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Original article

Synthesis, *in vitro* antitumor evaluation and DNA-binding study of novel tetrahydroquinolines and some derived tricyclic and tetracyclic ring systems

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ABSTRACT

The synthesis of some new tetrahydroquinolines, tetrahydropyrimido[4,5-*b*]quinolines, and tetrahydropentaazacyclopenta[*a*]anthracenes structurally related to some DNA intercalators is described. Fifteen compounds were evaluated for their antitumor activity by the National Cancer Institute (NCI), *in vitro* disease oriented antitumor screening. The most active tricyclic pyrimido[4,5-*b*]quinolines **3b**, **6b**, **7b** and **8b** were further subjected to DNA-binding investigation in an attempt to rationalize their activity. Compound **8b** proved to be the most active member in this study as evidenced from its remarkable growth inhibitory potential against some individual cell lines, and its broad spectrum of antitumor activity (GI_{50}, TGI and LC_{50} values 46.9, 85.3 and 97.4, respectively), together with a good DNA-binding affinity.

1. Introduction

Over the past two decades, chemotherapy has emerged as one of the major therapeutic disciplines of clinical oncology. The view that cancer is a multistep genetic process, together with the enormous influx of information about its molecular biology, have provided a basis for rational design of more selective and safer anticancer agents [1]. Nevertheless, empiric screening continues to be essential for discovering new lead structures with anticancer potential, and the NCI is still pursuing influential efforts in this field, where great interest is given to the originality of the chemical structures that have not been immensely investigated for such activity [2].

Among the wide range of the currently investigated anticancer agents, DNA intercalating agents have received particular attention [3]. They all comprise a planer or semi-planer pharmacophore consisting of two-four linearly-fused polyaromatic ring systems, together with side chains and/or substituents rich in hydrogen-bonding functionalities. Their mechanism of action involves perpendicular insertion between the base pairs of DNA through the formation of hydrogen bonding and Van der Waal forces [4a,5a]. This action disrupts the topology of the double helix leading to interference with DNA synthesis and transcription [6]. Moreover, G-quadruplexes are considered also potential targets for intercalators, where they act as telomerase inhibitors. Telomerase is a reverse transcriptase enzyme expressed profoundly in cancer cells and responsible for DNA elongation, and hence is essential for tumor growth [5b,7,8]. Consequently, an interesting target for cancer therapeutic intervention involves directly targeting the telomeric DNA substrate of telomerase [9]. Interestingly, all the structurally distinct G-quadruplex-binding ligands identified by high-throughput screening share the same structural feature of extended planarity as represented by acridines and some structurally relevant polycyclic compounds. Meanwhile, they appear to be readily taken up by cells, possibly as a consequence of their hydrophobic aromatic rings [4b,10,11].

Comprehensive literature survey revealed that quinolines, partially reduced quinolines and their derived polycyclic ring systems have attracted considerable synthetic interest owing to their versatile biological potentials including antiinflammatory [12], antimicrobial [13,14], antimalarial [15,16] antileishmanial [17], antiviral [18,19] and anticancer [20–22] activities. Among these, particular attention has been given to the chemotherapeutic activity of pyrimidoquinolines being an essential scaffold in many antitumor [23,24], antiproliferative [25] and anticancer [26] agents.

Tempted by the above-mentioned findings, and in continuation of our efforts linked with discovering and exploring novel lead

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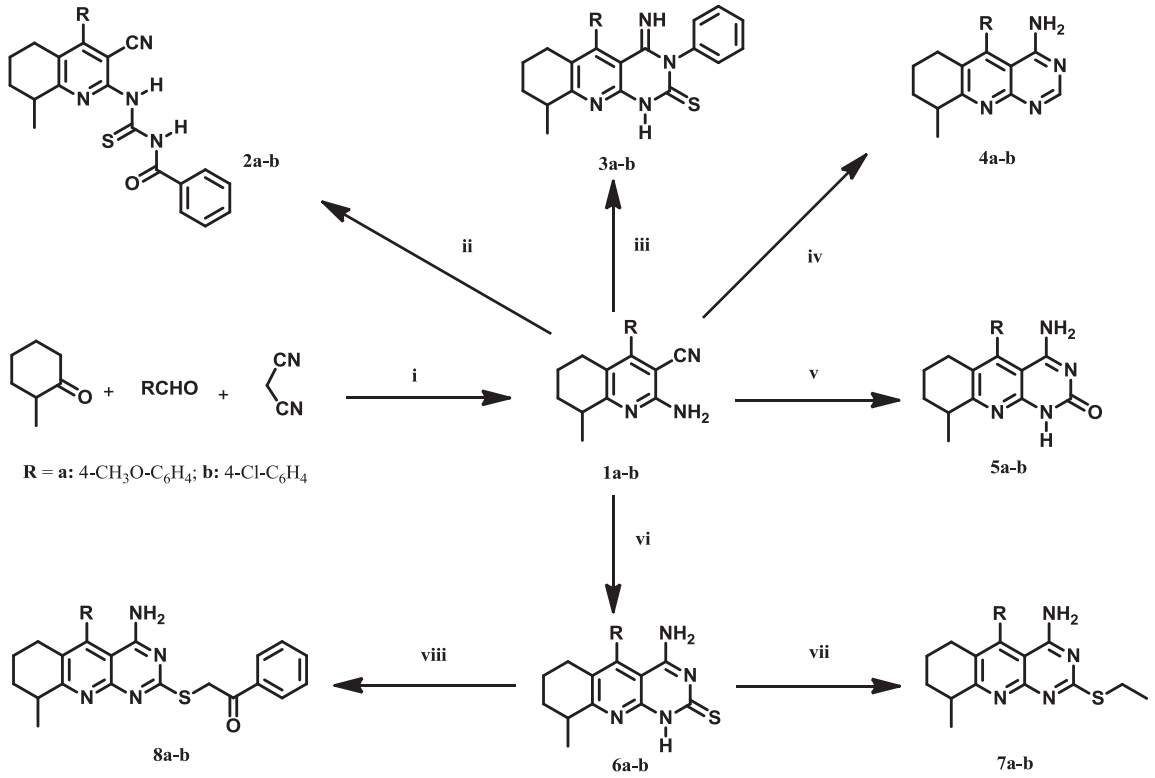
structures as potent chemotherapeutic agents [27–38], new derivatives of tetrahydroquinolines and some derived tricyclic tetrahydropyrimido[4,5-*b*]-quinolines structurally related to some DNA intercalators, have been designed and synthesized to be evaluated for their *in vitro* anticancer activity according to the current protocol of the NCI's disease-oriented human cells screening panel assay [39–41]. The target compounds were designed so as to comprise various substituents, pharmacophores and functionalities that would act as hydrogen-bond forming centers, such as the carbonyl, thione, thioether, thioureido, cyano, amino, and imino groups. Besides, the aryl substitution fashion of the main scaffold was attempted to grant variable electronic and lipophilic environment that might assist in hydrophobic bonding and hence affect the anticipated anticancer activity. Furthermore, annulation of some tricyclic tetrahydropyrimido[4,5-*b*]quinolines into their corresponding tetracyclic tetrahydropentaazacyclopenta[*a*]anthracenes was considered as an interesting structure variation that might have an impact on the targeted biological activity. Due to the observed structure similarities between the newly synthesized compounds and some DNA intercalators and G-quadruplex stabilizers, the most active compounds were subjected to DNA binding assay in an attempt to rationalize their anticancer potential.

2. Results and discussion

2.1. Chemistry

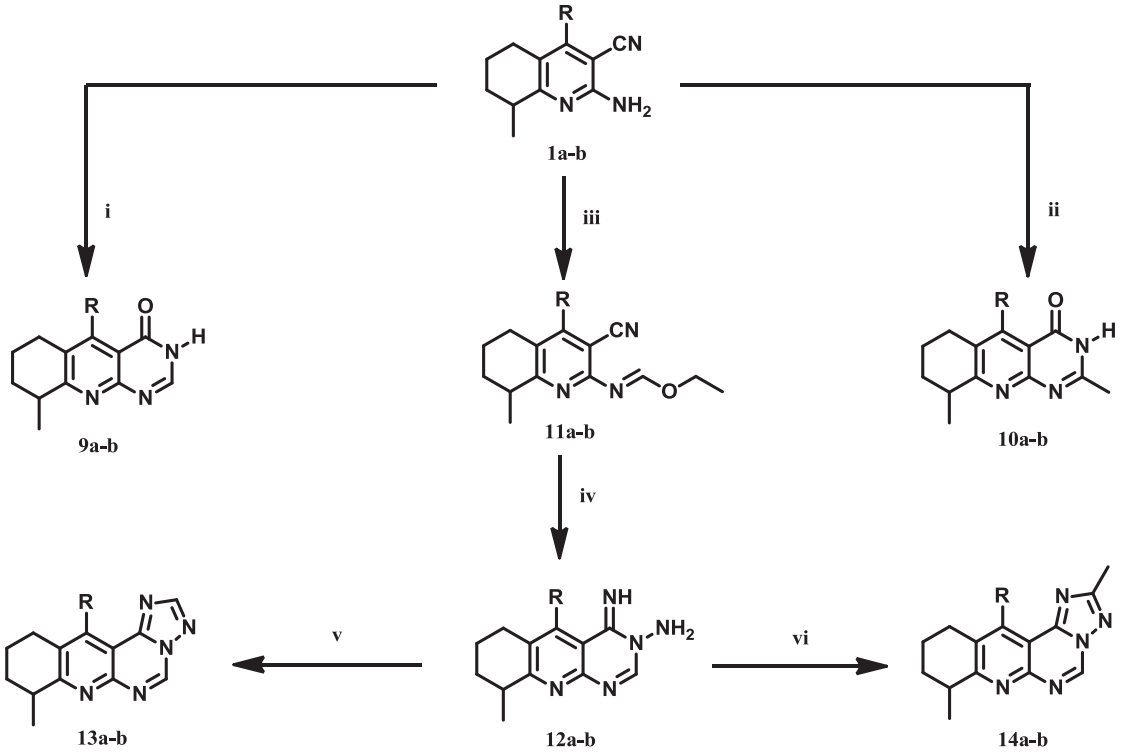
The proposed synthetic routes adopted to obtain the intermediate as well as target compounds are illustrated in Schemes 1 and 2. In Scheme 1, the key intermediates 2-amino-3-cyano-8-methyl-4-substituted-5,6,7,8-tetrahydroquinolines **1**; could be easily synthesized via multi-component one-put reaction of the 2-methylcyclohexanone, the appropriate aromatic aldehyde, malononitrile and an excess of ammonium acetate in boiling ethanol. The IR spectra of these compounds revealed absorption bands at 3450–3210 and 2226–2214 cm^{-1} characteristic for the NH_2 and the CN groups, respectively. Reacting compounds **1** with benzoyl isothiocyanate in the presence of anhydrous K_2CO_3 in acetone gave rise to the thiourea analogs **2**. Their IR spectra maintained the characteristic CN absorptions in addition to the new bands at 1710–1700 and 1185–1168 cm^{-1} attributed to the C=O and C=S groups, respectively. However, when the starting compounds **1** were reacted with phenyl isothiocyanate under different reaction conditions, the corresponding substituted tricyclic thiones **3** were obtained. Their IR spectra missed the CN absorption and revealed new bands at 1629–1615 cm^{-1} due to the (C=N) moieties and at 1210–1195 cm^{-1} corresponding to the (C=S) groups. Cyclization of the key intermediates **1** with formamide resulted in the formation of the targeted 4-amino-9-methyl-5-substituted-6,7,8,9-tetrahydropyrimido[4,5-*b*]quinolines **4**. Furthermore, fusion of the tetrahydroquinoline derivatives **1** either with urea or thiourea was utilized as fruitful way for a one-step synthesis of the target tricyclic compounds **5** and **6**, respectively. Thioalkylation of the 2-thione derivatives **6** with ethyl iodide or phenacyl bromide in the presence of sodium hydroxide afforded the corresponding alkylthio derivatives **7** and **8**, respectively. ^1H NMR spectra of **7** revealed the characteristic triplets and quartets of the thioethyl group, whereas, compounds **8** showed new singlets at δ 3.95 and 4.05 ppm attributed to the newly introduced methylene protons.

Shifting to Scheme 2, when compounds **1** were reacted with either formic acid or acetic anhydride, the targeted tetrahydropyrimido[4,5-



Reagents and reaction conditions: i: amm. acetate, ethanol, reflux, 3-6h; **ii:** C_6H_5-CONCS, K_2CO_3, acetone, reflux, 3h; **iii:** C_6H_5NCS, pyridine, reflux, 2-3h; **iv:** $HCONH_2$, reflux, 2-3h; **v:** urea, fusion, 1h; **vi:** thiourea, fusion, 1h; **vii:** CH_3CH_2I, 1N NaOH; r.t., 2-3h; **viii:** phenacyl bromide, 1N NaOH; r.t., 6-8h..

Scheme 1. Synthesis of the target compounds **1**–**8**.



R = **a:** 4-CH_3O-C_6H_4; **b:** 4-Cl-C_6H_4

Reagents and reaction conditions: i: HCOOH, heat, 30 min; **ii:** $(CH_3CO)_2O$, heat, 30 min; **iii:** $(EtO_3)CH$, $(CH_3CO)_2O$, reflux, 8-10h; **iv:** $NH_2NH_2.H_2O$ 99%, benzene, reflux, 2-4h; **v:** HCOOH, reflux, 6-8h; **vi:** $(CH_3CO)_2O$, reflux, 6-8h.

Scheme 2. Synthesis of the target compounds **9**–**14**.

b]quinolin-4-ones **9** and their 2-methyl analogs **10**, respectively, were successfully obtained. The IR spectra of the latter compounds showed the absence of the CN group absorption and the appearance of a new sharp absorption bands at 1710–1695 cm^{-1} due to the new C=O groups at position-4. Meanwhile, the ^1H NMR spectra of compounds **10** showed new singlets at δ 2.49–2.54 ppm due to the new CH_3 group introduced. Moreover, reacting **1** with triethyl orthoformate gave the 2-ethoxymethylidineamino derivatives **11**. Their IR spectra showed in addition to the characteristic cyano groups absorptions, new absorption bands at 1630–1620 cm^{-1} due to the C=N moiety, whereas their ^1H NMR were characterized by the presence of the CH_2CH_3 groups quartets and triplets at their respective chemical shifts in addition to the CH=N singlets at δ 8.05–8.11 ppm. Compound **11**, in its turn, was allowed to react with hydrazine hydrate to produce the tricyclic 3-amino-4-imino-9-methyl-5-substituted-6,7,8,9-tetrahydro-4*H*-pyrimido[4,5-*b*]quinolines **12**. The latter compounds were subjected to reaction either with formic acid to afford the targeted tetracyclic tetrahydropentaazacyclopenta[*a*]anthracenes **13**, or with acetic anhydride to give their methyl analogs **14**.

2.2. Preliminary in vitro one-dose antitumor screening

Out of the newly synthesized compounds, fifteen derivatives namely: **1a**,**b**, **2b**, **3b**, **4a**,**b**, **5b**, **6b**, **7a**,**b**, **8b**, **9a**, **10a**, **12a** and **13a** were selected by the National Cancer Institute (NCI) *in-vitro* disease-oriented human cells screening panel assay to be evaluated for their *in-vitro* antitumor activity. An effective one-dose assay has been added to the NCI-60 cell screen in order to increase compound throughput and reduce data-turnaround time to suppliers while maintaining efficient identification of active compounds [39–41]. All compounds submitted to the NCI-60 cell screen are tested initially at a single high dose (10 μM) in the full NCI-60 cell panel including leukemia, non-small cell lung, colon, CNS melanoma, ovarian, renal, prostate, and breast cancer cell lines. Only compounds which satisfy pre-determined threshold inhibition criteria would proceed to the five-dose screen. Data are reported as a mean graph of the percent growth of treated cells, and presented as percentage growth inhibition (GI %) caused by the test compounds (Table 1).

The obtained data revealed that, some of the tested subpanel tumor cell lines exhibited diverse sensitivity profiles against most of the tested compounds. Among these, the non-small cell lung cancer HOP-92 cell line exhibited various degree of sensitivity toward eleven out of the tested fifteen compounds, particularly toward compound **8b** (GI value 90.0%). Colon cancer HCT-15 proved to be highly sensitive to compound **8b** with GI value of 71.5%. Furthermore, the growth of the breast cancer T-47D cell line was variably affected by the presence of most of the tested compounds especially **8b** (GI value 72.6%), whereas the analogs **1a**, **2b**, **3b**, **4a**, **4b**, **7a** and **7b** exhibited moderate inhibitory activity against the same cell line with GI range of 38.8–50.0%. Regarding the leukemia subpanel, most of the tested compounds revealed an observable broad spectrum of growth inhibition on most of the tested cell lines, especially the CCRF-CEM cell line, which was noticeably affected by compound **8b**

Table 1
In vitro growth inhibitory percentage (GI %) of the tested compounds against some selected tumor cell lines at the single-dose assay.[a]

Cell lines	**1a**	**1b**	**2b**	**3b**	**4a**	**4b**	**5b**	**6b**	**7a**	**7b**	**8b**	**9a**	**10a**	**12a**	**13a**
NSCLS[b]															
A549/ATCC	15.7	–[c]	16.0	18.9	–	–	11.5	–	16.0	18.1	17.6	–	10.0	–	–
EKVX	–	39.5	16.7	31.0	11.5	–	–	14.5	–	12.3	26.7	–	–	21.5	–
HOP-92	20.0	10.0	38.2	26.2	20.4	25.3	27.7	–	26.8	35.2	**90.0**	–	21.3		–
NCI-H23	11.0	–	10.0	11.5	–	–	10.0	10.1	–	15.3	24.7	13.8	–	14.4	–
NCI-H522	14.6	–	27.5	31.0	–	11.0	12.9	10.0	13.2	21.9	17.5	20.8	22.0	26.0	–
Colon cancer															
HCT-116	15.7	–	20.0	34.0	14.2	14.0	14.0	10.5	20.0	18.8	38.4	–	–	22.3	–
HCT-15	25.3	–	11.6	10.0	20.0	10.0	10.0	14.3	23.8	18.8	**71.5**	13.7	11.0	25.0	–
HT29	–	–	–	–	–	13.3	–	–	23.3	27.7	21.5	–	–	–	–
Breast cancer															
MCF7	10.0	31.5	12.1	–	–	–	–	10.4	–	–	30.0	–	–	–	–
NCI/ADR-RES	24.1	–	–	11.4	16.3	–	–	–	–	12.8	12.8	20.0	–	–	–
HS 578T	–	25.0	13.8	–	–	14.7	19.4	–	36.2	14.2	38.2	–	–	–	–
MDA-MB-435	–	–	–	–	–	–	–	–	–	14.5	13.0	–	19.0	19.0	–
MDA-MB-468	15.2	36.5	14.5		11.3	20.0	10.0		13.8	30.8	22.9	21.5	–	–	–
T-47D	41.0	17.2	38.8	50.0	47.7	40.0	29.5	15.2	41.0	39.5	**72.6**	–	18.2	18.1	–
Ovarian cancer															
IGROV1	–	–	32.0	33.3	–	–	–	10.0	–	39.0	–	–	–	–	10.6
OVCAR-4	17.2	10.1	21.4	14.5	12.6	17.8	13.6	–	26.8	26.8	20.0	–	–	20.0	–
OVCAR-5	–	–	–	14.3	–	–	–	–	–	12.3	10.0	–	11.4	–	–
SK–OV-3	–	–	20.0	10.4	–	12.0	–	–	–	15.2	–	–	–	–	–
Leukemia															
CCRF-CEM	16.3	13.3	27.6	20.7	23.0	22.2	–	28.8	26.4	28.4	**60.1**	–	16.5	–	–
HL-60(TB)	11.7	–	30.0	24.2	–	12.4	24.6	10.0	34.5	–	22.0	–	–	–	–
K-562	28.4	11.8	36.3	28.5	23.4	25.0	36.4	15.0	32.5	32.4	27.7	–	11.2	–	–
MOLT-4	21.3	–	31.0	40.0	14.3	–	–	26.2	29.0	32.5	44.6	–	23.3	–	19.0
RPMI-8226	34.1	18.2	55.0	66.0	25.0	21.3	23.0	37.0	25.5	33.8	**69.2**	–	21.8	–	31.0
SR	36.6	–	–	–	17.3	–	–	25.8	–	37.9	–	–	46.8	–	17.8
Renal cancer															
A 498	–	21.7	39.0	25.2	–	27.7	–	11.5	–	21.7	21.2	–	–	–	13.5
ACHN	–	–	14.0	11.3	10.0	–	–	–	30.8	30.8	29.3	–	11.3	14.5	–
CAKI-1	–	–	22.5	–	–	–	–	31.3	–	–	11.8	–	–	10.0	–
RXF 393	11.1	–	–	17.8	20.3	11.0	–	–	25.9	26.6	**62.2**	–	12.5	–	–
UO-31	–	–	33.2	44.4	10.4	10.1	10.0	–	10.5	59.0	**63.0**	–	–	–	–
Melanoma															
LOX IMVI	12.3	–	11.1	13.1	13.0	–	–	–	10.6	–	14.6	–	17.3	–	15.0
M14	–	–	–	17.3	–	–	–	–	–	–	35.0	–	–	–	–
SK-MEL-2	–	15.0	10.3	11.3	–	–	–	11.4	–	–	14.5	–	–	11.0	–
SK-MEL-5	14.5	–	17.7	12.0	10.0	10.2	–	10.3	11.4	–	**67.0**	–	13.6	–	–
UACC-62	20.0	24.2	25.3	25.3	20.9	13.8	25.4	15.8	25.0	21.8	**70.8**	–	14.8	24.0	18.3
Prostate cancer															
PC-3	16.2	–	20.3	21.0	15.0	24.2	12.7	56.7	31.4	25.3	**66.8**	–	14.2	–	13.0
DU-145	–	–	–	13.0	–	–	14.0	–	24.5	–	17.0	–	–	–	–
CNS cancer															
SF-268	–	11.3	–	–	–	12.3	–	18.8	–	–	23.7	–	–	–	–
SF-295	–	–	17.2	12.8	–	–	–	–	–	–	23.9	–	–	–	–
SNB-19	11.0	20.5	14.1	–	10.0	21.3	16.6	10.0	12.5	–	–	–	–	–	–
SNB-75	25.3	26.8	15.7	13.9	20.5	21.5	11.0	10.2	–	33.0	–	34.3	–	14.3	–
U251	20.0	–	20.0	15.2	18.5	14.5	10.2	–	16.0	13.6	21.5	19.0	–	16.7	–

Bold figures denotes the most distinctive biological values.
[a] The data obtained from NCI's *in vitro* disease-oriented human tumor cell screen at 10 μM conc.
[b] NSCLC: Non-Small Cell Lung Cancer.
[c] GI < 10%.

(GI value of 60.1%). Meanwhile, the growth of the RPMI-8226 cell line was remarkably inhibited by the presence of compounds **2b**, **3b** and **8b** with GI values of 55.0, 66.0 and 69.2%, respectively. Other leukemia subpanel cell lines were moderately affected by the presence of compounds **1a**, **2b**, **5b**, **7b** and **8b** with GI range of 31.0–44.6%. Referring to the renal cancer subpanel, compound **8b** was able to remarkably inhibit the growth of the RXF 393 and UO-31 cell lines with GI values of 62.2 and 63.0%, respectively. The latter cell line showed also a noticeable sensitivity toward the analogs **2b**, **3b** and **7b** (GI values 33.2, 44.4 and 59.0%, respectively). Melanoma SK-MEL-5 and UACC-62 cell lines proved to be selectively sensitive to compound **8b** with GI values of 67.0 and 70.8%, respectively. It is to be noted that, compounds **6b** and **8b** were able to remarkably inhibit the growth of the prostate cancer PC-3 cell line with GI values of 56.7 and 66.8%, respectively.

Concerning the broad spectrum of antitumor activity, the 1*H*-pyrimido[4,5-*b*]quinoline-2-thiones **3b** and **6b** together with the thioether congeners **7b** and **8b** are the most active antitumor members, showing effectiveness toward a variety of cell lines belonging to different tumor subpanels. Compounds **3b**, **6b** and **7b** possessed moderate antitumor activity, with special selective potency against the leukemia cell lines. On the other hand, the 1-phenylethanone thioether analog **8b** proved to be the most active member in this bioassay as evidenced by its ability to exert a broad spectrum of growth inhibitory activity against most of the tested nine subpanel tumor cell lines. Consequently, **8b** was able to successfully meet the threshold inhibition criteria determined by the Development Therapeutic Program (DTP), passed this primary anticancer assay, and was carried over to the 5-dose screen against a panel of about 60 different tumor cell lines.

2.3. Full in vitro five-dose antitumor assay for compound **8b**

About 60 cell lines of nine tumor subpanels, including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer cell lines, were incubated with five concentrations (0.01–100 μM) for the tested compound and were used to create log concentration % growth inhibition curves. Three response parameters: GI_{50} (growth inhibitory activity), TGI (cytostatic activity), and LC_{50} (cytotoxic activity) were calculated for each cell line, using 5-flourouracil (5-FU) as a positive control. Subpanel and full panel mean-graph midpoint values (MG-MID) for certain agents are the average of individual real and default GI_{50}, TGI, or LC_{50} values of all cell lines in the subpanel or the full panel, respectively [39–41].

At the GI_{50} (MG-MID) level, compound **8b** showed almost 50% of the activity of the positive control 5-FU (46.9 vs 22.6 μM, respectively). Individually, it revealed about 50% of 5-FU activity against the leukemia subpanel (34.7 vs 15.1 μM, respectively), whereas, it was almost equipotent with 5-FU against the renal and breast cancer subpanels (51.8 vs 45.6 and 79.3 vs 76.4 μM, respectively). Furthermore, **8b** was able to induce mild cytostatic and cytotoxic activities with TGI and LC_{50} (MG-MID) concentrations of 85.3 and 97.4 μM, respectively (Table 2). The ratio obtained by dividing the compound's full panel MG-MID (μM) by its individual subpanel MG-MID (μM) is considered as a measure of compound's selectivity. Ratios between 3 and 6 refer to moderate selectivity, ratios >6 indicate high selectivity toward the corresponding cell line, while compounds meeting neither of these criteria are rated non-selective [42]. In this context, **8b** was found to be non-selective with broad spectrum antitumor activity against the nine tumor subpanels with selectivity ratios range of 0.58–1.35 at the GI_{50} MG-MID level.

2.4. Structure–activity correlation

Structurally, the biologically active compounds belong to three series: bicyclic tetrahydroquinolines (**1**, **2** and **11**), tricyclic tetrahydropyrimido[4,5-*b*]quinolines (**3**–**10** and **12**) and tetracyclic tetrahydropentaazacyclopenta[*a*]anthracenes (**13** and **14**) (Schemes 1 and 2). Based on the number of tumor cell lines that showed sensitivity toward the individual active compounds (Table 1), it could be realized that better antitumor activity was confined basically to the tricyclic tetrahydropyrimido[4,5-*b*]quinoline analogs, particularly those substituted with a 4-chlorophenyl moiety at position-5 of this scaffold. While the bicyclic tetrahydroquinolines **1a** and **1b** showed mild antitumor activity, the synthesis of the benzoylthioureido derivative **2b** resulted only in an increase in the overall antitumor spectrum against a larger number of tumor cell lines. Annulation of the tricyclic tetrahydropyrimido [4,5-*b*]quinoline ring system as in compounds **3**–**8** led to a noticeable improvement of the anticipated bioactivity. In this view, the 3-phenyl-1*H*-pyrimido[4,5-*b*]quinoline-2-thione **3b** showed better antitumor activity than the starting **1b**. Cyclization to the 4-amino-pyrimido[4,5-*b*]quinolines **4a**,**b** resulted in an obvious reduction in activity. Moreover, introduction of a carbonyl group at position-2 produced compound **5b** with moderate antitumor potency. Meanwhile, isosteric replacement of the 2-oxo with 2-thio group as in **6b** led to a slight improvement in activity especially against the leukemia subpanel. Alkylation of the thione function in the latter compounds to the corresponding ethyl thioether group as in **7a**,**b** resulted in a significant enhancement in both the antitumor spectrum and potential. Interestingly, alkylation of **6b** with a 1-phenylethanone counterpart furnished the analog **8b** which proved to be the most active member in this study, with remarkable antitumor potency and spectrum against 36 different subpanel tumor cell lines. On the other hand, introduction of a carbonyl function at position-4 in the pyrimido[4,5-*b*]quinoline scaffold as in **9a** and **10a** pulled the activity toward the weak side. The same could also be noticed upon replacement of the carbonyl with an imino function at the same position as in **12b**. It is worth-mentioning that, extention of the tricyclic tetrahydropyrimido [4,5-*b*]quinoline structure to the corresponding tetracyclic tetrahydropentaazacyclopenta[*a*]anthracene ring system as in **13a**, led to a dramatic loss in the overall antitumor spectrum and potential.

2.5. DNA-binding activity

2.5.1. DNA-binding assay on TLC-plate

In an attempt to rationalize their antitumor activity, compounds **3b**, **6b**, **7b** and **8b** (Fig. 1) were subjected to DNA-binding investigations. The principle of this method depends on the ability of DNA to migrate after being applied to RP18 F_{254} TLC plates, predeveloped with methanol/water mixture (8:2) as an elution system. When DNA was mixed with compounds with which it has been known to intercalate (e.g. ethidium bromide), the complex was retained at the base line using the same elution system, and the spots were visualized after spraying with anisaldehyde reagent [43,44]. In the presence of high affinity DNA-intercalator, higher amount of DNA is bound to form a complex that is retained on the base line. On the other hand, inactive compounds caused DNA to leave the origin and move along the plate. The results revealed that, compound **8b** displayed the highest affinity toward DNA as demonstrated from retention of the DNA-compound's complex at the base line. Meanwhile, the analogs **3b**, **6b** and **7b** showed moderate to weak binding activity.

2.5.2. Methyl green-DNA displacement colorimetric assay

It has been well-documented that methyl green reversibly binds polymerized DNA to form a stable complex at neutral pH for a long period of time. On the other hand, the color of methyl green alone

Table 2
Median growth inhibitory (GI_{50}, μM), total growth inhibitory (TGI, μM) and lethal (LC_{50}, μM) concentrations of *in vitro* subpanel tumor cell lines[a] for compound **8b**.

Compd.	Activity parameter	Subpanel tumor cell lines[b]									MG-MID[c]
		I	II	III	IV	V	VI	VII	VIII	IX	
8b	GI_{50}	34.7	46.1	–[d]	62.3	58.0	80.6	51.8	75.5	79.3	46.9
	TGI	65.1	–	86.5	84.1	–	95.5	–	–	89.6	85.3
	LC_{50}	93.3	–	–	–	–	–	–	–	–	97.4
5-FU	GI_{50}	15.1	–	8.40	72.1	70.6	61.4	45.6	22.7	76.4	22.6
	TGI	–	–	–	–	–	–	–	–	–	–
	LC_{50}	–	–	–	–	–	–	–	–	–	–

[a] Median values calculated according to the data obtained from NCI's *in vitro* disease-oriented human tumor cell screen.
[b] I, Leukemia; II, non-small cell lung cancer; III, colon cancer; IV, CNS cancer; V, melanoma; VI, ovarian cancer; VII, renal cancer; VIII, prostate cancer; IX, breast cancer.
[c] GI_{50}, TGI and LC_{50} (μM), full panel mean-graph midpoint (MG-MID) = the average sensitivity of all cell lines toward the test agent.
[d] Values > 100 μM.

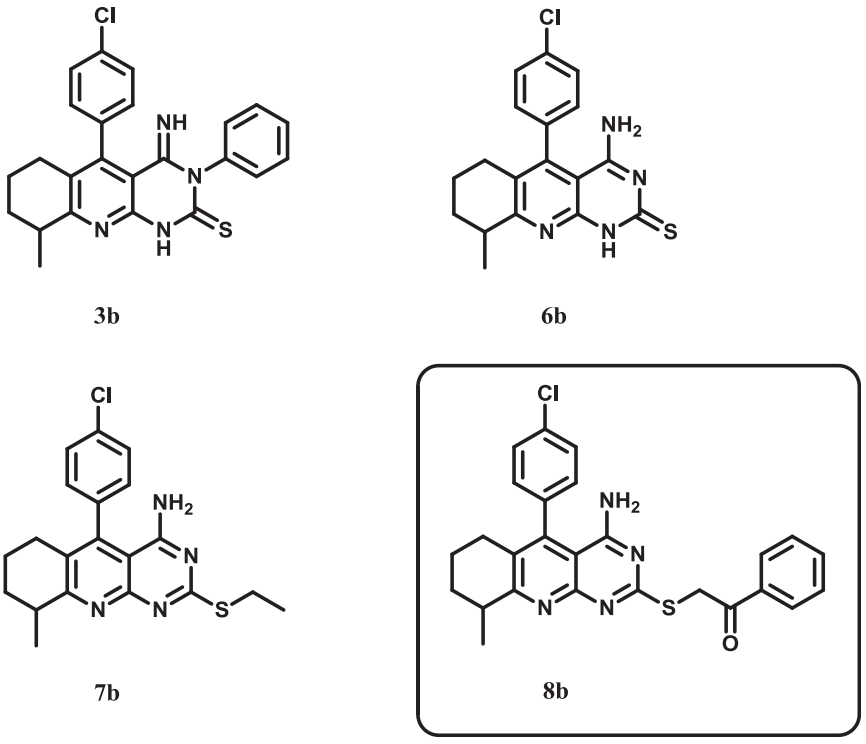


Fig. 1. Structures of the most active antitumor agents **3b**, **6b**, **7b** and **8b**.

fades when incubated for 24 h in the buffer used for displacement reactions in this investigation. This assay [45] was used to determine the degree of displacement of methyl green from DNA by the tested compounds colorimetrically, through measuring the decrease in the absorbance of the DNA/methyl green solution. The results were recorded in the form of IC_{50}, which is the sample concentration required to produce 50% reduction in the initial absorbance of the DNA-methyl green solution.

The results obtained were fairly in agreement with those obtained from the DNA-binding investigation on TLC plates (Table 3). Compound **8b** proved to be the most active member with (IC_{50} = 0.061 μmol/mL), when compared with ethidium bromide (IC_{50} = 0.004 μmol/mL), whereas the analogs **3b**, **6b** and **7b** showed moderate activity (IC_{50} = 0.097, 0.151 and 0.127 μmol/mL, respectively). Nevertheless, all of them were less active than ethidium bromide.

3. Conclusion

The principal objective of this investigation was to synthesize and investigate the antitumor activity of some novel polycyclic ring systems structurally related to the general template of DNA intercalators. Such goal has been verified by the synthesis of three novel series of bicyclic tetrahydroquinolines, tricyclic tetrahydropyrimido[4,5-*b*]quinolines, and tetracyclic tetrahydropentaazacyclopenta[*a*]anthracenes. The obtained NCI's *in vitro* antitumor data revealed that better antitumor activity was confined basically to the tricyclic tetrahydropyrimido[4,5-*b*]quinoline analogs, particularly those substituted with a 4-chlorophenyl counterpart and either thione and/or thioether functionalities. Compounds **3b**, **6b** and **7b** possessed moderate antitumor activity, whereas the thioether analog **8b** proved to be the most active member in this study as seen from its obvious broad spectrum of growth inhibitory activity against most of the tested nine subpanel tumor cell lines. Compound **8b** succeeded to pass over to the 5-dose screen, where it showed a broad spectrum of antitumor activity (GI_{50}, TGI and LC_{50} values of 46.9, 85.3 and 97.4, respectively), with special high selectivity profile against some individual cell lines. DNA-binding assays of the most active compounds **3b**, **6b**, **7b** and **8b** were in agreement with the obtained antitumor activity, where compound **8b** displayed the highest DNA-binding affinity. Finally, the broad spectrum antitumor effects expressed by the active compounds against most of the tested tumor cell lines increases the likelihood of their future derivatization in order to explore the scope and limitations of their activities.

Table 3
DNA binding affinity and DNA-methyl green displacement assay of compounds **3b**, **6b**, **7b** and **8b**.

Compound	DNA-binding affinity	IC_{50} (μg/mL)[a]
3b	Moderate	0.097 ± 0.005
6b	Moderate	0.151 ± 0.008
7b	Moderate	0.127 ± 0.004
8b	**High**	**0.061 ± 0.003**
Ethidium bromide	High	0.004 ± 0.006

Bold figures denotes the most distinctive biological values.
[a] Values represent the concentration (mean ± SD, $n = 6$ separate determinations) required to cause 50% decrease in the initial absorbance of DNA-methyl green complex.

4. Experimental

4.1. Chemistry

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu FT-IR 8400S infrared spectrophotometer using the KBr pellet technique. ^1H and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT NMR spectrometer using tetramethylsilane as the internal standard and a mixture of $CDCl_3$ and DMSO-d_6 as a solvent (Chemical shifts in δ, ppm). Splitting patterns

were designated as follows: s: singlet; d: doublet; m: multiplet; q: quartet. Mass spectra were recorded on Agilent LC-MS 6120 single quad. Elemental analyses were performed on a 2400 Perkin Elmer Series 2 analyzer and the found values were within ±0.4% of the theoretical values. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254.

*4.1.1. 2-Amino-3-cyano-8-methyl-4-substituted-5,6,7,8-tetrahydroquinolines (**1a**,**b**)*

A mixture of the 2-methylcyclohexanone (1.12 g, 10 mmol), the appropriate aldehyde (10 mmol), malononitrile (0.66 g, 10 mmol) and ammonium acetate (6.2 g, 80 mmol) in absolute ethanol (50 mL) was heated under reflux for 3–6 h. The reaction mixture was cooled and the formed precipitate was filtered, washed with water, dried and recrystallized from the appropriate solvent. IR (cm^{-1}): 3450–3210 (NH), 2226–2214 (CN).

*4.1.1.1. 2-Amino-3-cyano-8-methyl-4-(4-methoxyphenyl)-5,6,7,8-tetrahydroquinoline (**1a**).* Yield: 68%, mp: 177–9 °C, ^{1}H NMR (δ, ppm): 1.36 (s, 3H, CH_3), 1.68–2.39 (m, 6H, $C_{5,6,7}$–H), 2.88 (m, 1H, C_8–H), 3.85 (s, 3H, OCH_3), 5.09 (s, 2H, NH_2), 6.99–7.26 (m, 4H, Ar–H). ^{13}C NMR (δ, ppm): 21.1 (CH_3), 22.1, 27.6, 31.2 38.4.4 (cyclohexyl C), 56.2 (OCH_3), 116.7 (CN), 88.1, 117.3, 145.7, 163.8, 164.2 (pyridone C), 114.2, 127.8, 131.5, 162.4 (Ar C). MS *m*/*z* (relative intensity) 293 (M^+, 45). Anal. Calcd. for $C_{18}H_{19}N_3O$ (293.36): C, 73.69; H, 6.53; N, 14.32. Found: C, 73.42; H, 6.71; N, 14.09.

*4.1.1.2. 2-Amino-3-cyano-8-methyl-4-(4-chlorophenyl)-5,6,7,8-tetrahydroquinoline (**1b**).* Yield: 81%, mp: 180–2 °C, ^{1}H NMR (δ, ppm): 1.36 (s, 3H, CH_3), 1.62–2.51 (m, 6H, $C_{5,6,7}$–H), 2.96 (m, 1H, C_8–H), 5.12 (s, 2H, NH_2), 7.12–7.48 (m, 4H, Ar–H). ^{13}C NMR (δ, ppm): 21.6 (CH_3), 22.3, 27.0, 30.9, 36.5 (cyclohexyl C), 116.8 (CN), 89.7, 120.3, 153.1, 157.2, 165.8 (pyridone C), 127.5, 128.8, 132.8, 133.3 (Ar C). MS *m*/*z* (relative intensity) 297 (M^+, 25). Anal. Calcd. for $C_{17}H_{16}ClN_3$ (297.78): C, 68.57; H, 5.42; N, 14.11. Found: C, 68.65; H, 5.28; N, 14.03.

*4.1.2. 1-Benzoyl-3-(3-cyano-8-methyl-4-substituted-5,6,7,8-tetrahydroquinolin-2-yl)-thioureas (**2a**,**b**)*

To a mixture of the appropriate starting compound **1a**,**b** (2 mmol) and K_2CO_3 (0.35 g, 2.5 mmol) in dry acetone (20 mL), a solution of benzoyl isothiocyanate (0.33 g, 2 mmol) in dry acetone (10 mL) was added. The resultant solution was heated under reflux for 3 h. The reaction mixture was left for an overnight at room temperature, concentrated to half its volume and allowed to cool in the refrigerator for 4 h. The separated crystalline product was filtered, washed with diethyl ether and crystallized from ethanol. IR (cm^{-1}): 3450–3345 (NH), 2220–2210 (CN), 1710–1700 (C=O), 1185–1168 (C=S).

*4.1.2.1. 1-Benzoyl-3-(3-cyano-8-methyl-4-(4-methoxyphenyl)-5,6,7,8-tetrahydroquinolin-2-yl)-thiourea (**2a**).* Yield: 83%, mp: 176–8 °C, ^{1}H NMR (δ, ppm): 1.35 (s, 3H, CH_3), 1.63–2.61 (m, 6H, $C_{5,6,7}$–H), 3.08 (m, 1H, C_8–H), 3.87 (s, 3H, OCH_3), 5.04 (s, 1H, NH), 7.11–7.82 (m, 9H, Ar–H), 7.95 (s, 1H, NH). ^{13}C NMR (δ, ppm): 22.3 (CH_3), 25.4, 31.5, 32.2, 39.1 (cyclohexyl C), 56.1 (OCH_3), 91.6, 114.6, 125.2, 126.2, 128.4, 130.4, 131.8, 133.5, 156.7, 162.5, 163.2, 166.1 (Ar C), 118.0 (CN), 179.8 (CS), 169.5 (CO). MS *m*/*z* (relative intensity) 456 (M^+, 21). Anal. Calcd. for $C_{26}H_{24}N_4O_2S$ (456.56): C, 68.40; H, 5.30; N, 12.27. Found: C, 68.85; H, 5.17; N, 12.09.

*4.1.2.2. 1-Benzoyl-3-(3-cyano-8-methyl-4-(4-chlorophenyl)-5,6,7,8-tetrahydroquinolin-2-yl)-thiourea (**2b**).* Yield: 89%, mp: 138–9 °C, ^{1}H NMR (δ, ppm): 1.36 (s, 3H, CH_3), 1.69–2.68 (m, 6H, $C_{5,6,7}$–H), 3.08 (m, 1H, C_8–H), 4.85 (s, 1H, NH), 7.13–7.80 (m, 9H, Ar–H), 8.09 (s, 1H, NH). ^{13}C NMR (δ, ppm): 22.1(CH_3), 25.7, 31.3, 32.4, 37.9 (cyclohexyl C), 91.6, 123.3, 126.8, 127.4, 128.4, 129.1, 131.2, 132.7, 133.5, 137.2, 156.7, 163.2, 166.2 (Ar C), 117.2 (CN), 179.1 (CS), 168.7 (CO). MS *m*/*z* (relative intensity) 461 (M^+, 16). Anal. Calcd. for $C_{25}H_{21}N_4ClOS$ (460.98): C, 65.14; H, 4.59; N, 12.15. Found: C, 64.87; H, 4.71; N, 12.03.

*4.1.3. 4-Imino-9-methyl-3-phenyl-5-substituted-3,4,6,7,8,9-hexahydro-1H-pyrimido [4,5-b]quinoline-2-thiones (**3a**,**b**)*

A mixture of **1a**,**b** (1 mmol), phenyl isothiocyanate (0.15 g, 1.5 mol) in pyridine (15 mL) was refluxed for 2 h. After cooling, the solid product separated was filtered off, washed thoroughly with water, dried and recrystallized from acetic acid. IR (cm^{-1}): 3327–3180 (NH), 1629–1615 (C=N), 1210–1195 (C=S).

*4.1.3.1. 4-Imino-9-methyl-3-phenyl-5-(4-methoxyphenyl)-3,4,6,7,8,9-hexahydro-1H-pyrimido[4,5-b]quinoline-2-thione (**3a**).* Yield: 80%, mp: 134–6 °C, ^{1}H NMR (δ, ppm): 1.37 (s, 3H, CH_3), 1.68–2.43 (m, 6H, $C_{6,7,8}$–H), 3.10 (m, 1H, C_9–H), 3.91 (s, 3H, OCH_3), 4.87 (s, 1H, NH), 6.76–7.46 (m, 9H, Ar–H), 7.95 (s, 1H, NH). ^{13}C NMR (δ ppm): 21.9 (CH_3), 26.0, 31.8, 32.5, 39.4 (cyclohexyl C), 56.6 (OCH_3), 109.8, 114.4, 124.3, 125.2, 127.6, 128.6, 130.5, 139.7, 148.8, 157.1, 162.3, 163.2 (Ar C), 164.7 (C=NH), 178.4 (CS). MS 428 *m*/*z* (relative intensity) (M^+, 32). Anal. Calcd. for $C_{25}H_{24}N_4OS$ (428.55): C, 70.07; H, 5.64; N, 13.07. Found: C, 69.91; H, 5.87; N, 12.89.

*4.1.3.2. 4-Imino-9-methyl-3-phenyl-5-(4-chlorophenyl)-3,4,6,7,8,9-hexahydro-1H-pyrimido[4,5-b]quinoline-2-thione (**3b**).* Yield: 80%, mp: 197–9 °C, ^{1}H NMR (δ, ppm): 1.36 (s, 3H, CH_3), 1.63–2.58 (m, 6H, $C_{6,7,8}$–H), 3.08 (m, 1H, C_9–H), 4.88 (s, 1H, NH), 7.01–7.59 (m, 9H, Ar–H), 8.01 (s, 1H, NH). ^{13}C NMR (δ, ppm): 18.0 (CH_3), 25.3, 31.4, 32.3, 39.2 (cyclohexyl C), 109.5, 123.2, 124.6, 125.3, 126.8, 128.8, 129.3, 132.3, 137.3, 139.4, 148.9, 156.7, 163.4 (Ar C), 165.4 (C=NH), 180.5 (CS). MS 433 *m*/*z* (relative intensity) (M^+, 28). Anal. Calcd. for $C_{24}H_{21}ClN_4S$ (432.97): C, 66.58; H, 4.89; N, 12.94. Found: C, 66.81; H, 4.65; N, 12.74.

*4.1.4. 4-Amino-9-methyl-5-substituted-6,7,8,9-tetrahydropyrimido [4,5-b]quinolines (**4a**,**b**)*

A mixture of the appropriate intermediate **1a**,**b** (1 mmol) and formamide (10 mL) was heated under reflux for 2–3 h. The reaction mixture was allowed to cool and the precipitated solid product was collected, washed with cold ethanol and recrystallized from acetic acid containing few drops of water. IR (cm^{-1}): 3330–3265 (NH).

*4.1.4.1. 4-Amino-9-methyl-5-(4-methoxyphenyl)-6,7,8,9-tetrahydropyrimido[4,5-b] quinoline (**4a**).* Yield: 79%, mp: 191–3 °C, ^{1}H NMR (δ, ppm): 1.39 (s, 3H, CH_3), 1.56–2.36 (m, 6H, $C_{6,7,8}$–H), 2.93 (m, 1H, C_9–H), 3.85 (s, 3H, OCH_3), 5.26 (s, 2H, NH_2), 6.98–7.68 (m, 5H, Ar–H and C_2–H). ^{13}C NMR (δ, ppm): 21.9 (CH_3), 25.6, 31.4, 32.3, 38.7 (cyclohexyl C), 56.0 (OCH_3), 106.7, 114.4, 127.9, 130.3, 135.4, 149.5, 157.2, 158.3, 162.4, 167.4 (Ar C). MS 320 *m*/*z* (relative intensity) (M^+, 48). Anal. Calcd. for $C_{19}H_{20}N_4O$ (320.39): C, 71.23; H, 6.29; N, 17.49. Found: C, 71.12; H, 6.47; N, 17.60.

*4.1.4.2. 4-Amino-9-methyl-5-(4-chlorophenyl)-6,7,8,9-tetrahydropyrimido[4,5-b]quinoline (**4b**).* Yield: 77%, mp: 185–7 °C, ^{1}H NMR (δ, ppm): 1.38 (s, 3H, CH_3), 1.61–2.29 (m, 6H, $C_{6,7,8}$–H), 2.98 (m, 1H, C_9–H), 5.18 (s, 2H, NH_2), 7.14–7.46 (m, 5H, Ar–H and C_2–H). ^{13}C NMR (δ, ppm): 21.7 (CH_3), 25.3, 31.4, 32.2, 38.6 (cyclohexyl C), 106.1, 123.7, 129.0, 132.3, 135.2, 137.3, 149.7, 157.4, 158.1, 162.4, 167.7 (Ar C). MS 324 *m*/*z* (relative intensity) (M^+, 33). Anal. Calcd. for $C_{18}H_{17}ClN_4$ (324.81): C, 66.56; H, 5.28; N, 17.25. Found: C, 66.43; H, 5.47; N, 17.08.

*4.1.5. General method for the preparation of 4-amino-9-methyl-5-substituted-6,7,8,9-tetrahydro-1H-pyrimido[4,5-b]quinoline-2-ones (**5a**,**b**) and 4-amino-9-methyl-5-substituted-6,7,8,9-tetrahydro-1H-pyrimido[4,5-b]quinoline-2-thiones (**6a**,**b**)*

A mixture of the start **1a**,**b** (1 mmol) and either urea (0.3 g, 5 mmol) or thiourea (0.4 g, 5 mmol), was fused at 260–300 °C using sand bath for 1 h. The reaction mixture was allowed to attain room temperature, crude solid product was treated with water, then rubbed with cold ethanol, filtered and recrystallized from DMF containing few drops of water.

*4.1.5.1. 4-Amino-9-methyl-5-(4-methoxyphenyl)-6,7,8,9-tetrahydro-1H-pyrimido[4,5-b]-quinoline-2-one (**5a**).* Yield: 71%, mp: 234–6 °C, ^{1}H NMR (δ, ppm): 1.41 (s, 3H, C*H*$_3$), 1.53–2.64 (m, 6H, C$_{6,7,8}$–*H*), 3.05 (m, 1H, C$_9$–*H*), 3.87 (s, 3H, OC*H*$_3$), 4.19 (s, 2H, N*H*$_2$), 6.97–7.59 (m, 5H, Ar–*H* and N–*H*). ^{13}C NMR (δ, ppm): 21.8 (CH$_3$), 25.1, 30.8, 31.9, 37.9 (cyclohexyl C), 56.2 (OCH$_3$), 109.3, 114.5, 124.4, 126.9, 129.0, 138.2, 149.3, 157.2, 163.3, 164.4 (Ar C), 163.2 (CO). MS 336 *m*/*z* (relative intensity) (M$^+$, 38). Anal. Calcd. for $C_{19}H_{20}N_4O_2$ (336.39): C, 67.84; H, 5.99; N, 16.66. Found: C, 68.06; H, 6.14; N, 16.49.

*4.1.5.2. 4-Amino-9-methyl-5-(4-chlorophenyl)-6,7,8,9-tetrahydro-1H-pyrimido[4,5-b]quinoline-2-one (**5b**).* Yield: 78%, mp: 219–21 °C, ^{1}H NMR (δ, ppm): 1.40 (s, 3H, C*H*$_3$), 1.57–2.62 (m, 6H, C$_{6,7,8}$–*H*), 3.14 (m, 1H, C$_9$–*H*), 4.24 (s, 2H, N*H*$_2$), 7.12–7.68 (m, 5H, Ar–*H* and N–*H*). ^{13}C NMR (δ, ppm): 22.1 (CH$_3$), 25.1, 30.8, 31.9, 37.9 (cyclohexyl C), 109.3, 124.4, 128.4, 129.0, 134.4, 136.2, 149.3, 157.2, 163.3, 164.4 (Ar C), 163.8 (CO). MS 341 *m*/*z* (relative intensity) (M$^+$, 29). Anal. Calcd. for $C_{18}H_{17}ClN_4O$ (340.81): C, 63.44; H, 5.03; N, 16.44. Found: C, 63.61; H, 4.82; N, 16.53.

*4.1.5.3. 4-Amino-9-methyl-5-(4-methoxyphenyl)-6,7,8,9-tetrahydro-1H-pyrimido[4,5-b]-quinoline-2-thione (**6a**).* Yield: 73%, mp: 207–9 °C, ^{1}H NMR (δ, ppm): 1.40 (s, 3H, C*H*$_3$), 1.59–2.49 (m, 6H, C$_{6,7,8}$–*H*), 3.20 (m, 1H, C$_9$–*H*), 3.86 (s, 3H, OC*H*$_3$), 4.90 (s, 2H, N*H*$_2$), 6.91–7.32 (m, 5H, Ar–*H* and N–*H*). ^{13}C NMR (δ, ppm): 21.8 (CH$_3$), 25.3, 31.3, 32.4, 38.7 (cyclohexyl C), 56.0 (OCH$_3$), 109.7, 114.4, 124.6, 127.8, 130.3, 149.6, 156.9, 162.5, 163.4, 164.3 (Ar C), 182.8 (CS). MS 352 *m*/*z* (relative intensity) (M$^+$, 17). Anal. Calcd. for $C_{19}H_{20}N_4OS$ (352.45): C, 64.75; H, 5.72; N, 15.90. Found: C, 64.88; H, 5.59; N, 16.07.

*4.1.5.4. 4-Amino-9-methyl-5-(4-chlorophenyl)-6,7,8,9-tetrahydro-1H-pyrimido[4,5-b]quinoline-2-thione (**6b**).* Yield: 82%, mp: 210–2 °C, ^{1}H NMR (δ, ppm): 1.37 (s, 3H, C*H*$_3$), 1.68–2.59 (m, 6H, C$_{6,7,8}$–*H*), 3.10 (m, 1H, C$_9$–*H*), 4.68 (s, 2H, N*H*$_2$), 7.07–7.48 (m, 5H, Ar–*H* and N–*H*). ^{13}C NMR (δ, ppm): 22.1 (CH$_3$), 25.4, 31.2, 32.3, 37.6 (cyclohexyl C), 109.5, 124.4, 128.7, 129.2, 132.3, 137.1, 148.9, 157.3, 163.2, 164.4 (Ar C), 183.2 (CS). MS 357 *m*/*z* (relative intensity) (M$^+$, 12). Anal. Calcd. for $C_{18}H_{17}ClN_4S$ (356.87): C, 60.58; H, 4.80; N, 15.70. Found: C, 60.34; H, 5.07; N, 15.62.

*4.1.6. General method for the preparation of 2-ethylthio-4-amino-9-methyl-5-substituted-6,7,8,9-tetrahydro-1H-pyrimido[4,5-b]-quinolines (**7a**,**b**) and 2-(4-amino-9-methyl-5-substituted-6,7,8,9-tetrahydro-1H-pyrimido[4,5-b]quinolin-2-ylthio)-1-phenylethanones (**8a**,**b**)*

To a stirred solution of the proper thione **6a**,**b** (2 mmol) in a mixture of 1 N NaOH (5 mL) and ethanol (2 mL), the appropriate alkyl halide (2.8 mmol) was added. The reaction mixture was stirred at room temperature for 2–8 h, and the precipitated product was filtered, washed with aqueous ethanol, dried and recrystallized from ethanol. IR (cm^{-1}): 3440–3150 (NH). IR (cm^{-1}); for compounds **8a**,**b**: 1685–1680 (ketonic C=O).

*4.1.6.1. 2-Ethylthio-4-amino-9-methyl-5-(4-methoxyphenyl)-6,7,8,9-tetrahydro-1H-pyrimido-[4,5-b]quinoline (**7a**).* Yield: 59%, mp: 176–8 °C, ^{1}H NMR (δ, ppm): 1.23 (t, *J* = 9 Hz, 3H, ethyl–C*H*$_3$), 1.38 (s, 3H, C*H*$_3$), 1.48–2.28 (m, 6H, C$_{6,7,8}$–*H*), 3.14 (m, 1H, C$_9$–*H*), 3.62 (q, *J* = 9 Hz, 2H, ethyl–C*H*$_2$), 3.89 (s, 3H, OC*H*$_3$), 4.26 (s, 2H, N*H*$_2$), 6.97–7.26 (m, 4H, Ar–*H*). ^{13}C NMR (δ, ppm): 15.5 (CH$_3$), 21.6 (CH$_3$), 28.2 (CH$_2$), 25.4, 31.1, 32.2, 38.6 (cyclohexyl C), 56.1 (OCH$_3$), 102.3, 114.4, 127.9, 130.4, 135.4, 149.9, 158.0, 162.2, 162.5, 167.2, 167.6 (Ar C). MS 380 *m*/*z* (relative intensity) (M$^+$, 10). Anal. Calcd. for $C_{21}H_{24}N_4OS$ (380.51): C, 66.29; H, 6.36; N, 14.72. Found: C, 66.12; H, 6.53; N, 14.62.

*4.1.6.2. 2-Ethylthio-4-amino-9-methyl-5-(4-chlorophenyl)-6,7,8,9-tetrahydro-1H-pyrimido[4,5-b]quinoline (**7b**).* Yield: 72%, mp: 168–9 °C, ^{1}H NMR (δ, ppm): 1.26 (t, *J* = 9 Hz, 3H, ethyl–C*H*$_3$), 1.39 (s, 3H, C*H*$_3$), 1.68–2.33 (m, 6H, C$_{6,7,8}$–*H*), 3.17 (m, 1H, C$_9$–*H*), 3.68 (q, *J* = 9 Hz, 2H, ethyl–C*H*$_2$), 4.31 (s, 2H, N*H*$_2$), 6.81–7.24 (m, 4H, Ar–*H*). ^{13}C NMR (δ, ppm): 15.8 (CH$_3$), 21.7 (CH$_3$), 28.5 (CH$_2$), 25.1, 31.2, 32.3, 38.8 (cyclohexyl C), 102.1, 123.7, 129.1, 132.4, 135.3, 137.1, 148.7, 158.4, 162.4, 167.3, 167.7 (Ar C). MS 386 *m*/*z* (relative intensity) (M^{+1}, 10) Anal. Calcd. for $C_{20}H_{21}ClN_4S$ (384.93): C, 62.41; H, 5.50; N, 14.56. Found: C, 62.65; H, 5.37; N, 14.71.

*4.1.6.3. 2-(4-Amino-9-methyl-5-(4-methoxyphenyl)-6,7,8,9-tetrahydro-1H-pyrimido[4,5-b]quinolin-2-ylthio)-1-phenylethanone (**8a**).* Yield: 76%, mp: 140–2 °C, ^{1}H NMR (δ, ppm): 1.39 (s, 3H, C*H*$_3$), 1.68–2.33 (m, 6H, C$_{6,7,8}$–*H*), 3.20 (m, 1H, C$_9$–*H*), 3.88 (s, 3H, OC*H*$_3$), 4.05 (s, 2H, CO–C*H*$_2$), 4.26 (s, 2H, N*H*$_2$), 6.99–7.61 (m, 9H, Ar–*H*). ^{13}C NMR (δ, ppm): 21.8 (CH$_3$), 44.1 (CH$_2$), 25.4, 31.1, 32.2, 38.6 (cyclohexyl C), 56.1 (OCH$_3$), 102.1, 114.6, 127.8, 128.4, 128.7, 130.1, 132.8, 135.4, 137.3, 149.5, 158.2, 162.3, 162.6, 167.3, 167.8 (Ar C), 196.4 (CO). MS 470 *m*/*z* (relative intensity) (M$^+$, 18). Anal. Calcd. for $C_{27}H_{26}N_4O_2S$ (470.59): C, 68.91; H, 5.57; N, 11.91. Found: C, 68.67; H, 5.73; N, 12.14.

*4.1.6.4. 2-(4-Amino-9-methyl-5-(4-chlorophenyl)-6,7,8,9-tetrahydro-1H-pyrimido[4,5-b]quinolin-2-ylthio)-1-phenylethanone (**8b**).* Yield: 80%, mp: 150–1 °C, ^{1}H NMR (δ, ppm): 1.42 (s, 3H, C*H*$_3$), 1.74–2.45 (m, 6H, C$_{6,7,8}$–*H*), 3.17 (m, 1H, C$_9$–*H*), 3.95 (s, 2H, CO–C*H*$_2$), 4.18 (s, 2H, N*H*$_2$), 7.06–7.83 (m, 9H, Ar–*H*). ^{13}C NMR (δ, ppm): 21.3 (CH$_3$), 43.9 (CH$_2$), 25.2, 31.5, 32.4, 38.0 (cyclohexyl C), 102.1, 128.2, 128.4, 128.6, 129.7, 132.1, 134.8, 136.4, 149.5, 158.2, 162.3, 162.6, 167.3, 167.8 (Ar C), 196.4 (CO). MS 475 *m*/*z* (relative intensity) (M$^+$, 15). Anal. Calcd. for $C_{26}H_{23}ClN_4OS$ (475.01): C, 65.74; H, 4.88; N, 11.79. Found: C, 65.43; H, 5.07; N, 11.50.

*4.1.7. 9-Methyl-5-substituted-6,7,8,9-tetrahydro-3H-pyrimido[4,5-b]quinolin-4-ones (**9a**,**b**)*

A mixture of the appropriate compound **1a**,**b** (1 mmol) and formic acid (5 mL) was heated in a boiling water bath for 30 min. After being cooled to room temperature, the reaction mixture was poured onto ice-cold water, the precipitated solid product was filtered, washed with water and recrystallized from ethanol. IR (cm^{-1}): 3258–3150 (NH), 1710–1695 (C=O).

*4.1.7.1. 9-Methyl-5-(4-methoxyphenyl)-6,7,8,9-tetrahydro-3H-pyrimido[4,5-b]quinolin-4-one (**9a**).* Yield: 72%, mp: 186–8 °C, ^{1}H NMR (δ, ppm): 1.36 (s, 3H, C*H*$_3$), 1.52–2.28 (m, 6H, C$_{6,7,8}$–*H*), 3.12 (m, 1H, C$_9$–*H*), 3.91 (s, 3H, OC*H*$_3$), 6.97–7.56 (m, 5H, 4 Ar–*H* and C$_2$–*H*), 7.91 (s, 1H, N*H*). ^{13}C NMR (δ, ppm): 20.7 (CH$_3$), 21.7, 27.4, 31.8, 39.0 (cyclohexyl C), 56.1 (OCH$_3$), 114.2, 118.8127.4, 131.5, 135.2, 138.5, 152.6, 162.7, 163.0, 171.0 (Ar C), 170.5 (CO). MS 321 *m*/*z* (relative intensity) (M$^+$, 11). Anal. Calcd. for $C_{19}H_{19}N_3O_2$ (321.37): C, 71.01; H, 5.96; N, 13.08. Found: C, 71.32; H, 5.73; N, 12.91.

*4.1.7.2. 9-Methyl-5-(4-chlorophenyl)-6,7,8,9-tetrahydro-3H-pyrimido[4,5-b]quinolin-4-one (**9b**).* Yield: 68%, mp: 166–8 °C, ^{1}H NMR (δ, ppm): 1.39 (s, 3H, C*H*$_3$), 1.62–2.35 (m, 6H, C$_{6,7,8}$–*H*), 3.07 (m, 1H, C$_9$–*H*), 6.89–7.43 (m, 5H, 4 Ar–*H* and C$_2$–*H*), 7.98 (s, 1H, N*H*). ^{13}C NMR (δ, ppm): 20.8 (CH_3), 21.6, 27.5, 31.2, 36.8 (cyclohexyl C), 118.6, 126.4, 129.2, 129.3, 136.4, 138.2, 153.2, 162.6, 163.0, 170.3 (Ar C), 169.2 (CO). MS 325 *m*/*z* (relative intensity) (M^+, 28). Anal. Calcd. For $C_{18}H_{16}ClN_3O$ (325.79): C, 66.36; H, 4.95; N, 12.90. Found: C, 66.43; H, 5.09; N, 12.61.

*4.1.8. 2,9-Dimethyl-5-substituted-6,7,8,9-tetrahydro-3H-pyrimido[4,5-b]quinolin-4-ones (**10a**,**b**)*

A mixture of the start **1a**,**b** (1 mmol), acetic anhydride (5 mL) and conc. H_2SO_4 (0.5 mL) was heated in a boiling water bath for 10 min. The reaction mixture was cooled, poured carefully onto ice-cold water, treated with 20% NaOH solution till alkaline. The precipitated crude solid product was filtered, washed with water, dried and recrystallized from ethanol. IR (cm^{-1}): 3431–3226 (NH), 1715–1707 (C=O).

*4.1.8.1. 2,9-Dimethyl-5-(4-methoxyphenyl)-6,7,8,9-tetrahydro-3H-pyrimido[4,5-b]quinolin-4-one (**10a**).* Yield: 41%, mp: 122–4 °C, ^{1}H NMR (δ, ppm): 1.39 (s, 3H, C*H*$_3$), 1.47–2.15 (m, 6H, C$_{6,7,8}$–*H*), 2.54 (s, 3H, C$_2$–C*H*$_3$), 3.11 (m, 1H, C$_9$–*H*), 3.94 (s, 3H, OC*H*$_3$), 6.99–7.58 (m, 4H, Ar–*H*),8.0 (s, 1H, N*H*). ^{13}C NMR (δ, ppm): 19.8 (CH_3), 20.7 (CH_3), 21.7, 27.4, 31.8, 39.0 (cyclohexyl C), 56.2 (OCH_3), 114.2, 119.7, 130.5, 135.2, 138.5, 152.6, 162.0, 162.4, 163.9, 171.6 (Ar C), 170.2 (CO). MS 334 *m*/*z* (relative intensity) (M^{-1}, 26). Anal. Calcd. for $C_{20}H_{21}N_3O_2$ (335.40): C, 71.62; H, 6.31; N, 12.53. Found: C, 71.96; H, 6.17; N, 12.71.

*4.1.8.2. 2,9-Dimethyl-5-(4-chlorophenyl)-6,7,8,9-tetrahydro-3H-pyrimido[4,5-b]quinolin-4-one (**10b**).* Yield: 52%, mp: 135–7 °C, ^{1}H NMR (δ, ppm): 1.41 (s, 3H, C*H*$_3$), 1.61–2.21 (m, 6H, C$_{6,7,8}$–*H*), 2.49 (s, 3H, C$_2$–C*H*$_3$), 3.07 (m, 1H, C$_9$–*H*), 6.88–7.51 (m, 4H, Ar–*H*), 8.06 (s, 1H, N*H*). ^{13}C NMR (δ, ppm): 19.9 (CH_3), 20.9 (CH_3), 22.3, 27.4, 31.2, 37.4 (cyclohexyl C), 119.8, 123.5, 129.1, 132.3, 135.2, 137.2, 152.4, 162.4, 164.0, 170.6 (Ar C), 168.9 (CO). MS 339 *m*/*z* (relative intensity) (M^{-1}, 37). Anal. Calcd. for $C_{19}H_{18}ClN_3O$ (339.82): C, 67.15; H, 5.34; N, 12.37. Found: C, 66.93; H, 5.12; N, 12.68.

*4.1.9. 2-Ethoxymethylidineamino-3-cyano-8-methyl-4-substituted-5,6,7,8-tetrahydroquinolines (**11a**,**b**)*

A mixture of the appropriate intermediate **1a**,**b** (2 mmol) and triethyl orthoformate (10 mL) in acetic anhydride (10 mL), was heated under reflux for 8–10 h. The reaction mixture was allowed to cool, diluted with water and the precipitated solid was collected by filtration, washed with water and recrystallized from ethanol/benzene mixture 3:1. IR (cm^{-1}): 2223–2217 (CN), 1630–1620 (C=N).

*4.1.9.1. 2-Ethoxymethylidineamino-3-cyano-8-methyl-4-(4-methoxyphenyl)-5,6,7,8-tetrahydroquinoline (**11a**).* Yield: 74%, mp: 142–4 °C, ^{1}H NMR (δ, ppm): 1.22 (t, J = 8.6, 3H, ethyl–C*H*$_3$), 1.38 (s, 3H, C*H*$_3$), 1.70–2.26 (m, 6H, C$_{5,6,7}$–*H*), 3.14 (m, 1H, C$_8$–*H*), 3.71 (q, J = 8.6, 2H, ethyl–C*H*$_2$), 3.89 (s, 3H, OC*H*$_3$), 6.90–7.26 (m, 4H, Ar–*H*), 8.05 (s, 1H, N=C*H*). ^{13}C NMR (δ, ppm): 14.5 (CH_3), 20.9 (CH_3), 22.3, 27.4, 31.2, 37.4 (cyclohexyl C), 56.1 (CH_3O), 59.3 (CH_2), 118.0 (CN), 100.2, 114.3, 127.2, 130.3, 134.7, 155.5, 162.3, 168.2, 172.4 (Ar C), 163.8 (N=C). MS 349 *m*/*z* (relative intensity) (M^+, 8). Anal. Calcd. for $C_{21}H_{23}N_3O_2$ (349.43): C, 72.18; H, 6.63; N, 12.03. Found: C, 71.87; H, 6.81; N, 11.85.

*4.1.9.2. 2-Ethoxymethylidineamino-3-cyano-8-methyl-4-(4-chlorophenyl)-5,6,7,8-tetrahydroquinoline (**11b**).* Yield: 52%, mp: 135–7 °C, ^{1}H NMR (δ, ppm): 1.25 (t, J = 8.6, 3H, ethyl–C*H*$_3$), 1.42 (s, 3H, C*H*$_3$), 1.74–2.31 (m, 6H, C$_{5,6,7}$–*H*), 3.17 (m, 1H, C$_8$–*H*), 3.77 (q, J = 8.6, 2H, ethyl–C*H*$_2$), 7.01–7.49 (m, 4H, Ar–*H*), 8.11 (s, 1H, N=C*H*). ^{13}C NMR (δ, ppm): 14.5 (CH_3), 20.9 (CH_3), 22.3, 27.4, 31.2, 37.4 (cyclohexyl C), 59.3 (CH_2), 117.8 (CN), 100.4, 128.3, 129.2, 133.8, 134.4, 136.6, 155.5, 168.0, 172.2 (Ar C), 163.4 (N=C). MS 354 *m*/*z* (relative intensity) (M^+, 11) Anal. Calcd. for $C_{20}H_{20}ClN_3O$ (353.85): C, 67.89; H, 5.70; N, 11.88. Found: C, 67.96; H, 5.39; N, 11.56.

*4.1.10. 3-Amino-4-imino-9-methyl-5-substituted-6,7,8,9-tetrahydro-4H-pyrimido[4,5-b]-quinolines (**12a**,**b**)*

A mixture of the appropriate intermediate **11a**,**b** (10 mmol) and hydrazine hydrate 99% (15 mmol) in dry benzene (20 mL), was refluxed for 2–4 h. On cooling, the solid product thus precipitated was filtered, dried and recrystallized from toluene. IR (cm^{-1}): 3347–3243 (NH), 1632–1618 (C=N).

*4.1.10.1. 3-Amino-4-imino-9-methyl-5-(4-methoxyphenyl)-6,7,8,9-tetrahydro-4H-pyrimido[4,5-b]-quinoline (**12a**).* Yield: 69%, mp: 161–3 °C, ^{1}H NMR (δ, ppm): 1.35 (s, 3H, C*H*$_3$), 1.55–2.24 (m, 6H, C$_{6,7,8}$–*H*), 2.90 (m, 1H, C$_9$–*H*), 3.85 (s, 3H, OC*H*$_3$), 4.90 (s, 2H, N*H*$_2$), 6.79–7.36 (m, 5H, Ar–*H* and C$_2$–*H*), 7.87 (s, 1H, N*H*). ^{13}C NMR (δ, ppm): 21.5 (CH_3), 22.4, 27.4, 32.2, 39.4 (cyclohexyl C), 56.2 (OCH_3), 114.2, 118.3, 127.8, 130.4, 133.6, 147.9, 162.4, 163.6, 164.1, 165.0, 166.2 (Ar C). MS 336 *m*/*z* (relative intensity) (M^{+1}, 19). Anal. Calcd. for $C_{19}H_{21}N_5O$ (335.40): C, 68.04; H, 6.31; N, 20.88. Found: C, 67.84; H, 6.59; N, 20.93.

*4.1.10.2. 3-Amino-4-imino-9-methyl-5-(4-chlorophenyl)-6,7,8,9-tetrahydro-4H-pyrimido[4,5-b]-quinoline (**12b**).* Yield: 72%, mp: 157–9 °C, ^{1}H NMR (δ, ppm): 1.37 (s, 3H, C*H*$_3$), 1.56–2.29 (m, 6H, C$_{6,7,8}$–*H*), 2.96 (m, 1H, C$_9$–*H*), 4.95 (s, 2H, N*H*$_2$), 6.83–7.44 (m, 5H, Ar–*H* and C$_2$–*H*), 7.92 (s, 1H, N*H*). ^{13}C NMR (δ, ppm): 21.6 (CH_3), 22.7, 27.2, 31.8, 39.0 (cyclohexyl C), 118.8, 128.3, 129.5, 134.2, 136.5, 148.3, 161.4, 162.9, 163.5, 165.2, 166.8 (Ar C). MS 340 *m*/*z* (relative intensity) (M^+, 34). Anal. Calcd. for $C_{18}H_{18}ClN_5$ (339.82): C, 63.62; H, 5.34; N, 20.61. Found: C, 63.81; H, 5.77; N, 20.36.

*4.1.11. 7-Methyl-11-substituted-7,8,9,10-tetrahydro-1,3,3a,5,6-pentaazacyclopenta[a]anthracenes (**13a**,**b**)*

A solution of the appropriate compound **12a**,**b** (10 mol) in formic acid (10 mL) was heated under reflux for 6–8 h. The reaction mixture was cooled to room temperature, diluted with cold water and the precipitated solid was collected by filtration, washed with water, dried and recrystallized from benzene. IR (cm^{-1}): 1640–1625 (C=N).

*4.1.11.1. 7-Methyl-11-(4-methoxyphenyl)-7,8,9,10-tetrahydro-1,3,3a,5,6-pentaazacyclopenta[a]anthracenes (**13a**).* Yield: 74%, mp: 176–8 °C, ^{1}H NMR (δ, ppm): 1.39 (s, 3H, C*H*$_3$), 1.57–2.48 (m, 6H, C$_{8,9,10}$–*H*), 3.01 (m, 1H, C$_7$–*H*), 3.91 (s, 3H, OC*H*$_3$), 6.98–7.36 (m, 4H, Ar–*H*), 8.72 (s, 1H, C$_2$–*H*), 9.23 (s, 1H, C$_4$–*H*). ^{13}C NMR (δ, ppm): 21.8 (CH_3), 22.2, 27.8, 32.6, 39.1 (cyclohexyl C), 56.2 (OCH_3), 114.6, 121.4, 127.8, 130.4, 135.3, 147.8, 148.9, 156.2, 157.9, 162.5, 162.9 (Ar C). MS 345 *m*/*z* (relative intensity) (M^+, 48). Anal. Calcd. for $C_{20}H_{19}N_5O$ (345.40): C, 69.55; H, 5.54; N, 20.28. Found: C, 69.41; H, 5.66; N, 20.42.

*4.1.11.2. 7-Methyl-11-(4-chlorophenyl)-7,8,9,10-tetrahydro-1,3,3a,5,6-pentaazacyclopenta[a]anthracenes (**13b**).* Yield: 61%, mp: 183–5 °C, ^{1}H NMR (δ, ppm): 1.36 (s, 3H, C*H*$_3$), 1.55–2.36 (m, 6H, C$_{8,9,10}$–*H*), 2.98 (m, 1H, C$_7$–*H*), 7.06–7.42 (m, 4H, Ar–*H*), 8.77 (s, 1H, C$_2$–*H*), 9.33 (s, 1H, C$_4$–*H*). ^{13}C NMR (δ, ppm): 21.5 (CH_3), 22.4, 26.9, 31.9, 38.4 (cyclohexyl C), 121.3, 128.3, 129.5, 134.2, 136.5, 147.8, 148.9, 156.2, 157.9, 162.5, 162.9 (Ar C). MS 350 *m*/*z* (relative

intensity) (M^+, 39). Anal. Calcd. for $C_{19}H_{16}ClN_5$ (349.82): C, 65.24; H, 4.61; N, 20.02. Found: C, 65.41; H, 4.76; N, 20.25.

*4.1.12. 2,7-Dimethyl-11-substituted-7,8,9,10-tetrahydro-1,3,3a,5,6-pentaazacyclopenta[a]anthracenes (**14a**,**b**)*

A mixture of the appropriate **12a**,**b** (10 mmol) and acetic anhydride (10 mL) was heated under reflux for 6–8 h. The reaction mixture was allowed to attain room temperature, diluted with water, and the precipitated solid was filtered, washed with water, dried and recrystallized from acetic acid. IR (cm^{-1}): 1626–1612 (C=N).

*4.1.12.1. 2,7-Dimethyl-11-(4-methoxyphenyl)-7,8,9,10-tetrahydro-1,3,3a,5,6-pentaazacyclopenta[a]anthracenes (**14a**).* Yield: 80%, mp: 154–6 °C, ^{1}H NMR (δ, ppm): 1.44 (s, 3H, CH_3), 1.52–2.55 (m, 6H, $C_{8,9,10}$–H), 2.51 (s, 3H, C_2–CH_3), 3.01 (m, 1H, C_7–H), 3.94 (s, 3H, OCH_3), 6.87–7.46 (m, 4H, Ar–H), 8.96 (s, 1H, C_4–H). ^{13}C NMR (δ, ppm): 14.4 (CH_3), 21.6 (CH_3), 22.4, 27.7, 31.6, 38.5 (cyclohexyl C), 56.3 (OCH_3), 114.4, 121.2, 127.4, 131.0, 135.1, 147.2, 148.6, 156.0, 157.5, 161.0, 162.7 (Ar C). MS 359 *m*/*z* (relative intensity) (M^+, 41). Anal. Calcd. for $C_{21}H_{21}N_5O$ (359.42): C, 70.17; H, 5.89; N, 19.48. Found: C, 69.93; H, 6.08; N, 19.61.

*4.1.12.2. 2,7-Dimethyl-11-(4-chlorophenyl)-7,8,9,10-tetrahydro-1,3,3a,5,6-pentaazacyclopenta[a]anthracenes (**14b**).* Yield: 84%, mp: 135–7 °C, ^{1}H NMR (δ, ppm): 1.41 (s, 3H, CH_3), 1.55–2.47 (m, 6H, $C_{8,9,10}$–H), 2.56 (s, 3H, C_2–CH_3), 2.98 (m, 1H, C_7–H), 6.91–7.43 (m, 4H, Ar–H), 9.01 (s, 1H, C_4–H). ^{13}C NMR (δ, ppm): 14.0 (CH_3), 21.3 (CH_3), 21.8, 27.3, 32.6, 38.8 (cyclohexyl C), 121.4, 128.0, 129.7, 134.8, 135.1, 136.7, 147.2, 148.5, 156.4, 157.3, 160.4, 162.6 (Ar C). MS 364 *m*/*z* (relative intensity) (M^+, 36). Anal. Calcd. for $C_{20}H_{18}ClN_5$ (363.84): C, 66.02 H, 4.99; N, 19.25. Found: C, 65.87; H, 5.13; N, 19.44.

4.2. In vitro antitumor screening

Out of the newly synthesized compounds, fifteen derivatives namely: **1a**,**b**, **2b**, **3b**, **4a**,**b**, **5b**, **6b**, **7a**,**b**, **8b**, **9a**, **10a**, **12a** and **13a**; were selected by the National Cancer Institute (NCI) *in-vitro* disease-oriented human cells screening panel assay to be evaluated for their *in-vitro* antitumor activity. Primary *in vitro* one dose anticancer assay was performed using the full NCI 60 cell panel in accordance with the current protocol of the Drug Evaluation Branch, NCI, Bethesda, Maryland, USA. These cell lines were incubated with one concentration (10 μM) for each tested compound. A 48 h continuous drug exposure protocol was used, and a sulphorhodamine B (SRB) protein assay was employed to estimate cell viability or growth. Data are reported as a mean graph of the percent growth of treated cells, and presented as percentage growth inhibition (GI %) caused by the test compounds (Table 1).

Compound **8b**, passed this primary anticancer assay and consequently was carried over to the 5-dose screen against a panel of about 60 different tumor cell lines of nine tumor subpanels, including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer cell lines. These cell lines were incubated with five concentrations (0.01–100 μM) for the tested compound and were used to create log concentration % growth inhibition curves. Three response parameters: GI_{50} (growth inhibitory activity), TGI (cytostatic activity), and LC_{50} (cytotoxic activity) were calculated for each cell line, using 5-flourouracil (5-FU) as a positive control. Subpanel and full panel mean-graph midpoint values (MG-MID) for certain agents are the average of individual real and default GI_{50}, TGI, or LC_{50} values of all cell lines in the subpanel or the full panel, respectively [39–41] (Table 2).

4.3. DNA-binding assay on TLC-plate

Analysis of the DNA binding affinity of the tested compounds was performed using RP-TLC plates (RP-18 F_{254}; 0.25 mm Merck). TLC plates were pre-developed with methanol/water mixture (8:2). Test compounds were spotted (5 mg/mL in methanol) at the base line, followed by introducing DNA (1 mg/mL in water and methanol mixture 8:2) at the same positions at the origin. The plates were then developed with the above-mentioned elution system, and the location of DNA was visualized by spraying with anisaldehyde, which gives a blue color with DNA. Ethidium bromide was utilized as positive control (Table 3).

4.4. Methyl green-DNA displacement colorimetric assay

DNA-methyl green complex (20 mg) was suspended in 100 mL of 0.05 M Tris–HCl buffer, pH 7.5, containing 7.5 mmol $MgSO_4$ and stirred at 37 °C for 24 h. Compounds to be tested were dissolved in EtOH in Eppendorff tubes and the solvent was then removed under reduced pressure, and 200 mL of the DNA-methyl green solution was added to each tube. The initial absorbance of each sample was measured at 630 nm, and the samples were incubated in the dark at room for 24 h. Thereafter, the final absorbance of each sample was measured and the readings were corrected for initial absorbance and normalized as a percentage of the untreated DNA-methyl green absorbance value. Results were recorded as IC_{50} for each compound which is the sample concentration required to produce 50% reduction in the initial absorbance of the DNA-methyl green solution (Table 3).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.02.006.

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