Bioorganic & Medicinal Chemistry 19 (2011) 1987–1998

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and antitumor evaluation of phenyl *N*-mustard-quinazoline conjugates

Bhavin Marvania [a,d], Pei-Chih Lee [a], Ravi Chaniyara [a,d], Huajin Dong [b], Sharda Suman [a], Rajesh Kakadiya [a], Ting-Chao Chou [b], Te-Chang Lee [a], Anamik Shah [d], Tsann-Long Su [a,c,*]

[a] *Institute of Biomedical Sciences, Academia Sinica, Taipei 11529, Taiwan*
[b] *Preclinical Pharmacology Core Laboratory, Molecular Pharmacology and Chemistry Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA*
[c] *Graduate Institute of Pharmaceutical Chemistry, China Medical University, Taichung, Taiwan*
[d] *Department of Chemistry, Saurashtra University, Rajkot, Gujarat, India*

ARTICLE INFO

Article history:
Received 13 December 2010
Revised 25 January 2011
Accepted 26 January 2011
Available online 2 February 2011

Keywords:
Anticancer agents
Cell cycle
DNA-directed alkylating agents
DNA interstrand cross-linking agents
Nitrogen mustards

ABSTRACT

A series of *N*-mustard-quinazoline conjugates was synthesized and subjected to antitumor studies. The *N*-mustard pharmacophore was attached at the C-6 of the 4-anilinoquinazolines via a urea linker. To study the structure–activity relationships of these conjugates, various substituents were introduced to the C-4 anilino moiety. The preliminary antitumor studies revealed that these agents exhibited significant antitumor activity in inhibiting various human tumor cell growths in vitro. Compounds **21b**, **21g**, and **21h** were selected for further antitumor activity evaluation against human breast carcinoma MX-1 and prostate PC-3 xenograft in animal model. These agents showed 54–75% tumor suppression with low toxicity (5–7% body-weight changes). We also demonstrate that the newly synthesized compounds are able to induce DNA cross-linking through alkaline agarose gel shift assay and inhibited cell cycle arrest at G2/M phase.

1. Introduction

Designing DNA-directed alkylating agents is one of the most effective strategies to overcome the general drawbacks of DNA alkylating agents. The common drawbacks of these agents include high reactivity and the lack of DNA sequence-specific binding, resulting in side effects and carcinogenicity.[1,2] DNA-directed alkylating agents are synthesized by linking DNA-affinic molecules (carrier) to a *N*-mustard pharmacopore (warhead, such as alkyl *N*-mustard or phenyl *N*-mustard). The generally useful DNA-affinic molecules are DNA-intercalating agents (e.g., 9-anilinoacridines and acridines), binding agents (quinolines or quinazolines), and DNA minor groove binding agents (e.g., distamycin A and netropsin). Most of these carriers also exhibit anticancer activity by inhibiting Topoisomerases I and II.[3] Emerging evidence shows that DNA-directed alkylating agents are more cytotoxic than the used carrier itself. Consequently, connecting DNA-affinic molecules to alkylating agents usually results in improved therapeutic efficacy than the corresponding untargeted alkylating agents.[4–8]

We have previously synthesized a series of alkyl *N*-mustard-9-anilinoacridine conjugates having methylene (CH_2) or alkoxy [$O(CH_2)n$] spacer to the aniline, and/or acridine ring(s).[7–10] Although these conjugates (e.g., **1**, Fig. 1) exhibited significant cytotoxicity against various human tumor cell growth in vitro and potent antitumor activity in human tumor xenografted model, they have a narrow therapeutic window and low bioavailability (chemical instability with a short half-life) in mice, probably due to the inductive effect of the alkoxy linker, increasing the reactivity of the *N*-mustard moiety. To improve the poor bioavailability of these derivatives, we have synthesized a series of phenyl *N*-mustard-9-anilinoacridine conjugates bearing a urea, carbamate or carbonate linker.[11,12] We revealed that these derivatives (e.g., **2**) have more chemical stability with potent anticancer activity. The linkers used for the synthesis of phenyl *N*-mustard-9-anilinoacridine conjugates were previously applied in antibody-directed enzyme prodrug therapy (ADEPT) and melanocyte directed enzyme prodrug therapy (MDEPT) of *N*-mustard derivatives.

More recently, we utilized quinolines as carriers to prepare a series of *N*-mustard-quinoline conjugates having a urea or hydrazinecarboxamide linker (e.g., **3** and **4**).[13] Similarly, these conjugates possess potent antitumor activity against a variety of human tumor xenografts. Both linkers are also able to lower the reactivity of the *N*-mustard moiety, resulting in a longer half-life in rat plasma. The linkers in these derivatives are attached to the C-4 position of the 4-aminoquinolines, demonstrating that quinolines are also valuable carriers for building DNA-directed alkylating agents.

Aside from quinolines, 4-anilinoquinazoline derivatives [e.g., PD153035 (**5**) and EBEA22 (**6**)] were also reported to have high DNA binding affinity.[14] Particularly, compound **6** possesses higher

* Corresponding author. Tel.: +886 2 2789 9045; fax: +886 2 2782 5573.
E-mail address: tlsu@ibms.sinica.edu.tw (T.-L. Su).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.01.055

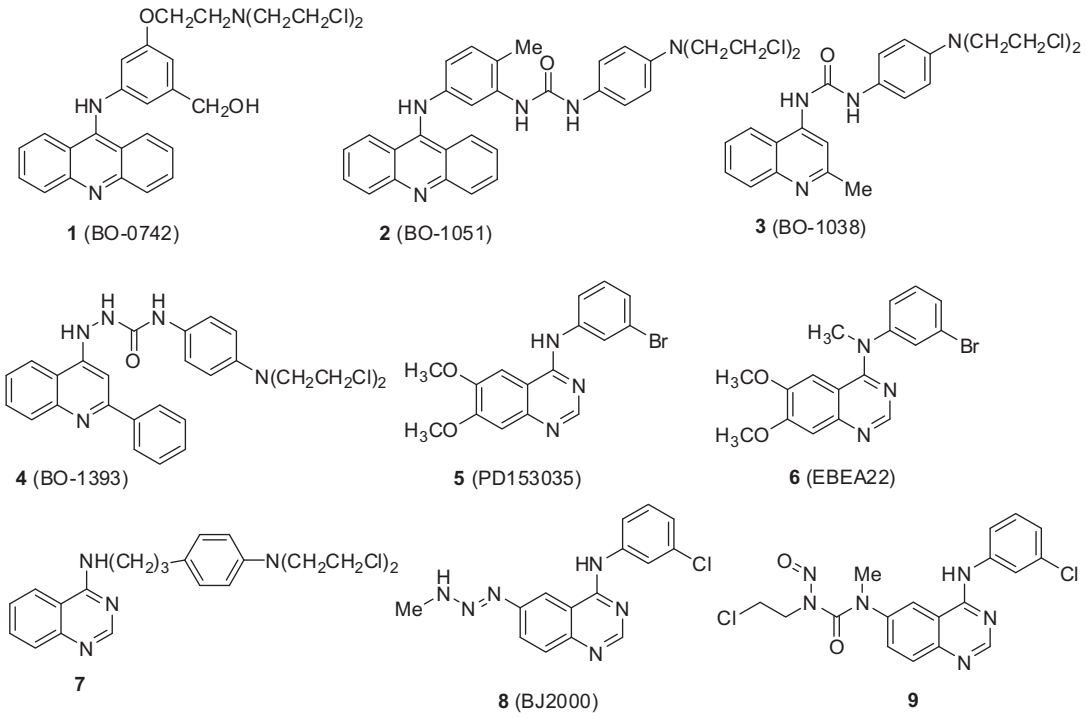


Figure 1. Chemical structures of *N*-mustard and quinazoline derivatives.

GC-selective binding than what was expected. It was also shown that both **5** and **6** have high affinities and selectivity toward the epidermal growth factor receptor tyrosine kinase (EGF-R TK) and exhibited potent cytotoxicity against several types of tumor cell growth in vitro.[15] Although compound **5** possessed potent cytotoxicity, this agent has low therapeutic efficacy in vivo because of its poor water solubility. Quinazolines have also been applied for synthesizing DNA-directed alkylating agents (e.g., **7**, Fig. 1). It was demonstrated that compound **7** is not a strong intercalator, but it may bind weakly at the major groove side.[16] These studies suggest that quinazolines can be applied for designing DNA-directed alkylating agents.

In order to discover new chemically stable DNA-directed alkylating agents, we therefore connected the phenyl *N*-mustard pharmacophore to quinazolines using a urea moiety as the linker. Initially, we attempted to attach the *N*-mustard to the 4-amino function of quinazolines bearing a urea linker; however, the product, *N*-mustard-4-aminoquinazoline **15** (Scheme 1) has very poor solubility, even does not dissolve in DMSO. Consequently, we prepared 6-amino-4-anilinoquinazolines for constructing new phenyl *N*-mustard-6-aminoquinazoline conjugates, in which the *N*-mustard moiety is linked to the 6-amino function via a urea linker. The studies will allow us to understand whether these conjugates have improved water-solubility and cytotoxicity. The results show that the new conjugates have better solubility in an intravenous injection (iv injection) vehicle and possess potent cytotoxicity in inhibiting various human lymphoblastic leukemia and solid tumor cell growth in vitro. It should be noted that some of the 4-anilinoquinazolines have already been used by the group of Jean-Claude to prepare degradable conjugates with various alkylating moieties such as N-nitroso ureas and triazenes (e.g., **8** and **9**).[17] We report herein the chemical synthesis, antitumor activity, and DNA cross-linking study of phenyl *N*-mustard-4-anilinoquinazoline conjugates.

2. Chemistry

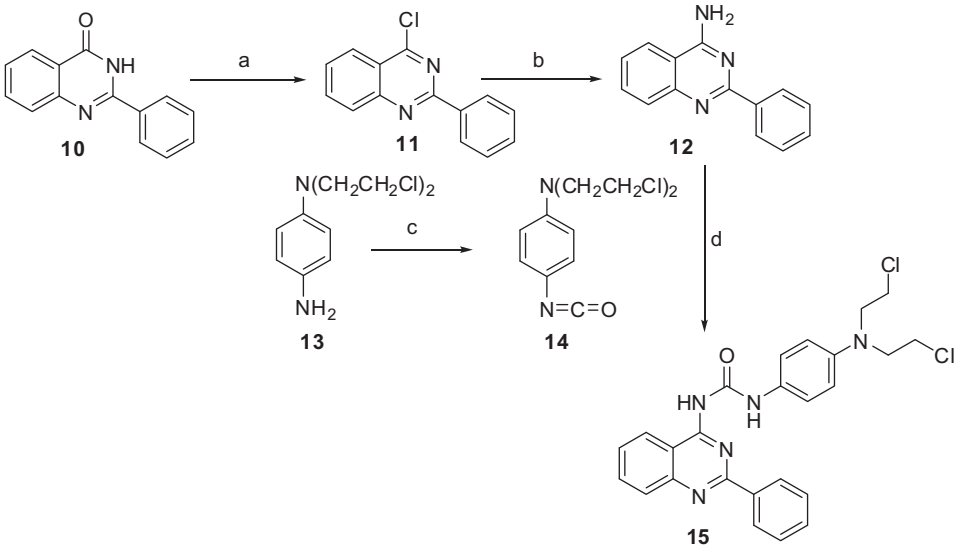
The synthesis of the *N*-mustard-quinazoline conjugates is shown in Schemes 1 and 2. The *N*-mustard-4-aminoquinazoline conjugate **15** was prepared starting from the known compound 2-phenylquinazolin-4(3*H*)-one (**10**, Scheme 1).[18,19] Compound **10** was treated with $POCl_3$ to produce 4-chloro-2-phenyl quinazoline (**11**), which was then reacted with ammonia in phenol at 170 °C to give 2-phenylquinazolin-4-amine (**12**). Reaction of **12** with the known 4-[*N*,*N*-bis(2-chloroethyl)-amino]phenylisocyanate **14**[20] [freshly prepared from *N*,*N*-bis(2-chloroethyl)benzene-1,4-diamine hydrochloride (**13**)[21]] in the presence of triethylamine afforded *N*-mustard-4-aminoquinazoline conjugate **15** in low yield.

Scheme 2 shows the synthesis of the *N*-mustard-6-aminoquinazoline conjugates (**21a–q**). The key starting materials, 6-amino-4-anilinoquinazolines (**20a–q**), were prepared by following the literature methods.[17] Among these derivatives, compounds **20b,c,d,f,h,j,o,p,q** are known, while all other compounds are new derivatives. Briefly, the commercially available 5-nitroanthranilonitrile **16** was treated with dimethylformamide dimethylacetal (DMF–DMA) in acetic acid to give (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide (**17**), which was then reacted with substituted anilines (**18a–q**) in acetic acid to afford 6-nitroquinazoline derivatives (**19a–q**). The nitro function in **19a–q** was converted into the corresponding 6-aminoquinazoline derivatives (**20a–q**) by treating with Fe/acetic acid. Reaction of **20a–q** with the freshly prepared **14** in the presence of triethylamine gave the desired *N*-mustard-6-aminoquinazoline conjugates (**21a–q**) bearing a urea linker.

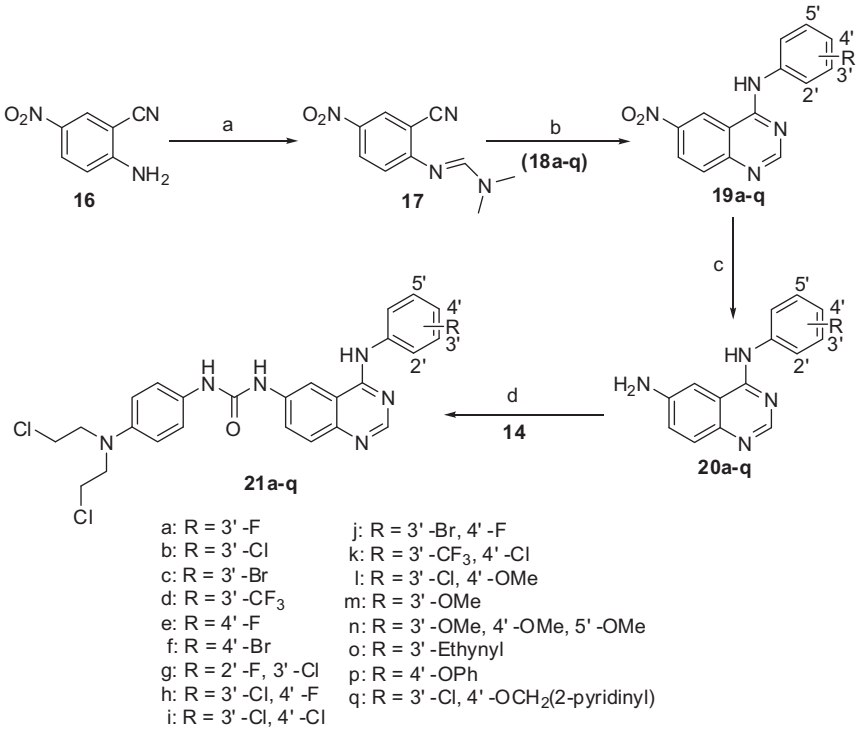
3. Biological result and discussion

3.1. In vitro cytotoxicity

To study the structure-activity relationships of the *N*-mustard-quinazoline conjugates, we have introduced electron-withdrawing halogen(s), electron-donating methoxy function(s), and other substituent to the 4-anilino ring. These derivatives were subjected to evaluating their cytotoxicities in inhibiting human lymphoblastic leukemia (CCRF-CEM) and its drug-resistant sublines resistant to Taxol (CCRF-CEM/Taxol) and Vinblastine (CCRF-CEM/VBL) cell



Scheme 1. Reagents and conditions: (a) $POCl_3$/reflux; (b) phenol/NH_3(g)/170 °C; (c) triphosgene/Et_3N/$CHCl_3$/THF, room temperature; (d) Et_3N/$CHCl_3$, room temperature.

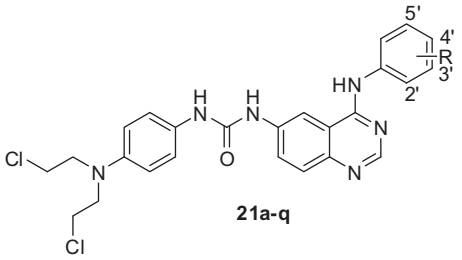


Scheme 2. Reagents and conditions: (a) DMF–DMA/reflux; (b) $ArNH_2$/AcOH/reflux; (c) Fe/AcOH/EtOH/H_2O/reflux; (d) Et_3N/THF, room temperature.

growth in vitro (Table 1). It demonstrated that the newly synthesized conjugates possess significant cytotoxicity with IC_{50} in micro molar range. The most cytotoxic compound of this series is the 3′,4′,5′-trimethoxyphenyl derivative **21n** with an IC_{50} value of 0.38 μM. However, the potency of the C-3′-OMe derivative (**21m**, IC_{50} >1.32 μM) is much weaker than the corresponding trimethoxy derivative **21n**. As for the halogen-substituted derivatives, it is clearly to see that order of the cytotoxicity of C-3′-halogen substituted compounds is C-3′–Br (**21c**) > C-3′–Cl (**21b**) > C-3′–F (**21a**). In contrast, the C-4′–F substituted derivative **21e** is apparently more potent than the corresponding C-4′–Br derivative **21f**. In the series of C-3′–C-4′ dihalogens substituted conjugates, the order of the cytotoxicity is **21g** > **21h** > **21i** ≅ **21j**. The SAR study shows that the position of the substitutions, numbers and types of halogen atom are critical for their activity. The cytotoxicity of compounds bearing other substituent, such as C-3′ or C-4′–CF_3 (**21d** and **21k**, respectively), C-3′-ethynyl (**21o**), C-4′-phenoxy (**21p**), and 3-chloro-4-(pyridin-2-ylmethoxy) (**21q**), were also evaluated. It reveals that C-3′-ethynyl (**21o**) is most cytotoxic among these conjugates with a IC_{50} value of 0.50 μM. Compounds having a CF_3 substituent (**21d** and **21k**) are less cytotoxic than other compounds tested.

The cytotoxicity of the newly synthesized compounds against human CCRF-CEM drug-resistant sublines (resistant to Vinblastine and Taxol, CCRF-CEM/VBL and CCRF-CEM/Taxol, respectively) were also studied. The results revealed that they generally have no or little cross-resistance to these two natural products except compounds **21n**, which has certain extent of cross-resistance

Table 1
The cytotoxicity of newly synthesized phenyl *N*-mustard-6-aminoquinazoline conjugates against human lymphoblastic leukemia (CCRF-CEM) and its drug-resistant sublines (CCRF-CEM/Taxol and CCRF-CEM/VBL)[a]



Compd	Substitute R				Cell growth inhibition (IC_{50} μM)		
	2′	3′	4′	5′	CCRF-CEM	CCRF-CEM/Taxol[b]	CCRF-CEM/VBL[b]
21a	H	F	H	H	2.51 ± 0.11	3.46 ± 0.18 [1.38×][c]	4.28 ± 0.01 [1.70×]
21b	H	Cl	H	H	1.66 ± 0.14	4.56 ± 0.0001 [2.75×]	3.26 ± 0.05 [1.96×]
21c	H	Br	H	H	0.95 ± 0.11	4.58 ± 0.05 [4.82×]	2.62 ± 0.01 [2.76×]
21d	H	CF_3	H	H	3.97 ± 0.22	4.57 ± 0.13 [1.15×]	4.68 ± 0.05 [1.17×]
21e	H	H	F	H	0.74 ± 0.02	3.16 ± 0.07 [4.27×]	1.24 ± 0.03 [1.67×]
21f	H	H	Br	H	4.64 ± 0.16	8.79 ± 0.16 [1.89×]	7.88 ± 0.36 [1.70×]
21g	F	Cl	H	H	0.75 ± 0.01	1.55 ± 0.02 [2.07×]	1.04 ± 0.03 [1.39×]
21h	H	Cl	F	H	1.00 ± 0.09	1.16 ± 0.14 [1.16×]	1.14 ± 0.03 [1.14×]
21i	H	Cl	Cl	H	2.60 ± 0.02	5.61 ± 0.16 [2.16×]	6.75 ± 0.53 [2.60×]
21j	H	Br	F	H	2.68 ± 0.11	4.40 ± 0.30 [1.64×]	4.74 ± 0.15 [1.77×]
21k	H	CF_3	Cl	H	4.77 ± 0.22	5.36 ± 0.14 [1.12×]	5.28 ± 0.58 [1.11×]
21l	H	Cl	OMe	H	0.80 ± 0.01	3.34 ± 0.02 [4.17×]	3.75 ± 0.04 [4.68×]
21m	H	OMe	H	H	1.32 ± 0.02	3.58 ± 0.12 [2.71×]	3.38 ± 0.17 [2.56×]
21n	H	OMe	OMe	OMe	0.38 ± 0.02	12.32 ± 0.79 [32.42×]	24.51 ± 1.72 [64.50×]
21o	H	3-ethynyl	H	H	0.50 ± 0.04	2.29 ± 0.02 [4.58×]	2.33 ± 0.34 [4.66×]
21p	H	H	OPh	H	4.77 ± 0.01	7.99 ± 0.18 [1.67×]	10.88 ± 0.02 [2.28×]
21q	H	Cl	OCH_2(2-pyridinyl)	H	1.41 ± 0.07	4.58 ± 0.08 [3.24×]	2.65 ± 0.13 [1.88×]
Taxol					0.003 ± 0.0003	0.43 ± 0.05 [143×]	1.27 ± 0.05 [423×]
Vinblastine					0.0007 ± 0.001	0.08 ± 0.01 [106.2×]	0.50 ± 0.12 [679.5×]
Carboplatin					3.4 ± 0.99		2.45 ± 0.65 [0.7×]

[a] Cell growth inhibition was measured by the XTT assay[25] for leukemic cells after 72-h incubation using a microplate spectrophotometer as described previously.[27] Similar in vitro results were obtained by using the Cell Counting Kit-8 for the CCK-8 assays as described by technical manual of Dojindo Molecular Technologies, Inc. (Gaithersburg, MD; Website: www.dojindo.com). IC_{50} values were determined from dose–effect relationship at six or seven concentrations of each drug by using the CompuSyn software by Chou and Martin[29] based on the median-effect principle and plot using the serial deletion analysis.[30,31] Ranges given for Taxol and vinblastine were mean ± SE (n = 4).
[b] CCRF-CEM/Taxol and CCRF-CEM/VBL are subcell lines of CCRF-CEM cells that are 143-fold resistant to Taxol, and 423-fold resistant to vinblastine, respectively, when comparing with the IC_{50} of the parent cell line.
[c] Numbers in the brackets are fold of cross-resistant determined by comparison with the corresponding IC_{50} of the parent cell line.

Table 2
The cytotoxicity of phenyl *N*-mustard-6-aminoquinazoline conjugates (**21a–q**) against human solid tumor (breast carcinoma MX-1, colon carcinoma HCT-116, lung carcinoma H1299 and prostate carcinoma PC3) cell growth in vitro[a]

Compd	Substitute R				Cell growth inhibition (IC_{50} μM)			
	2′	3′	4′	5′	MX-1[a]	HCT-116[a]	H1299[b]	PC3[b]
21a	H	F	H	H	10.11 ± 0.09	9.62 ± 0.12	ND	ND
21b	H	Cl	H	H	7.84 ± 0.03	7.46 ± 0.02	10.49 ± 2.04	10.37 ± 0.32
21c	H	Br	H	H	6.92 ± 0.05	2.17 ± 0.03	8.04 ± 1.26	10.13 ± 0.13
21d	H	CF_3	H	H	5.30 ± 0.63	5.97 ± 0.24	ND	ND
21e	H	H	F	H	3.43 ± 0.003	3.18 ± 0.03	11.60 ± 1.54	8.24 ± 1.76
21f	H	H	Br	H	6.71 ± 0.03	7.79 ± 0.36	ND	ND
21g	F	Cl	H	H	4.55 ± 0.02	3.84 ± 0.0005	5.52 ± 1.59	6.29 ± 1.12
21h	H	Cl	F	H	5.38 ± 0.05	3.36 ± 0.05	ND[c]	ND
21i	H	Cl	Cl	H	6.45 ± 0.50	2.79 ± 0.14	ND	ND
21j	H	Br	F	H	7.10 ± 0.14	5.99 ± 0.63	ND	ND
21k	H	CF_3	Cl	H	7.44 ± 0.58	5.34 ± 0.06	ND	ND
21l	H	Cl	OMe	H	5.51 ± 0.09	5.27 ± 0.18	6.94 ± 1.71	8.02 ± 2.08
21m	H	OMe	H	H	2.59 ± 0.13	2.44 ± 0.03	8.79 ± 1.32	9.44 ± 0.87
21n	H	OMe	OMe	OMe	4.02 ± 0.03	2.24 ± 0.01	10.28 ± 1.25	10.98 ± 1.89
21o	H	3-Ethynyl	H	H	6.27 ± 0.24	3.47 ± 0.13	ND	ND
21p	H	H	OPh	H	8.47 ± 0.15	12.41 ± 0.04	ND	ND
21q	H	Cl	OCH_2 (2-pyridinyl)	H	4.62 ± 0.20	2.22 ± 0.54	ND	ND
Cisplatin					4.95 ± 0.60	26.65 ± 4.19	ND	ND

[a] Cell growth inhibition was measured by the SRB assay[26] for solid tumor cells after 72-h incubation using a microplate spectrophotometer as described previously.[27]
[b] Cell growth inhibition was determined by the Alamar blue assay[28] in a 72 h incubation using a microplate spectrophotometer as described previously.
[c] Not determined.

(Table 1). It suggests that the *N*-mustard derivatives were neither a good substrate of *p*-glycoprotein nor mutated tubulin.

The selected compounds were further evaluated for their cytotoxicity in inhibiting other human solid tumors such as human

breast tumor (MX-1), colon cancer (HCT-116), human non-small cell lung cancer (H1299), and prostate cancer (PC3) cell growth in vitro. As shown in Table 2, one can see that these conjugates possess good to moderate cytotoxic effects against the growth of these cell lines tested in vitro. In comparison with the cytotoxicities of the *N*-mustard-quinoline conjugates, previously synthesized in our laboratory,[13] the *N*-mustard-quinazoline conjugates are less potent in inhibiting all tumor cell lines examined.

3.2. In vivo therapeutic activity

The in vitro cytotoxicity of the tested compound may not always directly reflex to its therapeutic efficacy in tumor xenograft model. In the present studies, we selected compounds **21b**, **21g**, and **21h** for evaluating their therapeutic efficacy in nude mice bearing human mammary carcinoma (MX-1) xenografts (Table 3). To find a maximal tolerable dose of compound tested, we administrated various doses to view its therapeutic effects via intravenous injection (iv inj.). The preliminary results show that compound **21h** possessed significant tumor growth inhibition (72%) in comparison with the untreated control when mice were treated successfully with the dose of 50 mg/kg, every two days for three times (Q2D × 3), 60 mg/kg (Q2D × 3), and then 70 mg/kg, every two days for two times (Q2D × 2). With the same drug administration route (iv inj.), compound **21b** showed a moderate tumor inhibition (62%), at the doses of 30 mg/kg (Q2D × 3) and 35 mg/kg, every two days for five times (Q2D × 5). Compound **21g** also showed to have moderate tumor suppression (52%) at the dose of 30 mg/kg (Q2D × 3) and then 40 mg/kg (Q2D × 5). Although all the tested compounds induced a 5–7% body-weight change during the treatment (Table 3), the body-weight of mice readily recovered after cessation of the treatment, suggesting that these agents have relatively low toxicity to the host.

Conjugates **21b** and **21g** were also selected for evaluating their therapeutic efficacy against human prostate PC-3 xenograft in nude mice. Table 3 shows that there were 27% and 69% tumor suppression by **21b** and **21g**, respectively, at the maximal tolerable dose of 55 mg/kg, every two days for six times (Q2D × 6) with acceptable toxicity (about 10–11% body weight loss). Similar observations were found that *N*-mustard-quinazoline conjugates are much less potent against human breast MX-1 xenograft in mice than that of the *N*-mustard-quinoline conjugates.[13]

3.3. DNA cross-linking study by alkaline agarose gel shift assay

The alkaline gel shift assay was performed to assess DNA cross-linking activity of compounds **21g**, **21l**, **21b**, and **21e** (Fig. 2). The pEGFP-N1 plasmid DNA was treated with compounds, **21g**, **21l**, **21b**, and **21e** at various concentrations as indicated (1, 10 and 20 μM). Melphalan was used as a positive control. The tested compounds show moderate cross-linking behavior at lower concentrations; however, at high concentrations, the cross-linking behavior was similar to melphalan. These results revealed that the newly synthesized *N*-mustard-quinazoline conjugates are capable to induce DNA cross-linking.

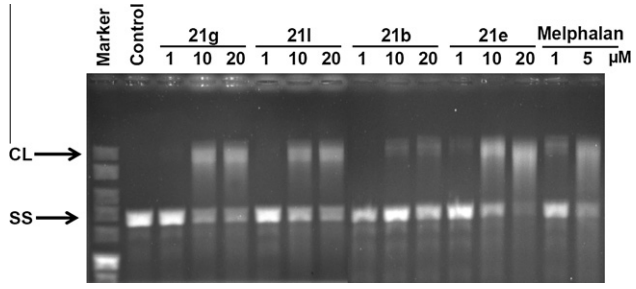


Figure 2. Representative DNA cross-linking gel shift assay for **21g**, **21l**, **21b**, and **21e** at various concentrations as indicated. Control lane shows single-stranded DNA (SS), while cross-linking (CL) shown in all tested lanes is DNA double-stranded cross-linking. Melphalan (1 and 5 μM) was used as a positive control.

3.4. Cell cycle inhibition

It was reported that DNA damage induced by DNA alkylating agents is known to cause cell cycle delay and arrest the cell cycle progression predominantly at the G2/M boundary.[22] We therefore studied the inhibitory effect of **21b** on cell cycle distribution (Table 4). The human non-small cell lung carcinoma H1299 cells were treated with **21b** at the concentrations of 5, 10, and 20 μM for 24 h. The cells were harvested, stained with propidium iodide (PI) and analyzed with a flow cytometer. It clearly shows that 5 μM of **21b** significantly accumulated the cells at G2/M phase, while 10 and 20 μM of **21b** prevented the cell cycle progression, which may be due to high level of DNA cross-linking. Similar G2/M arrest was previously observed in SW626 cells treated with melphalan.[23] Furthermore, increased sub-G1 populations were noticed in cells treated with **21b** at 20 μM.

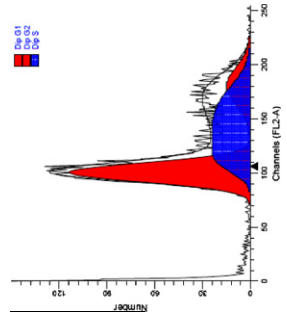
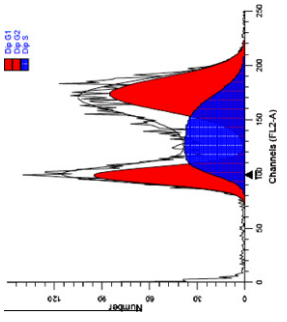
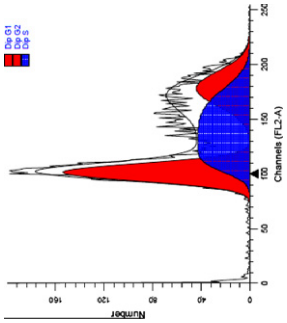
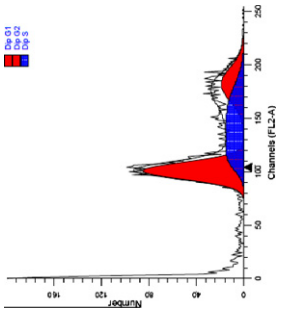
4. Conclusion

Recently, we have synthesized a series of *N*-mustard-quinoline conjugates bearing a urea or hydrazinecarboxamide linker for antitumor evaluation. We demonstrated that these conjugates exhibited potent in vitro cytotoxicity and therapeutic efficacy against human xenografts. To continue our research on the developing DNA-directed alkylating agents, we have synthesized a series of *N*-mustard-quinazoline conjugates, in which the *N*-mustard pharmacophore was attached at the C-6 of the 4-anilinoquinazolines via a urea linker. A variety of substituent(s) were introduced to the C-4 anilino moiety for studying their structure–activity relationships. The preliminary antitumor studies revealed that these agents exhibited significant antitumor activity in inhibiting various human tumor cell growths in vitro. Among compounds selected for evaluating their antitumor activity against human breast tumor (MX-1) xenograft in nude mice, 4-[3′-Cl,4′-F-phenylamino]quinazoline derivative (**21h**) is the most potent. We demonstrate that the newly synthesized *N*-mustard-quinazoline conjugates are generally less potent than the *N*-mustard-quinoline conjugates. Studies on the therapeutic efficacy against MX-1 xenograft in nude mice revealed that the tested compounds have moderate antitumor

Table 3
Therapeutic effects and toxicity of *N*-mustard-quinazoline conjugates in nude mice bearing human mammary carcinoma (MX-1) and prostate (PC-3) xenografts

Tumor used	Compd	Dose and schedule	Maximal tumor suppression (%)	Toxicity body-weight loss (%)	Death
MX-1	**21b**	30 mg/kg (Q2D × 3) and then 35 mg/kg (Q2D × 5)	62	3	0/3
	21g	30 mg/kg (Q2D × 3) and then 40 mg/kg (Q2D × 5)	52	6	0/3
	21h	50 mg/kg (Q2D × 3), 60 mg/kg (Q2D × 3), and then 70 mg/kg (Q2D × 2)	72	5	0/3
PC-3	**21b**	55 mg/kg (Q2D × 6)	27	11	0/5
	21g	55 mg/kg (Q2D × 6)	69	6	0/5

Table 4
Cell cycle inhibition in human non-small cell lung carcinoma H1299 by treating with compound **21b**

Concentration (μM)	0	5	10	20
				
sub G1	8.8 ± 6.9	8.9 ± 0.03	7.5 ± 0.2	35.3 ± 2.2
G1	44.8 ± 3.0	30.5 ± 0.4	34.8 ± 0.4	28.3 ± 1.0
S	24.9 ± 3.3	20.6 ± 4.0	28.5 ± 0.2	23.5 ± 3.5
G2/M	21.5 ± 0.6	40.0 ± 3.5	29.3 ± 0.01	12.9 ± 0.2

activity, but they are less toxic to the host based on the observation of the average body-weight changes. In the present studies we also show that the newly synthesized compounds are able to induce DNA cross-linking through alkaline agarose gel shift assay and inhibited cell cycle arrest at G2/M phase.

5. Experimental

5.1. General methods and materials

Compound solvents and reagents were reagent grade and used without purification unless otherwise noted. The melting points were recorded on a Fargo melting point apparatus and are uncorrected. Column chromatography was carried out on Silica Gel G60 (70–230 mesh, ASTM; Merck and 230–400 mesh, Silicycle Inc.). Reaction progress was monitored using analytical thin-layer chromatography (TLC) on 0.25 mm Merck F-254 silica gel glass plates. Visualization was achieved by UV light (254 nm). ^{1}H NMR spectra were recorded with a Bruker AVANCE 600 DRX and 400 MHz spectrometer. Chemical shifts are reported in parts per million (δ) using tetramethylsilane as the internal standard with coupling constants (J) reported in hertz (Hz). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, double doublet; br s, broad singlet. Elemental analyses were performed on a Heraeus CHN–O Rapid analyzer. High performance liquid chromatography analysis for checking purity of synthesized compounds were recorded on a Hitachi D-2000 Elite instrument: column, Mightysil RP-18 GP 250–4.6 (5 μL) mobile phase, MeCN/THF (70:30 v/v); flow rate, 1 mL/min; injected sample 10 μL, column temp, 27 °C; wavelength, 254 nm. The purity of all compounds was >95% based on analytical HPLC.

5.2. 4-Chloro-2-phenylquinazoline (11)

To a magnetically stirred solution of $POCl_3$ (25 mL) at 0 °C was added portion wise 2-phenylquinazolin-4(3*H*)-one **10** (5.0 g). The reaction mixture was refluxed for 2 h. After completion of the reaction, the excess $POCl_3$ was removed by vacuo. The residue was poured into a mixture of chloroform (50 mL) + ice cold water (80 mL) + ammonia solution (20 mL). The chloroform layer was separated and the aqueous layer was extracted with an additional 20 mL of chloroform. The united chloroform extracts were dried over Na_2SO_4 and filtered, and the solvent was removed by distillation to give **11**, 4.5 g (87%); mp 125–127 °C (lit.[24] 124–125 °C); ^{1}H NMR (DMSO-d_6) δ 7.58–7.69 (4H, m, 4 × ArH), 7.91–7.92 (2H, m, 2 × ArH), 8.16–8.21 (3H, m, 3 × ArH). Anal. ($C_{14}H_9ClN_2$): C, H, N.

5.3. 2-Phenylquinazolin-4-amine (12)

A mixture of 4-chloro-2-phenylquinazolin **11** (3.0 g, 12.0 mmol) and excess phenol was heated at 170 °C for 2 h. After completion of the reaction, the ammonia gas was passed into the reaction mass at 150 °C for 1 h. After that the reaction mixture was cooled to room temperature and poured into 5% sodium hydroxide solution. The solid was filtered and washed with water and dried to give **12**, 2.0 g (74%); mp 140–141 °C (lit.[25] 146–147 °C); ^{1}H NMR (DMSO-d_6) δ 7.45–7.53 (4H, m, 4 × ArH), 7.75–7.79 (2H, m, 2 × ArH), 7.83 (2H, br s, exchangeable, NH_2), 8.23–8.25 (1H, m, ArH), 8.45–8.48 (2H, m, 2 × ArH). Anal. ($C_{14}H_{11}N_3$): C, H, N.

5.4. 1-(4-Bis(2-chloroethyl)phenyl)-3-(2-phenylquinazolin-4-yl) urea (15)

A solution of isocyanate **14** (freshly prepared from **13**, 1.5 g 4.9 mmol) in chloroform (10 mL) was added dropwise to a solution

of 2-phenyl quinazolin-4-amine **12** (0.63 g, 2.8 mmol) in chloroform (30 mL) containing triethylamine (1 mL) at room temperature. The reaction mixture was stirred at room temperature for 1 h. The solid material was fall out from reaction mass. It was filtered and washed with chloroform to give 1-(4-bis(2-chloroethyl)phenyl)-3-(2-phenylquinazolin-4-yl)urea **15**, 0.2 g (17%); mp 263–264 °C; ^{1}H NMR (DMSO-d_6) δ 3.68–3.71 (8H, m, 4 × CH_2), 6.81 (2H, d, J = 8.6 Hz, 2 × ArH), 7.48 (2H, d, J = 8.6 Hz, 2 × ArH), 7.58–7.56 (4H, m, 4 × ArH), 7.94–7.95 (2H, m, 2 × ArH), 8.35–8.37 (2H, m, 2 × ArH), 8.72–8.74 (1H, m, ArH), 10.47, 12.02 (each 1H, s, exchangeable, 2 × NH). Anal. ($C_{25}H_{23}Cl_2N_5O$): C, H, N.

5.5. (*E*)-*N*′-(2-Cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide (17)

5-Nitroanthranilonitrile **16** (20.0 g, 122.5 mmol) was suspended in dimethylformamide dimethylacetal (43 mL, 360.0 mmol). The mixture was heated up to reflux temperature for 1.5 h. The resulting mixture was cooled to room temperature and refrigerated overnight. The yellow precipitated was filtered, washed with ethyl ether to give **17**, 25.0 g (96%); mp 153–154 °C (lit.[17] 153–155 °C); ^{1}H NMR (DMSO-d_6) δ 3.09 (3H, s, Me), 3.17 (3H, s, Me), 7.36–7.39 (1H, m, ArH), 8.25–8.28 (2H, m, 2 × ArH), 8.47–8.48 (1H, m, ArH). Anal. ($C_7H_5N_3O_2$): C, H, N.

5.6. *N*-(3-Fluorophenyl)-6-nitroquinazolin-4-amine (19a)

To a solution of (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (6.0 g, 2.7 mmol) and acetic acid (45 mL) was added 3-fluoroaniline (**18a**, 3.3 g, 3.0 mmol) at room temperature. The reaction mixture was heated up to reflux temperature for 1 h. After completion of the reaction, the resulting mixture was cooled to room temperature. The solid separated was filtered and washed with ether to give **19a**, 6.5 g (83%); mp 255–256 °C; ^{1}H NMR (DMSO-d_6) δ 6.98–7.03 (1H, m, ArH), 7.43–7.48 (1H, m, ArH), 7.68 (1H, d, J = 9.2 Hz, ArH), 7.89–7.95 (2H, m, 2 × ArH), 8.55 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.77 (1H, s, ArH), 9.65 (1H, d, J = 2.2 Hz, ArH), 10.48 (1H, s, exchangeable, NH). Anal. ($C_{14}H_9FN_4O_2$): C, H, N.

By following the same procedure as that for **19a** the following compound were synthesized.

5.6.1. *N*-(3-Chlorophenyl)-6-nitroquinazolin-4-amine (19b)

Compound **19b** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (5.0 g, 2.2 mmol) and 3-chloroaniline (**18b**, 3.2 g, 2.5 mmol) in acetic acid (40 mL): yield 6.2 g (90%); mp 285–286 °C (lit.[17] 278–281 °C); ^{1}H NMR (DMSO-d_6) δ 7.22–7.47 (1H, m, ArH), 7.43–7.47 (1H, m, ArH), 7.83–7.85 (1H, m, ArH), 7.95 (1H, d, J = 9.2 Hz, ArH), 8.07–8.08 (1H, m, ArH), 8.56 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.78 (1H, s, ArH), 9.65 (1H, d, J = 2.2 Hz, ArH), 10.48 (1H, s, exchangeable, NH). Anal. ($C_{14}H_9ClN_4O_2$): C, H, N.

5.6.2. *N*-(3-Bromophenyl)-6-nitroquinazolin-4-amine (19c)

Compound **19c** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (5.0 g, 2.2 mmol) and 3-bromoaniline (**18c**, 4.3 g, 2.5 mmol) in acetic acid (40 mL): yield 7.2 g (91%); mp 282–283 °C (lit.[17] 267–270 °C); ^{1}H NMR (DMSO-d_6) δ 7.35–7.41 (2H, m, 2 × ArH), 7.90–7.92 (1H, m, ArH), 7.94 (1H, d, J = 9.1 Hz, ArH), 8.18–8.19 (1H, m, ArH), 8.56 (1H, dd, J = 2.2 Hz, J = 9.1 Hz, ArH), 8.77 (1H, s, ArH), 9.64 (1H, d, J = 2.2 Hz, ArH), 10.46 (1H, s, exchangeable, NH). Anal. Calcd for $C_{14}H_9BrN_4O_2$: C, H, N.

5.6.3. *N*-(3-(Trifluoromethyl)phenyl)-6-nitroquinazolin-4-amine (19d)

Compound **19d** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (5.0 g, 2.2 mmol) and 3-(trifluoromethyl)aniline (**18d**, 4.0 g, 2.5 mmol) in acetic acid (40 mL): yield 6.5 g (85%); mp 210–211 °C (lit.[26] 209–211 °C); ^{1}H NMR (DMSO-d_6) δ 7.52–7.54 (1H, m, ArH), 7.65–7.69 (1H, m, ArH), 7.97 (1H, d, J = 9.1 Hz, ArH), 8.25–8.29 (2H, m, 2 × ArH), 8.59 (1H, dd, J = 2.2 Hz, J = 9.1 Hz, ArH), 8.79 (1H, s, ArH), 9.67 (1H, d, J = 2.2 Hz, ArH), 10.61 (1H, s, exchangeable, NH). Anal. ($C_{15}H_9F_3N_4O_2$): C, H, N.

5.6.4. *N*-(4-Fluorophenyl)-6-nitroquinazolin-4-amine (19e)

Compound **19e** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (4.0 g, 1.5 mmol) and 4-fluoroaniline (**18e**, 2.2 g, 2.0 mmol) in acetic acid (30 mL): yield 5.0 g (96%); mp 257–258 °C; ^{1}H NMR (DMSO-d_6) δ 7.28–7.32 (2H, m, 2 × ArH), 7.84–7.88 (2H, m, 2 × ArH), 7.96 (1H, d, J = 9.2 Hz, ArH), 8.58 (1H, dd, J = 2.5 Hz, J = 9.2 Hz, ArH), 8.72 (1H, s, ArH), 9.66 (1H, d, J = 2.5 Hz, ArH), 10.51 (1H, s, exchangeable, NH). Anal. ($C_{14}H_9FN_4O_2$): C, H, N.

5.6.5. *N*-(4-Bromophenyl)-6-nitroquinazolin-4-amine (19f)

Compound **19f** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (3.0 g, 1.3 mmol) and 4-bromoaniline (**18f**, 2.6 g, 1.5 mmol) in acetic acid (25 mL): yield 4.0 g (84%); mp 279–280 °C; ^{1}H NMR (DMSO-d_6) δ 7.62–7.65 (2H, m, 2 × ArH), 7.86–7.89 (2H, m, 2 × ArH), 7.97 (1H, d, J = 9.2 Hz, ArH), 8.57–8.60 (1H, dd, J = 2.4 Hz, J = 9.2 Hz, ArH), 8.76 (1H, s, ArH), 9.67 (1H, d, J = 2.4 Hz, ArH), 10.51 (1H, s, exchangeable, NH). Anal. ($C_{14}H_9BrN_4O_2$): C, H, N.

5.6.6. *N*-(2-Fluoro-3-chlorophenyl)-6-nitroquinazolin-4-amine (19g)

Compound **19g** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (5.0 g, 2.2 mmol) and 2-fluoro-3-chloroaniline (**18g**, 3.6 g, 2.5 mmol) in acetic acid (35 mL): yield 5.5 g (75%); mp 224–226 °C; ^{1}H NMR (DMSO-d_6) δ 7.30–7.34 (1H, m, ArH), 7.52–7.56 (2H, m, 2 × ArH), 7.74–8.01 (1H, m, ArH), 8.57–8.65 (2H, m, 2 × ArH), 9.55 (1H, s, ArH), 10.73 (1H, s, exchangeable, NH). Anal. ($C_{14}H_8ClFN_4O_2$): C, H, N.

5.6.7. *N*-(3-Chloro-4-fluorophenyl)-6-nitroquinazolin-4-amine (19h)

Compound **19h** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (3.0 g, 1.3 mmol) and 3-chloro-4-fluoroaniline (**18h**, 2.2 g, 1.5 mmol) in acetic acid (20 mL): yield 4.0 g (91%); mp 280–281 °C (lit.[27] 274–277 °C); ^{1}H NMR (DMSO-d_6) δ 7.45–7.50 (1H, m, ArH), 7.81–7.83 (1H, m, ArH), 7.94 (1H, d, J = 9.2 Hz, ArH), 8.14–8.17 (1H, m, ArH), 8.55 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.74 (1H, s, ArH), 9.60 (1H, d, J = 2.2 Hz, ArH), 10.50 (1H, s, exchangeable, NH). Anal. ($C_{14}H_8ClFN_4O_2$): C, H, N.

5.6.8. *N*-(3,4-Dichlorophenyl)-6-nitroquinazolin-4-amine (19i)

Compound **19i** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (6.0 g, 2.7 mmol) and 3,4-dichloroaniline (**18i**, 4.9 g, 3.0 mmol) in acetic acid (45 mL): yield 8.0 g (86%); mp 297–298 °C; ^{1}H NMR (DMSO-d_6) δ 7.65–7.67 (1H, m, ArH), 7.89–7.96 (2H, m, 2 × ArH), 8.27–8.28 (1H, m, ArH), 8.54–8.57 (1H, m, ArH), 8.79 (1H, s, ArH), 9.61–9.62 (1H, m, ArH), 10.49 (1H, s, exchangeable, NH). Anal. ($C_{14}H_8Cl_2N_4O_2$): C, H, N.

5.6.9. *N*-(3-Bromo-4-fluorophenyl)-6-nitroquinazolin-4-amine (19j)

Compound **19j** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (4.0 g, 1.5 mmol) and 3-bromo-4-fluoroaniline (**18j**, 4.0 g, 2.0 mmol) in acetic acid (30 mL): yield 6.0 g (90%); mp 260–261 °C (lit.[27] 257–258 °C); ^{1}H

NMR (DMSO-d_6) δ 7.43–7.47 (1H, m, ArH), 7.88–7.89 (1H, m, ArH), 7.95 (1H, d, J = 9.2 Hz, ArH), 8.25–8.26 (1H, m, ArH), 8.56 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.75 (1H, s, ArH), 9.61 (1H, d, J = 2.2 Hz, ArH), 10.48 (1H, s, exchangeable, NH). Anal. ($C_{14}H_8BrFN_4O_2$): C, H, N.

5.6.10. *N*-(4-Chloro-3-(trifluoromethyl)phenyl)-6-nitroquinazolin-4-amine (19k)

Compound **19k** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (3.0 g, 1.3 mmol) and 4-chloro-3-(trifluoromethyl)aniline (**18k**, 2.8 g, 1.4 mmol) in acetic acid (30 mL): yield 4.5 g (90%); mp 221–222 °C; ^{1}H NMR (DMSO-d_6) δ 7.77–7.79 (1H, m, ArH), 7.98 (1H, d, J = 9.2 Hz, ArH), 8.32–8.34 (1H, m, ArH), 8.43–8.44 (1H, m, ArH), 8.58 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.81 (1H, s, ArH), 9.63 (1H, s, J = 2.2 Hz, ArH), 10.53 (1H, s, exchangeable, NH). Anal. ($C_{15}H_8ClF_3N_4O_2$): C, H, N.

5.6.11. *N*-(3-Chloro-4-methoxyphenyl)-6-nitroquinazolin-4-amine (19l)

Compound **19l** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (5.0 g, 2.2 mmol) and 3-chloro-4-methoxyaniline (**18l**, 3.9 g, 2.5 mmol) in acetic acid (40 mL): yield 6.8 g (91%); mp 290–291 °C; ^{1}H NMR (DMSO-d_6) δ 3.90 (3H, s, Me), 7.21–7.23 (1H, m, ArH), 7.75–7.77 (1H, m, ArH), 7.91 (1H, d, J = 9.2 Hz, ArH), 7.99–8.00 (1H, m, ArH), 8.52 (1H, dd, J = 2.4 Hz, J = 9.2 Hz, ArH), 8.70 (1H, s, ArH), 9.60 (1H, d, J = 2.4 Hz, ArH), 10.38 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{11}ClN_4O_3$): C, H, N.

5.6.12. *N*-(3-Methoxyphenyl)-6-nitroquinazolin-4-amine (19m)

Compound **19m** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (5.0 g, 2.2 mmol) and 3-methoxyaniline (**18m**, 3.0 g, 2.5 mmol) in acetic acid (35 mL): yield 6.0 g (89%); mp 241–242 °C; ^{1}H NMR (DMSO-d_6) δ 3.82 (3H, s, Me), 6.78–6.81 (1H, m, ArH), 7.32–7.37 (1H, m, ArH), 7.49–7.53 (2H, m, 2 × ArH), 7.93 (1H, d, J = 9.2 Hz, ArH), 8.54–8.57 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.74 (1H, s, ArH), 9.66 (1H, d, J = 2.2 Hz, ArH), 10.38 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{12}N_4O_3$): C, H, N.

5.6.13. *N*-(3,4,5-Trimethoxyphenyl)-6-nitroquinazolin-4-amine (19n)

Compound **19n** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (5.0 g, 2.2 mmol) and 3,4,5-trimethoxyaniline (**18n**, 4.6 g, 2.5 mmol) in acetic acid (40 mL): yield 5.5 g (68%); mp 274–275 °C; ^{1}H NMR (DMSO-d_6) δ 3.81 (3H, s, Me), 3.69 (6H, s, 2 × Me), 7.27 (2H, s, 2 × ArH), 7.93 (1H, d, J = 9.2 Hz, ArH), 8.56 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.72 (1H, s, ArH), 9.64 (1H, d, J = 2.2 Hz, ArH), 10.32 (1H, s, exchangeable, NH). Anal. ($C_{17}H_{16}N_4O_5$): C, H, N.

5.6.14. *N*-(3-Ethynylphenyl)-6-nitroquinazolin-4-amine (19o)

Compound **19o** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (3.0 g, 1.3 mmol) and 3-ethynylaniline (**18o**, 1.6 g, 1.3 mmol) in acetic acid (30 mL): yield 3.8 g (95%); mp 271–272 °C; ^{1}H NMR (DMSO-d_6) δ 4.25 (1H, s, CH), 7.30–7.32 (1H, m, ArH), 7.44–7.48 (1H, m, ArH), 7.92–7.95 (2H, m, 2 × ArH), 8.05–8.06 (1H, m, ArH), 8.54–8.57 (1H, m, ArH), 8.76 (1H, s, ArH), 9.65–9.66 (1H, m, ArH), 10.48 (1H, s, exchangeable, NH). Anal. (C16H10N4O$_2$·0.5): C, H, N.

5.6.15. *N*-(4-Phenoxyphenyl)-6-nitroquinazolin-4-amine (19p)

Compound **19p** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformimidamide **17** (5.0 g, 2.2 mmol) and 4-phenoxyaniline (**18p**, 4.6 g, 2.5 mmol) in acetic acid (40 mL): yield 6.0 g (73%); mp 296–298 °C (lit.[27] 293–294 °C); ^{1}H NMR (DMSO-d_6) δ 7.06–7.08 (2H, m, 2 × ArH), 7.11–7.13 (2H, m, 2 × ArH), 7.17–7.19 (1H, m, ArH), 7.42–7.46 (2H, m, 2 × ArH), 7.85–7.87 (2H, m, 2 × ArH), 7.94 (1H, d, J = 9.2 Hz, ArH), 8.57 (1H, dd, J = 2.4 Hz, J = 9.2 Hz, ArH), 8.71 (1H, s, ArH), 9.67 (1H, d, J = 2.4 Hz, ArH), 10.55 (1H, s, exchangeable, NH). Anal. ($C_{20}H_{14}N_4O_3$): C, H, N.

5.6.16. *N*-(3-Chloro-4-(pyridin-2-ylmethoxy)phenyl)-6-nitroquinazolin-4-amine (19q)

Compound **19q** was synthesized from (*E*)-*N*-(2-cyano-4-nitrophenyl)- *N*,*N*-dimethylformimidamide **17** (3.0 g, 1.3 mmol) and 3-chloro-4-(pyridine-2-ylmethoxy)aniline (**18q**, 3.2 g, 1.3 mmol) in acetic acid (30 mL): yield 5.0 g (89%); mp 241–242 °C; ^{1}H NMR (DMSO-d_6) δ 5.32 (2H, s, CH_2), 7.30–7.33 (1H, m, ArH), 7.39–7.40 (1H, m, ArH), 7.60–7.62 (1H, m, ArH), 7.74–7.75 (1H, m, ArH), 7.88–7.95 (2H, m, 2 × ArH), 8.04–8.05 (1H, m, ArH), 8.55–8.57 (1H, m, ArH), 8.62–7.63 (1H, m, ArH), 8.73 (1H, s, ArH), 9.62–9.63 (1H, m, ArH), 10.43 (1H, s, exchangeable, NH). Anal. ($C_{20}H_{14}ClN_5O_3·2H_2O$): C, H, N.

5.7. N^4-(3-Fluorophenyl)quinazolin-4,6-diamine (20a)

A mixture of *N*-(3-fluorophenyl)-6-nitroquinazolin-4-amine **19a** (6.0 g, 21.1 mmol) and iron (8.13 g, 147.8 mmol) were suspended in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (16.3 mL, 295.4 mmol). The mixture was heated up to reflux temperature for 2 h. After completion of the reaction, the reaction mixture was cooled to room temperature and alkalinized by addition of concentrated ammonia solution (120 mL). The insoluble material was removed by filtration through Celite, and the filtrate was evaporated under reduce pressure. The resulting solid was washed with 10% K_2CO_3 solution and finally with water and dried to give **20a**, 4.0 g, (75%); mp 188–189 °C; ^{1}H NMR (DMSO-d_6) δ 5.62 (2H, s, exchangeable, NH_2), 6.84–6.88 (1H, m, ArH), 7.25–7.28 (1H, m, ArH), 7.35–7.40 (2H, m, 2 × ArH), 7.54–7.56 (1H, m, ArH), 7.67–7.69 (1H, m, ArH), 7.93–7.96 (1H, m, ArH), 8.39 (1H, s, ArH), 9.47 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{11}FN_4·0.8H_2O$): C, H, N.

By following the same procedure as that for **20a** the following compound were synthesized.

5.7.1. N^4-(3-Chlorophenyl)quinazolin-4,6-diamine (20b)

Compound **20b** was synthesized from *N*-(3-chlorophenyl)-6-nitroquinazolin-4-amine **19b** (5.0 g, 16.6 mmol) and iron (6.3 g, 116.2 mmol) in aqueous ethanol (400 mL, 70% v/v) containing acetic acid (13.3 mL, 232.4 mmol): yield 3.5 g (78%); mp 175–176 °C (lit.[17] 186–189 °C); ^{1}H NMR (DMSO-d_6) δ 5.62 (2H, s, exchangeable, NH_2), 7.09–7.11 (1H, m, ArH), 7.25–7.28 (1H, m, ArH), 7.34–7.39 (2H, m, 2 × ArH), 7.54–7.56 (1H, m, ArH), 7.82–7.85 (1H, m, ArH), 8.12–8.13 (1H, m, ArH), 8.39 (1H, s, ArH), 9.46 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{11}ClN_4$): C, H, N.

5.7.2. N^4-(3-Bromophenyl)quinazolin-4,6-diamine (20c)

Compound **20c** was synthesized from *N*-(3-bromophenyl)-6-nitroquinazolin-4-amine **19c** (6.0 g, 17.3 mmol) and iron (6.69 g, 121.7 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (13.9 mL, 243.4 mmol): yield 4.2 g (78%); mp 204–206 °C (lit.[17] 203–204 °C); ^{1}H NMR (DMSO-d_6) δ 5.62 (2H, s, exchangeable, NH_2), 7.22–7.35 (4H, m, 4 × ArH), 7.54–7.56 (1H, m, ArH), 7.88–7.90 (1H, m, ArH), 8.24–8.25 (1H, m, ArH), 8.38 (1H, s, ArH), 9.44 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{11}BrN_4$): C, H, N.

5.7.3. N^4-(3-(Trifluoromethyl)phenyl)quinazolin-4,6-diamine (20d)

Compound **20d** was synthesized from *N*-(3-(trifluoromethyl)phenyl)-6-nitroquinazolin-4-amine **19d** (6.0 g, 17.9 mmol) and iron (6.9 g, 125.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (14.3 mL, 250.0 mmol): yield 3.4 g (63%);

mp 174–175 °C; ^{1}H NMR (DMSO-d_6) δ 5.66 (2H, s, exchangeable, NH_2), 7.29–7.31 (1H, m, ArH), 7.39–7.42 (2H, m, 2 × ArH), 7.58–7.63 (2H, m, 2 × ArH), 8.24–8.26 (1H, m, ArH), 8.37–8.38 (1H, m, ArH), 8.42 (1H, s, ArH), 9.62 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{11}F_3N_4$): C, H, N.

5.7.4. N^4-(4-Fluorophenyl)quinazolin-4,6-diamine (20e)

Compound **20e** was synthesized from *N*-(4-fluorophenyl)-6-nitroquinazolin-4-amine **19e** (4.0 g, 14.0 mmol) and iron (5.4 g, 98.5 mmol) in aqueous ethanol (400 mL, 70% v/v) containing acetic acid (11.2 mL, 196.0 mmol): yield 3.5 g (97%); mp 185–186 °C; ^{1}H NMR (DMSO-d_6) δ 5.60 (2H, s, exchangeable, NH_2), 7.30–7.32 (2H, m, 2 × ArH), 7.40–7.56 (3H, m, 3 × ArH), 7.71–7.73 (2H, m, 2 × ArH), 8.30 (1H, s, ArH), 9.29 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{11}FN_4{\cdot}1.3H_2O$): C, H, N.

5.7.5. N^4-(4-Bromophenyl)quinazolin-4,6-diamine (20f)

Compound **20f** was synthesized from *N*-(4-bromophenyl)-6-nitroquinazolin-4-amine **19f** (4.0 g, 11.5 mmol) and iron (4.4 g, 80.5 mmol) in aqueous ethanol (400 mL, 70% v/v) containing acetic acid (5 mL, 162.0 mmol): yield 3.2 g (88%); mp 210–211 °C; ^{1}H NMR (DMSO-d_6) δ 5.63 (2H, s, exchangeable, NH_2), 7.27–7.29 (2H, m, 2 × ArH), 7.53–7.56 (3H, m, 3 × ArH), 7.90–7.92 (2H, m, 2 × ArH), 8.38 (1H, s, ArH), 9.45 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{11}BrN_4{\cdot}0.2H_2O$): C, H, N.

5.7.6. N^4-(2-Fluoro-3-chlorophenyl)quinazolin-4,6-diamine (20g)

Compound **20g** was synthesized from *N*-(2-fluoro-3-chlorophenyl)-6-nitroquinazolin-4-amine **19g** (5.0 g, 15.7 mmol) and iron (6.0 g, 110.0 mmol) in aqueous ethanol (500 mL, 70% v/v) containing acetic acid (12.5 mL, 219.0 mmol): yield 3.0 g (67%); mp 257–258 °C; ^{1}H NMR (DMSO-d_6) δ 5.65 (2H, s, exchangeable, NH_2), 7.25–7.27 (3H, m, 3 × ArH), 7.43–7.44 (1H, m, ArH), 7.54–7.56 (2H, m, 2 × ArH), 8.24 (1H, s, ArH), 9.42 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{10}ClFN_4$): C, H, N.

5.7.7. N^4-(3-Chloro-4-fluorophenyl)quinazolin-4,6-diamine (20h)

Compound **20h** was synthesized from *N*-(3-chloro-4-fluorophenyl)-6-nitroquinazolin-4-amine **19h** (2.0 g, 6.2 mmol) and iron (2.4 g, 44.0 mmol) in aqueous ethanol (200 mL, 70%v/v) containing acetic acid (5 mL, 57.9 mmol): yield 1.4 g (83%); mp 255–256 °C (lit.[27] 263–265 °C); ^{1}H NMR (DMSO-d_6) δ 5.62 (2H, s, exchangeable, NH_2), 7.24–7.27 (1H, m, ArH), 7.34–7.35 (1H, m, ArH), 7.38–7.43 (1H, m, ArH), 7.53–7.54 (1H, m, ArH), 7.83–7.84 (1H, m, ArH), 8.22–8.22 (1H, m, ArH), 8.36 (1H, s, ArH), 9.50 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{10}ClFN_4$): C, H, N.

5.7.8. N^4-(3,4,-Dichlorophenyl)quinazolin-4,6-diamine (20i)

Compound **20i** was synthesized from *N*-(3,4,-dichlorophenyl)-6-nitroquinazolin-4-amine **19i** (7.0 g, 20.8 mmol) and iron (8.0 g, 146.2 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (16.6 mL, 291.0 mmol): yield 5.5 g (86%); mp 243–244 °C; ^{1}H NMR (DMSO-d_6) δ 5.66 (2H, s, exchangeable, NH_2), 7.27–7.30 (1H, m, ArH), 7.34–7.35 (1H, m, ArH), 7.56–7.61 (2H, m, 2 × ArH), 7.91–7.93 (1H, m, ArH), 8.34–8.35 (1H, m, ArH), 8.42 (1H, s, ArH), 9.55 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{10}Cl_2N_4$): C, H, N.

5.7.9. N^4-(3-Bromo-4-fluorophenyl)quinazolin-4,6-diamine (20j)

Compound **20j** was synthesized from *N*-(3-bromo-4-fluorophenyl)-6-nitroquinazolin-4-amine **19j** (6.0 g, 16.5 mmol) and iron (6.36 g, 115.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (13.2 mL, 231.0 mmol): yield 3.5 g (64%); mp 225–226 °C (lit.[27] 224–225 °C); ^{1}H NMR (DMSO-d_6) δ 5.61 (2H, s, exchangeable, NH_2), 7.25–7.27 (1H, m, ArH), 7.31–7.32 (1H, m, ArH), 7.35–7.40 (1H, m, ArH), 7.53–7.55 (1H, m, ArH), 7.88–7.89 (1H, m, ArH), 8.30–8.31 (1H, m, ArH), 8.36 (1H, s, ArH), 9.45 (1H, s, exchangeable, NH). Anal. ($C_{14}H_{10}BrFN_4$): C, H, N.

5.7.10. N^4-(4-Chloro-3-(trifluoromethyl)phenyl)quinazolin-4,6-diamine (20k)

Compound **20k** was synthesized from *N*-(4-chloro-3-(trifluoromethyl)phenyl)-6-nitroquinazolin-4-amine **19k** (4.0 g, 10.8 mmol) and iron (4.2 g, 75.8 mmol) in aqueous ethanol (400 mL, 70% v/v) containing acetic acid (8.6 mL, 151.0 mmol): yield 2.0 g (56%); mp 265–266 °C; ^{1}H NMR (DMSO-d_6) δ 5.67 (2H, s, exchangeable, NH_2), 7.27–7.29 (1H, m, ArH), 7.33–7.34 (1H, m, ArH), 7.56–7.58 (1H, m, ArH), 7.68–7.70 (1H, m, ArH), 8.29–8.31 (1H, m, ArH), 8.40–8.41 (1H, m, ArH), 8.48 (1H, s, ArH), 9.69 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{10}ClF_3N_4$): C, H, N.

5.7.11. N^4-(3-Chloro-4-methoxyphenyl)quinazolin-4,6-diamine (20l)

Compound **20l** was synthesized from *N*-(3-chloro-4-methoxyphenyl)-6-nitroquinazolin-4-amine **19l** (6.0 g, 18.0 mmol) and iron (6.9 g, 126.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (14.4 mL, 252.0 mmol): yield 3.7 g (69%); mp 235–237 °C; ^{1}H NMR (DMSO-d_6) δ 3.58 (3H, s, Me), 5.57 (2H, s, exchangeable, NH_2), 7.15–7.16 (1H, m, ArH), 7.24–7.26 (1H, m, ArH), 7.33–7.34 (1H, m, ArH), 7.52–7.54 (1H, m, ArH), 7.73–7.54 (1H, m, ArH), 8.02–8.03 (1H, m, ArH), 8.32 (1H, s, ArH), 9.32 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{13}ClN_4O$): C, H, N.

5.7.12. N^4-(3-Methoxyphenyl)quinazolin-4,6-diamine (20m)

Compound **20m** was synthesized from *N*-(3-methoxyphenyl)-6-nitroquinazolin-4-amine **19m** (5.5 g, 18.5 mmol) and iron (7.2 g, 130.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (14.8 mL, 260.0 mmol): yield 3.2 g (75%); mp 182–183 °C; ^{1}H NMR (DMSO-d_6) δ 3.77 (3H, s, Me), 5.57 (2H, s, exchangeable, NH_2), 6.63–6.66 (1H, m, ArH), 7.23–7.27 (2H, m, 2 × ArH), 7.36–7.37 (1H, m, ArH), 7.49–7.58 (3H, m, 3 × ArH), 8.35 (1H, s, ArH), 9.28 (1H, s, exchangeable, NH). Anal. ($C_{15}H_{14}N_4O{\cdot}0.2H_2O$): C, H, N.

5.7.13. N^4-(3,4,5-Trimethoxyphenyl)quinazolin-4,6-diamine (20n)

Compound **20n** was synthesized from *N*-(3,4,5-trimethoxyphenyl)-6-nitroquinazolin-4-amine **19n** (5.0 g, 14.0 mmol) and iron (5.4 g, 98.3 mmol) in aqueous ethanol (500 mL, 70% v/v) containing acetic acid (11.2 mL, 196.5 mmol): yield 2.9 g (64%); mp 220–221 °C; ^{1}H NMR (DMSO-d_6) δ 3.66 (3H, s, Me), 3.79 (6H, s, 2 × Me), 5.54 (2H, s, exchangeable, NH_2), 7.22–7.25 (1H, m, ArH), 7.34–7.35 (3H, m, 3 × ArH), 7.51–7.53 (1H, m, ArH), 8.33 (1H, s, ArH), 9.19 (1H, s, exchangeable, NH). Anal. ($C_{17}H_{18}N_4O_3$): C, H, N.

5.7.14. N^4-(3-Ethynylphenyl)quinazolin-4,6-diamine (20o)

Compound **20o** was synthesized from *N*-(3-ethynylphenyl)-6-nitroquinazolin-4-amine **19o** (4.5 g, 15.5 mmol) and iron (5.9 g, 108.0 mmol) in aqueous ethanol (450 mL, 70% v/v) containing acetic acid (12.4 mL, 217.0 mmol): yield 2.5 g (62%); mp 110–111 °C; ^{1}H NMR (DMSO-d_6) δ 4.17 (1H, s, CH), 5.60 (2H, s, exchangeable, NH_2), 7.16–7.18 (1H, m, ArH), 7.24–7.27 (1H, m, ArH), 7.35–7.39 (2H, m, 2 × ArH), 7.53–7.56 (1H, m, ArH), 7.90–7.92 (1H, m, ArH), 8.08–8.09 (1H, m, ArH), 8.37 (1H, s, ArH), 9.39 (1H, s, exchangeable, NH). Anal. ($C_{16}H_{12}N_4{\cdot}1.2H_2O$): C, H, N.

5.7.15. N^4-(4-Phenoxyphenyl)quinazolin-4,6-diamine (20p)

Compound **20p** was synthesized from *N*-(4-phenoxyphenyl)-6-nitroquinazolin-4-amine **19p** (6.0 g, 16.7 mmol) and iron (6.4 g, 117.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing ace-

tic acid (13.3 mL, 233.0 mmol): yield 4.2 g (64%); mp 100–101 °C (lit.[27] 89–90 °C); ^{1}H NMR (DMSO-d_6) δ 5.40 (2H, s, exchangeable, NH_2), 6.97–6.99 (2H, m, 2 × ArH), 7.09–7.11 (2H, m, 2 × ArH), 7.30–7.39 (3H, m, 3 × ArH), 7.47–7.49 (2H, m, 2 × ArH), 7.67–7.78 (3H, m, 3 × ArH), 8.51 (1H, s, ArH), 9.50 (1H, s, exchangeable, NH). Anal. ($C_{20}H_{16}N_4O$): C, H, N.

5.7.16. N^4-(3-Chloro-4-(pyridin-2-ylmethoxy)phenyl) quinazolin-4,6-diamine (20q)

Compound **20q** was synthesized from *N*-(3-chloro-4-(pyridin-2-ylmethoxy)phenyl)-6-nitroquinazolin-4-amine **19q** (6.0 g, 14.7 mmol) and iron (5.6 g, 103.0 mmol) in aqueous ethanol (600 mL, 70% v/v) containing acetic acid (11.7 mL, 205.0 mmol): yield 2.5 g (40%); mp 238–239 °C; ^{1}H NMR (DMSO-d_6) δ 5.28 (2H, s, CH_2), 5.57 (2H, s, exchangeable, NH_2), 7.23–7.24 (2H, m, 2 × ArH), 7.31–7.37 (2H, m, 2 × ArH), 7.51–7.53 (1H, m, ArH), 7.58–7.60 (1H, m, ArH), 7.71–7.73 (1H, m, ArH), 7.86–7.88 (1H, m, ArH), 8.06–8.07 (1H, m, ArH), 8.32–8.33 (1H, m, ArH), 8.60 (1H, s, ArH), 9.33 (1H, s, exchangeable, NH). Anal. ($C_{20}H_{16}ClN_5O$): C, H, N.

5.8. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-fluorophenylamino)quinazolin-6-yl)urea (21a)

To a solution of N^4-(3-fluorophenylamino)quinazolin-4,6-diamine (**20a**, 0.99 g, 3.9 mmol) in dry THF (40 mL) containing triethylamine (2.7 mL) was added a solution of isocyanate **14** (freshly prepared from **13**, 3.00 g, 9.7 mmol) in dry THF (15 mL) at room temperature. After being stirred for 1 h at room temperature, the solid was filtered and washed with dry THF. The filtrate was evaporated to dryness in vacuo. The residue was purified by column chromatography using $CHCl_3$/MeOH (100:2 v/v) as an eluent. The fractions containing the main product were combined and evaporated to dryness. The residue was recrystallized from $CHCl_3$ to give 21a, 0.99 g, (50%); mp 190–191 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.71 (8H, m, 4 × CH_2), 6.73 (2H, d, J = 9.0 Hz, 2 × ArH), 6.89–6.94 (1H, m, ArH), 7.34 (2H, d, J = 9.0 Hz, 2 × ArH), 7.37–7.43 (1H, m, ArH), 7.66–7.67 (1H, m, ArH), 7.75–7.77 (1H, m, ArH), 7.87–7.92 (2H, m, 2 × ArH), 8.45–7.46 (1H, m, ArH), 8.55 (1H, s, ArH), 8.57, 8.83, 9.85 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{23}Cl_2FN_6O·H_2O$): C, H, N.

By following the same procedure as that for **21a** the following compound were synthesized.

5.8.1. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-chlorophenylamino)quinazolin-6-yl)urea (21b)

Compound **21b** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(3-chlorophenyl)quinazolin-4,6-diamine (**20b**, 0.70 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.90 g (65%); mp 172–173 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.71 (8H, m, 4 × CH_2), 6.73 (2H, d, J = 8.8 Hz, 2 × ArH), 7.14–7.17 (1H, m, ArH), 7.34 (2H, d, J = 8.8 Hz, 2 × ArH), 7.38–7.42 (1H, m, ArH), 7.76 (1H, d, J = 9.1 Hz, ArH), 7.81–7.83 (1H, m, ArH), 7.89 (1H, dd, J = 2.2 Hz, J = 9.1 Hz, ArH), 8.06–8.07 (1H, m, ArH), 8.46 (1H, d, J = 2.2 Hz ArH), 8.55 (1H, s, ArH), 8.59, 8.85, 9.83 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{23}Cl_3N_6O·H_2O$): C, H, N.

5.8.2. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-bromophenylamino)quinazolin-6-yl)urea (21c)

Compound **21c** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(3-bromophenyl)quinazolin-4,6-diamine (**20c**, 0.82 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.80 g (57%); mp 165–166 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.71–3.73 (8H, m, 4 × CH_2), 6.75–6.77 (2H, m, 2 × ArH), 7.32–7.38 (4H, m, 4 × ArH), 7.77–7.79 (1H, m, ArH), 7.91–7.94 (2H, m, 2 × ArH), 8.21–8.22 (1H, m, ArH), 8.48–8.49 (1H, m, ArH), 8.57 (1H, s, ArH), 8.61, 8.87, 9.85 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{23}BrCl_2N_6O·1.5H_2O$): C, H, N.

5.8.3. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-(trifluoromethyl)phenylamino)quinazolin-6-yl)urea (21d)

Compound **21d** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(3-(trifluoromethyl)phenyl)quinazolin-4,6-diamine (**20d**, 0.79 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.75 g (51%); mp 210–211 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.70–3.71 (8H, m, 4 × CH_2), 6.74 (2H, d, J = 8.8 Hz, 2 × ArH), 7.34 (2H, d, J = 8.8 Hz, 2 × ArH), 7.43–7.45 (1H, m, ArH), 7.60–7.64 (1H, m, ArH), 7.76–7.78 (1H, m, ArH), 7.87–7.89 (1H, m, ArH), 8.20–8.22 (1H, m, ArH), 8.29–8.31 (1H, m, ArH), 8.50–8.51 (1H, m, ArH), 8.56 (1H, s, ArH), 8.58, 8.85, 9.98 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{26}H_{23}Cl_2F_3N_6O·0.5H_2O$): C, H, N.

5.8.4. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(4-fluorophenylamino)quinazolin-6-yl)urea (21e)

Compound **21e** was synthesized from **14** (freshly prepared from **13**, 3.00 g, 9.7 mmol) and N^4-(4-fluorophenyl)quinazolin-4,6-diamine (**20e**, 0.99 g, 3.9 mmol) in dry THF (40 mL) containing triethylamine (2.7 mL): yield 1.0 g (53%); mp 223–224 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.71 (8H, m, 4 × CH_2), 6.74 (2H, d, J = 9.0 Hz, 2 × ArH), 7.19–7.24 (2H, m, 2 × ArH), 7.34 (2H, d, J = 9.0 Hz, 2 × ArH), 7.73 (1H, d, J = 9.0 Hz, ArH), 7.80–7.83 (2H, m, 2 × ArH), 7.88 (1H, dd, J = 2.0 Hz, J = 9.0 Hz, ArH), 8.42 (1H, d, J = 2.0 Hz, ArH), 8.46 (1H, s, ArH) 8.56, 8.79, 9.75 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{23}Cl_2FN_6O$): C, H, N.

5.8.5. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(4-bromophenylamino)quinazolin-6-yl)urea (21f)

Compound **21f** was synthesized from **14** (freshly prepared from **13**, 1.50 g, 4.8 mmol) and N^4-(4-bromophenyl)quinazolin-4,6-diamine (**20f**, 0.61 g, 1.9 mmol) in dry THF (30 mL) containing triethylamine (1.3 mL): yield 0.40 g (36%); mp 220–221 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.71 (8H, m, 4 × CH_2), 6.73 (2H, d, J = 9.0 Hz, 2 × ArH), 7.33 (2H, d, J = 9.0 Hz, 2 × ArH), 7.56 (2H, d, J = 8.8 Hz, 2 × ArH), 7.74 (1H, d, J = 9.0 Hz, ArH), 7.84 (2H, d, J = 8.8 Hz, 2 × ArH), 7.89 (1H, dd, J = 9.0 Hz, J = 2.0 Hz, ArH), 8.47 (1H, d, J = 2.0 Hz, ArH), 8.50 (1H, s, ArH), 8.59, 8.84, 9.80 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{23}BrCl_2N_6O·1.5H_2O$): C, H, N.

5.8.6. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(2-fluoro-3-chlorophenylamino)quinazolin-6-yl)urea (21g)

Compound **21g** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(2-fluoro-3-chlorophenyl)quinazolin-4,6-diamine (**20g**, 0.75 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.97 g (69%); mp 210–211 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.68–3.71 (8H, m, 4 × CH_2), 6.73 (2H, d, J = 9.0 Hz, 2 × ArH), 7.25–7.29 (1H, m, ArH), 7.33 (2H, d, J = 9.0 Hz, 2 × ArH), 7.45–7.54 (2H, m, 2 × ArH), 7.72–7.73 (1H, m, ArH), 7.82–7.84 (1H, m, ArH), 8.41–8.42 (1H, m, ArH), 8.46 (1H, s, ArH) 8.58, 8.87, 9.86 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{22}Cl_3FN_6O·0.5H_2O$): C, H, N.

5.8.7. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-chloro-4-fluorophenylamino)quinazolin-6-yl)urea (21h)

Compound **21h** was synthesized from **14** (freshly prepared from **13**, 0.53 g, 1.7 mmol) and N^4-(3-chloro-4-fluorophenyl)quinazolin-4,6-diamine **20h** (0.20 g, 0.6 mmol) in dry THF (20 mL) containing triethylamine (0.5 mL): yield 0.14 g (37%); mp 200–201 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.67–3.75 (8H, m, 4 × CH2), 6.73 (2H, d, J = 9.0 Hz, 2 × ArH), 7.34 (2H, d, J = 9.0 Hz, 2 × ArH), 7.41–7.46 (1H, m, ArH), 7.74–7.88 (3H, m, 3 × ArH), 8.14–8.17 (1H, m, ArH),

8.45–8.46 (1H, m, ArH), 8.52 (1H, s, ArH), 8.57, 8.83, 9.85 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{22}Cl_3FN_6O$): C, H, N.

5.8.8. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3,4-dichlorophenylamino)quinazolin-6-yl)urea (21i)

Compound **21i** was synthesized from **14** (freshly prepared from **13**, 1.00 g, 3.2 mmol) and N^4-(3,4,-dichlorophenyl)quinazolin-4,6-diamine (**20i**, 0.40 g, 1.3 mmol) in dry THF (25 mL) containing triethylamine (0.9 mL): yield 0.55 g (71%); mp 225–226 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.67–3.73 (8H, m, 4 × CH_2), 6.74 (2H, d, J = 8.8 Hz, 2 × ArH), 7.34 (2H, d, J = 8.8 Hz, 2 × ArH), 7.61–7.63 (1H, m, ArH), 7.75–7.77 (1H, m, ArH), 7.87–7.90 (2H, m, 2 × ArH), 8.28–8.29 (1H, m, ArH), 8.47–8.48 (1H, m, ArH), 8.57 (1H, s, ArH) 8.58, 8.86, 9.91 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{22}Cl_4N_6O$): C, H, N.

5.8.9. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-bromo-4-fluorophenylamino)quinazolin-6-yl)urea (21j)

Compound **21j** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(3-bromo-4-fluorophenyl)quinazolin-4,6-diamine (**20j**, 0.87 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.25 g (17%); mp 175–176 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.73–3.75 (8H, m, 4 × CH_2), 6.76 (2H, d, J = 8.8 Hz, 2 × ArH), 7.36 (2H, d, J = 8.8 Hz, 2 × ArH), 7.41–7.43 (1H, m, ArH), 7.77 (1H, d, J = 9.2 Hz, ArH), 7.87–7.90 (2H, m, 2 × ArH), 8.26 (1H, dd, J = 2.2 Hz, J = 9.2 Hz, ArH), 8.48 (1H, d, J = 2.2 Hz ArH), 8.54 (1H, s, ArH), 8.63, 8.89, 9.86 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{25}H_{22}BrCl_2FN_6O{\cdot}1.5H_2O$): C, H, N.

5.8.10. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(4-chloro-3-(trifluoromethyl)phenylamino)quinazolin-6-yl)urea (21k)

Compound **21k** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(4-chloro-3-(trifluoromethyl)phenyl) quinazolin-4,6-diamine (**20k**, 0.80 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.80 g (52%); mp 220–221 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.67–3.73 (8H, m, 4 × CH_2), 6.73 (2H, d, J = 8.8 Hz, 2 × ArH), 7.34 (2H, d, J = 8.8 Hz, 2 × ArH), 7.71–7.73 (1H, m, ArH), 7.76–7.92 (1H, m, ArH), 7.85–7.88 (1H, m, ArH), 8.27–8.31 (1H, m, ArH), 8.43–8.44 (1H, m, ArH), 8.51–8.52 (1H, m, ArH), 8.56 (1H, s, ArH) 8.57, 8.87, 10.05 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{26}H_{22}Cl_3F_3N_6O{\cdot}0.5H_2O$): C, H, N.

5.8.11. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-chloro-4-methoxyphenylamino)quinazolin-6-yl)urea (21l)

Compound **21l** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(3-chloro-4-methoxyphenyl)quinazolin-4,6-diamine (**20l**, 0.78 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 1.0 g (69%); mp 168–169 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.72 (8H, m, 4 × CH_2), 3.87 (3H, s, Me), 6.73 (2H, d, J = 8.6 Hz, 2 × ArH), 7.17–7.19 (1H, m, ArH), 7.34 (2H, d, J = 8.6 Hz, 2 × ArH), 7.72–7.73 (2H, m, 2 × ArH), 7.85–7.87 (1H, m, ArH), 7.96–7.97 (1H, m, ArH), 8.42–8.43 (1H, m, ArH), 8.47 (1H, s, ArH) 8.56, 8.79, 9.70 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{26}H_{25}Cl_3N_6O_2{\cdot}H_2O$): C, H, N.

5.8.12. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-methoxyphenylamino)quinazolin-6-yl)urea (21m)

Compound **21m** was synthesized from **14** (freshly prepared from **13**, 2.50 g, 8.1 mmol) and N^4-(3-methoxyphenyl)quinazolin-4,6-diamine (**20m**, 0.87 g, 3.2 mmol) in dry THF (35 mL) containing triethylamine (2.2 mL): yield 1.1 g (65%); mp 159–160 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.69–3.71 (8H, m, 4 × CH_2), 3.78 (3H, s, Me), 6.68–7.70 (1H, m, ArH), 6.73 (2H, d, J = 9.0 Hz, 2 × ArH), 7.26–7.28 (1H, m, ArH), 7.33 (2H, d, J = 9.0 Hz, 2 × ArH), 7.45–7.46 (1H, m, ArH), 7.52–7.53 (1H, m, ArH), 7.73 (1H, d, J = 9.0 Hz, ArH), 7.90 (1H, dd, J = 2.1 Hz, J = 9.0 Hz, ArH), 8.42 (1H, d, J = 2.1 Hz, ArH), 8.50 (1H, s, ArH) 8.57, 8.81, 9.65 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{26}H_{26}Cl_2N_6O_2{\cdot}H_2O$): C, H, N.

5.8.13. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3,4,5-trimethoxyphenylamino)quinazolin-6-yl)urea (21n)

Compound **21n** was synthesized from **14** (freshly prepared from **13**, 1.00 g, 3.2 mmol) and N^4-(3,4,5-trimethoxyphenyl)quinazolin-4,6-diamine (**20n**, 0.42 g, 1.3 mmol) in dry THF (25 mL) containing triethylamine (0.9 mL): yield 0.49 g (66%); mp 154–155 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.33 (3H, s, Me), 3.67–3.72 (8H, m, 4 × CH_2), 3.80 (6H, s, 2 × Me), 6.74 (2H, d, J = 9.0 Hz, 3 × ArH), 7.28 (2H, s, 2 × ArH), 7.34 (2H, d, J = 9.0 Hz, 2 × ArH), 7.73 (1H, d, J = 8.9 Hz, ArH), 7.85–7.88 (1H, dd, J = 2.0 Hz, J = 8.9 Hz, ArH), 8.42 (1H, d, J = 2.0 Hz, ArH), 8.49 (1H, s, ArH) 8.56, 8.81, 9.58 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{28}H_{30}Cl_2N_6O_4{\cdot}H_2O$): C, H, N.

5.8.14. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-ethynylphenylamino)quinazolin-6-yl)urea (21o)

Compound **21o** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(3-ethynylphenyl)quinazolin-4,6-diamine (**20o**, 0.68 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.75 g (52%); mp 164–165 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.67–3.73 (8H, m, 4 × CH_2), 4.19 (1H, s, CH), 6.73 (2H, d, J = 8.8 Hz, 2 × ArH), 7.20–7.21 (1H, m, ArH), 7.34 (2H, d, J = 8.8 Hz, 2 × ArH), 7.37–7.41 (1H, m, ArH), 7.73–7.76 (1H, m, ArH), 7.88–7.90 (2H, m, 2 × ArH), 8.03–8.04 (1H, m, ArH), 8.44–8.45 (1H, m, ArH), 8.52 (1H, s, ArH) 8.58, 8.82, 9.78 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{27}H_{24}Cl_2N_6O{\cdot}H_2O$): C, H, N.

5.8.15. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(4-phenoxyphenylamino)quinazolin-6-yl)urea (21p)

Compound **21p** was synthesized from **14** (freshly prepared from **13**, 2.00 g, 6.5 mmol) and N^4-(4-phenoxyphenyl)quinazolin-4,6-diamine (**20p**, 0.85 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.30 g (33%); mp 143–144 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.31–3.71 (8H, m, 4 × CH_2), 6.72–6.74 (2H, m, 2 × ArH), 7.01–7.07 (4H, m, 4 × ArH), 7.10–7.14 (1H, m, ArH), 7.32–7.41 (4H, m, 4 × ArH), 7.71–7.73 (1H, m, ArH), 7.81–7.90 (3H, m, 3 × ArH), 8.41–8.42 (1H, m, ArH), 8.46 (1H, s, ArH) 8.58, 8.80, 9.73 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{31}H_{28}Cl_2N_6O_2{\cdot}H_2O$): C, H, N.

5.8.16. 1-(4-(Bis(2-chloroethyl)amino)phenyl)-3-(4-(3-chloro-4-(pyridin-2-ylmethoxy)phenylamino)quinazolin-6-yl)urea (21q)

Compound **21q** was synthesized from **14** (freshly prepared from **13,** 2.00 g, 6.5 mmol) and N^4-(3-chloro-4-(pyridin-2-ylmethoxy)phenyl)quinazolin-4,6-diamine (**20q**, 0.98 g, 2.6 mmol) in dry THF (35 mL) containing triethylamine (1.8 mL): yield 0.90 g (56%); mp 156–157 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.68–3.74 (8H, m, 4 × CH_2), 5.30 (2H, s, CH_2), 6.74 (2H, d, J = 9.0 Hz, 2 × ArH), 7.25–7.27 (1H, m, ArH), 7.34 (2H, d, J = 9.0 Hz, 2 × ArH), 7.37–7.39 (1H, m, ArH), 7.58–7.60 (1H, m, ArH), 7.69–7.74 (2H, m, 2 × ArH), 7.86–7.91 (2H, m, 2 × ArH), 8.01–8.02 (1H, m, ArH), 8.42–8.42 (1H, m, ArH), 8.48 (1H, s, ArH), 8.60–8.61 (1H, m, ArH) 8.57, 8.80, 9.72 (each 1H, s, exchangeable, 3 × NH). Anal. ($C_{31}H_{28}Cl_3N_7O_2{\cdot}1.5H_2O$): C, H, N.

5.9. Biological experiments

5.9.1. Cytotoxicity assays

The effects of the newly synthesized compounds on cell growth were determined in T-cell acute lymphocytic leukemia CCRF-CEM) and their resistant subcell lines (CCRF-CEM/Taxol and CCRF-CEM/VBL) by the XTT assay[28] and human solid tumor cells (i.e., breast carcinoma MX-1 and colon carcinoma HCT-116) by the SRB assay[29] in a 72 h incubation using a microplate spectrophotometer as described previously.[30] After the addition of phenazine methosulfate-XTT solution at 37 °C for 6 h, absorbance at 450 and 630 nm was detected on a microplate reader (EL 340; Bio-Tek Instruments Inc., Winooski, VT). The cytotoxicity of the newly synthesized

compounds against non-small cell lung cancer H1299, human prostate cancer PC3, were determined by the Alamar blue assay[31] in a 72 h incubation using a microplate spectrophotometer as described previously. After the addition of Alamar blue solution, it was incubated at 37 °C for 6 h. Absorbance at 570 and 600 nm was detected on a microplate reader. IC_{50} values were determined from dose–effect relationship at six or seven concentrations of each drug using the CompuSyn software by Chou and Martin[32] based on the median-effect principle and plot.[33,34] Ranges given for Taxol and vinblastine were mean ± SE (n = 4).

5.9.2. In vivo studies

Athymic nude mice bearing the nu/nu gene were used for human breast tumor MX-1 and prostate PC-3 xenograft. Outbred Swiss-background mice were obtained from the National Cancer Institute (Frederick, MD). Male mice 8 weeks old or older weighing about 22 g were used for the experiments. Drug was administrated via the tail vein by iv injection.[27] Tumor volumes were assessed by measuring length × width × height (or width) by using caliper. Vehicle used was DMSO (50 μL) and Tween 80 (40 μL) in saline (160 μL). The maximal tolerable dose of the tested compound was determined and applied for the in vivo antitumor activity assay. All animal studies were conducted in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Animals and the protocol approved by the Memorial Sloan-Kettering Cancer Center's Institutional Animal Care and Use Committee.

5.9.3. Alkaline agarose gel shift assay

Formation of DNA cross-linking was analyzed by alkaline agarose gel electrophoresis. In brief, purified pEGFP-N1 plasmid DNA (1500 ng) was mixed with various concentrations (1–20 μM) of **21g**, **21l**, **21b** and **21e** in 40 μL binding buffer (3 mM sodium chloride/1 mM sodium phosphate, pH 7.4, and 1 mM EDTA). The reaction mixture was incubated at 37 °C for 2 h. At the end of reaction, the plasmid DNA was linearized by digestion with *Bam*HI and followed by precipitation with ethanol. The DNA pellets were dissolved and denatured in alkaline buffer (0.5 N NaOH–10 mM EDTA). An aliquot of 20 μL of DNA solution (1000 ng) was mixed with a 4 μL of 6× alkaline loading dye and then electrophoretically resolved on a 0.8% alkaline agarose gel with NaOH–EDTA buffer at 4 °C. The electrophoresis was carried out at 18 V for 22 h. After staining the gels with an ethidium bromide solution, and the DNA was then visualized under UV light.

5.9.4. Flow cytometric analysis

The effects of **21b** on cell cycle distribution were analyzed with a flow cytometer as described previously.[35] Briefly, human non-small cell lung carcinoma H1299 cells were treated with **21b** at 5, 10, and 20 μM for 24 h. The attached cells were then trypsinized, washed with phosphate buffer saline (PBS), and fixed with ice-cold 70% ethanol for 30 min. The cells were stained with 4 μg/mL propidium iodide (PI) in PBS containing 1% Triton X-100 and 0.1 mg/mL RNase A. The stained cells were then analyzed using the FACS SCAN flow cytometer (Becton Dickinson, San Joes, CA, USA). The percentage of the cells in each cell cycle phase was determined using the ModFit LT 2.0 software based on the DNA histograms.

Acknowledgments

We are grateful to the National Science Council (Grant No. NSC-99-2320-B-001-013) and Academia Sinica (Grant No. AS-100-TP-B13) for financial support. T.-C.C. was supported by the Sloan-Kettering Institute General Fund. The NMR spectra of synthesized compounds were obtained at High-Field Biomolecular NMR Core Facility supported by the National Research Program for Genomic Medicine (Taiwan). We would like to thank Dr. Shu-Chuan Jao in the Institute of Biological Chemistry (Academia Sinica) for providing the NMR service and the National Center for High-performance computing for computer time and facilities.

Supplementary data

Supplementary data (analysis data table of all unknown compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.01.055.

References and notes

1. Kaldor, J. M.; Day, N. E.; Hemminki, K. *Eur. J. Cancer Clin. Oncol.* **1988**, *24*, 703.
2. Osborne, M. R.; Lawley, P. D.; Crofton-Sleigh, C.; Warren, W. *Chem. Biol. Interact.* **1995**, *97*, 287.
3. Finlay, G. J.; Wilson, W. R.; Baguley, B. C. *Eur. J. Cancer Clin. Oncol.* **1989**, *25*, 1695.
4. Gourdie, T. A.; Valu, K. K.; Gravatt, G. L.; Boritzki, T. J.; Baguley, B. C.; Wakelin, L. P. G.; Wilson, W. R.; Woodgate, P. D.; Denny, W. A. *J. Med. Chem.* **1990**, *33*, 1177.
5. Fan, J.-Y.; Valu, K. K.; Woodgate, P. D.; Baguley, B. C.; Denny, W. A. *Anti-Cancer Drug Des.* **1997**, *12*, 181.
6. Wyatt, M. D.; Lee, M.; Hartley, J. A. *Anti-Cancer Drug Des.* **1997**, *12*, 49.
7. Bacherikov, V. A.; Chou, T.-C.; Dong, H.-J.; Zhang, X.; Chen, C.-H.; Lin, Y.-W.; Tsai, T.-J.; Lee, R.-Z.; Liu, L. F.; Su, T.-L. *Bioorg. Med. Chem.* **2005**, *13*, 3993.
8. Su, T.-L.; Lin, Y.-W.; Chou, T.-C.; Zhang, X.; Bacherikov, V. A.; Chen, C.-H.; Liu, L. F.; Tsai, T.-J. *J. Med. Chem.* **2006**, *49*, 3710.
9. Bacherikov, V. A.; Chou, T.-C.; Dong, H.-J.; Chen, C.-H.; Lin, Y.-W.; Tsai, T.-J.; Su, T.-L. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4719.
10. Chen, C.-H.; Lin, Y.-W.; Zhang, X.; Chou, T.-C.; Tsai, T.-J.; Kapuriya, N.; Kakadiya, R.; Su, T.-L. *Eur. J. Med. Chem.* **2009**, *44*, 3056.
11. Kapuriya, N.; Kapuriya, K.; Zhang, X.; Chou, T.-C.; Kakadiya, R.; Wu, Y.-T.; Tsai, T.-H.; Chen, Y.-T.; Lee, T.-C.; Shah, A.; Naliapara, Y.; Su, T.-L. *Bioorg. Med. Chem.* **2008**, *16*, 5413.
12. Kapuriya, N.; Kapuriya, K.; Dong, H.; Zhang, X.; Chou, T.-C.; Chen, Y.-T.; Lee, T.-C.; Lee, W.-C.; Tsai, T.-H.; Naliapara, Y.; Su, T.-L. *Bioorg. Med. Chem.* **2009**, *17*, 1264.
13. Kakadiya, R.; Dong, H.; Kumar, A.; Dodia, N.; Zhang, X.; Chou, T.-C.; Lee, T.-C.; Shah, A.; Su, T.-L. *Bioorg. Med. Chem.* **2010**, *18*, 2285.
14. Goossens, J.-E.; Bouey-Bencteux, E.; Houssin, R.; Hénichart, J. P.; Colson, P.; Houssier, C.; Laine, W.; Baldeyrou, B.; Bailly, C. *Biochemistry* **2001**, *40*, 4663.
15. Fry, D. W.; Kraker, A. J.; McMichael, A.; Ambroso, L. A.; Nelson, J. M.; Leopold, W. R.; Connors, R. W.; Bridges, A. J. *Science* **1994**, *265*, 1093.
16. Prakash, A. S.; William, A. D.; Gourdie, T. A.; Valu, K. K.; Woodgate, P. D.; Wakelin, L. P. G. *Biochemistry* **1990**, *29*, 9799.
17. Domarkas, J.; Dudouit, F.; Williams, C.; Qiyu, Q.; Banerjee, R.; Brahimi, F.; Jean-Claude, B. J. *J. Med. Chem.* **2006**, *49*, 3544.
18. Wang, G. W.; Miao, C. B.; Kang, H. *Bull. Chem. Soc. Jpn.* **2006**, *79*, 1426.
19. Hour, M. J.; Huang, L. J.; Kuo, S. C.; Xia, Y.; Bastow, K.; Nakanishi, Y.; Hamel, E.; Lee, K. H. *J. Med. Chem.* **2000**, *43*, 4479.
20. Dowell, R. I.; Springer, C. J.; Davies, D. H.; Hadley, E. M.; Burke, P. J.; Boyle, F. T.; Melton, R. G.; Connors, T. A.; Blakey, D. C.; Mauger, A. B. *J. Med. Chem.* **1996**, *39*, 1100.
21. Jordan, A. M.; Khan, T. H.; Malkin, H.; Helen, M. I.; Osborn, H. M. I. *Bioorg. Med. Chem.* **2002**, *10*, 2625.
22. O'Connell, M. J.; Walworth, N. C.; Carr, A. M. *Trends Cell Biol.* **2000**, *10*, 296–303.
23. Erba, E.; Mascellani, E.; Pifferi, A.; D'Incalci, M. C. *Int. J. Cancer* **1995**, *62*, 170.
24. Endicott, M.; Wick, E.; Mercury, M. L.; Sherrill, M. L. *J. Am. Chem. Soc.* **1946**, *68*, 1299.
25. Finch, N.; Gschwend, H. W. *J. Org. Chem.* **1971**, *11*, 1463.
26. Bridges, A. J.; Zhou, H.; Cody, D. R.; Rewcastle, G. W.; McMichael, A.; Showalter, H. D.; Fry, D. W.; Kraker, A. J.; Denny, W. A. *J. Med. Chem.* **1996**, *39*, 267.
27. Smaill, J. B.; Palmer, B. D.; Rewcastle, G. W.; Denny, W. A.; McNamara, D. J.; Dobrusin, E. M.; Bridges, A. J.; Zhou, H.; Showalter, H. D.; Winters, R. T.; Leopold, W. R.; Fry, D. W.; Nelson, J. M.; Slintak, V.; Elliot, W. L.; Roberts, B. J.; Vincent, P. W.; Patmore, S. J. *J. Med. Chem.* **1999**, *42*, 1803.
28. Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827.
29. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenny, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
30. Chou, T.-C.; O'Connor, O. A.; Tong, W. P.; Guan, Y.; Zhang, Z.-G.; Stachel, S. J.; Lee, C.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8113.
31. Al-Nasiry, S.; Geusens, N.; Hanssens, M.; Luyten, C.; Pijnenborn, R. *Hum. Reprod.* **2007**, *22*, 1304.
32. Chou, T.-C.; Martin, N. *Compusyn Software*; ComboSyn: Paramus, NJ, 2005.
33. Chou, T.-C.; Talalay, P. *Adv. Enzyme Regul.* **1984**, *22*, 27.
34. Chou, T.-C. *Pharmacol. Rev.* **2006**, *58*, 621.
35. Kakadiya, R.; Dong, H.; Lee, P.-C.; Kapuriya, N.; Zhang, X.; Chou, T.-C.; Lee, T.-C.; Kapuriya, K.; Shah, A.; Su, T.-L. *Bioorg. Med. Chem.* **2009**, *17*, 5614.