European Journal of Medicinal Chemistry 79 (2014) 446–454

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Design, synthesis and biological evaluation of some novel substituted quinazolines as antitumor agents

Amer M. Alanazi [a], Alaa A.-M. Abdel-Aziz [a,b], Ibrahim A. Al-Suwaidan [a], Sami G. Abdel-Hamide [c], Taghreed Z. Shawer [d], Adel S. El-Azab [a,e,*]

[a] Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia
[b] Department of Medicinal Chemistry, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt
[c] Department of Pharmaceutical Chemistry, College of Pharmacy, Salman Bin Abdulaziz University, Alkharj, Saudi Arabia
[d] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Al-Azhar University, Cairo 11884, Egypt
[e] Department of Organic Chemistry, Faculty of Pharmacy, Al-Azhar University, Cairo 11884, Egypt

ARTICLE INFO

Article history:
Received 3 February 2014
Received in revised form
6 April 2014
Accepted 8 April 2014
Available online 12 April 2014

Keywords:
Synthesis
Quinazoline
In-vitro antitumor evaluation
Selective activity
NCI

ABSTRACT

A novel series of 6-chloro-2-*p*-tolylquinazolinone and substituted-(4-methylbenzamido)benzamide (**1**–**20**) were designed, synthesized and evaluated for their *in-vitro* antitumor activity. Compounds **3, 14** and **16** possessed remarkable broad-spectrum antitumor activity. Compound **16** was found to be a particularly active growth inhibitor of the renal cancer (GI_{50} = 4.07 μM), CNS cancer (GI_{50} = 7.41 μM), ovarian cancer (GI_{50} = 7.41 μM) and non-small cell lung cancer (GI_{50} = 7.94 μM). Compound **16** ranks as nearly 1.5-fold more potent (mean GI_{50} = 15.8 μM) compared to 5-FU (mean GI_{50} = 22.6 μM).

1. Introduction

Cancer is a disease characterized by a shift in the controlled mechanisms that govern cell proliferation and differentiation [1]. Malignancy is caused by abnormalities in cells, which might be due to inherited genes or caused by outside exposure of the body to chemicals, radiation, or even infectious agents [2,3]. The development of new anti-cancer therapeutic tools has advanced greatly in the past decade; thus approaches for the treatment of cancer have moved towards targeting the specific molecular alterations that occur in tumor cells. This approach has been concentrated on the development of ideal anticancer drugs that eradicate cancer cells without harming normal tissues [4,5]. Unfortunately, no currently available agents meet this criterion and clinical use of drugs involves a weighing of benefits against toxicity in a search of favorable therapeutic index [6].

Many of chemotherapeutics currently used in cancer therapy are agents which inhibit tumor growth by inhibiting the replication and transcription of DNA. The practice of chemotherapy of cancer suffers from various drawbacks viz. the participation of a number of enzymes like ribonucleotides reductase (RNR), topoisomerase I (Topo I) and topoisomerase II (Topo II) at different stages of development of cancer [7], survival of cancer cells even under anaerobic conditions [8], and ultimately the problem of multidrug resistance [9–11] developed in the cancerous cells towards chemotherapeutic agents.

As an important pharmacophore, quinazoline has a variety of biological activities [12–29].

FDA has approved several quinazoline derivatives as anticancer drugs, such as Gefitinib, Erlotinib, Lapatinib and Vandetanib. Based on the good performances of quinazoline derivatives in anticancer application, development of novel quinazoline derivatives as anticancer drugs is a promising field.

In the present study, we have designed a number of new quinazoline and diamide derivatives (**1**–**20**) containing various substituent with different electronic environment which would affect the lipophilicity, and hence the activity of the target molecules and biologically evaluated there *in-vitro* antitumor activities. The

* Corresponding author. (Adel S. El-Azab), Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia.
E-mail addresses: adelazaba@yahoo.com, adelazaba@hotmail.com (A.S. El-Azab).

http://dx.doi.org/10.1016/j.ejmech.2014.04.029
0223-5234/

objective of forming these hybrids is an attempt to attain an active antitumor agent with potentiated activity and selectivity toward cancerous cells.

2. Results and discussion

2.1. Chemistry

6-Chloro-2-*p*-tolyl-4*H*-benzo[d][1,3]oxazin-4-one (**1**) was prepared by the reaction of 5-chloroanthranilic acid with 4-methylbenzoyl chloride in pyridine followed by boiling with acetic anhydride [27]. Treatment of **1** with formamide furnished 6-chloro-2-*p*-tolylquinazolin-4(3*H*)-one (**2**) in 73% yields. Boiling of compound **1** with various amines in anhydrous pyridine afforded 5-chloro-2-(4-methylbenzamido)-*N*-substituted benzamide (**3**–**5**) in 90–94% yields.

Additionally, reaction of 6-chloro-2-ptolyl-4*H*-benzo[d][1,3] oxazin-4-one (**1**) with ethyl 2-aminoacetate hydrochloride in boiling pyridine gave a mixture of ethyl 2-(5-chloro-2-(4-methylbenzamido)benzamido)acetate (**6**) and ethyl 2-(6-chloro-4-oxo-2-*p*-tolylquinazolin-3(4*H*)-yl)acetate (**7**) in 34% and 60% yields, respectively.

Moreover, 2-(6-chloro-4-oxo-2-*p*-tolylquinazolin-3(4*H*)-yl)acetohydrazide (**8**) and 5-chloro-*N*-(2-hydrazinyl-2-oxoethyl)-2-(4-methylbenzamido)benzamide (**9**) were obtained in a good yield (80% and 86%, respectively) via the reaction of compound **6** or **7** with hydrazine hydrate in boiling ethanol (Scheme 1).

IR spectra of compounds **3**–**5** showed absorption bands at 3173–3182 cm^{-1} and 1668–1670 cm^{-1} due to stretching vibration of the (CONH) group, moreover, ^{1}H NMR spectra showed the appearance of two (CONH) as a singlet signal at δ (12.46, 9.57 ppm), (11.57, 10.64 ppm) and (11.39, 11.14 ppm) respectively. The presence of a band at 1720–1716 cm^{-1} due to the (CO) group of the ester moiety as well as ^{1}H NMR spectra showed the appearance of two signals at δ 4.14–4.11 and 1.20–1.13 ppm of the ester function confirmed compounds **6**–**7**.

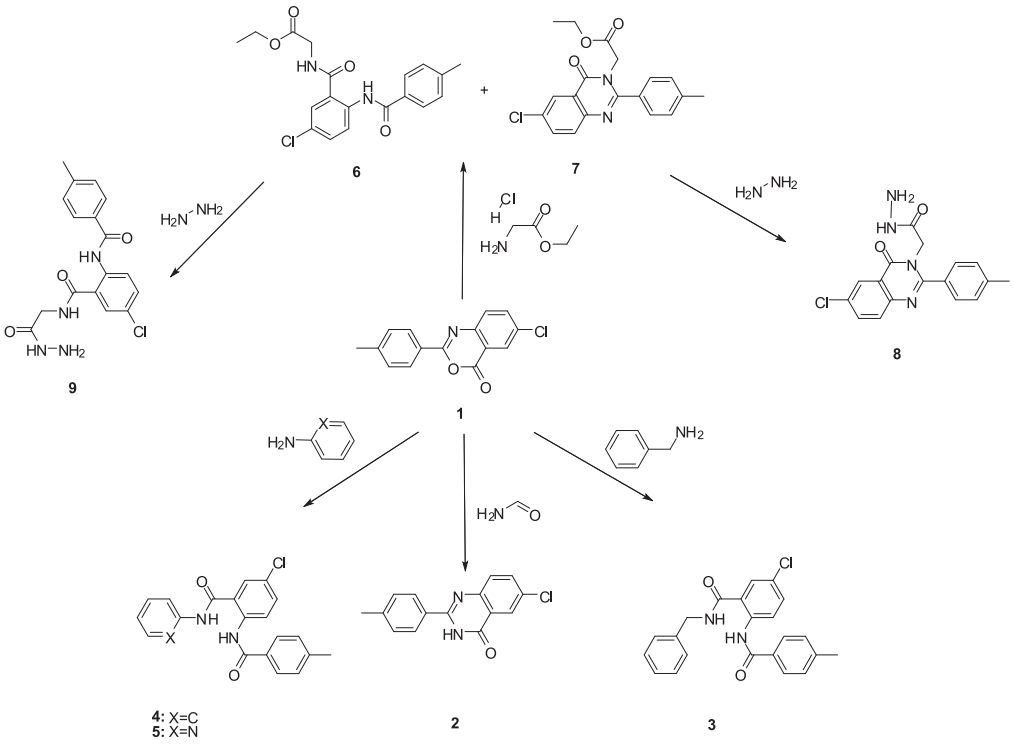
Furthermore, when compound **1** was reacted with hydrazine hydrate in ethanol at room temperature and/or boiling ethanol gave *N*-(4-chloro-2-(hydrazinecarbonyl)phenyl)-4-methylbenzamide (**10**) and/or 3-amino-6-chloro-2-*p*-tolylquinazolin-4(3*H*)-one (**11**) in 90% and 88% yield, respectively.

As well, the reaction of compound **11** with chloroacetylchloride and/or benzaldehyde provided 2-chloro-*N*-(6-chloro-4-oxo-2-*p*-tolylquinazolin-3(4*H*)-yl)acetamide (**12**) and 3-(benzylideneamino)-6-chloro-2-*p*-tolylquinazolin-4(3*H*)-one (**16**) in 89% and 92% yield, respectively. 2-Amino-*N*-(6-chloro-4-oxo-2-*p*-tolylquinazolin-3(4*H*)-yl)acetamide (**13**) and *N*-(6-chloro-4-oxo-2-*p*-tolylquinazolin-3(4*H*)-yl)-2-hydrazinylacetamide (**14**) were obtained by stirring of compound **12** with concentrated ammonia solution and/or hydrazine hydrate at room temperature in relatively good yield.

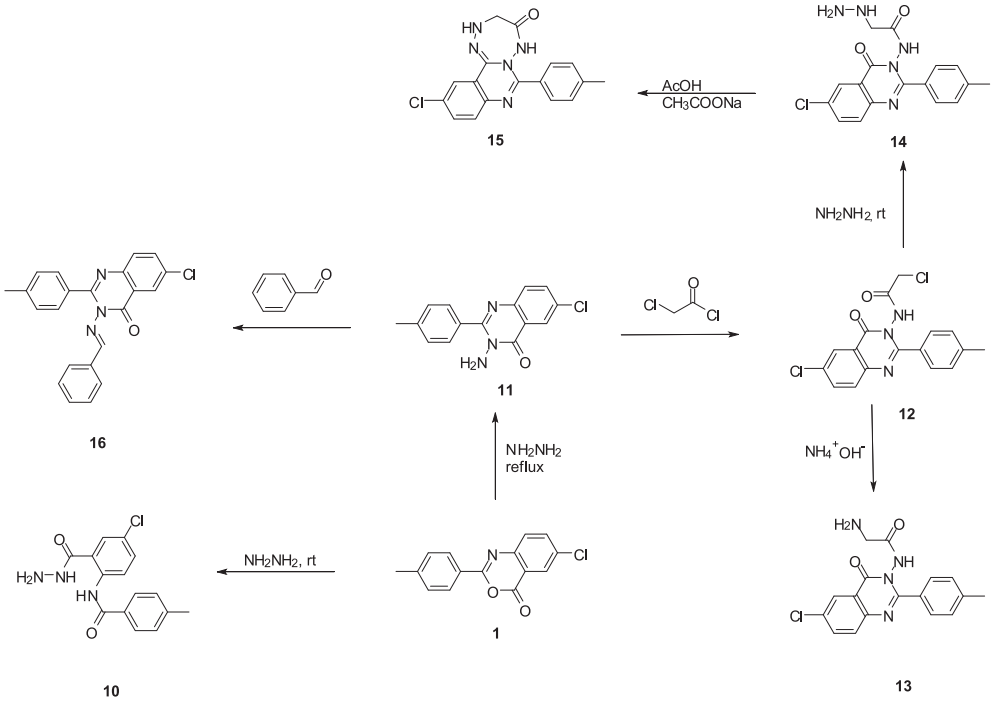
In addition, 11-chloro-7-*p*-tolyl-2,3-dihydro-[1,2,4,5]tetrazepino[2,3-c]quinazolin-4(5*H*)-one (**15**) was achieved via boiling of compound **14** with acetic acid in the presence of fused sodium acetate in 46% yield (Scheme 2).

Compound **10** showed a characteristic (CO) band of diamide moiety at 1671, 1668 cm^{-1} and (NH) absorption band at 3277, 3172, 3168 cm^{-1} as well as ^{1}H NMR showed a singlet signal at 11.20 and doublet signal at 8.11 ppm corresponding to (CONH) group. Additionally compound **11** revealed (NH_2) and (CO) groups at 3266, 3121 and 1680 cm^{-1} respectively, in addition to singlet signal at 5.70 ppm due to the presence of (NH_2) group in ^{1}H NMR spectrum. ^{1}H NMR spectra of compounds **12** and **16** were characterized by the disappearance of (NH_2) singlet signal at 5.70 ppm of compound **11** with appearance of new singlet signal at 9.01 ppm and 11.67 ppm for (HNCO) and (HC=N) moieties respectively, as well as the presence of additional aliphatic carbon at 40.4 ppm for compound **12** in ^{13}C NMR spectrum.

IR of compounds **13** showed absorption bands of (NH_2) at 3269, 3175 cm^{-1} and 1704, 1670 cm^{-1} due to stretching vibration of (CO) groups with the presence of (NH_2) signal at 7.27 ppm in ^{1}H NMR. Compound **15** showed characteristic (CO) band at 1702 cm^{-1} and (NH) absorption band at 3167 cm^{-1} due to the amide moiety as well



Scheme 1. Reactions of 6-chloro-2-*p*-tolyl-4H-benzo[d][1,3]oxazin-4-one.



Scheme 2. The synthesis and reactions of 3-amino-6-chloro-2-*p*-tolylquinazolin-4(3H)-one.

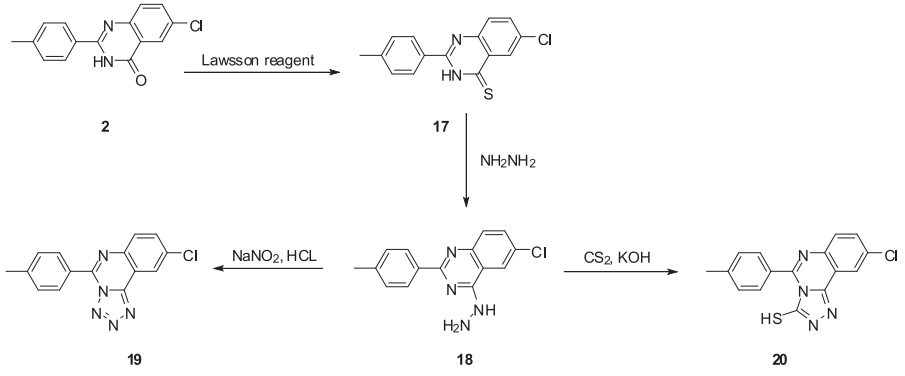
as ^{1}H NMR showed a singlet signals at 11.23 and 3.24 ppm corresponding to (CONH and $COCH_2$) groups.

On the other hand, 6-chloro-4-hydrazinyl-2-*p*-tolylquinazoline (**18**) was achieved by reaction of compound **2** with Lawsson reagent in boiling toluene followed by boiling with hydrazine hydrate in 62% overall yield. Correspondingly, reaction of compound **18** with carbon disulfide in ethanol containing potassium hydroxide and/or sodium nitrite in the presence of dilute hydrochloric acid produced 9-chloro-5-*p*-tolyltetrazolo[1,5-c]quinazoline (**19**) and 9-chloro-5-*p*-tolyl-[1,2,4]triazolo[4,3-c]quinazoline-3-thiol (**20**) in 61% and 64% yield, respectively (Scheme 3).

Compound **17** revealed a characteristic (CS) band at 1206 cm^{-1} with disappearance of (CO) group at 1668 $cm^{-1}$of compound **2.** Likewise, ^{1}H NMR of compound **17** showed fading singlet proton of amide moiety (CONH) at 12.41 ppm through the presence of singlet signal thioamide group (CSNH) at 13.98 ppm with existence of (CS) peak at 187.2 ppm in ^{13}C NMR. Compound **18** was confirmed by the loss of singlet signal of thioamide group (CSNH) at 13.98 ppm and (CS) peak at 187.2 ppm in NMR spectrum with the presence of new singlet signals at 9.69 and 4.92 ppm for (NH & NH_2) respectively. Compounds **19** and **20** were approved by the disappearance of singlet signals at 9.69 and 4.92 ppm for (NH & NH_2) groups with appearance of new typical singlet proton of (SH) at 14.60 ppm for compound **20**.

2.2. Antitumor activity

The selected synthesized compounds were submitted and evaluated *in vitro* at the single concentration of 10 μM toward a panel of approximately 60 cancer cell lines at the National Cancer Institute (NCI). The human tumor cell lines were derived from nine different cancer types: leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancers. Primary anticancer assays were performed according to the US NCI protocol (http://dtp.nci.nih.gov), as described elsewhere [30–33]. The compounds were added at the single concentration, and the cell culture was incubated for 48 h. The end point determinations were made with a protein binding dye, sulforhodamine B (SRB). The data reported as



Scheme 3. The synthesis and reactions of 6-chloro-4-hydrazinyl-2-*p*-tolylquinazoline.

mean-graph of the percent growth of the treated cells, and presented as percentage growth inhibition (GI %) caused by the test compounds (Table 1).

With regard to broad spectrum antitumor activity; close examination of the data presented in Table 1, revealed that compounds **3, 14** and **16** are the most active members of this study, showing effectiveness toward numerous cell lines belong to different tumor subpanels. Consequently, compound **16** was selected in advanced assay against a panel of approximately 60 tumor cell lines at 10-fold dilutions of five concentrations (100, 10, 1, 0.1, and 0.01 μM) [26]. Dose response curves obtained from the NCI's *in vitro* disease-oriented human tumor cells line of compound **16** on nine cancer disease at five concentrations were shown in Fig. 1. Based on the cytotoxicity assays, three antitumor activity dose–response parameters were calculated for experimental agents against each cell line: GI_{50}, molar concentration of the compound that inhibits 50% net cell growth; TGI, molar concentration of the compound leading to total inhibition; LC_{50}, molar concentration of the compound leading to 50% net cell death. Furthermore a mean graph midpoints (MG-MID) were calculated for each of the parameters, giving an average activity parameter overall cell lines for the tested compounds (Table 2). The NCI screening data analysis indicated that compound **16** possessed potent *in vitro* antiproliferative activity, with mean GI_{50} values across the 60 cell lines 15.8 μM. At the second assay (Table 2), the compound **16** was found to be a particularly active growth inhibitor of the renal cancer (GI_{50} = 4.07 μM), CNS cancer (GI_{50} = 7.41 μM), ovarian cancer (GI_{50} = 7.41 μM) and non-small cell lung cancer (GI_{50} = 7.94 μM). Compound **16** ranks as nearly 1.5-fold more potent (mean GI_{50} = 15.8 μM) compared with 5-FU (mean GI_{50} = 22.6 μM).

On the other hand, Compound **4** yielded selective activities toward CNS cancer cell lines, compound **5** showed selective activities toward leukemia cancer cell lines, whereas compound **19** revealed selective activities toward Non-small cell lung cancer. Compounds **13**, **15**, and **20** possess weak antitumor activity.

Regarding the activity toward individual cell lines; compound **3** and **5** showed selective activity against leukemia cell lines CCRF-CEM and K-562 with GI values of 27, 29% and 70, 35% respectively, whilst, HL-60(TB), MOLT-4, PRMI-8226 and SR cell lines were sensitive to compounds **3** and **16** with GI values of 49, 26, 49, 18, 23, 20 and 63, 20% respectively.

Non-small cell lung; A549/ATCC and HOP-62 cell lines proved to be selectively sensitive to **14**, **16** and **19** with GI values of 23, 87, 47 and 83, 98, 51% respectively, compounds **3**, **14** and **16** have fanatical activity against EKVX and NCI-H522 cell lines with GI values of 20, 34, 40 and 47, 61 and 52% respectively. In addition, compound **14** and **20** proved to susceptible to NCI-H226 cell line with GI values of 35 and 27%, compounds **4**, **5** and **19** have dedicated activity against NCI-H522 cell lines with GI values of 21, 22 and 21%. Meanwhile, NCI-H322M and NCI-H460 cell lines were susceptible to compound **14** and **16** with GI values of 29, 24 and 49 and 23% respectively, while HOP-62 cell lines having a tendency to compound **4** with GI values of 29% and compound **16** lethal to HOP-92 cell lines.

Respecting colon cancer; compounds **14** and **16** showed GI values of 28, 34, 46, 47, 28 and 23% with colon COLO 205, HCT-116 and SW-620 cell line respectively, while **3** and **14** demonstrated moderate activities against HT29 and KM12 cancer cell line with GI value 77, 33, 40 and 21% respectively. On the other hand, compound **3** verified sensitivity in 43 and 42% to colon HCT-15 and SW-620 cancer cells.

Concerning CNS cancer; compounds **14** and **16** showed selective activity through CNS cancer SF-268, SNB-19 and U251 cell line with GI values of 28, 40, 22, 25, 58 and 88%, respectively. Compounds **4**, **5**, **14** and **16** showed GI values of 29, 22, 54, 22% and 28, 24, 25 and

Table 1
Percentage growth inhibition (GI %) of *in vitro* subpanel tumor cell lines at 10 μM concentration.

Subpanel tumor cell lines	% Growth Inhibition (GI %)[a]								
	3	4	5	13	14	15	16	19	20
Leukemia									
CCRF-CEM	27	nt	29	14	–	–	–	–	12
HL-60(TB)	49	nt	16	–	12	–	26	–	–
K-562	70	nt	35	–	11	–	–	11	11
MOLT-4	49	nt		–	–	–	18	–	13
PRMI-8226	23	nt	15	–	13	–	20	–	–
SR	63	nt	–	–	13	–	20	–	–
Non-small cell lung cancer									
A549/ATCC	15	–	13	–	23	11	87	47	11
EKVX	20	–	12	22	34	12	40	11	11
HOP-62	12	29	–	15	83	–	98	51	–
NCI-H226	–	–	–	11	35	–	14	–	27
HOP-92	–	–	–	–	nt	–	L	–	–
NCI-H23	–	–	–	–	13	–	11	–	–
NCI-H322M	–	–	–	–	29	–	24	–	–
NCI-H460	–	–	–	–	49	–	23	–	–
NCI-H522	47	21	22	–	61	–	52	21	–
Colon cancer									
COLO 205	–	–	–	–	28	–	34	–	–
HCC-2998	14	–	–	–	–	–	–	–	–
HCT-116	11	–	–	11	46	–	47	–	–
HCT-15	43	11	14		16	–	14	–	–
HT29	77	–	14	12	33	–	17	–	–
KM12	40	–	11	–	21	–	18	–	11
SW-620	42	–	–	–	28	–	23	17	–
CNS cancer									
SF-268	–	–	13	–	28	–	40	–	–
SF-539	16	29	22	15	54	–	22	18	–
SNB-19	–	13	–	–	22	–	25	11	–
SNB-75	33	28	24	15	25	–	74	–	–
U251	19	14	–	–	58	–	88	–	–
Melanoma									
LOX IMVI	21	–	–	–	27	–	11	–	–
MALME-3M	12	12	–	–	14	–	35	–	–
M14	26	–	–	11	17	–	29	–	–
MDA-MB-435	98	11	19	–	–	–	34	13	–
SK-MEL-2	49	–	–	–	–	–	–	–	–
SK-MEL-28	17	–	–	–	23	–	–	–	–
SK-MEL-5	22	13	39	–	11	–	–	–	12
UACC-257	15	–	–	–	23	–	–	–	33
UACC-62	30		16	–	18	–	23	18	–
Ovarian cancer									
IGROV1	16	–	14	–	41	–	43	–	–
OVCAR-3	–	–	12	–	45	–	42	11	–
OVCAR-4	21	11	22	–	80	–	86	–	–
OVCAR-5	–	14	–	11	18	–	18	–	–
OVCAR-8	13	–	–	–	23	–	73	–	–
NCI/ADR-RES	28	–	–	–	24	–	30	–	–
SK-OV-3	–	12	–	–	84	–	64	12	–
Renal cancer									
786-0	–	–	–	–	22	–	30	–	–
A498	21	–	39		46	34	16	11	19
ACHN	14	17	–	14	42	–	74	–	–
CAKI-1	23	18	20	14	35	14	40	25	15
RXF 393	13	–	–	–	11	–	–	–	–
SN12C	–	–	–	–	33	–	32	16	–
TK-10	–	–	–	–	61	–	26	–	19
UO-31	24	–	13	11	25	18	33	24	–
Prostate cancer									
PC-3	24	15	23	–	33	–	30	18	–
DU-145	–	–	11	–	28	–	27	–	–
Breast cancer									
MCF7	47	11	19	12	22	–	20	–	–
MDA-MB-231/ATCC	–	16	–	–	62	11	41	27	16
HS 578T	21	–	–	–	40	–	44	–	–
BT-549	–	–	–	–	–	–	–	–	–
T-47D	–	20	20		52		25	23	–
MDA-MB-468	17	11	11	–	–	11		–	–

[a] nt = not tested; –, GI <10%; L, compound proved lethal to the cancer cell line.

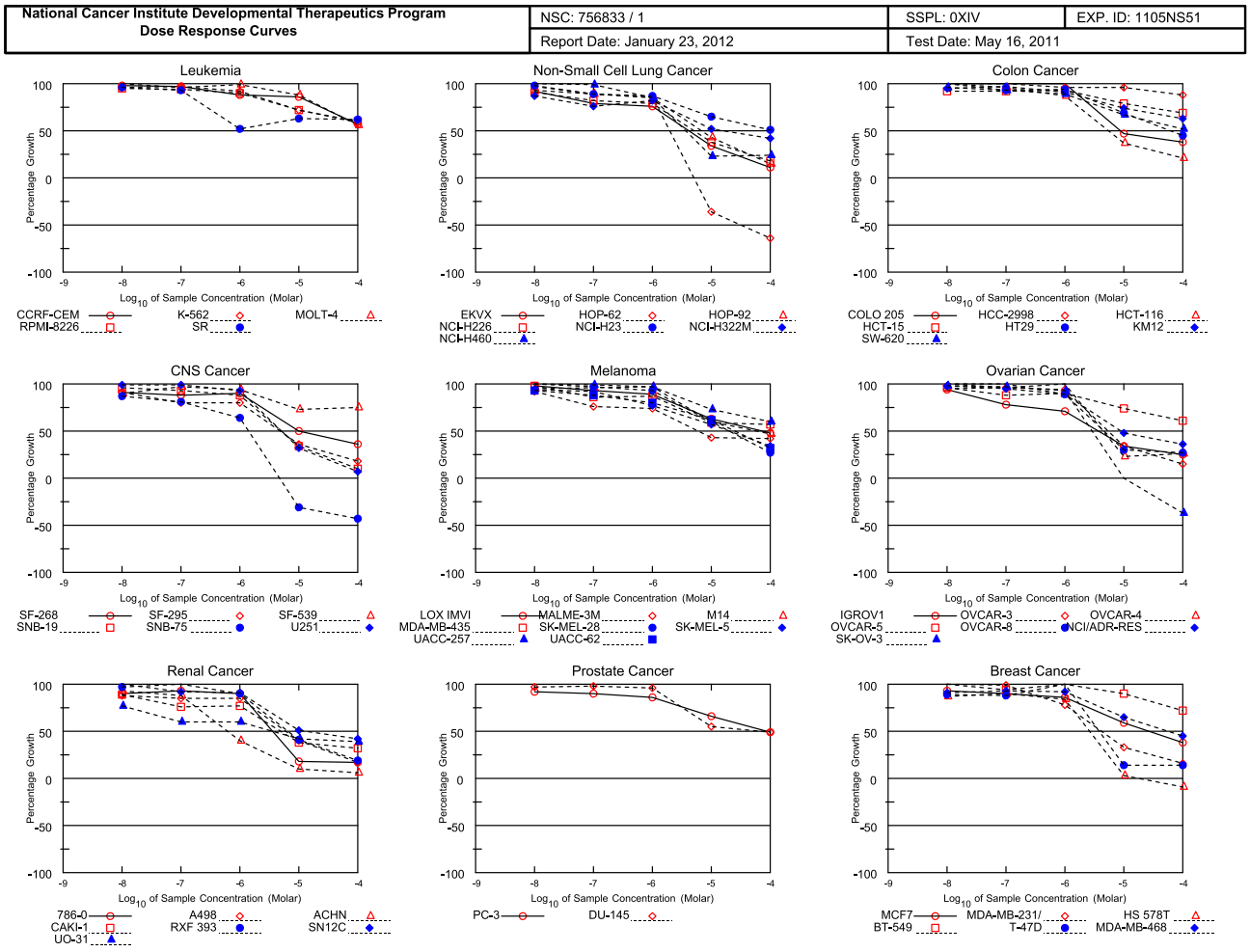


Fig. 1. Dose response curves (% growth verses sample concentration at NCI fixed protocol, μM) obtained from the NCI's *in vitro* disease-oriented human tumor cells line of compound (***16***) on nine cancer disease.

74% to CNS cancer SF-539, SNB-75 cell lines, respectively, while compound **3** showed activities against SNB-75 cell lines with GI values of 33%.

Regarding Melanoma; compounds **3** and **16** are active against M14, MDA-MB-435 and UACC-62 cell lines with GI values of 26, 29, 98, 34, 30 and 23% respectively, whereas, LOX IMVI cell lines sensitive to compounds **3** and **14** with GI values of 21% and 27%. Compounds **3** and **5** are active against SK-MEL-5 cell lines with GI values of 22 and 39%, while Compounds **14** and **20** showed moderate activity towards UACC-257 cell lines with GI values of 23% and 33%. Melanoma SK-MEL-2, SK-MEL-28 and MALME-3M cell lines showed selective activity to compounds **3**, **14** and **16** with GI values of 49%, 23 and 35%.

Respecting ovarian cancer; compounds **3**, **5, 14** and **16** showed activities against Ovarian OVCAR-4 cell line with GI values of 21, 22, 80 and 86%. Ovarian NCI/ADR-RES cell line responsive to compounds **3**, 1**4** and **16** with GI values of 28, 24 and 30%, while compounds **14** and **16** showed remarkable potency against IGROV1, OVCAR-3, OVCAR-8 and SK-OV-3 cell line with GI values of 41, 43, 45, 42, 23, 73, 84 and 64% respectively.

Relating to renal cancer; renal 786-0, ACHN, CAKI-1, SN12C, TK-10 and UO-31 cell line sensitive to compounds **14** and **16** with GI values of 22, 30, 42, 74, 35, 40, 33, 32, 61, 26, 25 and 33% respectively. Compounds **3**, **5**, **14** and **15** showed certain activity against renal A498 cell lines with GI values of 21, 39, 46 and 34%, whilst compounds **3**, **5** and **19** have certain sensitivity to CAKI-1 cell lines with GI values of 23, 20 and 25%. Additionally, Renal UO-31 cancer cell line sensitive to compounds **3** and **19** with GI values of 24 and 24%.

Prostate PC-3 cell lines proved to be selectively sensitive to compounds **3**, **5**, **14** and **16** with GI value of 24, 23, 33 and 30%, at the same time as compounds **14** and **16** active against prostate DU-145 cell line with GI value of 28 and 27%.

Pertaining to breast cancer; breast MDA-MB-231/ATCC cell line convinced responsive to compounds **14**, **16** and **19** with GI value of 62, 41and 27%, respectively. Compounds **4**, **5**, **14**, **16** and **19** showed GI effectiveness against breast T-47D cell line with values of 20, 20, 52, 25 and 23%, concomitantly, breast cancer MCF7 and HS 578T cell lines demonstrated sensitivity to compounds **3**, **14** and **16** with GI values of 47, 22, 20% and 21, 40 and 44%, respectively.

2.3. Structure activity correlations

Compounds of the present investigation belong to *N*-substituted-5-chloro-2-(4-methylbenzamido)benzamide and 3-substituted-6-chloro-2-*p*-tolylquinazolin-4(3*H*)-one. This study revealed that compounds **13** and **15** devoid of any significant antitumor potency.

Structure–activity correlation revealed that reaction of 6-chloro-2-*p*-tolyl-4*H*-benzo[d][1,3]oxazin-4-one (**1**) with various amines gave *N*-substituted benzamide (**3**–**5)** analogs with variable potency, therefore ***N***-benzyl-5-chloro-2-(4-methylbenzamido)benzamide (**3**) possessed broad spectrum antitumor activity compared to *N*-

Table 2
Compound **16** median growth inhibitory (GI_{50}, μM), total growth inhibitory (TGI, μM) and median lethal (LC_{50}, μM) concentration of *in-vitro* subpanel tumor cell lines.

Compound	Activity	Subpanel tumor cell lines									MG-MID[a]
		Leukemia	NSC lung cancer	Colon cancer	CNS cancer	Melanoma	Ovarian cancer	Renal cancer	Prostate cancer	Breast cancer	
16	GI_{50}	[b]	7.94	43.65	7.41	35.48	7.41	4.07	69.9	13.6	15.8
	TGI										83.1
	LC_{50}										97.7
5-FU	GI_{50}	15.1	[b]	8.4	72.1	70.6	61.4	45.6	22.7	76.4	22.60
	TGI										[b]
	LC_{50}										[b]

[a] Full panel mean-graph midpoint (μM).
[b] Compounds showed values >100 μM.

phenyl benzamide (**4**) or *N*-pyridyl benzamide (**5**), also *N*-pyridyl benzamide **4** more active than *N*-phenyl benzamide **5**.

Replacement of 3-amino group of compound **11** with 2-aminoacetamide produced compound **13** with insignificant antitumor activity. Switch of 2-aminoacetamide group at position 3 of compound **13** with 2-hydrazinylacetamide at position 3 afforded compound **14** with dramatically advanced the antitumor activity; on the other hand, cyclization of compound **14** gave compound **15** with severely failure of the antitumor activity.

Exchange of 3-amino group of compound **11** with benzylidine moiety gave compound **16** enhanced the antitumor activity as compared to compound **13-15**.

Cyclization of compound **18** produced compounds 9-chloro-5-*p*-tolyltetrazolo[1,5-c]quinazoline (**19**) and 9-chloro-5-*p*-tolyl-[1,2,4]triazolo[4,3-c]quinazoline-3-thiol (**20**) with variable antitumor activity, consequently substitution of triazole moiety of compound **20** into tetrazole moiety gave compound **19** accompanied with mild improved the antitumor activity.

3. Conclusion

A novel series of 6-chloro-2-*p*-tolylquinazolinone and substituted (4-methylbenzamido)benzamide (**1**–**20**) were designed, synthesized and evaluated for their *in-vitro* antitumor activity. A single dose (10 μM) of the test compounds was used in the National Cancer Institute (NCI) 60 cell lines panel assay. The results of this study demonstrated that compound **4** yielded selective activities toward CNS cancer cell lines, compound **5** showed selective activities toward leukemia cancer cell lines, whereas compound **19** revealed selective activities toward Non-small cell lung cancer, in addition to, compounds **13**, **15**, and **20** possess weak antitumor activity. On the other hand, **3**, **14** and **16** possessed remarkable broad-spectrum antitumor activity. Compound **16** was carried over and tested against a panel of 60 different tumor cell lines at a 5-log dose rang. Three response parameters, GI_{50}, TGI and LC_{50} were calculated for each cell line, using the known drug 5-Fluorouracil (5-FU) as a positive control. Compound **16** was found to be a particularly active growth inhibitor of the renal cancer (GI_{50} = 4.07 μM), CNS cancer (GI_{50} = 7.41 μM), ovarian cancer (GI_{50} = 7.41 μM) and non-small cell lung cancer (GI_{50} = 7.94 μM). Compound **16** ranks as nearly 1.5-fold more potent (mean GI_{50} = 15.8 μM) compared with 5-FU (mean GI_{50} = 22.6 μM).

4. Experimental

4.1. Chemistry

Melting points (corrected) were recorded on Barnstead 9100 Electrothermal melting apparatus. IR spectra were recorded on a Perkin-Elmer spectrometer. ^{1}H NMR and ^{13}C NMR was recorded in DMSO-d_6 on a Jeol 500 MHz instrument using TMS as internal standard (chemical shifts in δ ppm). Microanalytical data (C, H, and N) were performed on Perkin-Elmer 240 analyzer and they agreed with proposed structures within ±0.4% of the calculated values. Mass spectra were recorded on a Shimadzu PQ-5000 GC–MS apparatus. Solvent evaporation was performed under reduced pressure using Buchan Rotatory Evaporator unless otherwise stated. T.L.C. was performed on precoated silica gel plates (60- F254, 0.2 mm), manufactured by E.M. Sciences, Inc, and shortwave UV (254) nm was used to detect the U.V. absorbing compounds (CH_2Cl_2, EtOH 10:1 v/v). Compounds ***1***, ***2***, ***11***, ***17*** and ***18*** were prepared according to reported procedure [27].

4.1.1. 6-Chloro-2-p-tolylquinazolin-4(3H)-one ***(2)***

Benzoxazine (***1***) (10 mmol, 2.71 g) was heated under reflux in formamide (50 ml) for 3 h. The solid obtained was filtered while hot and dried, mp 300–302 °C in 73% yield.

IR (KBr, cm^{-1}) ν: 3179 (NH), 1668 (CO); ^{1}H NMR (DMSO-d_6): δ 12.41 (s, 1H, NHCO), 8.11 (s, 1H, Ar.), 8.04 (d, 1H, *J* = 1.5 Hz), 7.82 (d, 2H, *J* = 8.0 Hz), 7.68 (d, 1H, *J* = 8.5 Hz), 7.37–7.22 (m, 2H), 2.33 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 167.7, 160.2, 147.9, 146.3, 143.4, 134.9, 129.9, 129.8, 128.2, 124.3, 125.3, 127.5, 21.6. MS: 252 ([M–28], 78% rel. abundance).

4.1.2. General procedure for the synthesis of compounds ***3***–***5***

A solution of benzoxazine (***1***) (2 mmol, 542 mg) and appropriate amine (2.1 mmol) in pyridine (10 ml) was heated under reflux for 10–12 h. The reaction mixture was cooled and the solvent was removed under reduced pressure; the residue was triturated with water and filtered. The solid obtained was dried and recrystallized from ethanol.

4.1.2.1. N-Benzyl-5-chloro-2-(4-methylbenzamido)benzamide ***(3)***. Mp 270–272 °C, IR (KBr, cm^{-1}) ν: 3182, 3173 (NH), 1670 (CO); ^{1}H NMR (DMSO-d_6): δ 12.46 (s, 1H), 9.57 (t, 1H, *J* = 5.5, 6.0 Hz), 8.68 (d, 1H, *J* = 9.0 Hz), 7.99 (d, 1H, *J* = 2.5 Hz), 7.64 (d, 2H, *J* = 7.0 Hz), 7.62 (dd, 1H, *J* = 2.0 Hz), 741–7.24 (m, 6H), 7.20 (d, 1H, *J* = 6.0 Hz), 4.38 (d, 2H, *J* = 6.0 Hz), 2.33 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 21.0, 42.7, 121.4, 122.0, 126.5, 127.0, 127.3, 127.9, 128.3, 129.9, 131.4, 132.0, 138.5, 138.6, 142.3, 164.3, 167.3. MS: 378 ($[M]^+$, 31% rel. abundance).

4.1.2.2. 5-Chloro-2-(4-methylbenzamido)-N-phenyl benzamide ***(4)***. Mp 260–263 °C, IR (KBr, cm^{-1}) ν: 3180, 3175 (NH), 1669 (CO); ^{1}H NMR (DMSO-d_6): δ 11.57 (s, 1H), 10.64 (s, 1H), 8.47 (d, 1H, *J* = 9.0 Hz), 7.98 (d, 1H, *J* = 2.0 Hz), 7.80 (d, 2H, *J* = 8.0 Hz), 7.73–7.67 (m, 3H), 7.42–7.37 (m, 4H), 7.16 (t, 1H, *J* = 7.0, 7.5 Hz), 2.41 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 21.0, 121.1, 123.0, 124.4, 126.9, 127.1, 127.8,

128.6, 128.7, 129.4, 131.3, 131.8, 137.5, 138.2, 166.0. MS: 364 ([M]$^+$, 27% rel. abundance).

*4.1.2.3. 5-Chloro-2-(4-methylbenzamido)-N-(pyridin-2-yl)benzamide **(5)**.* Mp 257–259 °C, IR (KBr, cm^{-1}) ν: 3179, 3176 (NH), 1668 (CO); ^{1}H NMR (DMSO-d$_6$): δ 11.39 (s, 1H), 11.14 (s, 1H), 8.40–8.36 (m, 2H), 8.08 (d, 1H, J = 8.5 Hz), 7.98 (d, 1H, J = 7.5 Hz), 7.86–7.83 (m, 1H), 7.80 (d, 2H, J = 7.5 Hz), 7.64 (dd, 1H, J = 2.5 Hz), 7.34 (d, 2H, J = 8.0 Hz), 7.20–7.18 (m, 1H), 2.48 (s, 3H). ^{13}C NMR (DMSO-d$_6$): δ 21.0, 115.4, 120.3, 123.2, 124.5, 127.0, 127.1, 129.2, 129.3, 131.3, 131.9, 137.4, 138.1, 142.2, 148.0, 151.6, 164.6, 166.6. MS: 365 ([M]$^+$, 24% rel. abundance).

*4.1.3. General procedure for the synthesis of compounds **6**–**7***

A solution of benzoxazine (***1***) (2 mmol, 542 mg) and ethyl 2-aminoacetate hydrochloride (2.1 mmol, 292 mg) in pyridine (10 ml) was heated under reflux for 10 h. The reaction mixture was cooled and the solvent was removed under reduced pressure; the residue was triturated with water and filtered. The solid obtained was dried and chromatographed (SiO_2, 20 g, elution with $CHCl_3$) afforded compounds ***6*** and ***7***.

*4.1.3.1. Ethyl 2-(5-chloro-2-(4-methylbenzamido)benzamido)acetate **(6)**.* Mp 233–235 °C, IR (KBr, cm^{-1}) ν: 3179, 3178 (NH), 1716, 1661 (CO); ^{1}H NMR (DMSO-d$_6$): δ 12.20 (s, 1H), 9.50 (t, 1H, J = 5.5, 5.75 Hz), 8.68 (d, 1H, J = 9.0 Hz), 7.94 (d, 1H, J = 2.5 Hz), 7.79 (d, 2H, J = 8.0 Hz), 7.68 (dd, 1H, J = 2.5 Hz), 7.38 (d, 2H, J = 8.0 Hz), 4.14 (dd, 2H, J = 7.0 Hz), 4.06 (d, 2H, J = 5.75 Hz), 2.38 (s, 3H), 1.20 (t, 3H, J = 7.0 Hz). ^{13}C NMR (DMSO-d$_6$): δ 14.0, 21.0, 41.4, 60.7, 121.0, 122.0, 126.6, 127.0, 127.9, 129.5, 131.3, 132.3, 138.4, 142.5, 164.4, 168.0, 169.3. MS: 374 ([M]$^+$, 12% rel. abundance).

*4.1.3.2. Ethyl 2-(6-chloro-4-oxo-2-p-tolylquinazolin-3(4H)-yl)acetate **(7)**.* Mp 247–248 °C, IR (KBr, cm^{-1}) ν: 1720, 1668 (CO); ^{1}H NMR (DMSO-d$_6$): δ 8.12–8.09 (m, 1H), 7.98–7.93 (m, 1H), 7.77–7.72 (m, 1H), 7.44 (d, 2H, J = 7.5 Hz), 7.34 (d, 2H, J = 7.0 Hz), 4.63 (s, 2H), 4.11 (q, 2H, J = 6.5 Hz), 2.39 (s, 3H), 1.13 (t, 3H, J = 6.5 Hz). MS: 356 ([M]$^+$, 36% rel. abundance).

*4.1.4. N-(4-Chloro-2-(hydrazinecarbonyl)phenyl)-4-methylbenzamide **(10)***

A mixture of benzoxazine (***1***) (10 mmol, 2.71 g) and hydrazine hydrate (750 mg, 15 mmol) in (50 ml) absolute ethanol was stirred at room temperature for 12 h. The reaction mixture was filtered and dried, mp 224–226 °C in 90% yield.

IR (KBr, cm^{-1}) ν: 3277, 3172, 3168 (NH), 1671, 1668 (CO); ^{1}H NMR (DMSO-d$_6$): δ 11.20 (s, 1H), 8.11 (d, 1H, J = 2.5 Hz), 7.93 (dd, 1H, J = 2.5 Hz), 7.77 (d, 1H, J = 9.0 Hz), 7.54 (d, 2H, J = 7.0 Hz), 7.30 (d, 2H, J = 8.0 Hz), 2.38 (s, 3H), 1.38 (s, 2H). ^{13}C NMR (DMSO-d$_6$): δ 21.0, 122.0, 125.4, 128.4, 128.5, 129.9, 130.3, 131.5, 135.3, 140.2, 145.4, 156.6, 158.6, 168.0. MS: 303 ([M]$^+$, 17% rel. abundance).

*4.1.5. 3-Amino-6-chloro-2-p-tolylquinazolin-4(3H)-one **(11)***

Benzoxazine **(1)** (2 mmol, 543 mg) was heated under reflux in hydrazine hydrate (5 ml) for 3 h. The solid obtained was filtered while hot and dried, mp 208–210 °C in 88% yield. IR (KBr, cm^{-1}) n: 3266, 3121 (NH), 1680 (CO); ^{1}H NMR (DMSO-d$_6$): d 8.10 (d, 1H, J = 2.5 Hz), 7.84 (dd, 1H, J = 2.5 Hz), 7.74–7.71 (m, 3H), 7.29 (d, 2H, J = 8.0 Hz), 5.70 (s, NH_2), 2.39 (s, 3H, CH_3). ^{13}C NMR (DMSO-d$_6$): d 160.7, 156.6, 145.9, 139.9, 134.8, 132.1, 131.2, 130.2, 130.1, 128.4, 125.3, 121.6, 21.4. MS: 285 ([M]$^+$, 60% rel. abundance and 287 [M+2] 20% rel. abundance).

*4.1.6. 2-Chloro-N-(6-chloro-4-oxo-2-p-tolylquinazolin-3(4H)-yl) acetamide **(12)***

3-Amino-6-chloro-2-*p*-tolylquinazolin-4(3*H*)-one (***11***) (3 mmol, 855 mg) was stirred at room temperature with chloroacetyl-chloride (3.1 mmol, 350 mg) in dichloromethane (10 ml) containing triethylamine (5 mmol, 505 mg) for 12 h. The reaction mixture was diluted with water and extract with chloroform, the extract was washed successively with water and brine, dried over anhydrous $MgSO_4$, evaporated under reduced pressure and chromatographed (SiO_2, 20 g, elution with $CHCl_3$–AcOEt, 10:1 v/v) afforded compound **12,** mp 217–218 °C in 89% yield.

IR (KBr, cm^{-1}) ν: 3183 (NH), 1708, 1668 (CO); ^{1}H NMR (DMSO-d$_6$): δ 11.67 (s, 1H), 8.13 (d, 1H, J = 2.5 Hz), 7.88 (dd, 1H, J = 2.5 Hz), 7.76 (d, 1H, J = 8.7 Hz), 7.58 (d, 2H, J = 8.0 Hz), 7.29 (d, 2H, J = 8.0 Hz), 4.21 (dd, 2H, J = 8.5 Hz), 2.49 (s, 3H). ^{13}C NMR (DMSO-d$_6$): δ 21.0, 40.4, 121.8, 125.4, 128.5, 128.6, 129.9, 131.7, 135.3, 140.3, 145.3, 156.2, 158.3, 165.5. MS: 361 ([M]$^+$, 32%, 363 [M+2], 11% rel. abundance).

*4.1.7. 2-Amino-N-(6-chloro-4-oxo-2-p-tolylquinazolin-3(4H)-yl) acetamide **(13)***

2-Chloro-*N*-(6-chloro-4-oxo-2-p-tolylquinazolin-3(4*H*)-yl)acetamide (***12***) (3 mmol, 1086 mg) was stirred at room temperature with ammonia solution (5 ml) for 12 h. The reaction mixture was filtered, washed with water, dried and recrystallized from ethanol, mp 222–223 °C in 74% yield.

IR (KBr, cm^{-1}) ν: 3269, 3175 (NH), 1704, 1670 (CO); ^{1}H NMR (DMSO-d$_6$): δ 12.95 (s, 1H), 8.85 (d, 2H, J = 5.5 Hz), 8.68 (t, 1H, J = 2.5, 3.0 Hz), 8.18 (t, 1H, J = 7.0, 7.5 Hz), 8.09 (d, 1H, J = 2.5 Hz), 8.01 (dd, 1H, J = 2.5 Hz), 7.77 (d, 1H, J = 9.0 Hz), 7.58 (d, 1H, J = 8.0 Hz), 7.27 (d, 2H, J = 8.0 Hz), 5.80 (d, 1H, J = 15.5 Hz), 5.68 (d, 1H, J = 15.5 Hz), 2.38 (s, 3H). ^{13}C NMR (DMSO-d$_6$): δ 21.0, 59.9, 121.7, 125.4, 127.9, 128.5, 128.6, 129.7, 130.0, 131.0, 140.4, 145.2, 145.7, 146.6, 155.8, 158.0, 164.2. MS: 342 ([M]$^+$, 18% rel. abundance).

*4.1.8. N-(6-Chloro-4-oxo-2-p-tolylquinazolin-3(4H)-yl)-2-hydrazinylacetamide **(14)***

2-Chloro-*N*-(6-chloro-4-oxo-2-*p*-tolylquinazolin-3(4*H*)-yl)acetamide (***12***) (3 mmol, 1.086 g) was stirred at room temperature with hydrazine hydrate (5 ml) for 12 h. The reaction mixture was filtered, washed with water, dried and recrystallized with ethanol, mp 243–244 °C in 75% yield.

*4.1.9. 11-Chloro-7-p-tolyl-2,3-dihydro-[1,2,4,5]tetrazepino[2,3-c] quinazolin-4(5H)-one **(15)***

N-(6-Chloro-4-oxo-2-*p*-tolylquinazolin-3(4*H*)-yl)-2-hydrazinylacetamide (***14***) (3 mmol, 1071 mg) was heated under reflux with acetic acid (10 ml) containing fused sodium acetate (1.0 g) for 12 h. The reaction mixture was cooled and the solvent was removed under reduced pressure; the residue was triturated with water and filtered. The solid obtained was washed with water, dried and recrystallized from ethanol, mp > 300 °C in 46% yield.

IR (KBr, cm^{-1}) ν: 3167 (NH), 1702 (CO); ^{1}H NMR (DMSO-d$_6$): δ 11.23 (s, 1H), 8.15 (d, 1H, J = 23.0 Hz), 7.90 (dd, 1H, J = 24.0 Hz), 7.76 (t, 1H, J = 87.0 Hz), 7.56 (d, 2H, J = 80.5 Hz), 7.29 (d, 2H, J = 79.5 Hz), 3.24 (s, 2H), 2.49 (s, 3H). ^{13}C NMR (DMSO-d$_6$): δ 21, 56.1, 121.9, 125.4, 128.4, 128.5, 129.4, 130.3, 131.5, 135.2, 140.1, 145.3, 156.6, 158.6, 168.6. MS: 339 ([M]$^+$, 29% rel. abundance).

*4.1.10. 3-(Benzylideneamino)-6-chloro-2-p-tolylquinazolin-4(3H)-one **(16)***

3-Amino-6-chloro-2-*p*-tolylquinazolin-4(3*H*)-one (**11**) (3 mmol, 855 mg) was stirred at room temperature with benzaldehyde (3.1 mmol, 320 mg) in methanol (10 ml) for 12 h. The reaction mixture was filtered and dried, mp 288–290 °C in 92% yield.

IR (KBr, cm^{-1}) ν: 1671(CO); ^1H NMR (DMSO-d_6): δ 9.01 (s, 1H), 8.14 (s, 1H), 7.91 (d, 1H, $J = 7.0$ Hz), 7.80 (d, 1H, $J = 7.0$ Hz), 7.75 (d, 2H, $J = 6.0$ Hz), 7.61–7.52 (m, 5H), 7.25 (d, 2H, $J = 6.0$ Hz), 2.34 (s, 3H).

4.1.11. 6-Chloro-2-p-tolylquinazolin-4(3H)-thione (17)

A mixture of 6-chloro-2-*p*-tolylquinazolin-4(3*H*)-one **(2)** (10 mmol, 2.70 g) and Lawsson reagent (5.0 g) was heated under reflux in anhydrous toluene (70 ml) for 6 h. The reaction mixture was filtered while hot, the solvent was evaporated and the residue was chromatographed (SiO_2, 30 g, elution with $CHCl_3$) gave compound **17**. Mp 265–267 °C in 80% yield. IR (KBr, cm^{-1}) ν: 3188 (NH), 1206 (CS); ^1H NMR (DMSO-d_6): δ 13.98 (s, 1H, Ar.), 8.49 (d, 1H, $J = 1.5$ Hz), 8.06 (d, 2H, $J = 7.5$ Hz), 7.87–7.83 (m, 1H), 7.75–7.69 (m, 1H), 7.33 (d, 2H, $J = 7.5$ Hz), 2.38 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 187.2, 161.8, 152.3, 147.9, 143.6, 135.7, 132.6, 129.5, 128.9, 128.3, 126.4, 122.6, 21.5. MS: 286 ($[M]^+$, 39% rel. abundance).

4.1.12. 6-Chloro-4-hydrazino-2-p-tolylquinazoline (18)

6-Chloro-2-*p*-tolylquinazolin-4(3*H*)-thione (**17**) (2 mmol, 773 mg) was heated under reflux in (5 ml) hydrazine hydrate for 3 h The solid obtained was filtered while hot, dried and recrystallized from ethanol, mp 273–275 °C in 62% yield.

^1H NMR (DMSO-d_6): δ 9.69 (s, 1H), 8.45 (d, 2H, $J = 8.0$ Hz), 8.32 (s, 1H), 7.74 (d, 2H, $J = 11.0$ Hz), 7.29 (d, 2H, $J = 8.0$ Hz), 4.92 (s, 2H), 2.37 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 160.2, 159.5, 148.8, 140.4, 136.0, 133.3, 130.1, 129.4, 129.2, 128.6, 122.2, 114.0, 21.5. MS: 285 ([M+1], 6% rel. abundance).

4.1.13. 9-Chloro-5-p-tolyltetrazolo[1,5-c]quinazoline (19)

A solution of 6-chloro-4-hydrazino-2-*p*-tolylquinazoline (**18**) (568 mg, 2 mmol) in EtOH–AcOH (10:1) (11 ml) at 0 °C, concentrated hydrochloric acid (1 ml) and $NaNO_2$ solution (742 mg, 10 mmol) (1 ml) were added, the mixture was stirred for 2 h at the same temperature and the stirring was continued for 10 h at room temperature. The mixture was diluted with water, extracted with EtOAc, the extract was washed successively with water and brine, dried over anhydrous $MgSO_4$, evaporated under reduced pressure and chromatographed (SiO_2, 50 g, elution with hexane–AcOEt, 10:1 v/v) afforded compound **19**, mp 277–278 °C in 61% yield.

^1H NMR (DMSO-d_6): δ 8.41 (d, 1H, $J = 8.0$ Hz), 8.08 (d, 2H, $J = 5.5$ Hz), 7.85 (dd, 1H, $J = 2.5$ Hz), 7.74 (d, 1H, $J = 8.75$ Hz), 7.53 (d, 1H, $J = 2.5$ Hz), 7.38 (d, 2H, $J = 4.5$ Hz), 2.39 (s, 3H). MS: 295 ($[M]^+$, 49% rel. abundance).

4.1.14. 9-Chloro-5-p-tolyl-[1,2,4]triazolo[4,3-c]quinazoline-3-thiol (20)

A solution of 6-chloro-4-hydrazino-2-*p*-tolylquinazoline (**18**) (568 mg, 2 mmol), carbon disulphide (760 mg, 10 mmol) and potassium hydroxide (170 mg, 3 mol) in ethanol (10 ml) was stirred at room temperature for 4 h the reaction mixture was heated under reflux for 6 h, cooled, the solvent was evaporated under reduced pressure. The residual solid obtained was dissolved in water, acidified with dilute hydrochloric acid and the precipitated solid was filtered, washed with water, and crystallized from ethanol, mp 263–265 °C in 64% yield.

^1H NMR (DMSO-d_6): δ 14.60 (s, 1H), 8.47 (d, 1H, $J = 8.5$ Hz), 8.24–8.85 (m, 3H), 8.60–8.51 (m, 1H), 7.35 (dd, 2H, $J = 6.5$ Hz), 2.40 (s, 3H). MS: 326 ($[M]^+$, 36% rel. abundance).

4.2. Antitumor screening

A primary anticancer assay was performed for an approximately 60 human tumor cell line panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda, MD [30–33].

Tested compounds were added to the culture at a single concentration (10^{-5} M), and the cultures were incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). The results for each tested compound were reported as the growth percentage of the treated cells when compared to that of the untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. The cytotoxic and/or growth inhibitory effects of the most active selected compounds were tested *in vitro* against the full panel of about 60 human tumor cell lines at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. A 48 h continuous drug exposure protocol was followed, and an SRB protein assay was used to estimate cell viability or growth. By use of the seven absorbance measurements [time zero, (T_z), control growth in the absence of drug (C), and test growth in the presence of drug at the five concentration levels (T_i)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as

$$[(T_i - T_z)/(C - T_z) \times 100] \text{for concentration of which } T_i \geq T_z$$

$$[((T_i - T_z)/T_z) \times 100] \text{for concentration of which } T_i \leq T_z$$

Three dose response parameters (GI_{50}, TGI, LC_{50}) were calculated for each compound. Growth inhibition of 50% (GI_{50}) was calculated from $[(T_i - T_z)/(C - T_z)] \times 100 = 50$, which was the drug concentration resulting in a 50% lower net protein increase in the treated cells (measured by SRB staining) compared to the net protein increase seen in the control cells. The drug concentration resulting in total growth inhibition (TGI) was calculated from $T_i = T_z$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment compared to that at the beginning) indicating a net loss of cells following treatment was calculated from $[(T_i - T_z)/T_z] \times 100 = -50$. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as more or less for the maximum or minimum concentration tested. The lowest values are obtained with the most sensitive cell lines. The compounds having $GI_{50} \leq 100$ μM were declared to be active.

Acknowledgments

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-VPP-163. The authors would like to express their gratitude and thanks to the National Cancer Institute (NCI), Bethesda Maryland, USA, http://dtp.cancer.gov/for doing the antitumor testing of the new compounds.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.029.

References

[1] C.C. Harris, M. Hollstein, New England Journal of Medicine 329 (1993) 1318.
[2] L.A. Liott, P.S. Steeg, W.G. Steller-Stevenson, Cell 64 (1991) 327.
[3] P. Mignatti, D.B. Rifkin, Physiological Reviews 73 (1993) 161.
[4] J. Baselga, S.M. Swain, Nature Reviews Cancer 9 (2009) 463.

[5] C.H.J. Brown, S. Lain, C.H.S. Verma, A.R. Fersht, D.P. Lane, Nature Reviews Cancer 9 (2009) 862.
[6] S.D. Auerbuch, in: B.A. Chabner, J.M. Collins (Eds.), Cancer Chemotherapy: Principles and Practice, Lippincott, Philadelphia, 1990, pp. 314–328.
[7] M. Ljungman, Chemical Reviews 109 (2009) 2929.
[8] G.L. Semenza, Cancer and Metastasis Reviews 26 (2007) 223.
[9] P.D.W. Eckford, F.J. Sharom, Chemical Reviews 109 (2009) 2989.
[10] M.F. Ullah, Asian Pacific Journal of Cancer Prevention 9 (2008) 1.
[11] P. Singh, K. Paul, W. Hozler, National Academy Science Letters 28 (2005) 365.
[12] A.M. Alanazi, I.A. Al-Suwaidan, A.A.-M. Abdel-Aziz, M.A. Mohamed, A.M. El-Morsy, A.S. El-Azab, Medicinal Chemistry Research 22 (2013) 5566.
[13] M.A. Aziza, M.W. Nassar, S.G. Abdel-Hamide, A.E. El-Hakim, A.S. El-Azab, Indian Journal of Heterocyclic Chemistry 6 (1996) 25.
[14] A.S. El-Azab, Phosphorus Sulfur Silicon Related Elements 183 (2007) 333.
[15] N.S. Habib, K.A. Ismail, A.A. El-Tombary, T. Abdel-Aziem, Pharmazie 55 (2000) 495.
[16] M.A. Al-Omar, A.S. El-Azab, H.A. El-Obeid, S.G.J. Abdel Hamide, Saudi Chemical Society 10 (2006) 113.
[17] A.M. Alafeefy, A.A. Kadi, A.S. El-Azab, S.G.J. Abdel Hamide, M.H. Daba, Archiv de Pharmazie 341 (2008) 377.
[18] A. Kumar, S. Sharma, A. Archana, K. Bajaj, S. Sharma, H. Panwar, T. Singh, V.K. Srivastava, Bioorganic & Medicinal Chemistry 11 (2003) 5293.
[19] V. Alagarsamy, V.R. Solomon, K. Dhanabal, Bioorganic & Medicinal Chemistry 15 (2007) 235.
[20] A.S. El-Azab, E.H. Kamal, S.M. Attia, Monatshefte fuer Chemie 142 (2011) 837.
[21] A.S. El-Azab, E.H. Kamal, Bioorganic & Medicinal Chemistry Letters 22 (2012) 1879.
[22] S.K. Kashaw, V. Kashaw, P. Mishra, N.K. Jain, J.P. Stables, European Journal of Medicinal Chemistry 44 (2009) 4335.
[23] V. Archana, K. Srivastava, A. Kumar, Bioorganic & Medicinal Chemistry 12 (2004) 1257.
[24] A.S. El-Azab, E.H. Kamal, Bioorganic & Medicinal Chemistry Letters 22 (2012) 327.
[25] F.A. Al-Omary, L.A. Abou-Zeid, M.N. Nagi, E.-S.E. Habib, A.A. Abdel-Aziz, A.S. El-Azab, S.G.J. Abdel Hamide, M.A. Al-Omar, A.M. Al-Obaid, H.I. El-Subbagh, Bioorganic & Medicinal Chemistry 18 (2010) 2849.
[26] A.M. Al-Obaid, S.G.J. Abdel Hamide, H.A. El-Kashef, M.A. Al-Omar, A.S. El-Azab, H.A. Al-Khamees, H.I. El-Subbagh, European Journal of Medicinal Chemistry 4 (2009) 2379.
[27] A.S. El-Azab, M.A. Al-Omar, A.A. Abdel-Aziz, N.I. El-Sayed, A.M. Aleisa, M.M. Sayed-Ahmed, S.G. Abdel-Hamid, European Journal of Medicinal Chemistry 45 (2010) 4188.
[28] I.A. Al-Suwaidan, A.M. Alanazi, A.A. Abdel-Aziz, M.A. Mohamed, A.S. El-Azab, Bioorganic & Medicinal Chemistry Letters 23 (2013) 3935.
[29] N.M. Abdel Gawad, H.H. Georgey, R.M. Youssef, N.A. El-Sayed, European Journal of Medicinal Chemistry 45 (2010) 6058.
[30] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo, M. Boyd, Journal of the National Cancer Institute 83 (11) (1991) 757.
[31] M.R. Boyd, K.D. Paull, Drug Development Research 34 (1995) 91.
[32] M.R. Boyd, in: B.A. Teicher, A.A. Paul (Eds.), Anticancer Drug Development Guide, second ed., Humana Press Inc., Totowa, NJ, 2004, pp. 41–62.
[33] R.H. Shoemaker, Nature Reviews Cancer 6 (2006) 813.