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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Synthesis and antitumor screening of new series of pyrimido-[4,5-*b*]quinolines and [1,2,4]triazolo[2′,3′:3,4]pyrimido[6,5-*b*]quinolines

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**Abstract** New series of pyrimido[4,5-*b*]quinolines and [1,2,4]triazolo[2′,3′:3,4]pyrimido[6,5-*b*]quinolines have been synthesized. Compounds **4a, 4e**, **4f**, **4h**, **5b**, **5d**, **6a**, **6d**, **6e**, **8c**, **8d**, **10c–e**, **10h**, **11a, 11b,** and **12a** were tested for in vitro antitumor activity against human breast carcinoma (MCF-7) cell line, where compound **8d** was found to be the most active member with $IC_{50}$ value of 3.62 μM. The DNA-binding affinity for the same compounds showed that compounds **8d** and **10d** exhibited the highest affinity to DNA. The detailed synthesis, spectroscopic, and biological data are reported.

**Keywords** Pyrimido[4,5-*b*]quinolines · [1,2,4]Triazolo[2′3′:3,4]pyrimido[6,5-*b*]quinolines · In vitro antitumor screening

## Introduction

Quinoline and pyrimidoquinoline derivatives continue to attract the interest of medicinal chemists due to the wide range of biological properties. Some of them showed antitumor activity (Al-Said *et al*., 2011; Abbas *et al*., 2011; Ghorab *et al*., 2010; Alqasoumi *et al*., 2010; Ghorab *et al*., 2009; Ali *et al*., 2008; Shrestha *et al*., 2008; Abouzid and Shouman, 2008; Ali *et al*., 2007; Wilson *et al*., 2007; Rhee *et al*., 2007), while other derivatives have been used as antimicrobial agents (El-Gazzar *et al*., 2008; Suresh *et al*., 2003; Selvi *et al*., 2006). Also, some of these derivatives showed antimalarial activity (Joshi *et al*., 2005), analgesic properties (El-Gazzar *et al*., 2008; Kidwai and Negi, 1997; El-Gazzar *et al*., 2009a, b) as well as anti-inflammatory activity (El-Gazzar *et al*., 2008, 2009a, b; Abd El-Salam *et al*., 2009). Considering these published data and our previous interests in the field of fused pyrimidines as antitumor agents (El-Sayed *et al*., 2011; El-Ashmawy *et al*., 2010; Mahran *et al*., 1998), it appeared worthwhile to prepare certain derivatives of pyrimido[4,5-*b*]quinoline and [1,2,4]triazolo[2′,3′:3,4]pyrimido[6,5-*b*]quinoline skeletons whose rigidity might contribute to a potential DNA-binding affinity and antitumor activity. Herein, we report their synthesis, preliminary DNA-binding assay and antineoplastic evaluation against human breast carcinoma (MCF-7) cell line.

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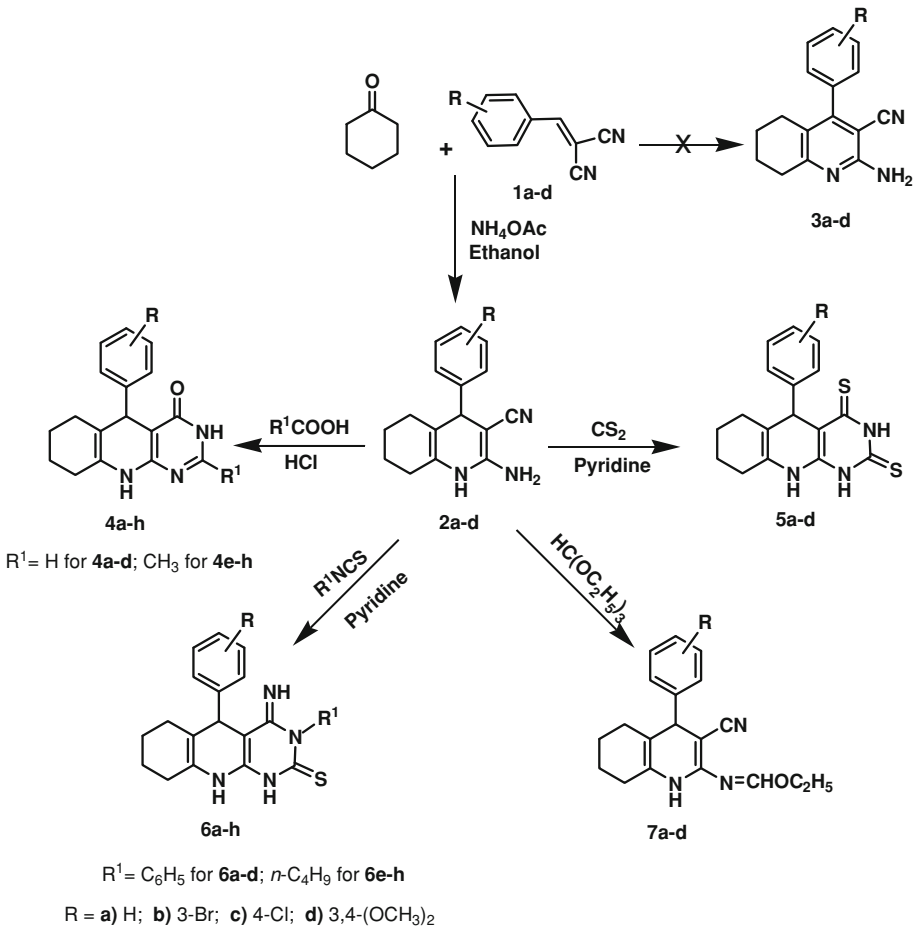
## Results and discussion

### Chemistry

2-Amino-1,4,5,6,7,8-hexahydro-4-(substituted)phenylquinoline-3-carbonitriles (**2a–d**) were prepared (Elkholy, 2007), via the reaction of cyclohexanone, ammonium acetate, and the appropriate benzylidenemalononitrile **1a–d** (Bigi *et al*., 2000). The $^1$H NMR spectra of these compounds revealed the presence of a singlet at $\delta$ 5.51–6.55 ppm significant for $C_4$–H. This excluded structures **3a–d** and supported the formation of the adducts **2a–d**. Compounds **2a–d**, typical $\beta$-enaminonitrile derivatives, were used as precursors for the synthesis of pyrimidoquinoline derivatives. Refluxing **2a–d** with aliphatic acids, namely formic and acetic acids in the presence of catalytic amount of concentrated hydrochloric acid yielded 6,7,8,9-tetrahydro-2-(unsubstituted or methyl)-5-(substituted)phenylpyrimido[4,5-*b*]quinolin-4(3*H*, 5*H*, 10*H*)-ones (**4a–h**) (Scheme 1). The reaction

**Scheme 1** Synthesis of compounds 2-7




proceeds via initial acid hydrolysis of the nitrile group to give the corresponding carboxamide derivatives, which then condensed with formic or acetic acid with loss of two molecules of water to yield the desired products **4a**–**h**. The spectral data (IR, MS and $^1$H NMR) of these compounds were in agreement with the assigned structures. The IR spectrum for compound **4d** showed absorption band at 1,663 cm$^{-1}$ for (C=O), also it showed the disappearance of the (C≡N) absorption band. Regarding the $^1$H NMR spectra of compounds **4e**–**h**, the protons of the ($CH_3$) group of the pyrimidine ring appeared as a singlet at $\delta$ 2.11 and 1.96 ppm for the two tested compounds **4f** and **4h**, respectively. The remaining protons were observed at the expected regions. The mass spectrum of compound **4e** showed a molecular ion peak of *m*/*z* 293 and a base peak of *m*/*z* 216.

On the other hand, the *ortho* aminonitriles **2a**–**d** were further utilized for another cyclocondensation reaction using carbon disulfide in pyridine to afford the 5,6,7,8,9,10-hexahydro-5-(substituted)phenyl-pyrimido[4,5-*b*]quinoline-2,4(1*H*, 3*H*)-dithiones (**5a**–**d**) (Scheme 1). The structural assignments of compounds **5a**–**d** were based on microanalytical and spectral data. The IR spectra showed the disappearance of the (C≡N) absorption band confirming the formation of the desired cyclic structures. The mass spectrum of compound **5b** showed peaks at *m*/*z* 408 ($M^+$+2), 407 ($M^+$+1), 406 ($M^+$) and a base peak at *m*/*z* 105. Regarding the $^1$H NMR spectrum of the same compound, two singlets at $\delta$ 6.57 and 8.60 ppm indicating ($C_5$–H) and (NH), respectively, were clearly identified. Also, it showed two triplets at $\delta$ 2.19 and 2.70 ppm for 2-($CH_2$) groups, in addition to a multiplet at $\delta$ 1.59–1.72 ppm that represents the four protons of the other 2-($CH_2$) groups. Aromatic protons appeared at the expected regions.

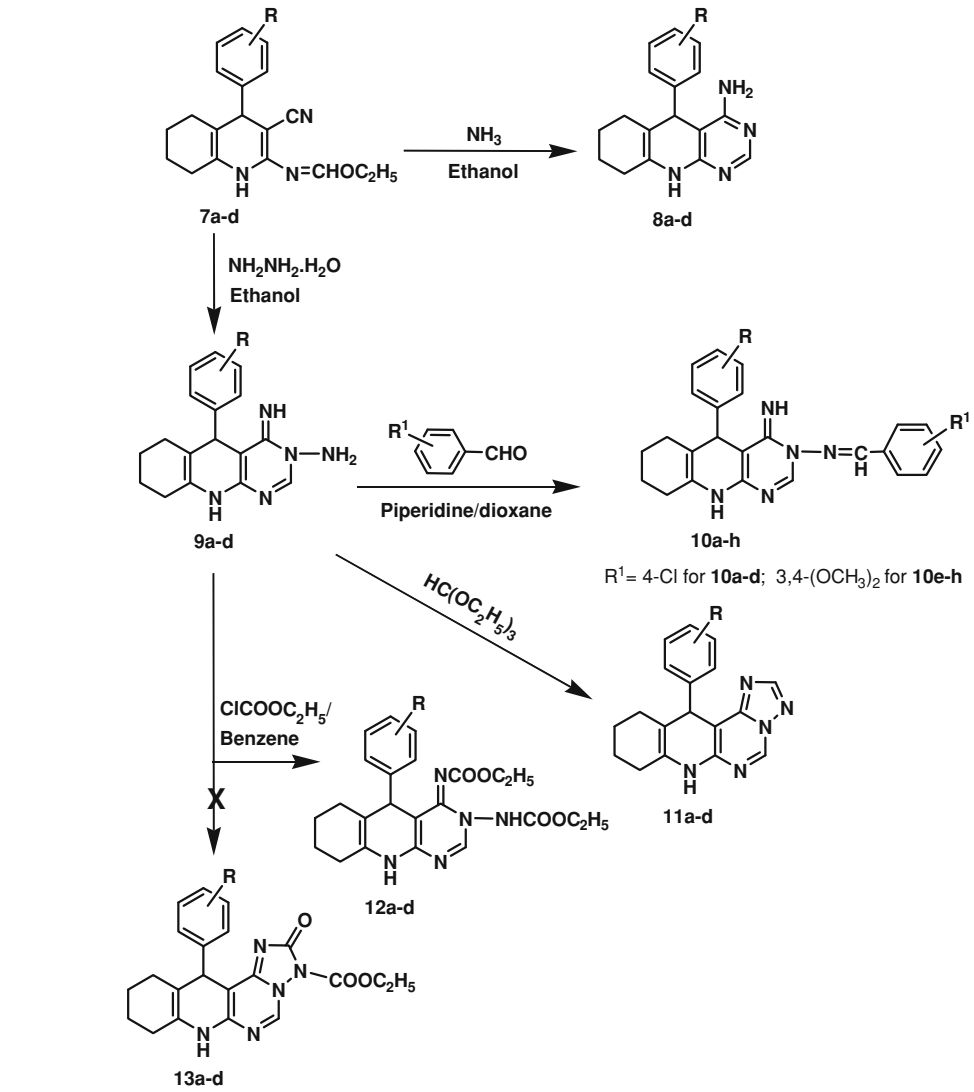
Upon heating compound **2a**–**d** with *n*-butyl or phenyl isothiocyanate in pyridine, 3,4,5,6,7,8,9,10-octahydro-4-imino-3-(*n*-butyl or phenyl)-5-(substituted)phenylpyrimido[4,5-*b*]quinoline-2(1*H*)-thiones (**6a**–**h**) were obtained (Scheme 1). The structures of these compounds were confirmed by elemental analyses, IR and $^1$H NMR spectra. The IR spectra of the tested compounds displayed absorption band at the range 3,399–3,425 cm$^{-1}$ for (3NH), also it showed the absence of the (C≡N) absorption band confirming the formation of the desired cyclic structures. The $^1$H NMR spectrum of **6h** exhibited a significant multiplet at $\delta$ 0.88–1.38 ppm corresponding to the butyl protons, in

addition to a singlet at $\delta$ 3.41 ppm for (2NH) groups. Moreover, the signals indicating ($4CH_2$), ($C_5$–H) and aromatic protons were observed at the expected regions.

Condensation of compound **2a–d** with triethyl orthoformate yielded the corresponding ethyl *N*-[3-cyano-1,4,5,6,7,8-hexahydro-4-(substituted)phenylquinolin-2-yl]formimidates (**7a–d**) (Scheme 1). The structural assignments of compounds **7a–d** were based on analytical and spectral data. The IR spectra displayed an absorption band at 2,208–2,213 $cm^{-1}$ for (C≡N). $^1$H NMR spectra showed the typical quartet-triplet pattern characteristic for the ethyl ester group. The $^1$H NMR spectrum of **7a** displayed a quartet signal at $\delta$ 4.34–4.38 ppm for (2H, $O\underline{CH_2}CH_3$), while the triplet signal was observed at $\delta$ 1.34 ppm for (3H, $OCH_2\underline{CH_3}$). Furthermore, the signals characteristic for 4-($CH_2$), (NH), (N=CH), ($C_4$–H) and the aromatic protons appeared at the expected regions.

Reaction of compound **7a–d** with 35 % ammonia solution in ethanol produced the corresponding pyrimdoquinoline derivatives **8a–d** rather than the opened analogs as evidenced from IR data (Scheme 2). The structures of these compounds were established based on their elemental analyses and spectral data. The IR spectra revealed the absence of the (C≡N) absorption band, confirming the formation of the desired cyclic structures. In addition, the $^1$H NMR spectra of these compounds showed the disappearance of the quartet-triplet pattern of the ethyl ester group and the appearance of a significant singlet at $\delta$ 3.01–3.03 ppm in all derivatives corresponding to the ($NH_2$) group. Treatment of compounds **7a–d** with hydrazine hydrate in ethanol yielded 3-amino-3,4,6,7,8,9-hexahydro-4-imino-5-(substituted)phenyl-5*H*, 10*H*-pyrimido[4,5-*b*] quinolines (**9a–d**) (Scheme 2). The IR spectrum of compound **9c** showed three absorption bands at 3427, 3353, and

**Scheme 2** Synthesis of compounds 8-12



3201 $cm^{-1}$ for ($NH_2$) and 2-(NH) groups, respectively. Also, it showed the disappearance of the (C≡N) absorption band. The $^1$H NMR spectrum of the same compound revealed the presence of three singlets at $\delta$ 5.46, 5.83, and 7.76 ppm indicating ($NH_2$), ($C_5$–H) and 2-(NH) groups, respectively. In addition, the remaining protons appeared at the expected regions.

The preparation of 3,4,6,7,8,9-hexahydro-4-imino-3-(substituted benzylidene)amino-5-(substituted)-phenyl-5*H*, 10*H*-pyrimido[4,5-*b*]quinolines (**10a**–**h**) has been accomplished by reacting equimolar amounts of compound **9a**–**d** with the appropriate benzaldehyde in dioxane in the presence of piperidine as a basic catalyst (Scheme 2). The elemental analyses and spectral data (IR, MS, and $^1$H NMR) of compounds **10a**–**h** were in agreement with the assigned structures. The mass spectrum of compound **10c** showed a molecular ion peak of *m*/*z* 450 and a base peak of *m*/*z* 283. The $^1$H NMR spectrum of compound **10a** exhibited three singlets at $\delta$ 3.25, 3.70, and 5.24 ppm indicating (NH), (NH), and ($C_5$–H), respectively. Also, it showed a multiplet at $\delta$ 7.24–7.52 ppm characteristic for (Ar–H, N=CH).

Condensation of compound **9a**–**d** with triethyl orthoformate afforded 7,8,9,10,11,12-hexahydro-12-(substituted) phenyl-[1,2,4]triazolo[2′,3′:3,4]pyrimido[6,5-*b*]quinolines (**11a**–**d**) (Scheme 2). The structures of compounds **11a**–**d** were ascertained through elemental analyses and $^1$H NMR spectra. The notable feature in the $^1$H NMR spectra is the presence of four singlets characteristic for ($C_2$–H), ($C_5$–H), (NH), and ($C_{12}$–H). For example, the $^1$H NMR spectrum of **11b** showed four singlets at $\delta$ 5.08, 8.20, 8.41, and 9.40 ppm corresponding to ($C_{12}$–H), (NH), ($C_5$–H), and ($C_2$–H), respectively. In addition, the signals representing the ($4CH_2$) groups and the aromatic protons appeared at the expected regions. The mass spectrum of compound **11d** showed a molecular ion peak of *m*/*z* 364 and a base peak of *m*/*z* 309.

It is worth mentioning that reaction of compound **9a**–**d** with ethyl chloroformate in benzene afforded the unexpected opened structures **12a**–**d** rather than the desired cyclic compounds **13a**–**d** as evidenced by analytical and spectroscopic data (Scheme 2). The IR spectra showed an intense carbonyl ester absorption band at 1,710–1,729 $cm^{-1}$ in all derivatives. The IR spectrum of **12b** as an example displayed absorption bands at 3,332 and 1,729 $cm^{-1}$ indicating (2NH) and (C=O) groups, respectively. The notable feature in the $^1$H NMR spectra is the presence of two multiplets at $\delta$ 1.26–1.42 and 4.21–4.31 ppm corresponding to ($2COOCH_2\underline{CH_3}$) and ($2COO\underline{CH_2}CH_3$), respectively, confirming the formation of compounds **12a**–**d**.

## Biological investigation

### The in vitro antitumor activity against breast cancer (MCF-7) cell line

Potential antitumor activities of compounds **4a, 4e**, **4f**, **4h**, **5b**, **5d**, **6a**, **6d**, **6e**, **8c**, **8d**, **10c**–**e**, **10h**, **11a, 11b,** and **12a** were screened at the National Cancer Institute (NCI), Cancer Biology Department, Pharmacology Unit, Cairo, Egypt, adopting the sulforhodamine B (SRB) assay, method of (Skehan *et al*., 1990) using doxorubicin as a reference antitumor agent.

The results of in vitro antitumor activity of the tested compounds indicated that compound **8d** exhibited the highest cytotoxic activity against human breast cancer cells (MCF-7) with $IC_{50}$ value of 3.62 μM. Compounds **4e**, **4f**, **5b**, **6a**, **6d**, **6e, 8c**, **10d**, **10e, 10h,** and **12a** showed lower activity with $IC_{50}$ values of 4.97, 4.03, 4.50, 6.98, 5.30, 5.70, 3.89, 3.76, 5.37, 6.17, and 5.44 μM, respectively. On the other hand, compounds **4a**, **4h**, **5d**, **10c**, **11a,** and **11b** are the least active members with $IC_{50}$ values greater than 10 μM (Table 1).

**Table 1** In vitro antitumor activity of the selected compounds and doxorubicin against human breast cancer cells (MCF-7)

| Comp. no. | Antitumor activity $IC_{50}$ (μM)[a, b] | Comp. no. | Antitumor activity $IC_{50}$ (μM)[a, b] |
|---|---|---|---|
| **4a** | >10.00 | **8d** | 3.62 |
| **4e** | 4.97 | **10c** | >10.00 |
| **4f** | 4.03 | **10d** | 3.76 |
| **4h** | >10.00 | **10e** | 5.37 |
| **5b** | 4.50 | **10h** | 6.17 |
| **5d** | >10.00 | **11a** | >10.00 |
| **6a** | 6.98 | **11b** | >10.00 |
| **6d** | 5.30 | **12a** | 5.44 |
| **6e** | 5.70 | Doxorubicin | 0.70 |
| **8c** | 3.89 | | |

[a] $IC_{50}$, compound concentration required to inhibit tumor cell proliferation by 50 %

[b] Values are means of three experiments

**Table 2** DNA-binding affinity of the selected compounds and bleomycin using methyl green/DNA displacement assay

| Comp. no. | Methyl green/DNA $IC_{50}$ (μM[a]) | Comp. no. | Methyl green/DNA $IC_{50}$ (μM) | Comp. no. | Methyl green/DNA $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| 4a | 88 ± 2 | **6c** | 55 ± 2 | **10d** | 14 ± 2 |
| 4e | 22 ± 2 | **6d** | 40 ± 2 | **10e** | 32 ± 2 |
| 4f | 32 ± 2 | **6e** | 72 ± 1 | **10h** | 38 ± 2 |
| 4h | 25 ± 2 | **6g** | 88 ± 2 | **11a** | 88 ± 2 |
| 5a | 72 ± 1 | **8c** | 25 ± 2 | **11b** | 90 ± 2 |
| 5b | 76 ± 1 | **8d** | 12 ± 1 | **12a** | 40 ± 2 |
| 5d | 38 ± 2 | **10a** | 88 ± 2 | Bleomycin | 10 ± 2 |
| 6a | 25 ± 2 | **10c** | 70 ± 2 | | |

[a] Values represent the concentration (mean ± SD, $n = 3$–5 separate determinations) required for 50 % decrease in the initial absorbance of DNA/methyl green solution

### DNA-binding assay

The mechanism by which several known antitumor agents produce their effects involves interaction with DNA. Alkylating agents and intercalating agents represent two major classes of antitumor drugs that act by direct interaction with DNA. Based on this fact, some short-term procedures have been designed to be applied for the discovery and evaluation of both naturally-occurring and synthetic compounds that function by this mechanism. DNA-binding assay (Pezzuto *et al*., 1983, 1991) and methyl green/DNA displacement assay (Burres *et al*., 1992) were applied for determination of the interaction of small molecular weight compounds with DNA.

### DNA-binding assay on TLC plates

It was demonstrated that when DNA was applied to RP-18 TLC plates, migration was observed when MeOH/$H_2O$ (8:2) was used as the elution solvent. However, when DNA was mixed with compounds with which it interacts (e.g., ethidium bromide), the complex was retained at the origin with the same elution solvent. On the other hand, compounds with no affinity did not retain the DNA at the origin (Pezzuto *et al*., 1983, 1991).

Results from DNA-binding assay revealed that compounds **8d** and **10d** displayed the highest DNA-binding affinity which was demonstrated by retaining the complex at the origin or by its migration for a very short distance. Compounds **4e**, **4f**, **4h**, **5d**, **6a**, **6d**, **8c**, **10e**, **10h,** and **12a** showed moderate affinity, while compounds **5a**, **5b**, **6c**, **6e,** and **10c** exhibited weak affinity. On the other hand, compounds **4a**, **6g**, **10a**, **11a,** and **11b** were proved to be inactive.

### Methyl green/DNA displacement assay

Methyl green reversibly binds polymerized DNA forming a stable complex at neutral pH. The maximum absorption for methyl green/DNA complex is 642.5–645 nm. This assay (Burres *et al*., 1992) was used to measure the displacement of methyl green from DNA by compounds with ability to bind DNA. The degree of displacement was determined spectrophotometrically by measuring the change in initial absorbance of methyl green/DNA solution in the presence of reference compound.

The obtained data indicated that compounds **8d** and **10d** are the most active members with $IC_{50}$ values of 12 and 14 μM, while compounds **4e**, **4f**, **4h**, **5d**, **6a**, **6d**, **8c**, **10e**, **10h** and **12a** showed moderate affinity with $IC_{50}$ values of 22, 32, 25, 38, 25, 40, 25, 32, 38, and 40 μM, respectively (Table 2).

Some structural features were found to be beneficial to the antitumor activity of such compounds. In particular, the pyrimido[4,5-*b*]quinoline derivatives were more active than those possessing the [1,2,4]triazolo[2′,3′:3,4]pyrimido[6,5-*b*]quinoline skeleton. Also, the presence of 3, 4-dimethoxyphenyl moiety at the 5-position of the pyrimidoquinoline ring system may contribute in the DNA-binding affinity as in compounds **4h**, **5d**, **6d**, **8d**, **10d,** and **10h**. This indicates that better affinity could be attained by the presence of 5-phenyl moiety substituted with electron-donating groups. In addition, introduction of electron-donating groups such as methyl or amino at the 2- or 4-position, respectively, of the pyrimidine ring favored the activity as in compounds **4e**, **4f**, **4h,** and **8c**. On the other hand, incorporation of the triazole ring into the pyrimidoquinoline system completely abolished the activity as in compounds **11a** and **11b**.

In summary, the present results suggest that the synthesized compounds possess moderate antitumor activity comparable to the activity of the commonly used anticancer drug, doxorubicin.

## 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 ($\nu$ in $cm^{-1}$) at Faculty of Science, Mansoura University. Nuclear magnetic resonance ($^1H$ NMR) spectra were obtained on 300 MHz FT-NMR spectrometer at Faculty of Science, Cairo University. The chemical shifts are expressed in $\delta$ ppm using tetramethylsilane (TMS) as internal reference and DMSO-$d_6$ or $CDCl_3$ as solvents. 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 Micro-analytical 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).

Compounds **1a–d** were prepared according to the reported procedure (Bigi *et al*., 2000) compounds, **2a–d** were prepared adopting the described procedure (Elkholy, 2007). The following materials were used in the biological screening: Breast cancer (MCF-7) cell line (American Type Culture Collection, Rockville, MD, USA). TLC plates (RP-$18_{F254}$, 0.25 mm, Merck). DNA and Anisaldehyde (Sigma-Aldrich Co., USA). Methyl green/DNA (Sigma, St. Louis, MO, USA).

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

A mixture of cyclohexanone (4.9 g, 0.05 mol), ammonium acetate (5.78 g, 0.075 mol) and the appropriate benzylidenemalononitrile **1a–d** (0.05 mol) in absolute ethanol (50 mL) was heated at reflux temperature for 6 h. The precipitated solid was collected by filtration, dried and crystallized from ethanol to yield the desired hexahydroquinoline derivatives **2a–d**.

*2-Amino-1,4,5,6,7,8-hexahydro-4-phenylquinoline-3-carbonitrile (**2a**)* Yield 75 %, m.p. 225–227 °C. IR spectrum (KBr, $\nu$, $cm^{-1}$): 3419, 3306 ($NH_2$); 3150 (NH); 2928, 2861 (CH), 2211 (C≡N). $^1H$ NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.57–1.76 (m, 4H, $2CH_2$), 2.21 (t, 2H, $CH_2$), 2.71 (t, 2H, $CH_2$), 6.55 (s, 1H, $C_4$–H), 7.28–7.53 (m, 8H, Ar–H, $NH_2$, NH). MS *m/z* (%): 250 (6.30, $M^+-1$), 249 (43.80, $M^+-2$), 51 (100.00). Anal. Calcd. for $C_{16}H_{17}N_3$ (251.33): C 76.46, H 6.82, N 16.72. Found: C 76.70, H 6.65, N 16.43.

*2-Amino-4-(3-bromophenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (**2b**)* Yield 70 %, m.p. 232–234 °C. IR spectrum (KBr, $\nu$, $cm^{-1}$): 3420, 3302 ($NH_2$); 3147 (NH); 2943, 2863 (CH), 2213 (C≡N). MS *m/z* (%): 331 (3.30, $M^+$+1), 330 (20.18, $M^+$), 329 (100.00, $M^+-1$). Anal. Calcd. for $C_{16}H_{16}BrN_3$ (330.22): C 58.19, H 4.88, N 12.72. Found: C 58.40, H 5.18, N 12.53.

*2-Amino-4-(4-chlorophenyl)-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (**2c**)* Yield 85 %, m.p. 252–253 °C. $^1H$ NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.59–1.74 (m, 4H, $2CH_2$), 2.20 (t, 2H, $CH_2$), 2.71 (t, 2H, $CH_2$), $CH_2$), 6.61 (s, 1H, $C_4$–H), 7.34–7.59 (m, 7H, Ar–H, $NH_2$, NH). MS *m/z* (%): 288 (0.17, $M^+$+2), 287 (0.77, $M^+$+1), 283 (100.00, $M^+-3$). Anal.Calcd. for $C_{16}H_{16}ClN_3$ (285.77): C 67.25, H 5.64, N 14.70. Found: C 67.00, H 5.80, N 14.53.

*2-Amino-1,4,5,6,7,8-hexahydro-4-(3,4-dimethoxyphenyl) quinoline-3-carbonitrile (**2d**)* Yield 80 %, m.p. 254–255 °C. IR spectrum (KBr, $\nu$, $cm^{-1}$): 3429, 3303 ($NH_2$); 3146 (NH); 2936, 2837 (CH); 2208 (C≡N). $^1H$ NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.69–1.85 (m, 4H, $2CH_2$), 2.39 (t, 2H, $CH_2$), 2.87 (t, 2H, $CH_2$), 3.87 (s, 3H, $OCH_3$), 3.94 (s, 3H, $OCH_3$), 5.51 (s, 1H, $C_4$–H), 6.76–6.99 (m, 6H, Ar–H, NH, $NH_2$). MS *m/z* (%): 313 (0.02, $M^+$+2), 312 (0.31, $M^+$+1), 311 (3.27, $M^+$), 309 (100.00, $M^+-2$). Anal. Calcd. for $C_{18}H_{21}N_3O_2$ (311.38): C 69.43, H 6.80, N 13.49. Found: C 69.17, H 6.58, N 13.23.

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

A mixture of compound **2a–d** (0.005 mol), formic acid or glacial acetic acid (10 mL) and a catalytic amount of concentrated hydrochloric acid (2 mL) was heated at reflux temperature for 48 h. The reaction mixture was allowed to cool to room temperature, poured onto crushed ice and neutralized with 1N sodium hydroxide solution. The precipitated solid was collected by filtration, dried, and crystallized from the appropriate solvent.

*6,7,8,9-Tetrahydro-5-phenylpyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**4a**)* Crystallized from DMF, yield 76 %, m.p. 198–200 °C. IR spectrum (KBr, $\nu$, $cm^{-1}$): 3,420 (2NH); 2975, 2899 (CH); 1,654 (C=O). $^1H$ NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.56–1.70 (m, 4H, $2CH_2$), 2.17–2.68 (m, 4H, $2CH_2$), 5.44 (s, 1H, $C_5$–H), 6.49 (s, 1H, NH), 7.26–7.46 (m, 7H, Ar–H, NH). Anal.Calcd. for $C_{17}H_{17}N_3O$ (279.34): C 73.10, H 6.13, N 15.04. Found: C 72.85, H 6.31, N 15.23.

*5-(3-Bromophenyl)-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**4b**)* Crystallized from DMF, yield 82 %, m.p.117–119 °C. IR spectrum (KBr, $\nu$, $cm^{-1}$): 3426, 3313 (2NH); 2955, 2875 (CH); 1667 (C=O). $^1H$ NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.63–1.82 (m, 4H, $2CH_2$),

2.23–2.88 (m, 4H, $2CH_2$), 5.54 (s, 1H, $C_5$–H), 6.67 (s, 1H, NH), 7.45–7.73 (m, 6H, Ar–H, NH). MS *m/z* (%): 356 (5.70, $M^+-2$), 327 (100.00). Anal. Calcd. for $C_{17}H_{16}BrN_3O$ (358.23): C 57.00, H 4.50, N 11.73. Found: C 56.75, H 4.21, N 11.53.

*5-(4-Chlorophenyl)-6,7,8,9-tetrahydropyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**4c**)* Crystallized from DMF, yield 67 %, m.p. decomp. 220 °C. MS *m/z* (%): 315 (0.40, $M^+$+1), 314 (0.70, $M^+$), 60 (100.00). Anal. Calcd. for $C_{17}H_{16}ClN_3O$ (313.78): C 65.07, H 5.14, N 13.39. Found: C 65.32, H 4.91, N 13.61.

*6,7,8,9-Tetrahydro-5-(3,4-dimethoxyphenyl)pyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**4d**)* Crystallized from DMF, yield 78 %, m.p. 257–259 °C. IR spectrum (KBr, *v*, $cm^{-1}$): 3336, 3164 (2NH); 2936, 2858 (CH); 1663 (C=O). $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.52–1.72 (m, 4H, $2CH_2$), 2.17–2.65 (m, 4H, $2CH_2$), 3.89 (s, 3H, $OCH_3$), 3.92 (s, 3H, $OCH_3$), 5.44 (s, 1H, $C_5$–H), 6.62–7.27 (m, 6H, Ar–H, 2NH). MS *m/z* (%): 341 (0.10, $M^+$+2), 340 (0.70, $M^+$+1), 339 (2.50, $M^+$), 216 (100.00). Anal. Calcd. for $C_{19}H_{21}N_3O_3$ (339.39): C 67.24, H 6.24, N 12.38. Found: C 67.46, H 6.21, N 12.53.

*6,7,8,9-Tetrahydro-2-methyl-5-phenylpyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**4e**)* Crystallized from DMF, yield 80 %, m.p.200–202 °C. IR spectrum (KBr, *v*, $cm^{-1}$): 3338, 3167 (2NH); 2934, 2859 (CH); 1664 (C=O). $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.57–1.76 (m, 4H, $2CH_2$), 2.05 (s, 3H, $CH_3$), 2.19–2.72 (m, 4H, $2CH_2$), 5.52 (s, 1H, $C_5$–H), 6.50 (s, 1H, NH), 7.13–7.54 (m, 6H, Ar–H, NH). MS *m/z* (%): 293 (61.3, $M^+$), 216 (100.00). Anal. Calcd. for $C_{18}H_{19}N_3O$ (293.36): C 73.69, H 6.53, N 14.32. Found: C 73.95, H 6.27, N 14.63.

*5-(3-Bromophenyl)-6,7,8,9-tetrahydro-2-methylpyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**4f**)* Crystallized from ethanol/DMF(3:2), yield 85 %, m.p. 170–172 °C. IR spectrum (KBr, *v*, $cm^{-1}$): 3338, 3152 (2NH); 2928, 2858 (CH); 1654 (C=O).$^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.52–1.72 (m, 4H, $2CH_2$), 2.11 (s, 3H, $CH_3$), 2.19–2.69 (m, 4H, $2CH_2$), 5.46 (s, 1H, $C_5$–H), 6.50 (s, 1H, NH), 7.07–7.47 (m, 5H, Ar–H, NH). Anal. Calcd. for $C_{18}H_{18}BrN_3O$ (372.26): C 58.08, H 4.87, N 11.29. Found: C 58.34, H 4.62, N 11.15.

*5-(4-Chlorophenyl)-6,7,8,9-tetrahydro-2-methylpyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**4g**)* Crystallized from ethanol/DMF (3:2), yield 72 %, m.p. 223–225 °C. MS *m/z* (%): 328 (66.70, $M^+$), 327 (55.60, $M^+-1$), 283 (100.00). Anal. Calcd. for $C_{18}H_{18}ClN_3O$ (327.81): C 65.95, H 5.53, N 12.82. Found: C 65.61, H 5.70, N 12.67.

*6,7,8,9-Tetrahydro-5-(3,4-dimethoxyphenyl)-2-methylpyrimido[4,5-b]quinolin-4(3H, 5H, 10H)-one (**4h**)* Crystallized from DMF, yield 80 %, m.p. 210–212 °C. $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.68–1.91 (m, 4H, $2CH_2$), 1.96 (s, 3H, $CH_3$), 2.41 (t, 2H, $CH_2$), 2.90 (t, 2H, $CH_2$), 3.91 (s, 3H, $OCH_3$), 3.95 (s, 3H, $OCH_3$), 5.61 (s, 1H, $C_5$–H), 6.77–7.00 (m, 4H, Ar–H, NH), 8.64 (s, 1H, NH). Anal. Calcd. for $C_{20}H_{23}N_3O_3$ (353.41): C 67.97, H 6.56, N 11.89. Found: C 67.70, H 6.21, N 11.63.

*General procedure for the preparation of 5,6,7,8,9,10-hexahydro-5-(substituted)phenylpyrimido[4,5-b]quinoline-2,4(1H, 3H)-dithiones (**5a–d**)*

A mixture of compound **2a–d** (0.005 mol) and carbon disulfide (3.8 g, 0.05 mol) in pyridine (5 mL) was heated at reflux temperature on a water bath (80 °C) for 18 h. The reaction mixture was allowed to cool to 0 °C. Ethanol (20 mL) was added to the reaction mixture and the precipitated solid was filtered, washed with ethanol, dried, and crystallized from acetone.

*5,6,7,8,9,10-Hexahydro-5-phenylpyrimido[4,5-b]quinoline-2,4(1H, 3H)-dithione (**5a**)* Yield 70 %, m.p. 228–230 °C.$^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.58–1.71 (m, 4H, $2CH_2$), 2.19 (t, 2H, $CH_2$), 2.70 (t, 2H, $CH_2$), 5.48 (s, 1H, NH), 6.56 (s, 1H, $C_5$–H), 7.19–7.58 (m, 7H, Ar–H, 2NH). Anal. Calcd. for $C_{17}H_{17}N_3S_2$ (327.47): C 62.35, H 5.23, N 12.83. Found: C 62.50, H 5.41, N 12.65.

*5-(3-Bromophenyl)-5,6,7,8,9,10-hexahydropyrimido[4,5-b]quinoline-2,4(1H, 3H)-dithione (**5b**)* Yield 65 %, m.p. 235–237 °C. $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.59–1.72 (m, 4H, $2CH_2$), 2.19 (t, 2H, $CH_2$), 2.70 (t, 2H, $CH_2$), 6.57 (s, 1H, $C_5$–H), 7.29–7.68 (m, 6H, Ar–H, 2NH), 8.64 (s, 1H, NH). MS *m/z* (%): 408 (14.00, $M^+$+2), 407 (14.00, $M^+$+1), 406 (14.00, $M^+$), 105 (100.00). Anal. Calcd. for $C_{17}H_{16}BrN_3S_2$ (406.36): C 50.25, H 3.97, N 10.34. Found: C 50.48, H 4.21, N 10.53.

*5-(4-Chlorophenyl)-5,6,7,8,9,10-hexahydropyrimido[4,5-b]quinoline-2,4(1H, 3H)-dithione (**5c**)* Yield 80 %, m.p. 250–252 °C. IR spectrum (KBr, *v*, $cm^{-1}$): 3,396 (3NH); 2952, 2866 (CH). $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.57–1.63 (m, 4H, $2CH_2$), 2.19 (t, 2H, $CH_2$), 2.68 (t, 2H, $CH_2$), 6.56 (s,1H, $C_5$–H), 7.32–7.58 (m, 6H, Ar–H, 2NH), 8.62 (s, 1H, NH). Anal. Calcd. for $C_{17}H_{16}ClN_3S_2$ (361.91): C 56.42, H 4.46, N 11.61. Found: C 56.10, H 4.21, N 11.83.

*5,6,7,8,9,10-Hexahydro-5-(3,4-dimethoxyphenyl)pyrimido[4,5-b]quinoline-2,4(1H, 3H)-dithione (**5d**)* Yield 80 %, m.p. 239–240 °C. $^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm): 1.58–1.74 (m, 4H, $2CH_2$), 2.25 (t, 2H, $CH_2$), 2.69 (t, 2H,

$CH_2$), 3.76 (s, 3H, $OCH_3$), 3.81 (s, 3H, $OCH_3$), 5.58 (s, 1H, $C_5$–H), 6.66–7.07 (m, 5H, Ar–H, 2NH), 9.20 (s, 1H, NH). Anal. Calcd. for $C_{19}H_{21}N_3O_2S_2$ (387.52): C 58.89, H 5.46, N 10.84. Found: C 58.60, H 5.21, N 10.75.

*General procedure for the preparation of 3,4,5,6,7,8,9,10-octahydro-4-imino-3-(n-butyl or phenyl)-5-(substituted)phenylpyrimido[4,5-b]quinoline-2(1H)-thiones (**6a–h**)*

A mixture of compound **2a–d** (0.005 mol) and the appropriate isothiocyanate (0.005 mol) in pyridine (5 mL) was heated at reflux temperature for 12–16 h. The reaction mixture was allowed to cool to 0 °C and poured onto ethanol (20 mL). The precipitated solid was collected by filtration, washed with ethanol, dried and crystallized from ethanol/DMF (3:2).

*3,4,5,6,7,8,9,10-Octahydro-4-imino-3,5-diphenylpyrimido[4,5-b]quinoline-2(1H)-thione (**6a**)* Yield 70 %, m.p. 238–240 °C. $^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm): 1.77–1.83 (m, 4H, $2CH_2$), 2.89–2.96 (m, 4H, $2CH_2$), 6.92–7.69 (m, 13H, Ar–H, $C_5$–H, 2NH), 8.64 (s, 1H, NH). Anal. Calcd. for $C_{23}H_{22}N_4S$ (386.51): C 71.47, H 5.74, N 14.50. Found: C 71.25, H 5.97, N 14.33.

*5-(3-Bromophenyl)-3,4,5,6,7,8,9,10-octahydro-4-imino-3-phenylpyrimido[4,5-b]quinoline-2(1H)-thione (**6b**)* Yield 65 %, m.p. 165–167 °C. $^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm): 1.88–1.92 (m, 4H, $2CH_2$), 2.39–2.43 (m, 4H, $2CH_2$), 6.87–7.94 (m, 12H, Ar–H, $C_5$-H, 2NH), 9.15 (s, 1H, NH). Anal. Calcd. for $C_{23}H_{21}BrN_4S$ (465.41): C 59.36, H 4.55, N 12.04. Found: C 59.65, H 4.81, N 11.93.

*5-(4-Chlorophenyl)-3,4,5,6,7,8,9,10-octahydro-4-imino-3-phenylpyrimido[4,5-b]quinoline-2(1H)-thione (**6c**)* Yield 75 %, m.p. 218–220 °C. IR spectrum (KBr, $\nu$, $cm^{-1}$): 3,399 (3NH), 3,055 (CH). $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.82–1.89 (m, 4H, $2CH_2$), 2.41–2.47 (m, 4H, $2CH_2$), 6.77–7.57 (m, 12H, Ar–H, $C_5$–H, 2NH), 8.66 (s, 1H, NH). Anal. Calcd. for $C_{23}H_{21}ClN_4S$ (420.96): C 65.62, H 5.03, N 13.31. Found: C 65.99, H 5.21, N 13.53.

*3,4,5,6,7,8,9,10-Octahydro-4-imino-5-(3,4-dimethoxyphenyl)-3-phenylpyrimido[4,5-b]quinoline-2(1H)-thione (**6d**)* Yield 80 %, m.p. 200–202 °C. $^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm): 1.27–2.40 (m, 8H, $4CH_2$), 3.89 (s, 6H, $2OCH_3$), 5.89 (s, 1H, $C_5$–H), 6.40–7.27 (m, 11H, Ar–H, 3NH). Anal. Calcd. for $C_{25}H_{26}N_4O_2S$ (446.56): C 67.24, H 5.87, N 12.55. Found: C 67.50, H 6.11, N 12.33.

*3-n-Butyl-3,4,5,6,7,8,9,10-octahydro-4-imino-5-phenylpyrimido[4,5-**b**]quinoline-2(1H)-thione (**6e**)* Yield 72 %, m.p. 200–202 °C. $^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm): 0.91–1.69 (m, 13H, $CH_3$, $5CH_2$), 2.88–2.96 (m, 4H, $2CH_2$), 3.35 (s, 2H, 2NH), 5.20 (s, 1H, $C_5$–H), 7.27–7.70 (m, 5H, Ar–H), 8.03 (s, 1H, NH). Anal. NH). Anal. Calcd. for $C_{21}H_{26}N_4S$ (366.52): C 68.82, H 7.15, N 15.29. Found: C 69.15, H 7.21, N 15.03.

*5-(3-Bromophenyl)-3-n-butyl-3,4,5,6,7,8,9,10-octahydro-4-iminopyrimido[4,5-b]quinoline-2(1H)-thione (**6f**)* Yield 70 %, m.p. >300 °C. IR spectrum (KBr, $\nu$, $cm^{-1}$): 3,398 (3NH), 3,024 (CH). $^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm): 0.83–1.68 (m, 13H, $CH_3$, $5CH_2$), 2.89–2.96 (m, 4H, $2CH_2$), 3.32 (s, 2H, 2NH), 5.40 (s, 1H, $C_5$–H), 7.46–7.71 (m, 4H, Ar–H), 8.03 (s, 1H, NH). Anal. Calcd. for $C_{21}H_{25}BrN_4S$ (445.42): C 56.63, H 5.66, N 12.58. Found: C 56.87, H 5.80, N 12.43.

*3-n-Butyl-5-(4-chlorophenyl)-3,4,5,6,7,8,9,10-octahydro-4-iminopyrimido[4,5-b]quinoline-2(1H)-thione (**6g**)* Yield 65 %, m.p. >300 °C. IR spectrum (KBr, $\nu$, $cm^{-1}$): 3,425 (3NH), 3064, 2955, 2929 (CH). $^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm): 0.93–1.86 (m, 13H, $CH_3$, $5CH_2$), 2.35 (t, 2H, $CH_2$), 2.84 (t, 2H, $CH_2$), 3.41 (s, 2H, 2NH), 5.10 (s, 1H, $C_5$–H), 7.25–7.57 (m, 4H, Ar–H), 8.63 (s, 1H, NH). Anal. Calcd. for $C_{21}H_{25}ClN_4S$ (400.97): C 62.90, H 6.28, N 13.97. Found: C 63.15, H 6.45, N 13.83.

*3-n-Butyl-3,4,5,6,7,8,9,10-octahydro-4-imino-5-(3,4-dimethoxyphenyl)pyrimido[4,5-b]quinoline-2(1H)-thione (**6h**)* Yield 50 %, m.p. >300 °C. $^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm): 0.88–1.38 (m, 9H, $CH_3$, $3CH_2$), 1.68–1.84 (m, 4H, $2CH_2$), 2.39 (t, 2H, $CH_2$), 2.83 (t, 2H, $CH_2$), 3.41 (s, 2H, 2NH), 5.06 (s, 1H, $C_5$–H), 6.76–7.05 (m, 4H, Ar–H, NH). Anal. Calcd. for $C_{23}H_{30}N_4O_2S$ (426.57): C 64.76, H 7.09, N 13.13. Found: C 65.15, H 7.21, N 12.93.

*General procedure for the preparation of ethyl N-[3-cyano-1,4,5,6,7,8-hexahydro-4-(substituted)-phenylquinolin-2-yl]formimidates (**7a–d**)*

A mixture of compound **2a–d** (0.01 mol) and triethyl orthoformate (10 mL) was heated at reflux temperature for 10–12 h. Excess reagent was evaporated under reduced pressure, and the solid obtained was triturated with ice-water, filtered, washed with water, dried and crystallized from ethanol.

*Ethyl N-[3-cyano-1,4,5,6,7,8-hexahydro-4-phenylquinolin-2-yl]formimidate (**7a**)* Yield 70 %, m.p. 101–103 °C. IR spectrum (KBr, $\nu$, $cm^{-1}$): 3,421 (NH); 2940, 2873 (CH); 2,210 (C≡N). $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.34 (t, 3H, $OCH_2CH_3$), 1.66–1.78 (m, 4H, $2CH_2$), 2.36–2.88 (m, 4H, $2CH_2$), 4.34–4.38 (q, 2H, $OCH_2CH_3$), 6.54

(s, 1H, $C_4$–H), 7.37–7.51 (m, 6H, Ar–H, N=CH), 8.55 (s,1H, NH). Anal. Calcd. for $C_{19}H_{21}N_3O$ (307.39): C 74.24, H 6.89, N 13.67. Found: C 73.95, H 6.61, N 13.83.

*Ethyl N-[4-(3-bromophenyl)-3-cyano-1,4,5,6,7,8-hexahydroquinolin-2-yl]formimidate (**7b**)* Yield 72 %, m.p. 110–112 °C. IR spectrum (KBr, $\nu$, $cm^{-1}$): 3,425 (NH); 2927, 2862 (CH); 2,237 (C≡N). $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.39 (t, 3H, $OCH_2CH_3$), 1.84–1.87 (m, 4H, $2CH_2$), 2.43–2.94 (m, 4H, $2CH_2$), 4.34–4.37 (q, 2H, $OCH_2CH_3$), 6.59 (s, 1H, $C_4$–H), 7.35–7.63 (m, 5H, Ar–H, N=CH), 8.51 (s, 1H, NH). Anal. Calcd. for $C_{19}H_{20}BrN_3O$ (386.29): C 59.08, H 5.22, N 10.88. Found: C 59.30, H 5.36, N 11.13.

*Ethyl N-[4-(4-chlorophenyl)-3-cyano-1,4,5,6,7,8-hexahydroquinolin-2-yl]formimidate (**7c**)* Yield 75 %, m.p. 125–127 °C. IR spectrum (KBr, $\nu$, $cm^{-1}$): 3,420 (NH), 2932, 2882 (CH), 2213 (C≡N). $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.34 (t, 3H, $OCH_2CH_3$), 1.67–1.77 (m, 4H, $2CH_2$), 2.36–2.88 (m, 4H, $2CH_2$), 4.44–4.47 (q, 2H, $OCH_2CH_3$), 5.08 (s, 1H, $C_4$–H), 7.19–7.61 (m, 5H, Ar–H, N=CH), 8.42 (s, 1H, NH). Anal. Calcd. for $C_{19}H_{20}ClN_3O$ (341.83): C 66.76, H 5.90, N 12.29. Found: C 66.45, H 5.71, N 12.46.

*Ethyl N-[3-cyano-1,4,5,6,7,8-hexahydro-4-(3,4-dimethoxyphenyl)quinolin-2-yl]formimidate (**7d**)* Yield 70 %, m.p. 174–175 °C. IR spectrum (KBr, $\nu$, $cm^{-1}$): 3,429 (NH); 2936, 2837 (CH); 2,208 (C≡N). $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.34 (t, 3H, $OCH_2CH_3$), 1.66–1.77 (m, 4H, $2CH_2$), 2.51 (t, 2H, $CH_2$), 2.87 (t, 2H, $CH_2$), 3.77 (s, 3H, $OCH_3$), 3.83 (s, 3H, $OCH_3$), 4.32–4.39 (q, 2H, $OCH_2CH_3$), 6.46 (s, 1H, $C_4$–H), 6.86–7.11 (m, 4H, Ar–H, N=CH), 8.48 (s, 1H, NH). MS *m/z* (%): 367 (5.70, $M^+$), 366 (26.30, $M^+-1$), 365 (100.00). Anal. Calcd. for $C_{21}H_{25}N_3O_3$ (367.44): C 68.64, H 6.86, N 11.44. Found: C 68.36, H 6.71, N 11.38.

*General procedure for the preparation of 4-amino-5,6,7,8,9,10-hexahydro-5-(substituted)phenylpyrimido[4,5-b]quinolines (**8a–d**)*

A mixture of compound **7a–d** (0.005 mol) and ammonia solution 35 % (10 mL) in absolute ethanol (15 mL) was heated at reflux temperature for 12 h. The solvent was evaporated under reduced pressure and the solid obtained was triturated with ice-water, filtered, washed with water, dried and crystallized from ethanol.

*4-Amino-5,6,7,8,9,10-hexahydro-5-phenylpyrimido[4,5-b]quinoline (**8a**)* Yield 70 %, m.p. 262–263 °C. $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.59–1.72 (m, 4H, $2CH_2$), 2.21–2.71 (m, 4H, $2CH_2$), 3.03 (s, 2H, $NH_2$), 6.55 (s, 1H, $C_5$–H), 7.29–7.62 (m, 6H, Ar–H), 8.45 (s, 1H, NH). Anal. Calcd. for $C_{17}H_{18}N_4$ (278.35): C 73.35, H 6.52, N 20.13. Found: C 73.10, H 6.31, N 20.43.

*4-Amino-5-(3-bromophenyl)-5,6,7,8,9,10-hexahydropyrimido[4,5-b]quinoline (**8b**)* Yield 65 %, m.p. 185–187 °C. IR spectrum (KBr, $\nu$, $cm^{-1}$): 3,396 ($NH_2$, NH); 3055, 2933, 2827 (CH). $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.72–1.85 (m, 4H, $2CH_2$), 2.19–2.71 (m, 4H, $2CH_2$), 3.02 (s, 2H, $NH_2$), 6.59 (s, 1H, $C_5$–H), 7.32–7.72 (m, 5H, Ar–H), 8.46 (s,1H, NH). Anal. Calcd. for $C_{17}H_{17}BrN_4$ (357.25): C 57.15, H 4.80, N 15.68. Found: C 56.96, H 4.68, N 15.83.

*4-Amino-5-(4-chlorophenyl)-5,6,7,8,9,10-hexahydropyrimido[4,5-b]quinoline (**8c**)* Yield 75 %, m.p. 228–229 °C. IR spectrum (KBr, $\nu$, $cm^{-1}$): 3,399 ($NH_2$, NH); 3055, 2924, 2887 (CH). $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.71–1.80 (m, 4H, $2CH_2$), 2.19–2.70 (m, 4H, $2CH_2$), 3.02 (s, 2H, $NH_2$), 6.59 (s, 1H, $C_5$–H), 7.32–7.72 (m, 5H, Ar–H), 8.45 (s, 1H, NH). MS *m/z* (%): 314 (6.10, $M^+$+1), 313 (4.40, $M^+$), 117 (100.00). Anal. Calcd. for $C_{17}H_{17}ClN_4$(312.80): C 65.28, H 5.48, N 17.91. Found: C 65.50, H 5.62, N 17.75.

*4-Amino-5,6,7,8,9,10-hexahydro-5-(3,4-dimethoxyphenyl)pyrimido[4,5-b]quinoline (**8d**)* Yield 68 %, m.p. 197–199 °C. IR spectrum (KBr, $\nu$, $cm^{-1}$): 3,405 ($NH_2$, NH); 3055, 2929 (CH). $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.71–1.88 (m, 4H, $2CH_2$), 2.27–2.69 (m, 4H, $2CH_2$), 3.01 (s, 2H, $NH_2$), 3.81 (s, 6H, $2OCH_3$), 6.49 (s, 1H, $C_5$–H), 6.88–7.16 (m, 4H, Ar–H), 8.43 (s, 1H, NH). Anal.Calcd. for $C_{19}H_{22}N_4O_2$ (338.40): C 67.44, H 6.55, N 16.56. Found: C 67.25, H 6.82, N 16.73.

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

A mixture of compound **7a–d** (0.005 mol) and hydrazine hydrate 98 % (2.5 g, 0.05 mol) in absolute ethanol (15 mL) was heated at reflux temperature for 12 h. The reaction mixture was cooled and the precipitated solid was collected by filtration, washed with water, dried and crystallized from dioxane.

*3-Amino-3,4,6,7,8,9-hexahydro-4-imino-5-phenyl-5H, 10H-pyrimido[4,5-b]quinoline (**9a**)* Yield 85 %, m.p. 266-267 °C. $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.58–1.70 (m, 4H, $2CH_2$), 2.10–2.64 (m, 4H, $2CH_2$), 5.45 (s, 2H, $NH_2$), 6.45 (s,1H, $C_5$–H), 7.19–7.47 (m, 6H, Ar–H), 8.15 (s, 2H, 2NH). Anal. Calcd. for $C_{17}H_{19}N_5$ (293.37): C 69.60, H 6.53, N 23.87. Found: C 69.30, H 6.81, N 23.68.

*3-Amino-5-(3-bromophenyl)-3,4,6,7,8,9-hexahydro-4-imino-5H, 10H-pyrimido[4,5-b]quinoline (**9b**)* Yield 86 %, m.p. 260–261 °C. $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.65–1.76 (m, 4H, $2CH_2$), 2.13–2.86 (m, 4H, $2CH_2$), 5.52 (s, 2H, $NH_2$), 6.11 (s, 1H, $C_5$–H), 7.32–7.64 (m, 5H, Ar–H), 7.76 (s, 2H, 2NH). MS *m/z* (%): 374 (9.80, $M^+$+2), 373 (22.10, $M^+$+1), 372 (14.60, $M^+$), 216 (100.00). Anal. Calcd. for $C_{17}H_{18}BrN_5$ (372.26): C 54.85, H 4.87, N 18.81. Found: C 55.12, H 4.68, N 18.63.

*3-Amino-5-(4-chlorophenyl)-3,4,6,7,8,9-hexahydro-4-imino-5H, 10H-pyrimido[4,5-b]quinoline (**9c**).* Yield 89 %, m.p. IR spectrum (KBr, *v*, $cm^{-1}$): 3427, 3353 ($NH_2$); 3201 (2NH); 2932, 2860 (CH). $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.58–1.70 (m, 4H, $2CH_2$), 2.09–2.87 (m, 4H, $2CH_2$), 5.46 (s, 2H, $NH_2$), 5.83 (s, 1H, $C_5$–H), 7.21–7.57 (m, 5H, Ar–H), 8.23 (s, 2H, 2NH). Anal. Calcd. for $C_{17}H_{18}ClN_5$ (327.81): C 62.29, H 5.53, N 21.36. Found: C 62.50, H 5.31, N 21.43.

*3-Amino-3,4,6,7,8,9-hexahydro-4-imino-5-(3,4-dimethoxyphenyl)-5H, 10H-pyrimido[4,5-b]quinoline (**9d**)* Yield 70 %, m.p. 254–255 °C. IR spectrum (KBr, *v*, $cm^{-1}$): 3428, 3354 ($NH_2$); 3,203 (2NH); 2932, 2860 (CH). $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.58–1.70 (m, 4H, $2CH_2$), 2.22–2.66 (m, 4H, $2CH_2$), 3.74 (s, 6H, $2OCH_3$), 5.46 (s, 2H, $NH_2$), 5.83 (s,1H, $C_5$–H), 6.32–6.83 (m, 4H, Ar–H), 8.12 (s, 2H, 2NH). Anal. Calcd. for $C_{19}H_{23}N_5O_2$ (353.42): C 64.57, H 6.56, N 19.82. Found: C 64.70, H 6.31, N 19.64.

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

A mixture of compound **9a–d** (0.005 mol), the appropriate benzaldehyde (0.005 mol) and a catalytic amount of piperidine (0.5 mL) in dioxane (10 mL) was heated at reflux temperature for 24 h. The precipitated solid was collected by filtration, washed several times with cold ethanol, dried and crystallized from acetone.

*3-(4-Chlorobenzylideneamino)-3,4,6,7,8,9-hexahydro-4-imino-5-phenyl-5H, 10H-pyrimido[4,5-b]quinoline (**10a**)* Yield 72 %, m.p. 215–217 °C. $^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm): 1.64–1.89 (m, 4H, $2CH_2$), 2.35 (t, 2H, $CH_2$), 2.86 (t, 2H, $CH_2$), 3.25 (s,1H, NH), 3.70 (s,1H, NH), 5.24 (s, 1H, $C_5$–H), 7.24–7.52 (m, 11H, Ar–H, N=CH). Anal. Calcd. for $C_{24}H_{22}ClN_5$ (415.92): C 69.31, H 5.33, N 16.84. Found: C 69.65, H 5.21, N 16.73.

*5-(3-Bromophenyl)-3-(4-chlorobenzylideneamino)-3,4,6,7,8,9-hexahydro-4-imino-5H, 10H-pyrimido-[4,5-b]quinoline (**10b**)* Yield 60 %, m.p. 158–159 °C. MS *m/z* (%): 493 (4.73, $M^+$−2), 125 (100.00). Anal. Calcd. for $C_{24}H_{21}BrClN_5$ (494.81): C 58.26, H 4.28, N 14.15. Found: C 58.40, H 4.45, N 14.33.

*3-(4-Chlorobenzylideneamino)-5-(4-chlorophenyl)-3,4,6,7,8,9-hexahydro-4-imino-5H, 10H-pyrimido[4,5-b]quinoline (**10c**)* Yield 65 %, m.p. 250–251 °C. MS *m/z* (%): 451 (0.03, $M^+$+1), 450 (0.05, $M^+$), 283 (100.00). Anal.Calcd. for $C_{24}H_{21}Cl_2N_5$ (450.36): C 64.01, H 4.70, N 15.55. Found: C 64.30, H 4.46, N 15.63.

*3-(4-Chlorobenzylideneamino)-3,4,6,7,8,9-hexahydro-4-imino-5-(3,4-dimethoxyphenyl)-5H, 10H-pyrimido [4,5-b]quinoline (**10d**)* Yield 50 %, m.p. 251–253 °C. IR spectrum (KBr, *v*, $cm^{-1}$): 3,426 (2NH); 3024, 2922 (CH). $^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm): 1.67–1.87 (m, 4H, $2CH_2$), 2.39 (t, 2H, $CH_2$), 2.84 (t, 2H, $CH_2$), 3.71 (s, 2NH), 3.90 (s, 3H, $OCH_3$), 3.94 (s, 3H, $OCH_3$), 5.18 (s, 1H, $C_5$–H), 6.76–6.99 (m, 8H, Ar–H), 7.27 (s, 1H, N=CH). MS *m/z* (%): 478 (0.83, $M^+$+2), 477 (2.07, $M^+$+1), 476 (2.26, $M^+$), 84 (100.00). Anal. Calcd. for $C_{26}H_{26}ClN_5O_2$ (475.97): C 65.61, H 5.51, N 14.71. Found: C 65.90, H 5.21, N 14.53.

*3,4,6,7,8,9-Hexahydro-4-imino-3-(3,4-dimethoxybenzylideneamino)-5-phenyl-5H, 10H-pyrimido[4,5-b]quinoline (**10e**)* Yield 65 %, m.p. 227–229 °C. $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.76–1.88 (m, 4H, $2CH_2$), 2.43 (t, 2H, $CH_2$), 2.92 (t, 2H, $CH_2$), 3.58 (s, 2NH), 3.89 (s, 3H, $OCH_3$), 3.96 (s, 3H, $OCH_3$), 5.44 (s, 1H, $C_5$–H), 6.73–7.40 (m, 9H, Ar–H, N=CH). MS *m/z* (%): 441 (1.94, $M^+$), 84 (100.00). Anal. Calcd. for $C_{26}H_{27}N_5O_2$ (441.52): C 70.73, H 6.16, N 15.86. Found: C 70.90, H 6.31, N 15.73.

*5-(3-Bromophenyl)-3,4,6,7,8,9-hexahydro-4-imino-3-(3,4-dimethoxybenzylideneamino)-5H, 10H-pyrimido[4,5-b]quinoline (**10f**)* Yield 65 %, m.p. 217–219 °C. $^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm): 1.75–1.92 (m, 4H, $2CH_2$), 2.41 (t, 2H, $CH_2$), 2.87 (t, 2H, $CH_2$), 3.64 (s, 2NH), 3.86 (s, 3H, $OCH_3$), 3.97 (s, 3H, $OCH_3$), 5.36 (s, 1H, $C_5$–H), 6.68–7.35 (m, 8H, Ar–H, N=CH). MS *m/z* (%): 519 (0.25, $M^+$−1), 518 (0.24, $M^+$−2), 329 (100.00). Anal. Calcd. for $C_{26}H_{26}BrN_5O_2$ (520.42): C 60.00, H 5.04, N 13.46. Found: C 60.27, H 4.98, N 13.71.

*5-(4-Chlorophenyl)-3,4,6,7,8,9-hexahydro-4-imino-3-(3,4-dimethoxybenzylideneamino)-5H, 10H-pyrimido[4,5-b]quinoline(**10g**)* Yield 70 %, m.p. 240–242 °C. MS *m/z* (%): 476 (2.10, $M^+$), 475 (3.30, $M^+$−1), 283 (100.00). Anal. Calcd. for $C_{26}H_{26}ClN_5O_2$ (475.97): C 65.61, H 5.51, N 14.71. Found: C 65.97, H 5.85, N 14.50.

*3,4,6,7,8,9-Hexahydro-4-imino-3-(3,4-dimethoxybenzylideneamino)-5-(3,4-dimethoxyphenyl)-5H,10H-pyrimido[4,5-b]quinoline (**10h**)* Yield 55 %, m.p. 217–218 °C. MS *m/z*

(%): 503 (0.31, $M^+$+1), 258 (100.00). Anal.Calcd. for $C_{28}H_{31}N_5O_4$ (501.58): C 67.05, H 6.23, N 13.95. Found: C 67.20, H 6.29, N 13.73.

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

A mixture of compound **9a–d** (0.005 mol) and triethyl orthoformate (5 mL) was heated at reflux temperature for 8–10 h. The reaction mixture was cooled and diluted with ethanol (5 mL). The precipitated solid was collected by filtration, dried and crystallized from ethanol.

*7,8,9,10,11,12-Hexahydro-12-phenyl-[1,2,4]triazolo[2′,3′:3,4] pyrimido[6,5-b]quinoline (**11a**)* Yield 50 %, m.p. 110–112 °C. $^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm): 1.22–3.26 (m, 8H, $4CH_2$), 5.15 (s, 1H, $C_{12}$–H), 7.12–7.55 (m, 5H, Ar–H), 8.18 (s, 1H, $C_5$–H), 9.38 (s, 1H, $C_2$–H), 10.90 (s, 1H, NH). Anal. Calcd. for $C_{18}H_{17}N_5$ (303.36): C 71.27, H 5.65, N 23.09. Found: C 71.50, H 5.41, N 23.33.

*12-(3-Bromophenyl)-7,8,9,10,11,12-hexahydro-[1,2,4]triazolo[2′,3′:3,4]pyrimido[6,5-b]quinoline (**11b**)* Yield 55 %, m.p.120–122 °C.$^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm): 1.26–2.96 (m, 8H, $4CH_2$), 5.08 (s, 1H, $C_{12}$–H), 7.21–7.58 (m, 4H, Ar–H), 8.20 (s, 1H, NH), 8.41 (s, 1H, $C_5$–H), 9.40 (s, 1H, $C_2$–H). Anal. Calcd. for $C_{18}H_{16}BrN_5$ (382.26): C 56.56, H 4.22, N 18.32. Found: C 56.70, H 4.28, N 18.49.

*12-(4-Chlorophenyl)-7,8,9,10,11,12-hexahydro-[1,2,4]triazolo[2′,3′:3,4]pyrimido[6,5-b]quinoline (**11c**)* Yield 62 %, m.p. 125–127 °C. $^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm):1.30–2.79 (m, 8H, $4CH_2$), 5.15 (s, 1H, $C_{12}$–H), 7.26–7.46 (m, 4H, Ar–H), 8.04 (s, 1H, NH), 8.41 (s, 1H, $C_5$–H), 9.55 (s,1H, $C_2$–H). Anal. Calcd. for $C_{18}H_{16}ClN_5$ (337.81): C 64.00, H 4.77, N 20.73. Found: C 64.23, H 4.91, N 20.57.

*7,8,9,10,11,12-Hexahydro-12-(3,4-dimethoxyphenyl)-[1,2,4]triazolo[2′,3′:3,4]pyrimido[6,5-b]quinoline (**11d**)* Yield 55 %, m.p.175–176 °C. $^1$H NMR spectrum: (DMSO-$d_6$, $\delta$ ppm): 1.30–2.79 (m, 8H, $4CH_2$), 3.87 (s, 3H, $OCH_3$), 3.91 (s, 3H, $OCH_3$), 5.15 (s, 1H, $C_{12}$–H), 6.73–7.15 (m, 3H, Ar–H), 8.22 (s, 1H, NH), 8.63 (s, 1H, $C_5$–H), 9.47 (s,1H, $C_2$–H). MS *m/z* (%): 364 (4.93, $M^+$+1), 309 (100.00). Anal. Calcd. for $C_{20}H_{21}N_5O_2$ (363.41): C 66.10, H 5.82, N 19.27. Found: C 65.96, H 5.71, N 19.46.

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

A mixture of compound **9a–d** (0.005 mol) and ethyl chloroformate (1.08 g, 0.01 mol) in benzene (15 mL) was heated at reflux temperature for 24 h. The reaction mixture was concentrated, the precipitated solid was collected by filtration, dried and crystallized from ethanol.

*3-(Ethoxycarbonylamino)-4-(ethoxycarbonylimino)-3,4,6,7,8,9-hexahydro-5-phenyl-5H, 10H-pyrimido[4,5-b]quinoline (**12a**)* Yield 60 %, m.p.110–112 °C. IR spectrum (KBr, *v*, $cm^{-1}$): 3313, 3208 (2NH), 2933, 2861 (CH), 1710 (2C=O). MS *m/z* (%): 438 (0.09, $M^+$+1), 391 (0.10, $M^+$–$C_2H_5OH$), 249 (100.00). Anal. Calcd. for $C_{23}H_{27}N_5O_4$ (437.49): C 63.14, H 6.22, N 16.01. Found: C 63.40, H 5.97, N 16.23.

*5-(3-Bromophenyl)-3-(ethoxycarbonylamino)-4-(ethoxycarbonylimino)-3,4,6,7,8,9-hexahydro-5H, 10H-pyrimido[4,5-b]quinoline (**12b**)* Yield 60 %, m.p.104–105 °C. IR spectrum (KBr, v, $cm^{-1}$): 3,332 (2NH); 2933, 2863 (CH); 1729 (2C=O).$^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm): 1.27–1.42 (m, 6H, $2COOCH_2\underline{CH_3}$), 1.73–1.88 (m, 4H, $2CH_2$), 2.29–2.99 (m, 4H, $2CH_2$), 4.21–4.31 (m, 4H, $2COO\underline{CH_2}CH_3$), 6.50 (s, 1H, $C_5$–H), 7.21–7.70 (m, 7H, $C_2$–H, Ar–H, 2NH). Anal. Calcd. for $C_{23}H_{26}BrN_5O_4$ (516.39): C 53.50, H 5.07, N 13.56. Found: C 53.12, H 4.92, N 13.29.

*5-(4-Chlorophenyl)-3-(ethoxycarbonylamino)-4-(ethoxycarbonylimino)-3,4,6,7,8,9-hexahydro-5H, 10H-pyrimido[4,5-b]quinoline (**12c**)* Yield 55 %, m.p. 112–113 °C. IR spectrum (KBr, v, $cm^{-1}$): 3380, 3297 (2NH), 2935, 2863 (CH), 1,725 (2C=O). $^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm): 1.28–1.41 (m, 6H, $2COOCH_2\underline{CH_3}$), 1.76–1.95 (m, 4H, $2CH_2$), 2.32–2.96 (m, 4H, $2CH_2$), 4.19–4.33 (m, 4H, $2COO\underline{CH_2}CH_3$), 6.65 (s, 1H, $C_5$–H), 7.32–7.70 (m, 7H, $C_2$–H, Ar–H, 2NH). MS *m/z* (%): 426 (0.84, $M^+$–$C_2H_5OH$), 283 (100.00). Anal. Calcd. for $C_{23}H_{26}ClN_5O_4$ (471.94): C 58.53, H 5.55, N 14.84. Found: C 58.73, H 5.31, N 14.67.

*3-(Ethoxycarbonylamino)-4-(ethoxycarbonylimino)-3,4,6,7,8,9-hexahydro-5-(3,4-dimethoxyphenyl)-5H,10H-pyrimido[4,5-b]quinoline (**12d**)* Yield 65 %, m.p. 155–156 °C. IR spectrum (KBr, v, $cm^{-1}$): 3,334 (2NH), 2935, 2863 (CH), 1,724 (2C=O).$^1$H NMR spectrum: ($CDCl_3$, $\delta$ ppm):

1.26–1.36 (m, 6H, $2COOCH_2\underline{CH_3}$), 1.74–1.91 (m, 4H, $2CH_2$), 2.50 (t, 2H, $CH_2$), 2.99 (t, 2H, $CH_2$), 3.90 (s, 3H, $OCH_3$), 3.94 (s, 3H, $OCH_3$), 4.23–4.30 (m, 4H, $2COO\underline{CH_2}CH_3$), 6.76–6.99 (m, 6H, $C_5$–H, Ar–H, 2NH), 7.36 (s, 1H, $C_2$–H). Anal. Calcd. for $C_{25}H_{31}N_5O_6$ (497.54): C 60.35, H 6.28, N 14.08. Found: C 60.53, H 5.99, N 14.13.

## Biological screening

### The in vitro antitumor activity against breast cancer (MCF-7) cell line

Cells were placed in 96-multiwell microtiter plate ($10^4$ cell/well) for 24 h before treatment with the compounds to allow attachment of the cells to the wall of the plate. Different concentrations of the tested compounds (0, 1, 2.5, 5 and 10 μM) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5 % $CO_2$. After 48 h, cells were fixed, washed and stained with SRB stain. Unbound dye was removed by four washes with 1 % acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for breast tumor cell line after the specified time (Skehan *et al*., 1990). The concentration required for 50 % inhibition of cell viability ($IC_{50}$) was calculated for each compound, and the results are given in Table 1.

### DNA-binding assay on TLC plates

TLC plates were predeveloped with MeOH/$H_2O$ (8:2). The tested compounds were then applied (5 mg/mL in MeOH) at the origin, followed by the addition of DNA (1 mg/mL in MeOH/$H_2O$ mixture) at the same positions at the origin. The plates were then developed with the same solvent system and the position of the DNA was determined by spraying with anisaldehyde reagent (Pezzuto *et al*., 1983, 1991). The reagent yields a blue color spot with DNA, and the intensity of the color was proportional to the quantity of DNA added to the plate. Bleomycin was used as a reference antitumor agent.

### Methyl green/DNA displacement assay

Methyl green/DNA (20 mg) was suspended in 100 mL of 0.05 M Tris–HCl buffer (pH 7.5) containing 7.5 mM $MgSO_4$ and stirred at 37 °C with a magnetic stirrer for 24 h. Samples to be tested were placed in eppendorff tubes, and 200 μl of DNA/methyl green solution was added to each tube. Samples were incubated in the dark at ambient temperature. After 24 h, the final absorbance was determined. Readings were corrected for initial absorbance and normalized as a % of the unreacted DNA/methyl green absorbance value (Burres *et al*., 1992). Bleomycin was used as a reference antitumor agent. The activity of compounds **4e**, **4f**, **4 h**, **5d**, **6a**, **6d**, **8c**, **8d**, **10d**, **10e**, **10h,** and **12a** which showed DNA-binding affinity have been determined and expressed as $IC_{50}$ (concentration required for 50 % decrease in the initial absorbance of the methyl green/DNA solution) (Table 2).

## Conclusion

New series of pyrimido[4,5-*b*]quinolines and [1,2,4]triazolo[2′,3′:3,4]pyrimido[6,5-*b*]quinolines have been synthesized and evaluated for in vitro antitumor activity against human breast carcinoma (MCF-7) cell line and DNA-binding affinity. The results of in vitro antitumor testing against MCF-7 breast cancer cells and DNA-binding assay indicated that compounds, **4f**, **6d**, **8c**, **8d**, **10d**, **10e**, **10h,** and **12a** exhibited better activities among compounds tested.

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## References

Abbas HAS, Hafez HN, El-Gazzar A-RBA (2011) Synthesis, in vitro antimicrobial and in vivo antitumor evaluation of novel pyrimidoquinolines and its nucleoside derivatives. Eur J Med Chem 46:21–30

Abd El-Salam OI, Abou El Ella DA, Ismail NSM, Abdullah M (2009) Synthesis, docking studies and anti-inflammatory activity of some 2-amino-5,6,7,8-tetrahydroquinoline-3-carbonitriles and related compounds. Pharmazie 64:147–155

Abouzid K, Shouman S (2008) Design, synthesis and in vitro antitumor activity of 4-aminoquinoline and 4-aminoquinazoline derivatives targeting EGFR tyrosine kinase. Bioorg Med Chem 16:7543–7551

Ali HI, Ashida N, Nagamatsu T (2007) Antitumor studies. Part 3: design, synthesis, antitumor activity, and molecular docking study of novel 2-methylthio-, 2-amino-, and 2-(*N*-substituted amino)-10-alkyl-2-deoxo-5-deazaflavins. Bioorg Med Chem 15:6336–6352

Ali HI, Tomita K, Akaho E, Kunishima M, Kawashima Y, Yamagishi T, Ikeya H, Nagamatsu T (2008) Antitumor studies-Part 2: structure-activity relationship study for flavin analogues including investigations on their in vitro antitumor assay and docking simulation into protein tyrosine kinase. Eur J Med Chem 43:1376–1389

Alqasoumi SI, Al-Taweel AM, Alafeefy AM, Noaman E, Ghorab MM (2010) Novel quinolines and pyrimido[4,5-*b*]quinolines

bearing biologically active sulfonamide moiety as a new class of antitumor agents. Eur J Med Chem 45:738–744

Al-Said MS, Ghorab MM, Al-Dosari MS, Hamed MM (2011) Synthesis and in vitro anticancer evaluation of some novel hexahydroquinoline derivatives having a benzene sulfonamide moiety. Eur J Med Chem 46:201–207

Bigi F, Conforti ML, Maggi R, Piccinno A, Sartori G (2000) Clean synthesis in water: uncatalysed preparation of ylidenemalononitriles. Green Chem 2:101–103

Burres NS, Frigo A, Rasmussen RR, McAlpine JB (1992) A colorimetric microassay for the detection of agents that interact with DNA. J Nat Prod 55:1582–1587

El-Ashmaway MB, El-Sherbeny MA, El-Sayed NS (2010) Synthesis, in vitro antitumor activity and DNA-binding affinity of novel thiadiazolopyrimidine and thiadiazoloquinazoline derivatives. Mans J Pharm Sci 26:60–68

El-Gazzar ABA, El-Enany MM, Mahmoud MN (2008) Synthesis, analgesic, anti-inflammatory, and antimicrobial activity of some novel pyrimido[4,5-*b*]quinolin-4-ones. Bioorg Med Chem 16:3261–3273

El-Gazzar ABA, Hafez HN, Abu-Hashem AA, Aly AS (2009a) Synthesis and antioxidant, anti-inflammatory, and analgesic activity of novel polycyclic pyrimido[4,5-*b*]quinolines. Phosphorus, Sulfur, Silicon Relat Elem 184:379–405

El-Gazzar ABA, Youssef MM, Youssef AMS, Abu-Hashem AA, Badria FA (2009b) Design and synthesis of azolopyrimidoquinolines, pyrimidoquinazolines as anti-oxidant, anti-inflammatory and analgesic activities. Eur J Med Chem 44:609–624

Elkholy YM (2007) An efficient synthesis of pyrazolo[3,4-*b*]quinolin-3-amine and benzo[*b*][1,8]naphthyridine derivatives. Molecules 12:361–372

El-Sayed NS, El-Bendary ER, El-Ashry SM, El-Kerdawy MM (2011) Synthesis and antitumor activity of new sulfonamide derivatives of thiadiazolo[3,2-*a*]pyrimidines. Eur J Med Chem 46: 3714–3720

Ghorab MM, Ragab FA, Hamed MM (2009) Design, synthesis and anticancer evaluation of novel tetrahydroquinoline derivatives containing sulfonamide moiety. Eur J Med Chem 44:4211–4217

Ghorab MM, Ragab FA, Heiba HI, Arafa RK, El-Hossary EM (2010) In vitro anticancer screening and radiosensitizing evaluation of some new quinolines and pyrimido[4,5-*b*]quinolines bearing a sulfonamide moiety. Eur J Med Chem 45:3677–3684

Joshi AA, Narkhede SS, Viswanathan CL (2005) Design, synthesis and evaluation of 5-substituted amino-2,4-diamino-8-chloropyrimido-[4,5-*b*]quinolines as novel antimalarials. Bioorg Med Chem Lett 15:73–76

Kidwai M, Negi N (1997) Synthesis of some novel substituted quinolines as potent analgesic agents. Monatash Chem 128:85–89

Mahran MA, El-Sherbeny MA, El-Obaid AMA, Badria FA (1998) Heterocyclic systems containing pyrimidine nucleus as potential antimicrobial and antitumor agents Alexandria. J Pharm Sci 12:39–44

Pezzuto JM, Antosiak SK, Messmer WM, Slaytor MB, Honig GR (1983) Interaction of the antileukemic alkaloid, 2-hydroxy-3,8,9-trimethoxy-5-methylbenzo[*c*]phenanthridine (fagaronine) with nucleic acids. Chem Biol Interact 43:323–339

Pezzuto JM, Che CT, McPherson DD, Zhu JP, Topcu G, Erdelmeier CAJ, Cordell GA (1991) DNA as affinity probe useful in the detection and isolation of biologically active natural products. J Nat Prod 54:1522–1530

Rhee HK, Park HJ, Lee SK, Lee Ch, Choo HY (2007) Synthesis, cytotoxicity, and DNA topoisomerase II inhibitory activity of benzofuroquinolinediones. Bioorg Med Chem 15:1651–1658

Selvi ST, Nadaraj V, Mohan S, Sasi R, Hema M (2006) Solvent free microwave synthesis and evaluation of antimicrobial activity of pyrimido[4,5-b] and pyrazolo[3,4-b]quinolines. Bioorg Med Chem 14:3896–3903

Shrestha AR, Ali HI, Ashida N, Nagamatsu T (2008) Antitumor studies. Part 5: synthesis, antitumor activity and molecular docking study of 5-(monosubstituted amino)-2-deoxo-2-phenyl-5-deazaflavins. Bioorg Med Chem 16:9161–9170

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR (1990) New coloremetric cytotoxicity assay for anticancer drug screening. J Natl Cancer Inst 82:1107–1112

Suresh T, Kumar RN, Magesh S, Mohan PS (2003) Synthesis, characterization and antimicrobial activity of 4-phenyl-3-thiopyrimido[4,5-*b*]quinolines (III). Ind J Chem B 42:2133–2135

Wilson JM, Henderson G, Black F, Sutherland A, Ludwig RL, Vousden KH, Robins DJ (2007) Synthesis of 5-deazaflavin derivatives and their activation of p53 in cells. Bioorg Med Chem 15:77–86