Med Chem Res
DOI 10.1007/s00044-013-0519-2

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and in vitro antitumor activity of new quinoline, pyrimido[4,5-*b*]quinoline, [1,2,3]triazino[4,5-*b*]quinoline, and [1,2,4]triazolo[2′,3′:3,4]pyrimido[6,5-*b*]quinoline analogs

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Received: 6 October 2012 / Accepted: 29 January 2013

Abstract New series of quinoline, pyrimido[4,5-*b*]quinoline, [1,2,3]triazino[4,5-*b*]quinoline, and [1,2,4]triazolo[2′,3′:3,4]pyrimido[6,5-*b*]quinoline analogs have been synthesized and characterized by analytical and spectrometrical methods (IR, ^{1}H NMR, ^{13}C NMR, MS). Fifteen of the newly synthesized compounds; namely, **3a**, **b**, **4b**, **6a**, **b**, **10a–f**, and **14a–d** were evaluated for their in vitro antitumor activity at the National Cancer Institute (NCI) 60 cell lines panel assay. Compounds **4b** and **10f** are the most active members in this study, demonstrating significant broad spectrum antitumor activity against most of the tested sub-panel tumor cell lines. The detailed synthesis, spectroscopic, and biological data are described.

Keywords Synthesis · Quinolines · Pyrimidoquinolines · Triazinoquinolines · Triazolopyrimidoquinolines · Antitumor activity

Introduction

Although there have been great advances in the detection and treatment of cancer, it remains one of the greatest medical challenges, with the incidence of some malignancies continuing to increase (Jemal *et al.*, 2007). Therefore, development of novel chemotherapeutic agents is an important and challenging task for the medicinal chemists. Hence, many research programs are directed toward the design and synthesis of new drugs for their chemotherapeutic usage.

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In the past few years, several studies have been devoted to the synthesis and biological activities of quinoline and pyrimidoquinoline derivatives. Some of them showed antitumor activity (Abbas *et al.*, 2011; Abouzid and Shouman, 2008; Abu-Hasem and Aly, 2012; Ali *et al.*, 2007, 2008; Al-Said *et al.*, 2011; Alqasoumi *et al.*, 2010; Ghorab *et al.*, 2009, 2010, 2011; Shrestha *et al.*, 2008; Yong *et al.*, 2012), while other derivatives have been used as antimicrobial agents (El-Gazzar *et al.*, 2008; Selvi *et al.*, 2006; Suresh *et al.*, 2003). Also, some of these derivatives displayed antimalarial activity (Joshi *et al.*, 2005), analgesic properties (El-Gazzar *et al.*, 2008; Kidwai and Negi, 1997) as well as anti-inflammatory activity (El-Gazzar *et al.*, 2008). In continuation to our previous work (El-Ashmaway *et al.*, 2012) directed toward facile synthesis of pyrimidoquinolines of potential antitumor activity, I thought to explore the utility of compounds **1a**, **b** as key precursors for the synthesis of new annulated quinoline derivatives to examine their antitumor activity.

Results and discussion

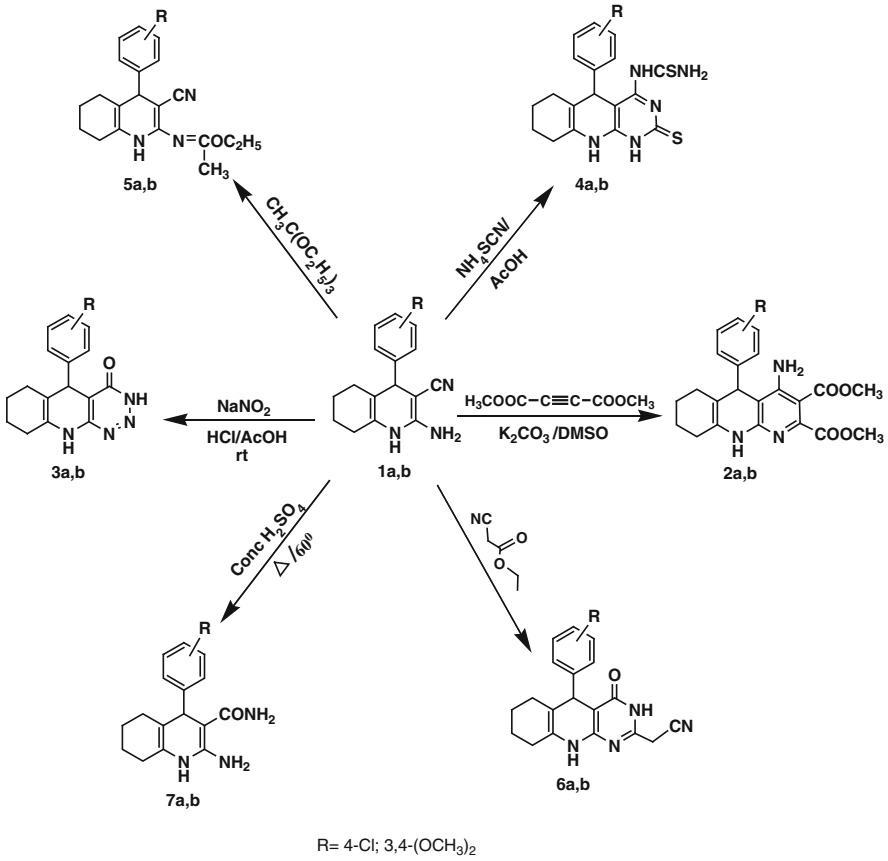
Chemistry

A general approach to synthesize the designed compounds is outlined in Schemes 1, 2, 3. 2-Amino-1,4,5,6,7,8-hexahydro-4-(substituted phenyl)quinoline-3-carbonitriles (**1a**, **b**), the key starting materials necessary for this study were previously prepared (El-Ashmaway *et al.*, 2012).

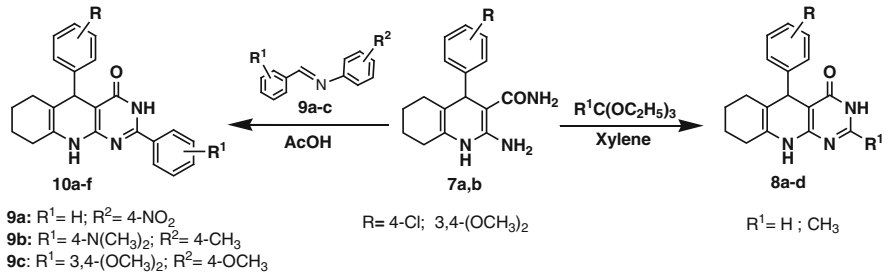
Condensation of compounds **1a**, **b** with dimethyl acetylenedicarboxylate (DMAD) in refluxing dimethyl sulfoxide (DMSO) and in the presence of potassium carbonate gave 4-amino-6,7,8,9-tetrahydro-2,3-*bis*(methoxycarbonyl)-5-(substituted phenyl)-5*H*, 10*H*-pyrimido[4,5-*b*]quinolines

Published online: 13 February 2013

Scheme 1 Synthesis of the target compounds **2a**, **b–7a**, **b**



Scheme 2 Synthesis of the target compounds **8a–d** and **10a–f**



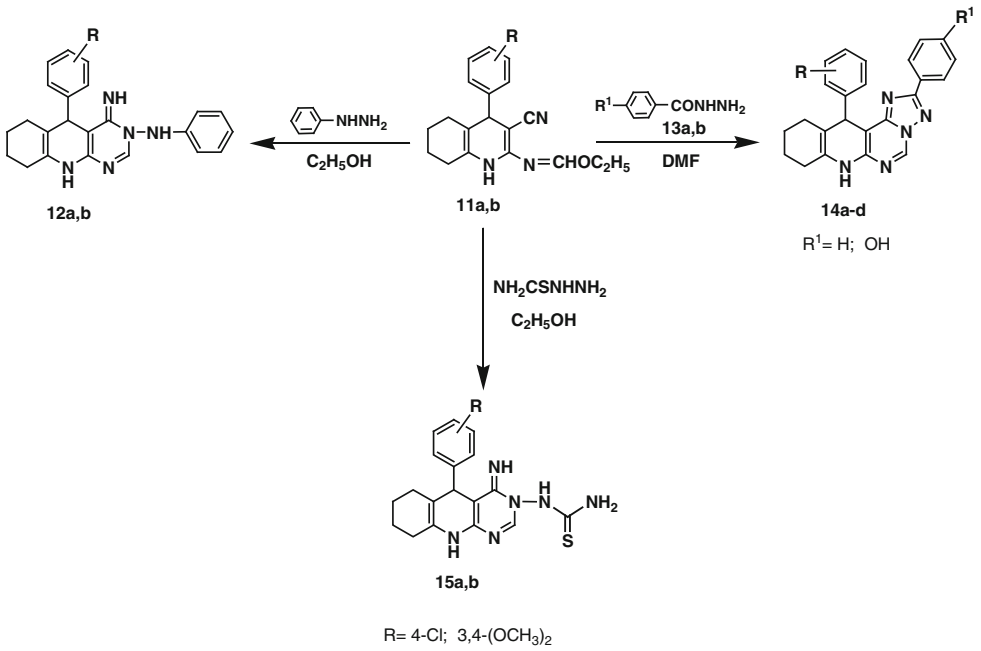
(2a, **b)** (Scheme 1). The IR spectra of these compounds showed characteristic carbonyl absorption band at 1679 and 1681 cm^{-1}, also it showed the absence of (C ≡ N) absorption band confirming the formation of the desired cyclic structures. The ^{1}H NMR spectrum of **2a** exhibited two significant singlets at δ 3.83 and 4.91 ppm corresponding to ($2OCH_3$) and (NH_2), respectively. Moreover, the signals for ($4CH_2$), (C_5–H) and aromatic protons were observed at the expected regions. ^{13}C NMR spectrum of the same compound displayed two characteristic signals at δ 52.1 and 52.2 ppm corresponding to ($2OCH_3$).

The triazinoquinolines **3a**, **b** were prepared by diazotization of the *ortho* aminonitriles **1a, b** via treatment with aqueous sodium nitrite and a mixture of concentrated hydrochloric/glacial acetic acids (3:1) (Scheme 1). IR spectrum of **3a** demonstrated the characteristic absorption bands for (2NH) and (C=O) groups at 3423, 3301, and 1643 cm^{-1}, respectively. In addition, the mass spectrum of **3b** showed a molecular ion peak at *m/z* 340 which is in agreement with its molecular formula $C_{18}H_{20}N_4O_3$.

The *ortho* aminonitriles **1a**, **b** were further utilized for another cyclocondensation reaction using ammonium thiocyanate in refluxing glacial acetic acid to afford the thiourea derivatives **4a**, **b** (Scheme 1). ^{1}H NMR spectrum of **4a** displayed two significant singlets at δ 10.52 and 11.16 ppm for (2NH), the remaining protons appeared at the expected regions. ^{13}C NMR spectrum of the same compound showed a characteristic signal at δ 183.2 ppm for (2C=S). The mass spectrum of **4b** showed a molecular ion peak at *m/z* 429 which is in agreement with the expected structure.

Condensation of compounds **1a**, **b** with triethyl orthoacetate yielded the ethyl acetimidate derivatives **5a**,

Scheme 3 Synthesis of the target compounds **12a**, **b**, **14a–d**, and **15a**, **b**



b (Scheme 1). The mass spectrum of **5a** showed a molecular ion peak at *m*/*z* 356 corresponding to its molecular weight. ^{1}H NMR spectrum of **5b** showed three significant singlets at δ 2.69, 6.45, and 8.48 ppm for (CH_3), (C_4–H), and (NH), respectively, in addition to the triplet-quartet pattern characteristic for the ethoxy protons at δ 1.34 and 4.32–4.39 ppm, respectively. ^{13}C NMR spectrum of the same compound displayed two characteristic signals for the ethyl carbons at δ 14.5 and 62.3 ppm.

Heating compounds **1a**, **b** with excess ethyl cyanoacetate yielded the 2-(cyanomethyl)pyrimido[4,5-*b*]quinoline derivatives **6a**, **b** (Scheme 1). The IR spectra of these compounds revealed the presence of the absorption bands characteristic for (C=O) and (C≡N) groups at the expected regions. Moreover, ^{1}H NMR spectra showed a characteristic singlet at δ 4.14 ppm for (CH_2CN). In addition, mass spectrum of compound **6b** showed a molecular ion peak at *m*/*z* 378 corresponding to its molecular weight along with a base peak at *m*/*z* 109.

Hydrolysis of compounds **1a**, **b** using 70 % sulfuric acid at 60 °C afforded 2-amino-1,4,5,6,7,8-hexahydro-4-(substituted phenyl)quinoline-3-carboxamides (**7a**, **b**) in 70 and 55 % yield, respectively, (Scheme 1). The IR spectra of these compounds showed the disappearance of (C≡N) absorption band with concomitant appearance of (C=O) absorption band confirming the formation of the desired structures. In addition, ^{13}C NMR spectrum of **7a** displayed significant signal at δ 169.0 ppm for (C=O).

Reaction of quinoline-3-carboxamides **7a**, **b** with triethyl orthesters in refluxing xylene yielded the pyrimido[4,5-*b*]quinolin-5-one derivatives **8a–d** (Scheme 2). These compounds were identical to the previously prepared ones (El-Ashmaway *et al.*, 2012) with respect to their melting points, mixed melting points, and TLC analysis using chloroform/methanol (9:1) as an elution solvent.

The versatility of arylideneanilines as aldehyde source-cum-dehydrating agents in heterocyclization reactions (Reddy *et al.*, 2005) was earlier demonstrated in benzimidazole (Sarojini *et al.*, 1972) and quinazolinone (Hanumanthu *et al.*, 1976) chemistry. Therefore, I assumed that benzylideneanilines **9a–c** may as well react with **7a**, **b** to provide **10a–f** in one step. Thus, reaction of **7a**, **b** with the benzylideneanilines **9a–c** (Singh *et al.*, 2010) in (1:2) molar ratio in refluxing glacial acetic acid afforded pyrimido[4,5-*b*]quinolin-5-one derivatives **10a–f**. Obviously, the second mole of benzylideneaniline acts as a dehydrating agent (Reddy *et al.*, 2005) (Scheme 2). Mass spectrum of compound **10a** showed a molecular ion peak at *m*/*z* 390 and a base peak at *m*/*z* 312. ^{1}H NMR spectrum of **10e** displayed three significant singlets at δ 3.04, 3.85, and 6.71 ppm for ($2CH_3$), ($2OCH_3$), and (C_5–H), respectively, in addition to two doublets at δ 6.76 and 7.68 ppm for (C_2-aromatic protons).

In conclusion, a facile general method for the synthesis of pyrimido[4,5-*b*]quinolin-5-one derivatives **8a–d** and **10a–f** from quinoline-3-carboxamides **7a**, **b** was developed by inserting one-carbon unit derived from suitable triethyl orthoesters and benzylidene anilines, respectively (Reddy *et al.*, 2005).

Treatment of ethyl formimidates **11a**, **b** (El-Ashmaway *et al.*, 2012) with phenylhydrazine in refluxing ethanol yielded 3,4,6,7,8,9-hexahydro-4-imino-3-phenylamino-5-(substituted phenyl)-5*H*, 10*H*-pyrimido[4,5-*b*]quinolines (**12a**, **b**) (Scheme 3). The disappearance of the (C≡N)

Table 1 Percentage growth inhibition (GI %) of in vitro sub-panel tumor cell lines at 10 μM concentration of compounds **3–14**

Subpanel tumor cell lines	% Growth inhibition (GI %)[a]														
	3a	**3b**	**4b**	**6a**	**6b**	**10a**	**10b**	**10c**	**10d**	**10e**	**10f**	**14a**	**14b**	**14c**	**14d**
Leukemia															
CCRF-CEM	–	–	–	–	–	–	–	–	–	–	11.7	–	13.2	–	–
HL-60(TB)	–	–	–	–	–	–	–	–	–	–	–	–	13.5	–	–
K-562	–	–	17.6	–	–	11.2	–	–	–	–	–	–	–	–	–
MOLT-4	–	–	–	–	–	13.6	–	–	–	–	–	11.0	–	19.8	12.3
RPMI-8226	–	–	–	–	–	–	–	–	–	–	10.8	12.3	13.2	10.5	–
SR	–	–	40.6	–	–	–	–	–	–	–	21.4	–	–	–	–
Non-small lung cancer															
A549/ATCC	–	–	–	–	–	–	–	–	–	–	–	–	–	12.3	–
EKVX	10.9	–	–	13.0	–	–	–	–	–	–	–	–	–	11.0	–
HOP-62	–	15.7	25.4	12.5	–	–	12.8	10.2	15.7	–	12.3	16.0	16.6	–	–
HOP-92	–	13.3	25.8	11.2	–	12.0	35.9	–	13.3	–	26.3	14.0	42.1	36.8	12.2
NCI-H226	10.3	15.9	18.6	–	11.3	12.3	–	11.0	15.9	13.6	13.3	15.2	–	19.6	15.3
NCI-H23	10.4	–	15.3	12.1	–	–	12.4	–	–	–	–	11.7	12.9	10.7	–
NCI-H460	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
NCI-H522	13.5	–	43.4	11.4	–	20.2	15.8	10.1	14.5	14.4	25.8	23.5	–	31.4	16.4
Colon cancer															
COLO 205	13.5	15.9	12.8	10.4	–	–	10.2	–	15.9	–	10.3	11.5	–	–	–
HCC-2998	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HCT-116	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HCT-15	–	–	10.2	–	–	–	–	–	–	–	10.7	10.2	–	13.1	–
HT29	–	–	–	–	–	–	–	–	–	–	–	14.4	–	–	–
KM 12	–	–	21.3	–	–	–	–	–	–	–	–	–	–	–	–
SW-620	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
CNS Cancer															
SF-268	–	–	–	–	–	–	–	–	–	–	12.2	–	–	–	–
SF-295	–	–	14.4	–	–	–	–	–	–	–	–	–	–	29.6	–
SF-539	–	–	21.1	–	–	–	–	–	–	–	14.5	–	–	–	–
SNB-19	–	10.7	10.4	–	–	–	–	–	10.7	–	–	–	–	–	–
SNB-75	–	28.3	20.3	20.4	–	15.5	12.0	11.2	28.4	–	22.6	10.5	20.5	–	19.1
Melanoma															
LOX IMVI	–	–	16.1	–	–	11.2	–	–	–	–	21.1	15.1	14.3	–	–
MALME-3 M	–	–	10.9	–	–	–	–	–	–	–	–	–	13.0	–	–
M14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
MDA-MB-435	–	–	**70.7**	–	–	–	–	–	–	–	–	–	–	–	–
SK-MEL-2	–	–	12.6	10.0	–	–	10.0	–	–	–	–	–	–	–	–
SK-MEL-28	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SK-MEL-5	13.0	10.9	25.5	–	–	17.8	–	13.0	10.9	–	18.6	–	–	–	23.2
UACC-257	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
UACC-62	nt	14.3	–	nt	–	–	nt	nt	14.4	nt	–	13.4	17.4	–	–
Ovarian cancer															
IGORV1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
OVCAR-3	–	–	–	–	–	–	11.1	–	–	–	–	–	–	–	–
OVCAR-4	–	–	–	–	–	–	–	–	–	–	10.8	–	–	–	–
OVCAR-5	–	12.6	–	–	–	–	–	–	12.6	–	–	–	–	11.7	–
OVCAR-8	–	–	–	–	–	–	–	–	–	–	12.1	–	–	14.7	–
NCI/ADR-RES	–	–	11.7	–	–	–	–	–	–	–	–	–	–	–	–

Table 1 continued

Subpanel tumor cell lines	% Growth inhibition (GI %)[a]														
	3a	**3b**	**4b**	**6a**	**6b**	**10a**	**10b**	**10c**	**10d**	**10e**	**10f**	**14a**	**14b**	**14c**	**14d**
SK-OV-3	–	–	18.6	–	–	–	–	–	–	–	–	–	–	–	–
Renal cancer															
786-0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A498	28.3	13.2	–	13.0	–	11.4	–	–	13.2	21.7	–	20.6	25.1	**53.6**	–
ACHN	–	16.0	11.4	13.8	–	–	13.1	–	18.0	–	28.8	–	–	20.0	–
CAKI-1	16.1	23.4	10.0	–	–	–	–	–	23.4	10.5	13.5	12.4	24.6	13.1	–
RXF 393	–	14.4	14.3	–	–	–	–	–	14.4	–	10.3	–	–	19.9	–
SN12C	–	10.8	–	15.5	–	–	14.6	12.0	10.8	12.9	15.0	–	–	–	–
TK-10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
UO-31	43.4	48.6	43.5	37.7	26.9	43.3	47.4	41.7	48.6	29.4	**66.3**	40.0	29.0	38.7	29.3
Prostate cancer															
PC-3	10.2	13.4	23.5	–	–	–	–	12.5	13.5	11.4	17.1	19.7	10.4	19.8	–
DU-145	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Breast cancer															
MCF-7	–	–	–	–	–	–	–	13.5	–	–	34.3	–	–	–	–
MDA-MB-231/ATCC	–	–	17.4	–	–	–	12.0	12.6	–	–	–	11.1	20.5	12.6	12.8
HS 578T	–	–	17.7	–	–	–	–	–	–	–	–	–	14.1	–	–
BT-549	–	–	–	–	–	–	–	–	–	–	nt	–	–	–	–
T-47D	13.3	21.1	27.5	14.1	–	15.5	17.6	–	21.1	14.9	28.3	14.7	15.8	26.2	18.8
MDA-MB-468	13.3	21.1	14.2	–	–	–	–	–	21.2	10.8	28.5	23.1	–	23.2	21.4

Bold values used to point out the active compounds and lethal effect

nt not tested

[a] –, GI <10 %

absorption band in IR spectra, disappearance of triplet–quartet pattern characteristic for ethyl protons in ^{1}H NMR spectra, and disappearance of ethyl carbons signals in ^{13}C NMR spectra confirmed the formation of the desired cyclic structures. Mass spectrum of compound **12a** showed a molecular ion peak at *m*/*z* 404 which is in agreement with its molecular formula $C_{23}H_{22}ClN_5$ along with a base peak at *m*/*z* 283. ^{1}H NMR spectrum of the same compound showed two significant singlets at δ 7.99 and 9.56 ppm for (2NH), the remaining protons appeared at the expected regions.

The triazolo[2′,3′:3,4]pyrimido[6,5-*b*]quinoline derivatives **14a–d** could be successfully obtained *via* the reaction of compounds **11a**, **b** with benzhydrazides **13a**, **b** (Deep *et al.*, 2010) in refluxing *N*,*N*-dimethylformamide (DMF) (Scheme 3). The IR spectrum of **14b** showed the disappearance of the (C ≡ N) absorption band. ^{1}H NMR spectrum of the same compound showed a characteristic singlet at δ 10.03 ppm for (OH), the remaining protons appeared at the expected regions. Mass spectrum of **14c** showed a molecular ion peak at *m*/*z* 439 corresponding to its molecular weight.

Finally, the {pyrimido[4,5-*b*]quinolin-3-yl}thiourea derivatives **15a**, **b** were synthesized through condensation of compounds **11a**, **b** with thiosemicarbazide in refluxing ethanol. Mass spectrum of **15a** showed a molecular ion peak at *m*/*z* 387 corresponding to its molecular weight along with a base peak at *m*/*z* 60. In addition, ^{1}H NMR spectrum of **15b** displayed five characteristic singlets at δ 4.50, 6.51, 7.51, 7.61, and 8.56 ppm indicating (NH_2), (C_5–H) and (3NH), respectively. The ^{13}C NMR spectrum of the same compound exhibited a significant signal at δ 181.0 ppm for (C=S).

Preliminary in vitro antitumor screening

Out of the newly synthesized compounds, fifteen derivatives; namely, **3a**, **b**, **4b**, **6a**, **b**, **10a–f**, and **14a–d** were selected by the National Cancer Institute (NCI) in vitro disease-oriented human cells screening panel assay to be evaluated for their antitumor activity. A single dose (10 μM) of the test compounds was used in the full NCI 60 cell lines panel assay which includes nine tumor subpanels, namely; leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cells (Boyd and Paull, 1995; Grever *et al.*, 1992; Monks *et al.*, 1991; Skahan *et al.*, 1990). The data reported as mean-graph of the percent growth of the treated cells, and presented as percentage growth inhibition

(GI %). The obtained results of the tested compounds (Table 1) showed distinctive potential pattern of selectivity, as well as broad-spectrum antitumor activity.

Regarding the activity toward individual cell lines, all tested compounds showed selective activity against renal cancer cell line (UO-31). Furthermore, compounds **3a**, **4b**, **6a**, **10a–f**, **14a**, **c**, **d** exhibited cytotoxic activity against non-small cell lung cancer cell line (NCI-H522). In addition, the compounds **3a**, **b**, **4b**, **6a**, **10a**, **b**, **d–f**, and **14a–d** were proved to be cytotoxic toward breast cancer cell line (T-47D). Meanwhile, melanoma cell line (MDA-MB-435) and leukemia cell line (SR) were proved to be selectively sensitive to **4b** with GI values of 70.7 and 40.6 %, respectively. Moreover, compound **10f** displayed promising activity against renal cancer cell line (UO-31) with GI value 66.3 %. In addition, the same compound exhibited moderate activity against breast cancer cell line (MCF-7) with GI value 34.3 %. Compound **14c** was proved to be the most active member in this study toward renal cancer cell line (A498) with GI value of 53.6 %. With regard to broad-spectrum antitumor activity; close examination of the data presented in Table 1, revealed that compounds **4b** and **10f** are the most active members in this study, showing effectiveness toward numerous cell lines belonging to different tumor subpanels.

Structure–activity correlation

Structure–activity correlation, based on the number of cell lines that were proved to be sensitive toward each of the tested compounds, revealed that, 5-(3,4-dimethoxyphenyl)-[1,2,3]triazino[4,5-*b*]quinoline **3b** and 5-(3,4-dimethoxyphenyl)pyrimido[4,5-*b*]quinoline derivatives **4b**, **10d**, **10f**, and **14c** are more active antitumor agents than their 5-(4-chlorophenyl) counterparts, **3a**, **10a–c,** and **14a**. The introduction of thioureido group at position 4- of 5-(3,4-dimethoxyphenyl)pyrimido[4,5-*b*]quinoline nucleus produced **4b** with the broadest spectrum antitumor activity and improved selectivity toward non-small cell lung, colon, CNS, melanoma, renal, and breast cancer cell lines. Meanwhile, the introduction of 3,4-dimethoxyphenyl moiety at position 2- of 5-(3,4-dimethoxyphenyl)pyrimido[4,5-*b*]quinoline nucleus produced **10f** with dramatic increase in antitumor activity and enhanced selectivity toward leukemia, non-small cell lung, CNS, renal, and breast cancer cell lines. On the other hand, introduction of cyanomethyl at position 2- of 5-(3,4-dimethoxyphenyl)pyrimido[4,5-*b*]quinoline nucleus produced **6b** with nearly abolished activity. Regarding the [1,2,4]triazolo[2′,3′:3,4]pyrimido[6,5-*b*]quinoline derivatives, the introduction of unsubstituted phenyl moiety at position 2- as in compounds **14a** and **14c** broaden the spectrum of antitumor activity in comparison with the corresponding 2-(4-hydroxyphenyl) analogs **14b** and **14d**.

Conclusion

Compounds **4b** and **10f** are broad-spectrum antitumor agents showing effectiveness toward numerous cell lines belonging to different tumor subpanels. Compound **4b** is the most active member in this study, while compound **6b** is almost inactive. From these results, I can conclude that not only 5-(3,4-dimethoxyphenyl) moiety is essential for antitumor activity but also other substituents on the pyrimidine nucleus play an important role on the activity of these compounds, i.e., the presence of 2-thioxo group and 2-(3,4-dimethoxyphenyl) moiety enhanced the antitumor activity as in compounds **4b** and **10f**, respectively. On the other hand, incorporation of 2-cyanomethyl moiety abolished the activity as in compound **6b**.

Experimental

Chemistry

All melting points (°C) were recorded on Fisher-Johns melting point apparatus and are uncorrected. The infrared spectra were recorded in KBr disk using a Unicam SP 1000 IR spectrometer (ν in cm^{-1}) at Faculty of Science, Mansoura University. Nuclear magnetic resonance (^1H and ^{13}C NMR) spectra were obtained on 300 MHz FT-NMR spectrometer at Faculty of Science, Cairo University. The chemical shifts are expressed in δ ppm using tetramethylsilane (TMS) as internal reference and DMSO-d_6 as solvent. Mass spectra were recorded on JEOL JMS-600H spectrometer using electron impact technique at 70 eV at Microanalytical Unit, Cairo University. Microanalyses (C, H, N) were performed at Microanalytical Unit, Cairo University, and were in agreement with the proposed structures within ±0.4 of the calculated values. Reaction times were monitored using TLC plates, Silica gel 60 F_{254} precoated (E. Merck) and the spots were visualized by UV (366 nm). Chloroform: methanol (9:1) was adopted as elution solvent. Compounds **1a**, **b** and **11a**, **b** were prepared according to the reported procedures (El-Ashmaway *et al.*, 2012), compounds **9a–c** were prepared adopting the described procedure (Singh *et al.*, 2010). Compounds **13a**, **b** were prepared following the literature procedure (Deep *et al.*, 2010).

*General procedure for the preparation of 4-amino-6,7,8,9-tetrahydro-2,3-bis(methoxycarbonyl)-5-(substituted phenyl)-5H, 10H-pyrimido[4,5-b]quinolines (**2a**, **b**)*

Dimethyl acetylenedicarboxylate (0.425 g, 0.003 mol) was added dropwise to a solution of *ortho* aminonitrile **1a**, **b** (0.002 mol), and potassium carbonate (0.83 g, 0.006 mol)

in DMSO (5 mL). The reaction mixture was heated at reflux temperature for 12 h then cooled, diluted with water (30 mL), and extracted with ether (3 × 15). The combined ether extract was evaporated and the remaining residue was purified by crystallization from ethanol/water (3:1) to give dark red crystals of compounds **2a**, **b**.

*4-Amino-5-(4-chlorophenyl)-6,7,8,9-tetrahydro-2,3-bis(methoxycarbonyl)-5H, 10H-pyrimido[4,5-b]quinoline (**2a**)* Yield 50 %, m.p. 232–234 °C. IR spectrum (KBr, *v*, cm^{-1}): 3444, 3403 (NH_2, NH), 1681 (2C=O). ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.83–2.30 (m, 4H, $2CH_2$), 2.65–2.99 (m, 4H, $2CH_2$), 3.83 (s, 6H, $2OCH_3$), 4.21 (s, 2H, NH_2, D_2O-exchangeable), 6.42 (s, 1H, C_5–H), 7.06–7.46 (m, 5H, Ar–H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.3, 22.8, 26.1, 26.6, 33.4, 52.1, 52.2, 103.2, 107.4, 111.3, 124.8 (2C), 129.4 (2C), 132.2, 135.8, 142.7, 147.6, 152.8, 155.3, 167.9 (2C). Anal. Calcd for $C_{22}H_{22}ClN_3O_4$ (427.88): C 61.75, H 5.18, N 9.82. Found: C 61.43, H 4.92, N 9.52.

*4-Amino-6,7,8,9-tetrahydro-2,3-bis(methoxycarbonyl)-5-(3,4-dimethoxyphenyl)-5H, 10H-pyrimido[4,5-b]quinoline (**2b**)* Yield 45 %, m.p. 220–222 °C. IR spectrum (KBr, *v*, cm^{-1}): 3446, 3367 (NH_2), 3212 (NH), 1679 (2C=O). ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.76–2.36 (m, 4H, $2CH_2$), 2.62–2.87 (m, 4H, $2CH_2$), 3.81 (s, 6H, $2OCH_3$), 3.87 (s, 6H, $2OCH_3$), 4.28 (s, 2H, NH_2, D_2O-exchangeable), 6.78–7.33 (m, 5H, C_5–H, Ar–H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.4 (2C), 25.3, 26.8, 32.6, 51.8, 51.9, 55.6, 55.7, 103.6, 106.7, 111.9, 112.2, 113.3, 121.8, 129.7, 145.8 (2C), 148.6 (2C), 153.1, 157.3, 168.7 (2C). MS *m/z* (%): 453 (0.03, M^+), 112 (100.00). Anal. Calcd for $C_{24}H_{27}N_3O_6$ (453.49): C 63.56, H 6.00, N 9.27. Found: C 63.87, H 5.78, N 9.57.

*General procedure for the preparation of 6,7,8,9-tetrahydro-5-(substituted phenyl)-[1,2,3]triazino[4,5-b]quinolin-4(3H, 5H, 10H)-ones (**3a**, **b**)*

A solution of sodium nitrite (0.21 g, 0.0025 mol) in water (2 mL) was added dropwise to a well-stirred cold suspension (−5 °C) of *ortho* aminonitrile **1a**, **b** (0.002 mol) in a mixture of concentrated hydrochloric acid (12 mL) and glacial acetic acid (4 mL). After complete addition, the ice bath was removed and stirring was continued at room temperature for 24 h. The precipitated solid was filtered, washed several times with water, dried and crystallized from dioxane/water (2:1).

*5-(4-Chlorophenyl)-6,7,8,9-tetrahydro-[1,2,3]triazino[4,5-b]quinolin-4(3H, 5H, 10H)-one (**3a**)* Yield 75 %, m.p. decomp. 202 °C. IR spectrum (KBr, *v*, cm^{-1}): 3423, 3301 (2NH), 1643 (C=O). ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.59–3.04 (m, 8H, $4CH_2$), 6.58 (s, 1H, C_5–H), 7.23–7.65 (m, 6H, Ar–H, 2NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.3 (2C), 25.6, 28.3, 31.8, 109.3, 111.5, 125.8 (2C), 130.1 (2C), 132.7, 135.1, 137.3 (2C), 161.7. MS *m/z* (%): 316 (16.98, M^++1), 81 (100.00). Anal. Calcd for $C_{16}H_{15}ClN_4O$ (314.77): C 61.05, H 4.80, N 17.80. Found: C 61.25, H 4.97, N 17.63.

*6,7,8,9-Tetrahydro-5-(3,4-dimethoxyphenyl)-[1,2,3]triazino[4,5-b]quinolin-4(3H, 5H, 10H)-one (**3b**)* Yield 70 %, m.p. 218–220 °C. IR spectrum (KBr, *v*, cm^{-1}): 3423, 3301 (2NH), 1642 (C=O). ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.60–2.09 (m, 4H, $2CH_2$), 2.30–2.87 (m, 4H, $2CH_2$), 3.76 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 6.62-7.43 (m, 6H, Ar–H, C_5-H, 2NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.1, 22.5, 25.8, 32.3 (2C), 55.5, 55.7, 109.3, 111.9, 116.8, 118.7, 128.5, 132.5, 136.4, 138.2, 148.4, 148.8, 160.7. MS *m/z* (%): 340 (0.03, M^+), 309 (100.00). Anal. Calcd for $C_{18}H_{20}N_4O_3$ (340.38): C 63.52, H 5.92, N 16.46. Found: C 63.75, H 5.71, N 16.13.

*General procedure for the preparation of 1,2,5,6,7,8,9,10-octahydro-N-{5-(substituted phenyl)-2-thioxo-pyrimido[4,5-b]quinolin-4-yl}thioureas (**4a**, **b**)*

Ammonium thiocyanate (0.456 g, 0.006 mol) was added to a solution of compound **1a**, **b** (0.002 mol) in glacial acetic acid (10 mL). The reaction mixture was heated at reflux temperature for 18 h, cooled and diluted with water. The separated solid was collected by filtration, dried, and crystallized from ethanol.

*N-{5-(4-Chlorophenyl)-1,2,5,6,7,8,9,10-octahydro-2-thioxopyrimido[4,5-b]quinolin-4-yl}thiourea (**4a**)* Yield 87 %, m.p. 249–250 °C. IR spectrum (KBr, *v*, cm^{-1}): 3441 (broad band NH_2, 3NH). ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.55–2.23 (m, 4H, $2CH_2$), 2.69–2.86 (m, 4H, $2CH_2$), 6.56 (s, 1H, C_5-H), 7.16–7.68 (m, 7H, Ar–H, NH, NH_2, D_2O-exchangeable), 10.52 (s, 1H, NH, D_2O-exchangeable), 11.16 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.1, 22.5, 26.1, 29.9 (2C), 81.6, 111.8, 126.9 (2C), 129.9 (2C), 132.1, 134.8, 137.1, 143.7, 166.3, 183.2 (2C). MS *m/z* (%): 405 (30.43, M^++1), 404 (26.09, M^+), 109 (100.00). Anal. Calcd for $C_{18}H_{18}ClN_5S_2$ (403.95): C 53.52, H 4.49, N 17.34. Found: C 53.18, H 4.70, N 17.11.

*1,2,5,6,7,8,9,10-Octahydro-N-{5-(3,4-dimethoxyphenyl)-2-thioxopyrimido[4,5-b]quinolin-4-yl}thiourea (**4b**)* Yield 83 %, m.p. 231–232 °C. IR spectrum (KBr, *v*, cm^{-1}): 3423, 3301 (NH_2, 3NH). ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.75–2.29 (m, 4H, $2CH_2$), 2.60–2.89 (m, 4H, $2CH_2$),

3.79 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.75–7.24 (m, 7H, Ar–H, C_5-H, NH, NH_2–D_2O-exchangeable), 9.38 (s, 1H, NH, D_2O-exchangeable), 10.55 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.3, 22.8, 26.1, 30.0 (2C), 55.6, 55.7, 81.3, 111.2, 112.6, 113.1, 122.7, 130.5, 135.9, 142.1, 144.3, 148.6, 167.6, 183.6 (2C). MS *m/z* (%): 429 (71.93, M^+), 428 (50.00, M^+-1), 246 (100.00). Anal. Calcd for $C_{20}H_{23}N_5O_2S_2$ (429.56): C 55.92, H 5.40, N 16.30. Found: C 55.64, H 5.11, N 15.96.

*General procedure for the preparation of ethyl N-[3-cyano-1,4,5,6,7,8-hexahydro-4-(substituted phenyl)quinolin-2-yl]acetimidates (**5a**, **b**)*

A mixture of compound **1a**, **b** (0.002 mol) and triethyl orthoacetate (5 mL) was heated at reflux temperature for 6–8 h. Excess reagent was evaporated in *vacuo* and the solid obtained was triturated with ice-water, filtered, washed with water, dried and crystallized from ethanol.

*Ethyl N-[4-(4-chlorophenyl)-3-cyano-1,4,5,6,7,8-hexahydroquinolin-2-yl]acetimidate (**5a**)* Yield 55 %, m.p. decomp. 235 °C. ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.34 (t, 3H, OCH_2CH_3), 1.78–1.83 (m, 4H, $2CH_2$), 2.36 (t, 2H, CH_2), 2.56 (s, 3H, CH_3), 2.80 (t, 2H, CH_2), 4.34–4.37 (q, 2H, OCH_2CH_3), 6.60 (s, 1H, C_4-H), 7.11–7.36 (m, 4H, Ar–H), 8.50 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 14.3, 15.1, 22.2 (2C), 25.6, 26.2, 33.6, 62.2, 84.2, 112.1, 116.8, 123.8 (2C), 129.6 (2C), 131.7, 133.4, 138.1, 153.6, 168.3. MS *m/z* (%): 358 (0.20, M^++2), 357 (0.33, M^++1), 356 (0.89, M^+), 283 (100.00). Anal. Calcd for $C_{20}H_{22}ClN_3O$ (355.86): C 67.50, H 6.23, N 11.81. Found: C 67.82, H 6.46, N 11.63.

*Ethyl N-[3-cyano-1,4,5,6,7,8-hexahydro-4-(3,4-dimethoxyphenyl)quinolin-2-yl]acetimidate (**5b**)* Yield 68 %, m.p. 163–164 °C. ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.34 (t, 3H, OCH_2CH_3), 1.78–1.83 (m, 4H, $2CH_2$), 2.43 (t, 2H, CH_2), 2.69 (s, 3H, CH_3), 2.85 (t, 2H, CH_2), 3.77 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 4.32–4.39 (q, 2H, OCH_2CH_3), 6.45 (s, 1H, C_4-H), 6.86–7.11 (m, 3H, Ar–H), 8.48 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 14.5, 15.1, 21.9, 22.3, 26.8 (2C), 33.9, 55.6, 55.7, 62.3, 83.9, 111.7, 112.3, 112.8, 116.5, 122.7, 135.2 (2C), 145.7, 148.5, 152.6, 167.9. MS *m/z* (%): 382 (1.08, M^++1), 381 (2.30, M^+), 309 (100.00). Anal. Calcd for $C_{22}H_{27}N_3O_3$ (381.47): C 69.27, H 7.13, N 11.02. Found: C 68.96, H 7.36, N 11.37.

*General procedure for the preparation of 2-(cyanomethyl)-6,7,8,9-tetrahydro-5-(substituted phenyl)pyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-ones (**6a**, **b**)*

A mixture of compound **1a**, **b** (0.002 mol) and ethyl cyanoacetate (5 mL) was heated at reflux temperature for 12 h. The solvent was evaporated to dryness in *vacuo* and the solid obtained was crystallized from dioxane.

*5-(4-Chlorophenyl)-2-(cyanomethyl)-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**6a**)* Yield 65 %, m.p. 185–187 °C. IR spectrum (KBr, *v*, cm^{-1}): 3353 (2NH), 2268 (C≡N), 1689 (C=O). ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.58–1.73 (m, 4H, $2CH_2$), 2.19 (t, 2H, CH_2), 2.70 (t, 2H, CH_2), 4.14 (s, 2H, CH_2CN), 6.57 (s, 1H, 2H, C_5–H), 7.33 (d, 2H, Ar–H), 7.56 (d, 2H, Ar–H), 7.69 (s, 2H, 2NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.1, 22.4, 25.8 (2C), 32.8 (2C), 87.8, 116.5, 118.2, 121.0 (2C), 128.7 (2C), 130.1, 133.4, 135.2, 152.7, 157.8, 161.9. MS *m/z* (%): 355 (0.36, M^++2), 354 (0.51, M^++1), 353 (1.47, M^+), 283 (100.00). Anal. Calcd for $C_{19}H_{17}ClN_4O$ (352.82): C 64.68, H 4.86, N 15.88. Found: C 64.44, H 5.03, N 15.53.

*2-(Cyanomethyl)-6,7,8,9-tetrahydro-5-(3,4-dimethoxyphenyl)pyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**6b**)* Yield 50 %, m.p. 173–175 °C. IR spectrum (KBr, *v*, cm^{-1}): 3409, 3312 (2NH), 2270 (C≡N), 1688 (C=O). ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.60–2.22 (m, 4H, $2CH_2$), 2.69–3.01 (m, 4H, $2CH_2$), 3.81 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.14 (s, 2H, CH_2CN), 6.46 (s, 1H, C_5-H), 6.88–7.63 (m, 5H, Ar–H, 2NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.3, 22.5, 25.6, 26.3, 32.4 (2C), 55.7, 55.9, 88.3, 112.2 (2C), 115.5, 117.1, 121.9, 136.2 (2C), 145.3, 149.1, 153.3, 156.9, 163.4. MS *m/z* (%): 379 (39.63, M^++1), 378 (31.71, M^+), 109 (100.00). Anal. Calcd for $C_{21}H_{22}N_4O_3$ (378.42): C 66.65, H 5.86, N 14.81. Found: C 66.39, H 5.57, N 14.61.

*General procedure for the preparation of 2-amino-1,4,5,6,7,8-hexahydro-4-(substituted phenyl)quinoline-3-carboxamides (**7a**, **b**)*

Ortho aminonitrile **1a, b** (0.01 mol) was heated at 60 °C with 70 % sulfuric acid (12 mL) for 8 h. The reaction mixture was cooled, diluted with cold water, and neutralized with 10 % aqueous sodium hydroxide solution. The precipitated solid was collected by filtration, washed with water, dried, and crystallized from ethanol.

*2-Amino-4-(4-chlorophenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide (**7a**)* Yield 70 %, m.p. 188–189 °C. IR spectrum (KBr, *v*, cm^{-1}): 3461, 3442 ($2NH_2$), 3338 (NH), 1664 (C=O). ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.56–1.70 (m, 4H, $2CH_2$), 2.30 (s, 2H, NH_2, D_2O-exchangeable), 2.48–2.63 (m, 4H, $2CH_2$), 5.96 (s, 2H, NH_2, D_2O-exchangeable), 6.17 (s, 1H, C_5-H), 7.12–7.43 (m, 5H, Ar–H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.4, 22.8, 25.7, 26.0, 32.2, 106.7, 115.2, 128.3 (2C), 130.0 (2C), 132.1, 136.5, 146.1, 156.3, 169.0. MS *m/z* (%): 304 (0.81, M^+), 258 (100.00). Anal. Calcd for $C_{16}H_{18}ClN_3O$ (303.79): C 63.26, H 5.97, N 13.83. Found: C 63.64, H 6.24, N 13.62.

*2-Amino-1,4,5,6,7,8-hexahydro-4-(3,4-dimethoxyphenyl)quinoline-3-carboxamide (**7b**)* Yield 55 %, m.p. 165–166 °C. IR spectrum (KBr, *v*, cm^{-1}): 3460, 3440 ($2NH_2$), 3336 (NH), 1663 (C=O). ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.59-1.90 (m, 4H, $2CH_2$), 2.20 (s, 2H, NH_2, D_2O-exchangeable), 2.64–2.75 (m, 4H, $2CH_2$), 3.82 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.65–7.05 (m, 6H, Ar–H, C_5–H, NH_2, D_2O-exchangeable), 7.75 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.1, 22.6, 26.3, 26.9, 33.1, 55.5, 55.6, 104.6, 109.7, 112.2, 115.3, 122.6, 133.7 (2C), 144.9, 148.6, 158.1, 168.7. MS *m/z* (%): 328 (4.30, M^+-1), 296 (100.00). Anal. Calcd for $C_{18}H_{23}N_3O_3$ (329.39): C 65.63, H 7.04, N 12.76. Found: C 65.31, H 7.36, N 12.48.

*General procedure for the preparation of 6,7,8,9-tetrahydro-2-(unsubstituted or methyl)-5-(substituted phenyl)pyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-ones (**8a–d**)*

A mixture of compound **7a,b** (0.002 mol) and the appropriate triethyl orthoester (0.002 mol) in xylene (10 mL) was heated at reflux temperature for 6 hours. The reaction mixture was cooled to room temperature and diluted with *n*-hexane (20 mL). The precipitated solid was collected by filtration, washed with *n*-hexane, dried, and crystallized from the appropriate solvent.

*5-(4-Chlorophenyl)-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**8a**)* (Crystallized from DMF), yield 78 %, m.p. decomp. 220 °C (El-Ashmaway *et al.*, 2012).

*6,7,8,9-Tetrahydro-5-(3,4-dimethoxyphenyl)pyrimido[4,5-b]quinolin-4(3H, 5H, 10H))-one (**8b**)* (Crystallized from DMF), yield 65 %, m.p. 257–259 °C (El-Ashmaway *et al.*, 2012).

*5-(4-Chlorophenyl)-6,7,8,9-tetrahydro-2-methylpyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**8c**)* (Crystallized from ethanol/DMF (3:2)), yield 76 %, m.p. 223–225 °C (El-Ashmaway *et al.*, 2012).

*6,7,8,9-Tetrahydro-5-(3,4-dimethoxyphenyl)-2-methylpyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**8d**)* (Crystallized from DMF), yield 70 %, m.p. 210–212 °C (El-Ashmaway *et al.*, 2012).

*General procedure for the preparation of 6,7,8,9-tetrahydro-2-(substituted phenyl)-5-(substituted phenyl)pyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-ones (**10a–f**)*

A mixture of compound **7a,b** (0.002 mol) and the appropriate benzylideneaniline **9a–c** (0.004 mol) in glacial acetic acid (10 mL) was heated at reflux temperature for 6–8 h. The reaction mixture was diluted with water (30 mL) and the separated solid was collected by filtration, dried, and crystallized from ethanol/water (2:1).

*5-(4-Chlorophenyl)-6,7,8,9-tetrahydro-2-phenylpyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**10a**)* Yield 80 %, m.p. 160–162 °C. IR spectrum (KBr, *v*, cm^{-1}): 3388 (2NH), 1664 (C=O). ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.58–1.77 (m, 4H, $2CH_2$), 2.32–2.80 (m, 4H, $2CH_2$), 6.18 (s, 1H, C_5-H), 7.19–8.22 (m, 10H, Ar–H, NH, D_2O-exchangeable), 10.55 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.6 (2C), 25.7, 26.2, 32.6, 105.3, 109.6, 127.1 (2C), 128.3, 128.7, 129.1 (2C), 130.3, 130.7, 131.2 (2C), 133.2 (2C), 138.6, 151.6, 153.4, 158.3. MS *m/z* (%): 391 (14.29, M^++1), 390 (17.69, M^+), 389 (37.69, M^+−1), 312 (100.00). Anal. Calcd for $C_{23}H_{20}ClN_3O$ (389.88): C 70.85, H 5.17, N 10.78. Found: C 70.56, H 4.93, N 11.05.

*5-(4-Chlorophenyl)-6,7,8,9-tetrahydro-2-(4-dimethylaminophenyl)pyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**10b**)* Yield 78 %, m.p. decomp. 183 °C. ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.78–2.79 (m, 8H, $4CH_2$), 3.06 (s, 6H, $2CH_3$), 6.60–7.46 (m, 9H, Ar–H, C_5-H), 9.84 (s, 1H, NH, D_2O-exchangeable), 10.47 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.1, 22.3, 25.6, 26.8, 31.1, 40.7 (2C), 104.9, 111.2, 112.6 (2C), 122.6, 124.8 (2C), 128.3 (2C), 129.1 (2C), 130.2, 135.3, 137.6, 149.4, 151.7, 154.6, 158.7. MS *m/z* (%): 433 (64.52, M^+), 429 (100.00). Anal. Calcd for $C_{25}H_{25}ClN_4O$ (432.95): C 69.35, H 5.82, N 12.94. Found: C 68.98, H 5.65, N 12.73.

*5-(4-Chlorophenyl)-6,7,8,9-tetrahydro-2-(3,4-dimethoxyphenyl)pyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**10c**)* Yield 72 %, m.p. 167–169 °C. ^{1}H NMR spectrum: (DMSO-d_6, δ ppm): 1.78–2.85 (m, 8H, $4CH_2$), 3.70 (s, 6H, $2OCH_3$), 6.84–7.48 (m, 8H, Ar–H, C_5-H), 9.77 (s, 1H, NH, D_2O-exchangeable), 10.45 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 21.6, 22.4, 26.8 (2C), 32.3, 55.6, 55.8, 101.1, 109.8, 112.2, 112.7, 120.4, 122.6, 124.7 (2C), 128.1 (2C), 131.7, 132.3, 138.6, 146.7, 148.3, 152.1, 156.6, 159.3. MS *m/z* (%): 450 (35.37, M^+), 164 (100.00). Anal. Calcd for $C_{25}H_{24}ClN_3O_3$ (449.93): C 66.74, H 5.38, N 9.34. Found: C 67.11, H 5.10, N 8.96.

*6,7,8,9-Tetrahydro-5-(3,4-dimethoxyphenyl)-2-phenylpyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**10d**)* Yield 65 %, m.p. 152–154 °C. IR spectrum (KBr, *v*, cm^{-1}): 3299, 3197 (2NH), 1652 (C=O). MS *m/z* (%): 416 (32.98, M^++1), 415 (7.82, M^+), 383 (100.00). Anal. Calcd for $C_{25}H_{25}N_3O_3$ (415.48): C 72.27, H 6.06, N 10.11. Found: C 72.47, H 5.85, N 10.34.

*6,7,8,9-Tetrahydro-5-(3,4-dimethoxyphenyl)-2-(4-dimethylaminophenyl)pyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**10e**)* Yield 60 %, m.p. 185–187 °C. ^{1}H NMR spectrum: (DMSO-d_6, δ ppm): 1.23–1.79 (m, 4H, $2CH_2$), 2.62–2.93 (m, 4H, $2CH_2$), 3.04 (s, 6H, $2CH_3$), 3.85 (s, 6H, $2OCH_3$), 6.71 (s, 1H, C_5-H), 6.79 (d, 2H, Ar–H), 7.10–7.27 (m, 3H, Ar–H), 7.68 (d, 2H, Ar–H), 7.96 (s, 1H, NH, D_2O-exchangeable), 9.67 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.1, 22.5, 26.6 (2C), 31.9, 41.1 (2C), 55.6, 55.7, 105.7, 111.5, 112.2 (2C), 112.7, 115.6, 122.1, 122.6, 128.3 (2C), 133.6, 135.2, 145.7, 147.3, 149.5, 152.3, 154.2, 158.6. MS *m/z* (%): 459 (1.57, M^++1), 458 (3.13, M^+), 296 (100.00). Anal. Calcd for $C_{27}H_{30}N_4O_3$ (458.55): C 70.72, H 6.59, N 12.22. Found: C 70.93, H 6.23, N 12.37.

*6,7,8,9-Tetrahydro-2,5-bis(3,4-dimethoxyphenyl)pyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**10f**)* Yield 60 %, m.p. 161–162 °C. ^{1}H NMR spectrum: (DMSO-d_6, δ ppm): 1.49–1.79 (m, 4H, $2CH_2$), 2.66–3.00 (m, 4H, $2CH_2$), 3.85 (s, 6H, $2OCH_3$), 3.87 (s, 6H, $2OCH_3$), 5.55 (s, 1H, C_5-H), 6.98–7.57 (m, 7H, Ar–H, NH, D_2O-exchangeable), 9.84 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.0, 24.6, 25.3, 26.8, 32.4, 55.6, 55.7, 55.8, 55.9, 106.1, 111.3, 111.7, 113.0, 115.2, 115.5, 119.1, 120.8, 121.0, 129.3, 134.9, 146.5, 148.5, 148.8, 151.5, 151.7, 152.5, 156.9. MS *m/z* (%): 476 (8.03, M^++1), 475 (3.55, M^+), 84 (100.00). Anal. Calcd for $C_{27}H_{29}N_3O_5$ (475.54): C 68.19, H 6.15, N 8.84. Found: C 68.44, H 6.30, N 8.67.

*General procedure for the preparation of 3,4,6,7,8,9-hexahydro-4-imino-3-phenylamino-5-(substituted phenyl)-5H, 10H-pyrimido[4,5-b]quinolines (**12a, b**)*

A mixture of compound **11a,b** (0.002 mol) and phenylhydrazine (0.216 g, 0.002 mol) in absolute ethanol (15 mL) was heated at reflux temperature for 10 h. The reaction mixture was concentrated and the precipitated solid was collected by filtration, washed with water, dried, and crystallized from dioxane.

*5-(4-Chlorophenyl)-3,4,6,7,8,9-hexahydro-4-imino-3-phenylamino-5H, 10H-pyrimido[4,5-b]quinoline (**12a**)* Yield 50 %, m.p. 222–223 °C. IR spectrum (KBr, *v*, cm^{-1}): 3421 (3NH). ^{1}H NMR spectrum: (DMSO-d_6, δ ppm): 1.59–1.74 (m, 4H, $2CH_2$), 2.19 (t, 2H, CH_2), 2.70 (t, 2H, CH_2), 6.58 (s, 1H, C_5–H), 7.32–7.58 (m, 11H, Ar–H, C_2–H, NH, D_2O-exchangeable), 7.99 (s, 1H, NH, D_2O-exchangeable), 9.56 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.8, 25.7, 26.0, 31.4, 32.2, 87.9, 111.9, 115.1, 116.8, 120.7, 128.2 (2C), 129.9 (2C), 132.1 (2C), 132.5, 136.0, 138.2, 146, 150.1, 153.2, 168.0. MS *m/z* (%): 404 (0.04, M^+), 403 (0.03, M^+−1), 283 (100.00). Anal. Calcd for $C_{23}H_{22}ClN_5$ (403.91): C 68.39, H 5.49, N 17.34. Found: C 68.11, H 5.21, N 17.60.

*3,4,6,7,8,9-Hexahydro-4-imino-5-(3,4-dimethoxyphenyl)-3-phenylamino-5H, 10H-pyrimido[4,5-b]quinoline (**12b**)* Yield 55 %, m.p. 190–192 °C. IR spectrum (KBr, *v*, cm^{-1}): 3429 (3NH). ^{1}H NMR spectrum: (DMSO-d_6, δ ppm): 1.59–1.86 (m, 4H, $2CH_2$), 2.32 (t, 2H, CH_2), 2.67 (t, 2H, CH_2), 3.75 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 6.52 (s, 1H, C_5–H), 6.79–7.06 (m, 10H, Ar–H, C_2–H, NH, D_2O-exchangeable), 8.70 (s, 1H, NH, D_2O-exchangeable), 9.65 (s, 1H, NH, D_2O-exchangeable). MS *m/z* (%): 430 (9.96, M^++1), 429 (17.57, M^+), 92 (100.00). Anal. Calcd for $C_{25}H_{27}N_5O_2$ (429.51): C 69.91, H 6.34, N 16.31. Found: C 69.64, H 6.58, N 15.97.

*General procedure for the preparation of 7,8,9,10,11,12-hexahydro-2,12-(substituted phenyl)-[1,2,4]triazolo[2′,3′:3,4]pyrimido[6,5-b]quinolines (**14a–d**)*

A mixture of compound **11a, b** (0.002 mol) and the appropriate benzhydrazide **13a, b** (0.002 mol) in DMF (10 mL) was heated at reflux temperature for 8–10 h. The solvent was evaporated in *vacuo* and the remaining solid was purified by crystallization from dioxane.

*12-(4-Chlorophenyl)-7,8,9,10,11,12-hexahydro-2-phenyl-[1,2,4]triazolo[2′,3′:3,4]pyrimido[6,5-b]quinoline (**14a**)* Yield 57 %, m.p. 162–164 °C. ^{1}H NMR spectrum:

(DMSO-d_6, δ ppm): 1.58–1.72 (m, 4H, $2CH_2$), 2.16 (t, 2H, CH_2), 2.71 (t, 2H, CH_2), 6.63 (s, 1H, C_{12}–H), 7.32–7.99 (m, 11H, Ar–H, C_5-H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.8 (2C), 26.2, 30.1, 33.7, 111.1, 113.5, 126.7 (2C), 127.2, 128.3, 128.9, 129.6 (2C), 130.1, 130.7, 131.3, 133.6, 136.8, 141.5, 148.2, 151.6, 157.3, 158.7. MS *m/z* (%): 415 (42.98, M^++1), 414 (51.24, M^+), 413 (73.55, M^+−1), 87 (100.00). Anal. Calcd for $C_{24}H_{20}ClN_5$ (413.90): C 69.64, H 4.87, N 16.92. Found: C 69.40, H 5.11, N 16.68.

*12-(4-Chlorophenyl)-7,8,9,10,11,12-hexahydro-2-(4-hydroxyphenyl)-[1,2,4]triazolo[2′,3′:3,4]pyrimido-[6,5-b]quinoline (**14b**)* Yield 52 %, m.p. 225–226 °C. IR spectrum (KBr, *v*, cm^{-1}): 3409 (OH), 3367 (NH). ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.60–1.89 (m, 4H, $2CH_2$), 2.32 (t, 2H, CH_2), 2.87 (t, 2H, CH_2), 6.57–6.93 (m, 5H, Ar–H, C_{12}–H), 7.27–7.95 (m, 6H, Ar–H, C_5-H, NH, D_2O-exchangeable), 10.03 (s, 1H, OH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.7 (2C), 25.9, 30.4, 33.2, 111.3, 112.7, 117.1 (2C), 123.4, 127.1 (2C), 129.3 (2C), 130.1 (2C), 130.9, 136.4, 139.7, 148.7, 151.3, 153.8, 156.9, 159.2. MS *m/z* (%): 429 (0.01, M^+-1), 283 (100.00). Anal. Calcd for $C_{24}H_{20}ClN_5O$ (429.90): C 67.05, H 4.69, N 16.29. Found: C 67.37, H 4.43, N 15.97.

*7,8,9,10,11,12-Hexahydro-12-(3,4-dimethoxyphenyl)-2-phenyl-[1,2,4]triazolo[2′,3′:3,4]pyrimido[6,5-b]-quinoline (**14c**)* Yield 45 %, m.p. 158–160 °C. ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.59–2.27 (m, 4H, $2CH_2$), 2.49–2.88 (m, 4H, $2CH_2$), 3.80 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.51 (s, 1H, C_{12}-H), 6.73–7.95 (m, 10H, Ar–H, C_5–H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.3, 22.7, 26.1 (2C), 32.9, 55.7, 55.9, 111.9, 112.2 (2C), 113.3, 120.3, 125.9, 126.7, 128.1, 128.4, 129.7, 130.3, 130.8, 136.3, 147.9 (2C), 148.3, 148.7, 157.6, 158.9. MS *m/z* (%): 441 (0.89, M^++2), 440 (3.58, M^++1), 439 (13.81, M^+), 105 (100.00). Anal. Calcd for $C_{26}H_{25}N_5O_2$ (439.51): C 71.05, H 5.73, N 15.93. Found: C 71.32, H 5.51, N 15.63.

*7,8,9,10,11,12-Hexahydro-2-(4-hydroxyphenyl)-12-(3,4-dimethoxyphenyl)-[1,2,4]triazolo[2′,3′:3,4]pyrimido[6,5-b] quinoline (**14d**)* Yield 40 %, m.p. 165–167 °C. ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.64–1.83 (m, 4H, $2CH_2$), 2.29 (t, 2H, CH_2), 2.98 (t, 2H, CH_2), 3.78 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.57–7.85 (m, 10H, Ar–H, C_5–H, C_{12}–H, NH, D_2O-exchangeable), 11.41 (s, 1H, OH, D_2O-exchangeable). MS *m/z* (%): 456 (6.40, M^++1), 455 (4.19, M^+), 453 (100.00, M^+-2). Anal. Calcd for $C_{26}H_{25}N_5O_3$ (455.51): C 68.56, H 5.53, N 15.37. Found: C 68.35, H 5.37, N 15.11.

*General procedure for the preparation of 1-{6,7,8,9-tetrahydro-4-imino-5-(substituted phenyl)pyrimido[4,5-b]quinolin-3(4H, 5H, 10H)-yl}thiourea (**15a, b**)*

A mixture of compound **11a,b** (0.002 mol) and thiosemicarbazide (0.182 g, 0.002 mol) in absolute ethanol (15 mL) was heated at reflux temperature for 18 h. The reaction mixture was concentrated and the precipitated solid was collected by filtration, washed with aqueous ethanol, dried, and crystallized from ethanol/water (2:1).

*1-{5-(4-Chlorophenyl)-6,7,8,9-tetrahydro-4-iminopyrimido[4,5-b]quinolin-3(4H, 5H, 10H)-yl}thiourea (**15a**)* Yield 78 %, m.p. 200–201 °C. ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.56–1.85 (m, 4H, $2CH_2$), 2.21 (t, 2H, CH_2), 2.70 (t, 2H, CH_2), 4.50 (s, 2H, NH_2, D_2O-exchangeable), 6.61 (s, 1H, C_5–H), 7.11–7.58 (m, 7H, Ar–H, C_2–H, 2NH, D_2O-exchangeable), 8.65 (s, H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.3, 22.7, 26.9, 32.6 (2C), 89.1, 111.2, 125.1 (2C), 129.8 (2C), 130.6, 136.4, 138.7, 148.1, 156.9, 161.2, 181.6. MS *m/z* (%): 387 (4.66, M^+), 60 (100.00). Anal. Calcd for $C_{18}H_{19}ClN_6S$ (386.9): C 55.88, H 4.95, N 21.72. Found: C 55.97, H 5.23, N 21.54.

*1-{6,7,8,9-Tetrahydro-4-imino-5-(3,4-dimethoxyphenyl)pyrimido[4,5-b]quinolin-3(4H, 5H, 10H)-yl}thiourea (**15b**)* Yield 75 %, m.p. 198–200 °C. ^1H NMR spectrum: (DMSO-d_6, δ ppm): 1.59–1.86 (m, 4H, $2CH_2$), 2.32–2.86 (m, 4H, $2CH_2$), 3.76 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.50 (s, 2H, NH_2, D_2O-exchangeable), 6.51 (s, 1H, C_5–H), 6.78–7.20 (m, 4H, Ar–H, C_2–H), 7.51 (s, 1H, NH, D_2O-exchangeable), 7.61 (s, 1H, NH, D_2O-exchangeable), 8.56 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 22.1, 22.5, 25.8, 32.8 (2C), 55.5, 55.7, 88.4, 111.6, 116.8, 118.7, 120.6, 128.5 (2C), 148.4 (2C), 148.8, 157.8, 160.7, 181.0. MS *m/z* (%): 412 (0.80, M^+), 60 (100.00). Anal. Calcd for $C_{20}H_{24}N_6O_2S$ (412.51): C 58.23, H 5.86, N 20.37. Found: C 58.47, H 5.62, N 20.55.

Antitumor screening

Under sterile conditions, cell lines were grown in RPMI 1640 media (Gibco, NY, USA) supplemented with 10 % fetal bovine serum (Biocell, CA, USA). The concentrations of the compounds ranging from 0.01 to 100 μM were prepared in phosphate buffer saline. Each compound was initially solubilized in DMSO, however, each final dilution contained less than 1% DMSO. Solutions of different concentrations (0.2 mL) were pipetted into separate well of 96-multi-well microtiter plate, duplicate wells were prepared for each individual dose. Cell culture (1.8 mL) containing a cell population of 6×10^4 cells/mL was

pipetted into each well. Controls containing only phosphate buffer saline and DMSO at identical dilutions, were also prepared in the same manner. The cultures were incubated with the compounds for 48 h in a humidified incubator at 37 °C and in atmosphere of 5 % CO_2. After 48 h, cells in each well were diluted 10 times with saline and counted using a coulter counter (Boyd and Paull, 1995; Grever *et al.*, 1992; Monks *et al.*, 1991; Skahan *et al.*, 1990). The data reported as mean-graph of the percent growth of the treated cells, and presented as percentage growth inhibition (GI %) (Table 1).

Acknowledgments The author is deeply thankful to the staff members of the Department of Health and Human Services, National Cancer Institute (NCI), Bethesda, Maryland, USA for carrying out the antitumor screening of the newly synthesized compounds.

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