrosine kinases (PTK) play important roles in regulating most cellular functions including cell cycle, proliferation, metabolism, survival, apoptosis, DNA repair and response to the microenvironment [2,3]. The development of PTK inhibitors has renovated the tactic to cancer therapy and is likely to affect other fields of medicine. The first step in the development of PTK inhibitors was from the family of benzene malononitrile (Fig. 1) [4,5]. Since then, many hundreds of such inhibitors were generated and the term tyrphostins was coined for such compounds by Levitki and Mishani [6]. In spite of the weaknesses among PTKs, one can develop small molecules that antagonize the activity of certain PTKs and that display much less toxicity than the currently available chemotherapeutic agents [6].

On the other hand, quinazoline containing compounds form an important class among heterocyclic pharmaceuticals and represent an attractive scaffold for designing anticancer agents. Research on quinazoline has led to the development and marketing of a new series of antitumor agents [7].

We thought that incorporating the tyrphostin structural feature on a quinazoline backbone might lead to interesting antitumor agents. Thus, in the present work certain new quinazoline bearing 2-cyano-3-substituted acrylamide derivatives, as tyrphostin analogs and/or their isosteres, were prepared and screened for their antitumor activity against three tumor cell lines. We anticipated that, these compounds would to inhibit tumor growth in a similar manner to, or even better than other tyrosine kinase inhibitors.

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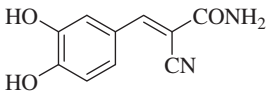


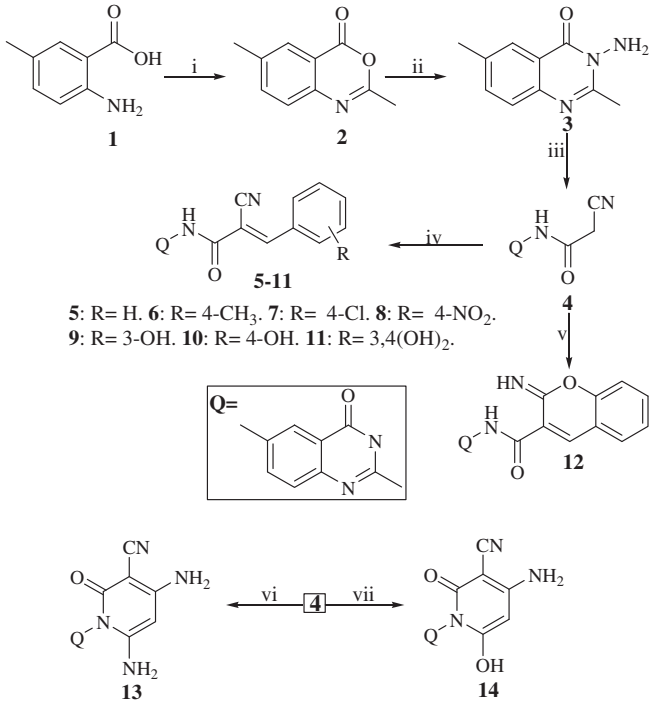
Tyrphostin AG 99

Fig. 1.

2. Results and discussion

2.1. Chemistry

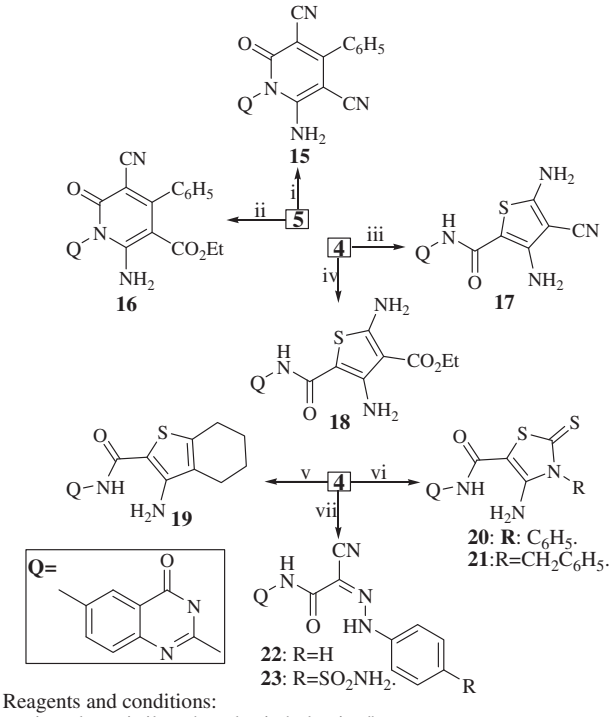
The synthetic strategies adopted for the synthesis of the intermediates and target compounds are depicted in Schemes 1 and 2. In Scheme 1, the 2,6-dimethyl-3-(2-cyanoacetylamino)-quinazoline-4(3*H*)-one **4** was prepared from the 3-aminoquinazoline derivative **3** according to a reported procedure [8,9]. IR spectrum of compound **4** showed the presence of two carbonyl groups at 1689 and 1678 cm^{-1}, in addition to another band at 2225 cm^{-1} due to the cyano group. ^1H NMR spectrum of compound **4** showed the presence of two singlets corresponding to $2CH_3$ groups, a singlet at δ 3.31 ppm for CH_2 group, in addition to the aromatic protons and a singlet at δ 8.10 ppm for an NH group. Moreover, the ^{13}C NMR data showed the presence of the three aliphatic, seven aromatic and two carbonyl carbon atoms at the appropriate δ values. Further elucidation for the structure of **4** was obtained through studying its chemical reactivity with some chemical reagents. Thus, the reaction of **4** with certain aromatic aldehydes gave the corresponding



5: R= H. **6**: R= 4-CH_3. **7**: R= 4-Cl. **8**: R= 4-NO_2.
9: R= 3-OH. **10**: R= 4-OH. **11**: R= 3,4$(OH)_2$.

Reagents and conditions:
i: Ac_2O/reflux.
ii: $N_2H_4.H_2O$/reflux.
iii: ethyl cyanoacetate/reflux.
iv: aldehyde, dioxane, piperidine/heat.
v: salicylaldehyde, dioxane, piperidine/heat.
vi: malononitrile, dioxane, triethylamine/heat.
vii: ethyl cyanoacetate, dioxane, triethylamine/heat.

Scheme 1.



Reagents and conditions:
i: malononitrile, ethanol, triethylamine/heat.
ii: ethyl cyanoacetate, ethanol, triethylamine/heat.
iii: malononitrile, dioxane, triethylamine/elemental sulfur/heat.
iv: ethyl cyanoacetate, dioxane, triethylamine/elemental sulfur/heat.
v: ethanol, cyclohexanone, triethylamine/elemental sulfur/heat.
vi: ethanol, RNCS, triethylamine/elemental sulfur/heat.
vii: aryldiazonium chloride/ethanol/0-5 °C/stirring/1 hr.

Scheme 2.

benzylidene derivatives, tyrphostin **5**–**11**. Analytical and spectral data for the later compounds were in agreement with the proposed structures. Generally, the IR spectra for these derivatives showed the presence of the NH group at around 3280 cm^{-1}, cyano group at around 2224 cm^{-1} and two carbonyl groups at around 1680 and 1670 cm^{-1}. Whereas the NMR spectra showed the absence of the active methylene protons in the ^1H spectrum and the appearance of the produced arylidene carbon at 107.0 δ value in the ^{13}C spectrum. On the other hand, the iminochromene derivative **12** was obtained through reaction of **4** with salicylaldehyde. The reaction goes in analogy with the reported literature [10]. The IR and ^{13}C NMR spectra of compound **12** were devoid of the cyano group and were consistent with the proposed structure. The reaction of **4** or **5** with either malononitrile or ethyl cyanoacetate gave the 2-pyridinone derivatives **13**–**16**. Analytical and spectral data are consistent with the proposed structures where the amino, cyano and carbonyl groups were common features of compounds **13**–**16** in the IR and NMR spectra.

The substituted thiophene derivatives **17** and **18** were obtained through reaction of compound **4** with either malononitrile or ethyl cyanoacetate and elemental sulfur in the presence of triethylamine as presented in Scheme 2. The reaction goes in parallel to the reported Gewald's thiophene synthesis [11]. Similarly, the reaction of **4** with cyclohexanone and elemental sulfur gave the 4,5,6,7-tetrahydrobenzothiophene derivative **19**. The later reaction took place in a similar way to the reported reactions of cyclohexanone with methylene reagents and elemental sulfur [12].

On the other hand the reaction of **4** with elemental sulfur and phenyl or benzylisothiocyanate, gave the thiazole derivative **20**, **21**, Scheme 2. Formation of the later products took place in parallel to

a reported reaction [13]. The reaction of **4** with either benzene diazonium chloride, 4-aminosulfonyl benzene diazonium chloride gave the aryl hydrazone derivatives **22** and **23**. Structures of all the new compounds were based on analytical and spectral data and were in accordance with the proposed structures.

2.2. In vitro anticancer screening

The newly synthesized compounds **4**–**23** were initially screened at the single concentration of 0.06 mM using the colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to test their *in vitro* cytotoxicity against human breast cancer cell line (MCF-7), cervix cancer (HeLa) and hepatocellular liver carcinoma (HepG2). Doxorubicin was used as the reference drug in this study. The cytotoxicity of the tested compounds was estimated in terms of percent growth inhibition compared to untreated control cells. All the compounds effected ≥70% inhibition and were retested by serial dilution from 0.06 to 0.002 mM. The results were expressed as IC_{50} (inhibitory concentration 50%), the concentration of the compound which inhibits the tumor cell growth by 50% and the data are presented in Table 1.

Close inspection of cytotoxic data revealed that most of the tested compounds showed remarkable antiproliferative activity against the three tested cell lines. This observation could be attributed to the synergistic effect that may result from combining the quinazoline core with the typical tyrphostin structural feature, 2-cyano-3-substituted acrylamide (Fig. 1).

Most of the compounds showed efficacy against human breast cancer cell line MCF-7 with IC_{50} values range of 0.06–0.002 mM compared to DOX IC_{50} (0.009 mM) Table 1. Human hepatocellular liver carcinoma cell line, HepG2 and human cervical cancer cells, HeLa proved to be sensitive toward compounds **5**–**11**, **13**, **15, 17** and **23** with IC_{50} concentration range of 0.009–0.017 mM compared to DOX (IC_{50} = 0.008, 0.009 mM, respectively).

Evidently, compounds comprising the frank tyrphostin feature (**5**–**11**), open chain, retained the highest activity against the three cell lines with IC_{50} ranging from 0.009 to 0.019 mM. However, rigidifying the structure and keeping the cyano group do not much affect the activity except for compound **15** which exhibited activity better than that of the standard drug against MCF-7 and HepG2, IC_{50} = 0.008 "and" 0.005 mM, respectively, whereas compounds **12**–**14** and **16**, **17** showed considerable activity against the three cell lines at somewhat higher IC_{50} values ranging from 0.010 to 0.026 mM. Conversely, cyclization with modification of the cyano group led to less active and more selective compounds. Compounds **19**–**21** were active only against human breast cancer cell line MCF-7 with IC_{50} 0.010–0.018 mM compared to DOX IC_{50} (0.009 mM). Compound **22** was active against MCF-7 and HepG2 while its sulfonamide congener **23** was active against the three tested cell lines mostly due to the impact of the well-known antitumor sulfonamide. This moiety was shown to selectively concentrate in tumor tissues in addition to their unique role in carbonic anhydrase inhibition [14,15].

Except for compounds **5**–**11** almost all the active compounds were devoid of the catechol moiety but had a considerable number of hydrogen bond donor and acceptor groups comparable to the model tyrphostin AG99. Such observation renders this family of quinazoline containing tyrphostin analogs worthy for further research and development.

Table 1
In vitro antitumor activity of the designed quinazoline derivatives **4**–**23**.

Compd. No.	IC_{50}(mM)		
	MCF-7[a]	HeLa[b]	HepG2[c]
4	0.040	0.030	0.055
5	0.009	0.013	0.014
6	0.010	0.009	0.012
7	0.012	0.012	0.009
8	0.012	0.015	0.010
9	0.015	0.014	0.014
10	0.014	0.013	0.013
11	0.008	0.008	0.008
12	0.017	0.038	0.023
13	0.010	0.015	0.017
14	0.026	0.045	0.041
15	0.008	0.005	0.010
16	0.017	0.021	0.052
17	0.010	0.015	0.015
18	0.016	0.023	0.022
19	0.018	0.031	0.022
20	0.013	0.035	0.027
21	0.010	0.026	0.032
22	0.019	0.079	0.012
23	0.009	0.012	0.009
DOX	0.009	0.008	0.009

[a] Human breast cancer cell line (MCF-7).
[b] Human cervical cancer cell line (HeLa).
[c] Human hepatocellular liver carcinoma cell line (HepG2).

3. POM virtual screenings and molecular properties calculations [16–23]

3.1. Molinspiration calculations

MiLog*P* (octanol/water partition coefficient) is calculated by the methodology developed by Molinspiration as a sum of fragment-based contributions and correction factors (Tables 2and 3). The method is very robust and is able to process practically all organic and most organometallic molecules. Total Polar Surface Area (TPSA) is calculated as a sum of fragment contributions [16]. *O*- and *N*-centered polar fragments are considered. PSA has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability and blood–brain barrier penetration. Prediction results of compounds **4**–**23** molecular properties (TPSA, GPCR ligand and ICM) are valued (Tables 2and 3). Lipophilicity (log*P*) and polar surface area (PSA) values are two important properties for the prediction of oral bioavailability of drug molecules [16–23]. Therefore log*P* and PSA values for compounds **4**–**23** we calculated using molinspiration software programs and compared with the values obtained for standard drug doxorubicin (DOX). For all the compounds, without exception, the calculated *c*log*P* values were around −1.5–2.3 (<5), which is the upper limit for the drugs to be able to penetrate through bio-membranes according to Lipinski's rule. Thus, these compounds, **4**–**23**, are expected to exhibit good bioavailability.

The lowest degree of lipophilicity among all the compounds was exhibited by compounds **4**–**23**, which is an indication for good water solubility. The (PSA) is calculated from the surface areas that are occupied by oxygen and nitrogen atoms and by hydrogen atoms attached to them. Thus, the PSA is closely related to the hydrogen bonding potential of a compound [16–23]. Molecules with PSA values around of 160 $Å^2$ or more are expected to exhibit poor intestinal absorption. Table 3 shows that all the compounds are within this limit. It has to be kept in mind that log*P* and PSA values are the most important two features, although not sufficient criteria for predicting oral absorption of a drug. It is worth to mention that all the compounds have zero violation of the rule of 5. Two or more violations suggest the probability of problems in bioavailability. Drug likeness of compounds **4**–**23** is tabulated in Table 3. Drug likeness may be defined as a complex balance of various molecular properties and structure features which determine whether particular molecule is similar to the known drugs.

Table 2
Osiris calculations of compounds **4**–**23**.

Compd.	Toxicity Risks[a]				Bioavailability and Drug Score[b]				
	MUT	TUM	IRRIT	RE	MW	CLP	S	DL	D-S
4					256	0.37	−2.81	−4.62	0.22
5					344	1.99	−4.2	−1.65	0.45
6					358	2.30	−4.55	−1.98	0.32
7					377	2.60	−4.94	0.13	0.40
8					389	1.86	−4.66	−10.80	0.21
9					360	2.30	−4.55	−0.79	0.47
10					360	1.69	−3.91	−0.41	0.27
11					376	1.39	−3.61	0.27	0.63
12					360	0.72	−3.08	2.97	0.84
13					322	−1.51	−3.89	−2.47	0.45
14					323	−0.98	−3.81	−2.35	0.45
15					408	0.66	−5.11	−0.19	0.37
16					455	0.64	−4.86	−3.72	0.25
17					354	0.27	−4.65	−1.72	0.42
18					401	0.87	−4.32	−1.30	0-44
19					368	2.22	−4.85	−2.78	0.36
20					423	1.78	−6.43	4.64	0.50
21					437	1.90	−5.91	5.36	0.53
22					360	2.10	−3.67	−1.07	0.24
23					439	0.71	−3.57	1.44	0.33
DOX					545	−0.95	−3.72	7.38	0.61

[a] MUT: mutagenic; TUM: tumorigenic; IRRIT: irritant; RE: reproductive effective.
[b] CLP: *cLogP*, S: Solubility, DL: Drug likeness, DS: Drug Score.

These properties, mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility and presence of various pharmacophoric features influence the behavior of molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and many others. Activity of all the 20 compounds and standard drug were rigorously analyzed under four criteria of known successful drug activity in the areas of GPCR ligand activity, ion channel modulation, kinase inhibition activity, and nuclear receptor ligand activity. Results are shown for all compounds in Table 3 by means of numerical assignment. Likewise all the compounds have consistent negative values in all categories and numerical values comparable to that of standard drug used for comparison. Therefore it is readily seen that all the compounds are expected to have close similar activity to standard drug used based upon these four rigorous criteria (GPCR ligand, ion channel modulator, kinase inhibitor and nuclear receptor ligand).

3.2. Osiris calculations

Structure based design is now fairly routine but many potential drugs fail to reach the clinic because of ADME-Tox liabilities. One very important class of enzymes, responsible for many ADMET problems, is the cytochromes P450. Inhibition of these or production of unwanted metabolites can result in many adverse drug reactions. With our recent work on the drug design by combination of various pharmacophoric sites using heterocyclic structure, it is now possible to predict activity and/or inhibition with increasing success in two targets (bacteria and HIV). This was done using a combined electronic/structure docking procedure and an example will be given here. The remarkably well behaved mutagenicity of diverse synthetic molecules classified in data base of Celeron Company of Swiss can be used to quantify the role played by various organic groups in promoting or interfering with the way a drug can associate with DNA. The Osiris calculations are tabulated in Table 2. Toxicity risks (mutagenicity, tumorogenicity, irritation, reproduction) and physicochemical properties (miLog*P*, solubility, drug likeness and drug score) of compounds **4**–**23** were calculated

Table 3
Drug likeness calculation of compounds **4**–**23** by using molinspiration.

Compd.	Molinspiration calculations[a]				Calculation of Bioactivity Scores[b]					
	cLogP	TPSA	OH−NH Interract	Volume	GPCRL	ICM	KI	NRL	PI	EI
4	0.103	88	1	226	−0.49	−1.08	−0.57	−1.36	−0.99	−0.66
5	2.719	88	1	308	−0.46	−0.71	−0.29	−0.71	−0.65	−0.45
6	3.17	88	1	325	−0.45	−0.68	−0.28	−0.68	−0.63	−0.44
7	3.40	88	1	322	−0.45	−0.69	−0.30	−0.70	−0.67	−0.47
8	2.68	134	1	332	−0.55	−0.68	−0.39	−0.73	−0.72	−0.51
9	3.14	88	1	325	−0.44	−0.68	−0.28	−0.67	−0.62	−0.44
10	2.24	108	2	316	−0.41	−0.64	−0.24	−0.55	−0.62	−0.39
11	1.75	128	3	324	−0.41	−0.64	−0.25	−0.56	−0.62	−0.38
12	1.26	101	2	312	−0.66	−1.10	−0.71	−1.19	−1.07	−0.44
13	0.60	133	4	277	−0.04	−0.18	0.14	−0.61	−0.34	−0.07
14	0.90	127	3	274	−0.06	−0.16	0.11	−0.35	−0.32	−0.06
15	2.59	131	2	354	−0.12	−0.29	0.15	0.45	−0.32	−0.12
16	3.05	133	2	398	−0.19	−0.25	−0.00	−0.37	−0.32	−0.18
17	0.957	140	5	294	−0.37	−1.11	−0.36	−1.28	−0.89	−0.66
18	1.41	142	5	339	−0.44	−1.03	−0.52	−1.21	−0.90	−0.69
19	2.84	90	3	323	−0.25	−0.83	−0.28	−1.05	−0.68	−0.42
20	2.27	95	3	351	−0.55	−1.21	−0.55	−1.36	−0.90	−0.62
21	2059	95	3	368	−0.46	−1.17	−0.45	−1.18	−0.74	−0.48
22	3.36	112	2	317	−0.45	−0.89	−0.58	−1.26	−0.78	−0.54
23	2.05	172	4	359	−0.47	−0.86	−0.53	−1.29	−0.55	−0.37
DOX[c]	−0.657	206	7	465	0.02	−0.16	−0.22	0.14	0.34	0.51

[a] TPSA: Total polar surface area.
[b] GPCRL: GPCR ligand; ICM: Ion channel modulator; KI: Kinase inhibitor; NRL: Nuclear receptor ligand; PI: Protease inhibitor; EI: Enzyme inhibitor.
[c] DOX: Doxorubicin.

by the methodology developed by Osiris. The toxicity risk predictor locates fragments within a molecule, which indicate a potential toxicity risk. Toxicity risk alerts are an indication that the drawn structure may be harmful concerning the risk category specified. From the data evaluated in Table 2 it is obvious that, 15 of 20 structures are supposed to be non-mutagenic, non-irritating with no reproductive effects when run through the mutagenicity assessment system in comparison with the standard drug. The log*P* value of a compound, which is the logarithm of its partition coefficient between *n*-octanol and water, is a well-established measure of the compound's hydrophilicity. Low hydrophilicities and therefore high log*P* values may cause poor absorption or permeation. It has been shown that for compounds to have a reasonable probability of good absorption, their log*P* value must not be greater than 5.0. On this basis, all the compounds **4**–**23** possessed log*P* values in the acceptable range. Along with this, compound **11**, which showed good antitumor screening results (IC_{50} = 0.008 mM against the three tested cell lines), is having the same log*P* value as compared to other compounds in the series.

3.3. The aqueous solubility

The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. Typically, a low solubility goes along with a bad absorption and therefore the general aim is to avoid poorly soluble compounds. Our estimated log*S* value is a unit stripped logarithm (base 10) of a compound's solubility measured in mol/liter. There are more than 80% of the drugs on the market have a (estimated) log*S* value greater than −4. In case of compounds **4**–**23**, values of log*S* are around −4. Further, Table 3 shows drug likeness of compounds **4**–**23** which is in the comparable zone with that of standard drug used for comparison. We have calculated overall drug score (DS) for the compounds **4**–**23** and compared with that of standard drug doxorubicin, Table 2. The drug score combines drug likeness, miLog*P*, log*S*, molecular weight and toxicity risks in one handy value than may be used to judge the compound's overall potential to qualify for a drug. This value is calculated by multiplying contributions of the individual properties with the equation (1):

$$DS = \prod(1/2 + 1/2Si)\prod ti \quad (1)$$

where; $S = 1/1 + e^{ap+b}$)

DS is the drug score. Si is the contributions calculated directly from miLog*P*; log*S*, molecular weight and drug likeness (pi) *via* the second equation, which describes a spline curve. Parameters *a* and *b* are (1, −5), (1, 5), (0.012, −6) and (1, 0) for miLog*P*, log*S*, molecular weight and drug likeness, respectively. The ti is the contributions taken from the four toxicity risk types and the values are 1.0, 0.8 and 0.6 for no risk, medium risk and high risk, respectively. The reported compounds **4**–**23** showed moderate to good drug score as compared with standard drug used.

4. Experimental protocols

4.1. Chemistry

Melting points (°C, uncorrected) were determined in open capillaries on a Gallenkamp melting point apparatus (Sanyo Gallenkamp, Southborough, UK) and were uncorrected. Precoated silica gel plates (silica gel 0.25 mm, 60G F254; Merck, Germany) were used for thin layer chromatography, dichloromethane/methanol (9.5:0.5) mixture was used as a developing solvent system and the spots were visualized by ultraviolet light and/or iodine. Infra red spectra were recorded in KBr discs using IR-470 Shimadzu spectrometer (Shimadzu, Tokyo, Japan). ^{1}H NMR spectra (DMSO-d_6) were recorded on Bruker AC-300 Ultra Shield NMR spectrometer (Bruker, Flawil, Switzerland, δ ppm) at 300 MHz for ^{1}H and 75 MHz for ^{13}C, using TMS as internal standard and peak multiplicities are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Electron Impact Mass Spectra were recorded on a Shimadzu GC-MS-QP 5000 instrument (Shimadzu, Tokyo, Japan). Elemental analyses were performed, on Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany), at the Microanalytical Unit, Faculty of Science, Cairo University, Cairo, Egypt, and the found results were within ±0.4% of the theoretical values.

4.1.1. 2-Cyano-N-(2,6-dimethyl-4-oxoquinazolin-3(4H)-yl) acetamide ***4***

This compound was prepared according to a reported procedure [8,9].

4: Yield: 88%, mp: 185–187 °C (toluene). IR (KBr, cm^{-1}): 3281 (NH), 3089 (CH arom.), 2989, 2886 (CH aliph.), 2225 (C≡N), 1689, 1678 (2C=O), 1624 (C=N). ^{1}H NMR: δ 1.30 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 3.31 (s, 2H, CH_2CN), 7.44 (d, J = 7.0 Hz, 1H, Ar–H), 7.64 (d, J = 7.0 Hz, 1H, Ar–H), 7.82 (s, 1H, Ar–H), 8.10 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR: δ 19.0 (CH_3), 24.2 (CH_3), 28.0 (CH_2), 115.1 (C≡N), 124.1, 126.4, 130.4, 134.2, 135.2, 144.8, 158.8 (Ar–C), 164.6, 168.2 (2C=O). MS *m*/*z* (Rel. Int.): 257 (M^+ + 1, 56), 105 (100). Analysis for $C_{13}H_{12}N_4O_2$.

4.1.2. 2-Cyano-N-(2,6-dimethyl-4-oxoquinazolin-3(4H)-yl)-3-(4-substituted phenyl) acrylamide ***5***–***11*** and N-(2,6-dimethyl-4-oxoquinazolin-3(4H)-yl)-2-imino-2H-chromene-3-carboxamide ***12***

General procedure: equimolecular mixture of **4** (2.56 g, 0.01 mol) and the selected aldehyde (0.01 mol), in 1,4-dioxane (20 mL) containing piperidine (0.5 mL) was heated under reflux for 3 h. The reaction mixture was left to cool then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

5: Yield: 82%, mp: 198–200 °C (dioxane). IR (KBr, cm^{-1}): 3283 (NH), 3092 (CH arom.), 2985, 2880 (CH aliph.), 2223 (C≡N), 1688, 1678 (2C=O), 1629 (C=N). ^{1}H NMR: δ 1.60 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 7.34 (bs, 1H, NH, D_2O exchangeable), 7.28 (d, J = 6.5 Hz, 2H, Ar–H), 7.32 (t, J = 6.0 Hz, 1H, Ar–H), 7.39 (d, J = 6.2 Hz, 2H, Ar–H), 7.44 (d, J = 6.0 Hz, 1H, Ar–H), 7.68 (d, J = 6.1 Hz, 1H, Ar–H), 7.78 (s, 1H, Ar–H), 8.1 (s, 1H, vinylic–H). ^{13}C NMR: δ 17.1 (CH_3), 22.2 (CH_3), 107.1 (arylidene carbon), 116.0 (C≡N), 123.8, 126.4, 126.9, 127.5, 128.0, 130.3, 134.2, 135.0, 135.3, 135.7, 144.8, 153.6, 158.8 (Ar–C), 164.5, 168.2 (2C=O). MS *m*/*z* (Rel. Int.): 345 (M^+ + 1, 34), 105 (100). Analysis for $C_{20}H_{16}N_4O_2$.

6: Yield: 88%, mp: 192–194 °C (acetic acid). IR (KBr, cm^{-1}): 3274 (NH), 3091 (CH arom.), 2984, 2880 (CH aliph.), 2222 (C≡N), 1685, 1677 (2C=O), 1622 (C=N). ^{1}H NMR: δ 1.58 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 7.33 (bs, 1H, NH, D_2O exchangeable), 7.40 (d, J = 7.1 Hz, 2H, Ar–H), 7.43 (d, J = 7.0 Hz, 2H, Ar–H), 7.47 (d, J = 7.3 Hz, 1H, Ar–H), 7.65 (d, J = 7.5 Hz, 1H, Ar–H), 7.82 (s, 1H, Ar–H), 8.0 (s, 1H, vinylic–H). ^{13}C NMR: δ 17.2 (CH_3), 22.2 ($2CH_3$), 107.2 (arylidene carbon), 116.2 (C≡N), 123.6, 126.3, 126.9, 127.5, 128.0, 130.3, 134.2, 135.0, 135.3, 135.7, 144.8, 153.6, 158.8 (Ar–C), 164.5, 168.2 (2C=O). MS *m*/*z* (Rel. Int.): 359 (M^+ + 1, 52). Analysis for $C_{21}H_{18}N_4O_2$.

7: Yield: 83%, mp: 189–191 °C (acetic acid). IR (KBr, cm^{-1}): 3277 (NH), 3092 (CH arom.), 2986, 2880 (CH aliph.), 2221 (C≡N), 1689, 1671 (2C=O), 1620 (C=N). ^{1}H NMR: δ 1.60 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 7.39 (d, J = 7.1 Hz, 2H, Ar–H), 7.41 (d, J = 7.0 Hz, 2H, Ar–H), 7.45 (d, J = 7.3 Hz, 1H, Ar–H), 7.66 (d, J = 7.5 Hz, 1H, Ar–H), 7.82 (s, 1H, Ar–H), 8.01 (bs, 1H, NH, D_2O exchangeable), 8.2 (s, 1H, vinylic–H). ^{13}C NMR: δ 18.3 (CH_3), 23.6 (CH_3), 106.7 (arylidene carbon), 115.0 (C≡N), 123.4, 126.0, 126.5, 127.8, 128.6, 130.2, 133.4,

135.3, 135.6, 147.8, 153.6, 158.8 (Ar–C), 164.3, 168.1 (2C=O). MS *m/z* (Rel. Int.): 380 (M^+ + 2, 24), 378 (M^+, 75). Analysis for $C_{20}H_{15}ClN_4O_2$.

8: Yield: 75%, mp: 194–196 °C (acetic acid). IR (KBr, cm^{-1}): 3275 (NH), 3091 (CH arom.), 2988, 2881 (CH aliph.), 2220 (C≡N), 1687, 1670 (2C=O), 1623 (C=N). ^{1}H NMR: δ 1.58 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 7.33 (bs, 1H, NH, D_2O exchangeable), 7.44 (d, J = 7.3 Hz, 1H, Ar–H), 7.57 (d, J = 7.4 Hz, 2H, Ar–H), 7.62 (d, J = 7.0 Hz, 1H, Ar–H), 7.83 (s, 1H, Ar–H), 8.02 (d, J = 6.0 Hz, 2H, Ar–H), 8.13 (s, 1H, vinylic–H). ^{13}C NMR: δ 17.8 (CH_3), 24.0 (CH_3), 106.6 (arylidene carbon), 114.8 (C≡N), 123.4, 126.0, 126.5, 127.9, 128.2, 130.2, 134.0, 135.1, 135.3, 135.6, 147.8, 153.6, 158.8 (Ar–C), 164.3, 168.1 (2C=O). MS *m/z* (Rel. Int.): 390 (M^+ + 1, 19). Analysis for $C_{20}H_{15}N_5O_4$.

9: Yield: 75%, mp: 208–210 °C (acetic acid). IR (KBr, cm^{-1}): 3376 (OH), 3282 (NH), 3084 (CH arom.), 2980, 2884 (CH aliph.), 2225 (C≡N), 1681, 1672 (2C=O), 1627 (C=N). ^{1}H NMR: δ 1.58 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 6.86 (s, 1H, Ar–H), 6.90 (d, J = 6.4 Hz, 1H, Ar–H), 6.99 (d, J = 6.5 Hz, 1H, Ar–H), 7.03 (t, J = 6.6 Hz, 1H, Ar–H), 7.41 (d, J = 7.0 Hz, 2H, Ar–H), 7.90 (s, 1H, Ar–H), 8.03 (s, 1H, vinylic–H), 8.11 (bs, 1H, NH, D_2O exchangeable), 10.50 (bs, 1H, OH, D_2O exchangeable). ^{13}C NMR: δ 18.9 (CH_3), 24.0 (CH_3), 107.7 (arylidene carbon), 116.2 (C≡N), 123.8, 126.4, 126.8, 127.2, 128.2, 131.6, 134.7, 135.3, 136.8, 145.8, 153.7, 158.5 (Ar–C), 164.3, 168.0 (2C=O). MS *m/z* (Rel. Int.): 360 (M^+, 64). Analysis for $C_{20}H_{16}N_4O_3$.

10: Yield: 75%, mp: 214–216 °C (ethanol). IR (KBr, cm^{-1}): 3370 (OH), 3278 (NH), 3095 (CH arom.), 2981, 2881 (CH aliph.), 2220 (C≡N), 1687, 1672 (2C=O), 1621 (C=N). ^{1}H NMR: δ 1.59 (s, 3H, CH_3), 2.35 (s, 3H, CH_3)), 6.70 (d, J = 6.5 Hz, 2H, Ar–H), 7.0 (d, J = 6.5 Hz, 2H, Ar–H), 7.34 (d, J = 7.0 Hz, 2H, Ar–H), 7.95 (s, 1H, Ar–H), 8.12 (s, 1H, vinylic–H), 8.40 (bs, 1H, NH, D_2O exchangeable), 10.26 (bs, 1H, OH, D_2O exchangeable). ^{13}C NMR: δ 19.1 (CH_3), 24.5 (CH_3), 108.3 (arylidene carbon), 116.5 (C≡N), 123.2, 126.4, 126.1, 127.4, 128.2, 130.5, 134.0, 135.2, 135.3, 136.1, 144.3, 153.7, 159.0 (Ar–C), 164.0, 166.2 (2C=O). MS *m/z* (Rel. Int.): 361 (M^+ + 1, 16). Analysis for $C_{20}H_{16}N_4O_3$.

11: Yield: 89%, mp: 251–253 °C (ethanol). IR (KBr, cm^{-1}): 3382, 3376 (2OH), 3276 (NH), 3095 (CH arom.), 2980, 2886 (CH aliph.), 2228 (C≡N), 1683, 1669 (2C=O), 1625 (C=N). ^{1}H NMR: δ 1.54 (s, 3H, CH_3), 2.35 (s, 3H, CH_3)), 6.79 (d, J = 7.40 Hz, 1H, Ar–H), 7.24 (s, 1H, Ar–H), 7.35 (d, J = 7.0 Hz, 1H, Ar–H), 7.45 (d, J = 7.0 Hz, 1H, Ar–H), 7.66 (d, J = 7.0 Hz, 1H, Ar–H), 7.94 (s, 1H, Ar–H), 8.15 (s, 1H, vinylic–H), 8.47 (bs, 1H, NH, D_2O exchangeable), 10.27 (bs, 2H, 2OH, D_2O exchangeable). ^{13}C NMR: δ 19.6 (CH_3), 22.9 (CH_3), 107.8 (arylidene carbon), 115.0, 116.2 (C≡N), 117.5, 121.0, 123.5, 125.5, 127.1, 129.6, 134.3, 136.9, 144.5, 145.4, 146.9, 151.4, 156.3 (Ar–C), 161.0, 164.7 (2C=O). MS *m/z* (Rel. Int.): 377 (M^+ + 1, 18). Analysis for $C_{20}H_{16}N_4O_4$.

12: Yield: 72%, mp: 229–231 °C (ethanol). IR (KBr, cm^{-1}): 3272 (NH), 3255 (NH), 3095 (CH arom.), 2886, 2871 (CH aliph.), 1671, 1668 (2C=O), 1622 (C=N). ^{1}H NMR: δ 1.60 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 5.70 (bs, 1H, =NH, D_2O exchangeable), 6.89 (t, J = 6.2 Hz, 1H, Ar–H), 7.04 (t, J = 6.3 Hz, 1H, Ar–H), 7.18 (d, J = 6.5 Hz, 1H, Ar–H), 7.22 (d, J = 6.4 Hz, 1H, Ar–H), 7.35 (d, J = 6.5 Hz, 1H, Ar–H), 7.59 (d, J = 6.5 Hz, 1H, Ar–H), 7.70 (s, 1H, Ar–H), 8.32 (s, 1H, Ar–H), 8.90 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR: δ 19.9 (CH_3), 23.2 (CH_3), 115.9, 117.0, 122.0, 123.4, 126.0, 126.5, 127.8, 128.6, 130.2, 131.8, 133.4, 135.3, 135.6, 147.8, 153.6 (Ar–C), 158.1, 162.5, 165.8 (2C=O and C=N). MS *m/z* (Rel. Int.): 361 (M^+ + 1, 14). Analysis for $C_{20}H_{16}N_4O_3$.

*4.1.3. 4,6-Diamino-1,2-dihydro-1-(2,6-dimethyl-4-oxoquinazolin-3(4H)-yl)-3-cyano-2-oxopyridine **13** and 4-amino-1,2-dihydro-6-hydroxy-1-(2,6-dimethyl-4-oxoquinazolin-3(4H)-yl)-3-cyano-2-oxopyridine **14***

Equimolecular amounts of compound **4** (2.56 g, 0.01 mol) and either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) in 1,4-dioxane (30 mL) containing triethylamine (1.5 mL) was heated under reflux for 5 h. The reaction mixture was left to cool and evaporated under vacuum. The remaining product was triturated with ethanol and the formed solid product was collected by filtration.

13: Yield: 67%, mp: 203–205 °C (ethanol). IR (KBr, cm^{-1}): 3381, 3355, 3326 ($2NH_2$), 3065 (CH arom.), 2884 (CH aliph.), 2225 (C≡N), 1677, 1661 (2C=O), 1620 (C=N). ^{1}H NMR: δ 1.46 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 4.37 (s, 1H, pyridine–H), 7.27 (d, J = 6.5 Hz, 1H, Ar–H), 7.30 (d, J = 6.5 Hz, 1H, Ar–H), 7.81 (s, 1H, Ar–H), 8.45 (bs, 4H, $2NH_2$, D_2O exchangeable). ^{13}C NMR: δ 19.5 (CH_3), 23.7 (CH_3), 70.0 (*C*–CN), 79.3 (pyridine–CH), 115.7 (C≡N), 121.0, 123.5, 128.4, 133.9, 136.4, 145.3 (Ar–C), 162.7, 166.5 (2C=O), 168.0, 175.7 (2C–NH_2). MS *m/z* (Rel. Int.): 323 (M^+ + 1, 13), 322 (M^+, 25). Analysis for $C_{16}H_{14}N_6O_2$.

14: Yield: 65%, mp: 179–181 °C (ethanol). IR (KBr, cm^{-1}): 3416 (OH), 3307, 3304 (NH_2), 3089 (CH arom.), 2884, 2867 (CH aliph.), 1677, 1661 (2C=O), 2220 (C≡N), 1620 (C=N). ^{1}H NMR: δ 1.50 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 5.02 (bs, 2H, NH_2, D_2O exchangeable), 6.37 (s, 1H, pyridine–H), 7.25 (d, J = 6.5 Hz, 1H, Ar–H), 7.31 (d, J = 6.5 Hz, 1H, Ar–H), 7.80 (s, 1H, Ar–H), 12.80 (bs, 1H, OH, D_2O exchangeable). ^{13}C NMR: δ 19.8 (CH_3), 23.6 (CH_3), 71.8 (*C*–CN), 74.5 (pyrimidine–CH), 115.8 (C≡N), 121.2, 123.7, 128.5, 133.4, 136.9, 145.0 (Ar–C), 162.5, 165.8 (2C=O), 171.0 (C–OH), 175.7 (C–NH_2). MS *m/z* (Rel. Int.): 324 (M^+ + 1, 12), 323 (M^+, 29). Analysis for $C_{16}H_{13}N_5O_3$.

*4.1.4. 6-Amino-1-(2,6-dimethyl-4-oxoquinazolin-3(4H)-yl)-2-oxo-4-phenyl-1,2-dihydro pyridine-3,5-dicarbonitrile **15**, ethyl 2-amino-5-cyano-1-(2,6-dimethyl-4-oxoquinazolin-3(4H)-yl)-6-oxo-4-phenyl-1,6-dihydropyridine-3-carboxylate **16***

To a solution of compound **5** (3.44 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (1 mL) the appropriate active methylene derivative (0.01 mol) was added. The reaction mixture was heated under reflux for 6 h then poured onto ice/water and the separated solid was filtered, washed with water, dried and crystallized.

15: Yield: 67%, mp: 196–198 °C (ethanol). IR (KBr, cm^{-1}): 3352, 3329 (NH_2), 3091 (CH arom.), 2888, 2865 (CH aliph.), 2228, 2225 (2C≡N), 1676, 1665 (2C=O), 1621 (C=N). ^{1}H NMR: δ 1.50 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 7.0 (d, J = 7.0 Hz, 2H, Ar–H), 7.14 (t, J = 7.0 Hz, 1H, Ar–H), 7.27 (d, J = 6.5 Hz, 1H, Ar–H), 7.40 (t, J = 7.0 Hz, 2H, Ar–H), 7.52 (d, J = 6.5 Hz, 1H, Ar–H), 7.81 (s, 1H, Ar–H), 8.61 (bs, 2H, NH_2, D_2O exchangeable). ^{13}C NMR: δ 20.0 (CH_3), 23.9 (CH_3), 72.0 (*C*–C≡N), 115.0 (*C*–C≡N), 115.3, 115.9 (2C≡N), 120.0, 121.3, 126.4, 128.3, 128.4, 129.3, 132.9, 133.6, 137.2, 144.5, 157.0, 158.7, 159.1 (Ar–C), 162.5, 165.8 (2C=O). MS *m/z* (Rel. Int.): 409 (M^+ + 1, 18), 408 (M^+, 65). Analysis for $C_{23}H_{16}N_6O_2$.

16: Yield: 70%, mp: 170–172 °C (ethanol). IR (KBr, cm^{-1}): 3341 (NH_2), 3090 (CH arom.), 2889, 2863 (CH aliph.), 2226 (C≡N), 1729, 1676, 1665 (3C=O), 1621 (C=N). ^{1}H NMR: δ 1.0 (s, 3H, CH_3), 2.13 (t, 3H, CH_3), 2.36 (s, 3H, CH_3), 4.63 (q, 2H, CH_2), 6.94 (d, J=6.6 Hz, 2H, Ar–H), 7.12 (t, 1H, J = 7.1 Hz, Ar–H), 7.28 (d, J = 6.5 Hz, 1H, Ar–H), 7.41 (t, J=6.5 Hz, 2H, Ar–H), 7.49 (d, J = 6.5 Hz, 1H, Ar–H), 7.81 (s, 1H, Ar–H), 8.45 (bs, 2H, NH_2, D_2O exchangeable). ^{13}C NMR: δ 15.2 (CH_3), 19.8 (CH_3), 24.0 (CH_3), 87.0 (*C*–CO_2Et), 110.8 (*C*–C≡N), 116.5 (C≡N), 120.3, 122.1, 126.7, 128.4, 128.9, 129.5, 132.2, 133.5, 138.0, 144.7, 156.8, 158.6, 159.4 (Ar–C), 161.7, 163.5, 168.0 (3C=O). MS *m/z* (Rel. Int.): 456 (M^+ + 1, 18), 455 (M^+, 65). Analysis for $C_{25}H_{21}N_5O_4$.

*4.1.5. 3,5-Diamino-4-cyano-N-(2,6-dimethyl-4-oxoquinazolin-3(4H)-yl) thiophene-2-carboxamide **17** and ethyl 5-(2,6-dimethyl-4-oxoquinazolin-3(4H)-ylcarbamoyl)2,4diaminothiophene-3-carboxylate **18***

4.1.5.1. General procedure. To a solution of compound **4** (2.56 g, 0.01 mol) in absolute ethanol (50 mL) containing triethylamine (1.0 mL) either malononitrile (0.66 g, 0.01 mol), ethyl cyanoacetate

(1.13 g, 0.01 mol) or cyclohexanone (0.98 g, 0.01 mol) together with elemental sulfur (0.32 g, 0.01 mol) were added. The whole reaction mixture was heated under reflux for 1 h then poured onto ice/water mixture and the formed solid product, in each case, was collected by filtration.

17: Yield: 76%, mp: 221–223 °C (ethanol). IR (KBr, cm^{-1}): 3374, 3298, 3275 ($2NH_2$), 3091 (CH arom.), 2888, 2865 (CH aliph.), 2226 (C≡N), 1676, 1665 (2C═O), 1621 (C═N). ^1H NMR: δ 2.16 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 6.48, 6.69 (2bs, 4H, $2NH_2$, D_2O exchangeable), 7.24 (d, J = 7.0 Hz, 1H, Ar–H), 7.27 (d, J = 7.0 Hz, 1H, Ar–H), 7.80 (s, 1H, Ar–H), 8.10 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR: δ 20.0 (CH_3), 23.8 (CH_3), 82.5 (*C*–C≡N), 116.3 (C≡N), 121.0, 122.8, 127.9, 133.7, 136.5, 137.3, 144.9 (Ar–C), 155.0, 158.6 (2*C*–NH_2), 161.7, 162.5 (2C═O). MS *m*/*z* (Rel. Int.): 355 (M^+ + 1, 20), 354 (M^+, 47). Analysis for $C_{16}H_{14}N_6O_2S$.

18: Yield: 76%, mp: 200–2002 °C (ethanol). IR (KBr, cm^{-1}): 3365, 3307, 3285 ($2NH_2$, NH), 3089 (CH arom.), 2984, 2867 (CH aliph.), 1721, 1672, 1666 (3C═O), 1620 (C═N). ^1H NMR: δ 1.03 (t, J = 7.36 Hz, 3H, CH_3), 2.25 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 4.25 (q, J = 7.60 Hz, 2H, CH_2), 5.74, 6.80 (2bs, 4H, $2NH_2$, D_2O exchangeable), 7.25 (d, J = 7.0 Hz, 1H, Ar–H), 7.32 (d, J = 7.0 Hz, 1H, Ar–H), 7.82 (s, 1H, Ar–H), 11.13 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR: δ 15.20 (CH_3), 19.9 (CH_3), 24.2 (CH_3), 60.7 (CH_2), 121.2, 122.7, 127.9, 130.6, 131.5, 135.0, 137.3, 138.0, 144.5, 158.6 (Ar–C), 161.2, 163.0, 167.20 (3C═O). MS *m*/*z* (Rel. Int.): 402 (M^+ + 1, 12), 401 (M^+, 24). Analysis for $C_{18}H_{19}N_5O_4S$.

4.1.6. 3-Amino-4,5,6,7-tetrahydro-N-(2,6-dimethyl-4-oxoquinazolin-3(4H)-yl)benzo[b]thiophene-2-carboxamide ***19****, 4-amino-2,3-dihydro-N-(2,6-dimethyl-4-oxoquinazolin-3(4H)-yl)-3-phenyl-2-thioxothiazole-5-carboxamide* ***20*** *and 4-amino-N-(2,6-dimethyl-4-oxoquinazolin-3(4H)-yl)-3-benzyl-2-thioxo-2,3-dihydrothiazole-5-carboxamide* ***21***

4.1.6.1. General procedure. To a solution of compound **4** (2.56 g, 0.01 mol) in absolute ethanol (50 mL) containing triethylamine (1.0 mL) cyclohexanone (0.98 g, 0.01 mol) and/or the isothiocyanate derivative together with elemental sulfur (0.32 g, 0.01 mol) were added. The whole reaction mixture was heated under reflux for 3 h then poured onto ice/water mixture and the formed solid product, in each case, was collected by filtration and crystallized from ethanol.

19: Yield: 66%, mp: 192–194 °C (ethanol). IR (KBr, cm^{-1}): 3377, 3292, 3245 (NH_2, NH), 3095 (CH arom.), 2962, 2880 (CH aliph.), 1686, 1675 (2C═O), 1638 (C═N). ^1H NMR: δ 1.45 (s, 3H, CH_3), 1.70–1.80 (t, J = 7.40, 4H, $2CH_2$), 2.24–2.25 (m, 4H, $2CH_2$), 2.30 (s, 3H, CH_3), 6.49 (bs, 2H, NH_2, D_2O exchangeable), 7.23 (d, J = 7.0 Hz, 1H, Ar–H), 7.27 (d, J = 7.0 Hz, 1H, Ar–H), 7.80 (s, 1H, Ar–H), 11.80 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR: δ 19.6 (CH_3), 23.2 (CH_3), 17.3, 21.0, 23.5, 25.2 ($4CH_2$), 120.7, 122.9, 126.8, 127.9, 133.5, 136.6, 137.4, 139.0, 144.1, 147.3, 159.0 (Ar–C), 161.7, 162.5 (2C═O). MS *m*/*z* (Rel. Int.): 369 (M^+ + 1, 14), 368 (M^+, 33). Analysis for $C_{19}H_{20}N_4O_2S$.

20: Yield: 73%, mp: 211–213 °C (ethanol). IR (KBr, cm^{-1}): 3325, 3289, 3252 (NH_2, NH), 3089 (CH arom.), 2888, 2865 (CH aliph.), 1676, 1661 (2C═O), 1640 (C═N), 1269 (C═S). ^1H NMR: δ 2.15 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 6.72 (bs, 2H, NH_2, D_2O exchangeable), 7.08 (d, J = 7.0 Hz, 2H, Ar–H), 7.22 (t, J = 7.0 Hz, 1H, Ar–H), 7.28 (d, J = 6.5 Hz, 1H, Ar–H), 7.35 (t, J = 7.0 Hz, 2H, Ar–H), 7.56 (d, J = 6.5 Hz, 1H, Ar–H), 7.80 (s, 1H, Ar–H), 9.55 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR: δ 20.0 (CH_3), 24.6 (CH_3), 78.0, 120.3, 127.7, 129.3, 132.1, 133.9, 136.7, 140.1, 143.5, 154.8, 158.1 (Ar–C), 161.7, 166.8 (2C═O), 177.2 (C═S). MS *m*/*z* (Rel. Int.): 424 (M^+ + 1, 11), 423 (M^+, 42). Analysis for $C_{20}H_{17}N_5O_2S_2$.

21: Yield: 81%, mp: 211–213 °C (ethanol). IR (KBr, cm^{-1}): 3330, 3282, 3257 (NH_2, NH), 3089 (CH arom.), 2890, 2868 (CH aliph.), 1669, 1663 (2C═O), 1640 (C═N), 1265 (C═S). ^1H NMR: δ 2.15 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 4.59 (s, 2H, CH_2), 6.80 (bs, 2H, NH_2, D_2O exchangeable), 7.20 (d, J = 7.0 Hz, 2H, Ar–H), 7.24 (t, J = 7.0 Hz, 1H, Ar–H), 7.27 (d, J = 6.5 Hz, 1H, Ar–H), 7.41 (t, J=7.0 Hz, 2H, Ar–H), 7.55 (d, J = 6.5 Hz, 1H, Ar–H), 7.81 (s, 1H, Ar–H), 9.15 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR: δ 20.1 (CH_3), 24.5 (CH_3), 50.5 (CH_2), 77.5, 120.7, 127.5, 129.2, 132.7, 133.5, 135.0, 138.1, 143.6, 154.0, 156.9 (Ar–C), 160.4, 162.9 (2C═O), 175.0 (C═S). MS *m*/*z* (Rel. Int.): 438 (M^+ + 1, 3). Analysis for $C_{21}H_{19}N_5O_2S_2$.

4.1.7. 2-(2-Phenylhydrazono)-2-cyano-N-(2,6-dimethyl-4-oxoquinazolin-3(4H)-yl)acetamide ***22*** *and 2-(2-(4-aminosulphonylphenyl)hydrazono)-2-cyano-N-(2,6-dimethyl-4-oxoquinazolin-3(4H)-yl)acetamide* ***23***

4.1.7.1. General procedure. To a solution of compound **4** (2.56 g, 0.01 mol) in ethanol (40 mL) containing sodium acetate (10.0 g), benzene diazonium chloride (1.40 g, 0.01 mol) and/or 4-sulphonylamino–benzene–diazonium– chloride (2.19 g, 0.01 mol) with continuous stirring at 0–5 °C for 1 h. The solid product, formed in each case, was collected by filtration and crystallized from ethanol.

22: Yield: 73%, mp: 207–209 °C (ethanol). IR (KBr, cm^{-1}): 3317, 3283 (2NH), 3087 (CH arom.), 2878, 2869 (CH aliph.), 2226 (C≡N) 1677, 1661 (2C═O), 1622 (C═N), 1375, 1190 (SO_2). ^1H NMR: δ 2.17 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 6.25 (s, 1H, NH, D_2O exchangeable), 6.88 (t, J = 6.5 Hz, 1H, Ar–H), 7.10 (d, J = 7.0 Hz, 2H, Ar–H), 7.24 (d, J = 7.0 Hz, 2H, Ar–H), 7.27 (d, J = 7.0 Hz, 1H, Ar–H), 7.32 (d, J = 7.0 Hz, 1H, Ar–H), 7.82 (s, 1H, Ar–H), 11.20 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR: δ 19.2 (CH_3), 24.5 (CH_3), 107.0 (*C*–CN), 115.6 (C≡N), 117.0, 119.5, 121.0, 122.4, 128.9, 129.6, 133.0, 138.0, 144.9, 148.3, 154.0 (Ar–C), 161.2, 163.0 (2C═O). MS *m*/*z* (Rel. Int.): 361 (M^+ + 1, 3), 360 (M^+, 7). Analysis for $C_{19}H_{16}N_6O_2$.

23: Yield: 58%, mp: 256–258 °C (ethanol). IR (KBr, cm^{-1}): 3332, 3275, 3251 (NH_2, 2NH), 3087 (CH arom.), 2880, 2864 (CH aliph.), 2227 (C≡N) 1674, 1667 (2C═O), 1630 (C═N), 1371, 1192 (SO_2). ^1H NMR: δ 2.13 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 6.97 (d, J = 7.0 Hz, 2H, Ar–H), 7.37 (d, J = 7.0 Hz, 1H, Ar–H), 7.42 (d, J = 7.0 Hz, 1H, Ar–H), 7.64 (d, J = 7.0 Hz, 2H, Ar–H), 7.76 (s, 1H, Ar–H), 7.85 (s, 2H, NH_2, D_2O exchangeable), 8.20 (s, 1H, NH, D_2O exchangeable), 11.70 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR: δ 20.0 (CH_3), 24.7 (CH_3), 107.1 (*C*–C≡N), 115.0 (C≡N), 117.0, 121.0, 122.4, 128.9, 129.5, 130.0, 133.4, 137.5, 144.6, 148.5, 154.2 (Ar–C), 161.5, 163.0 (2C═O). MS *m*/*z* (Rel. Int.): 440 (M^+ + 1, 5), 439 (M^+, 9). Analysis for $C_{19}H_{17}N_7O_4S$.

4.2. In vitro anticancer screening

The stock solutions of the tested compounds were prepared in DMSO and were used for serial dilutions in culture medium. Human breast cancer (MCF-7), cervical cancer (HeLa) and hepatocellular liver carcinoma (HepG2) cell lines were grown in RPMI-1640 medium supplemented with 10% calf serum. For growth assays, exponentially growing cells were suspended in the above-mentioned medium at a density of 4×10^4 cells per mL, seeded onto 96-well plates well (200 μL/well), and incubated at 37 °C in a humidified 5% CO_2 atmosphere for 24 h. After that, the cell medium in test wells was changed to new culture medium containing different concentrations of the tested compounds, while the cell medium in control wells was changed to new culture medium containing an equivalent volume of solvent. After incubation at 37 °C in a humidified 5% CO_2 atmosphere for 3 d, 100 μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 0.5 mg/mL) in serum-free medium was added to each well and incubated at 37 °C for an additional 4 h. Then, 100 μL of DMSO was added to each well and mixed thoroughly to dissolve the resulting formazan product. The cell viability was evaluated by measurement

of the optical densities at 540 nm using a Microelisa Reader. The percentage of cell growth inhibition was calculated as follows:

$$\%\text{Inhibition} = (\text{Mean}_{\text{OD control}} - \text{Mean}_{\text{OD test}})/\text{Mean}_{\text{OD control}} \times 100\%$$

The dose response curves of the compounds effecting ≥70% inhibition in one-dose prescreening for each cell line were measured with the concentrations of 0.06–0.002 mM, and the concentration causing 50% cell growth inhibition (IC_{50}) was calculated. The results are given in Table 1.

5. Conclusion

The present work, through simple synthetic approaches, led to the development of novel hybrids of quinazoline containing tyrphostin pharmacophore that exhibited remarkable antiproliferative activities against three tumor cell lines. The compounds showed suitable drug like properties and are expected to present good bioavailability profile. As new class of tyrphostin analogs, 10 of the obtained new compounds showed remarkable antiproliferative activity against three tumor cell lines. Most of the active compounds were devoid of the typical catechol feature of tyrphostin and so the activity could be attributed to some sort of synergism between quinazoline and tyrphostin coupled molecule.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2012.03.044. These data include MOL files and InChIKeys of the most important compounds described in this article.

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