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Synthesis and evaluation of cytotoxic activities of new guanidines derived from carbazoles

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ABSTRACT

Several new alkylguanidines derived from carbazole have been synthesized in a simple one-pot reaction starting from 3-aminocarbazole derivatives. The aminocarbazoles were reacted with ethoxycarbonylisothiocyanate, to give thiourea intermediates, followed by the addition of an alkylamine and $HgCl_2$ to give ethoxycarbonylguanidine intermediates. The reaction mixture was then heated at 160 °C to give the *N*-(1,4-dimethyl-9*H*-carbazol-3-yl)-*N*′-alkylguanidines.

The cytotoxic activity of all the synthesized guanidines was evaluated against different cell lines.

Carbazoles display a wide range of biological activities, making them attractive compounds to synthetic and medicinal chemists.[1] Many carbazole derivatives are used as organic materials, due to their photorefractive, photoconductive, whole transporting, and light emitting proprieties.[2] Several carbazoles are known for their potent antitumor, antibacterial, anti-inflammatory, psychotropic and anti-histaminic properties.[3]

Pyrido[4,3-*b*]carbazoles, such as compounds **I**–**VI** (Fig. 1), constitute an interesting nitrogen-containing tetracyclic heterocycles which exhibit broad antitumor activities. These molecules bind with DNA through intercalation and thus interfere with topoisomerase II activity. The presence of cationic side-chains in these carbazoles greatly increases their DNA affinity and biological potency.

For example, compound **VI** (S16020-2) was proven to be much more potent than the parent ellipticine **I**, and was selected for clinical trials.[4] Moreover, it has been shown that the presence of a basic side chain of the *N*,*N*-dialkylaminoalkyl type enhances the drugs' DNA affinity.[5] On the other hand, guanidine-containing heterocycles are known by their anticancer activities.[6–12]

In a preceding Letter of our laboratory, we have described the synthesis of several 2-dialkylamino-5,11-dimethyl-6*H*-pyrimido[5,4-*b*]carbazol-4(3*H*)-ones (**VII**)[13] as new analogs of ellipticine **I**, via a simple and efficient one-pot reaction. In this method a DMF solution of 3-aminocarbazole **1**, used as a starting material, was reacted with ethoxycarbonylisothiocyanate, to give the thiourea intermediate **2**, followed by the addition of the dialkylamine and $HgCl_2$ to give the ethoxycarbonylguanidine intermediates **3**. The latter intermediates were subjected to thermal cyclization at 160 °C in the same reaction medium to give the 2-dialkylamino-5,11-dimethyl-6*H*-pyrimido[5,4-*b*]carbazol-4(3*H*)-ones (**VII**) (Scheme 1).

In the course of this reaction, we have noticed that when propylamine, as a primary aliphatic amine, was used in place of the secondary amines the final cyclization step could not be achieved and no 2-propylpyrimidinocarbazole **VII** (R′ = H, R″ = propyl) was

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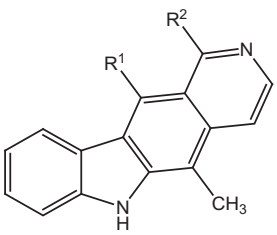
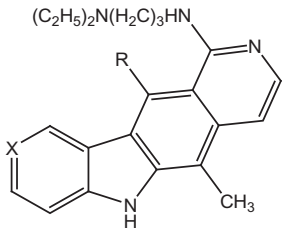
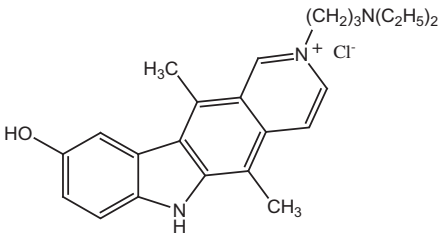


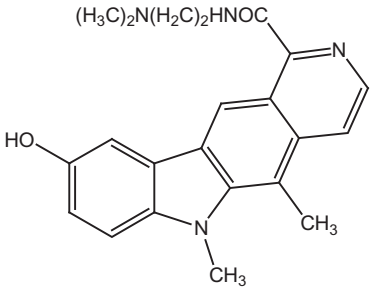
I Ellipticine $R^1 = CH_3$, $R^2 = H$
II Olivacine $R^1 = H$, $R^2 = CH_3$



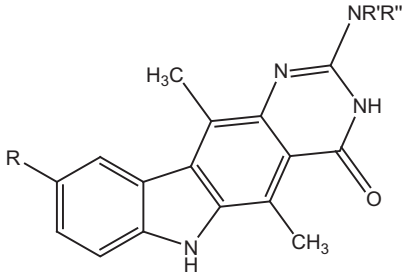
III Retelliptine X = CH_3O-C, R = CH_3
IV Pazelliptine X =N, R = H



V Datelliptium

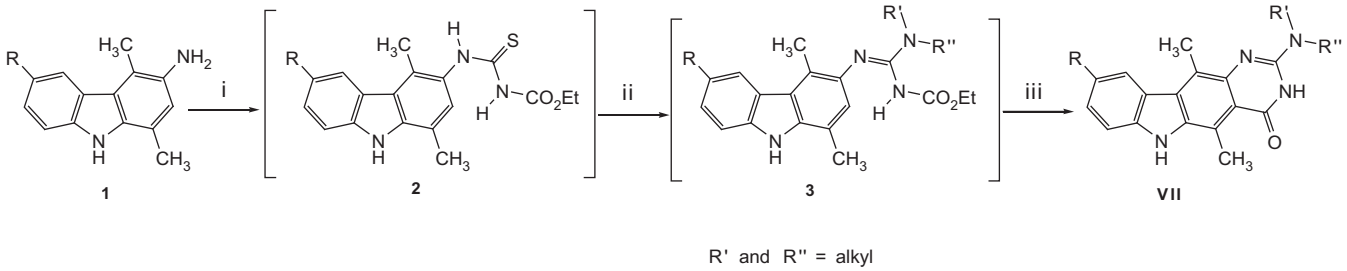


VI S16020-2

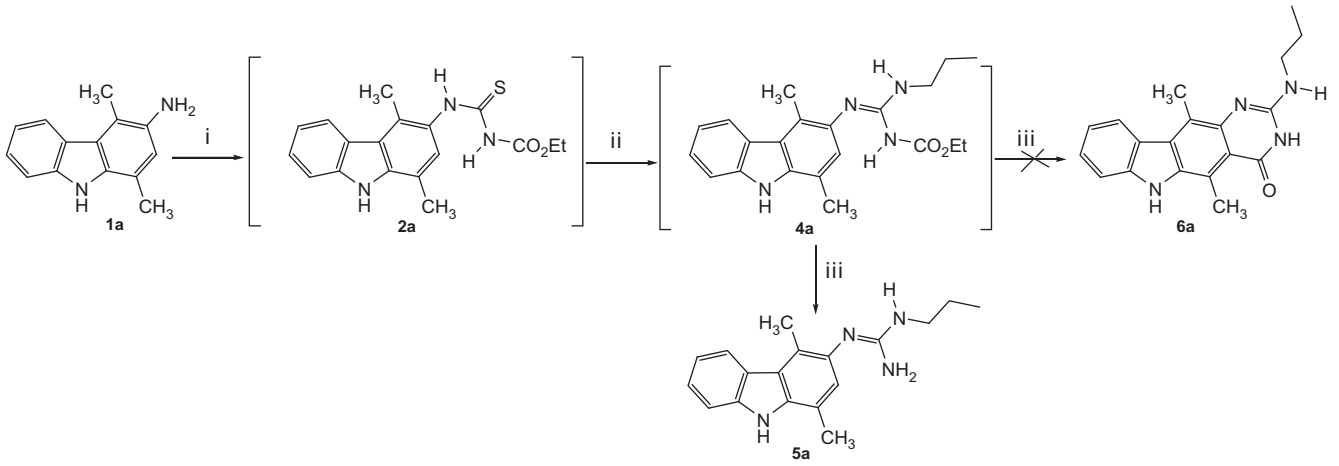


VII 2-Dialkylamino-5,11-dimethyl-6*H*-pyrimido[5,4-*b*]carbazol-4(3*H*)-ones

Figure 1. Pyridine- and pyrimidine-fused carbazoles.

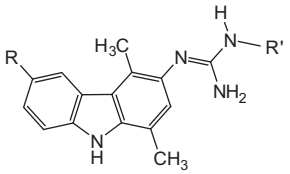


Scheme 1. Reagents and conditions: (i) EtOCONCS (1 equiv), DMF, 4 h, rt; (ii) *sec* amine (3 equiv), $HgCl_2$ (1 equiv), 10 min, 0 °C, then 18 h, rt; (iii) 2 h, 160 °C.



Scheme 2. Reagents and conditions: (i) EtOCONCS (1 equiv), DMF, 4 h, rt; (ii) propylamine (3 equiv), $HgCl_2$ (1 equiv), 10 min, 0 °C, then 18 h, rt; (iii) 2 h, 160 °C.

Table 1
N-(1,4-Dimethyl-9*H*-carbazol-3-yl)-*N*′-alkylguanidines **5a**–**g**



5a-g

Compounds	R	R′	Yield (%)
5a	H	$(CH_2)_2CH_3$	65
5b	H	$(CH_2)_3CH_3$	35
5c	Br	$HC(CH_2CH_3)_2$	30
5d	Br	$(CH_2)_3CH_3$	68
5e	Br	$C(CH_3)_3$	55
5f	$OCO_2CH_2CH_3$	H	40
5g	$OCO_2CH_2CH_3$	$(CH_2)_3CH_3$	30

obtained. The reaction ended, however, with the deprotection of the ethoxycarbonyl guanidine intermediate **3** giving the carbazol-3-ylpropylguanidine derivative **5a** (Scheme 2).

This observation prompted us to investigate further this reaction with other primary aliphatic amines and to evaluate its utility as a facile route for the synthesis of 2-alkylcarbazolylguanidines.

The reaction was then applied to aminocarbazole **1**, (where R = H, Br, OCOOEt),[14] using, in addition to propylamine, other aliphatic amines such as butylamine, *tert*-butylamine, 3-pentylamine, or ammonia. In all cases, the corresponding guanidines were obtained (Table 1). Thus, these rather unexpected results represent a new route for the preparation of unreported carbazolylguanidines **5a**–**g**.

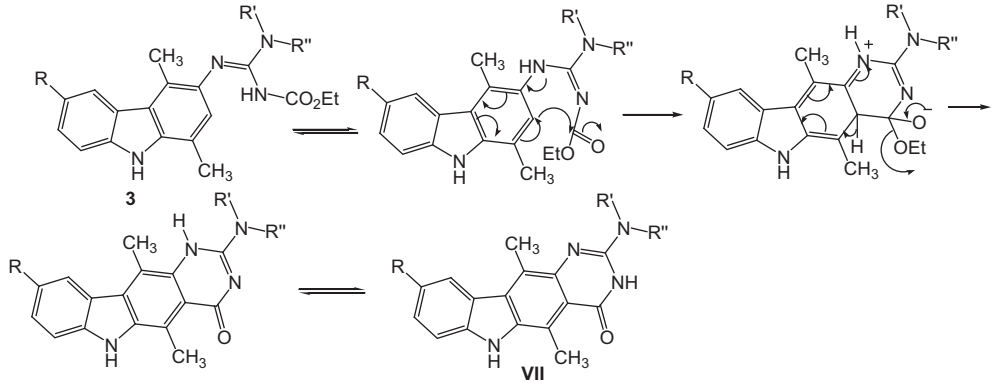
The reason for this behaviour could be understood if we look first at the intramolecular cyclization reaction of the intermediate **3**, formed in the case of using dialkylamines, to give the corresponding pyrimidocarbazoles **VII** (Scheme 3).

The above mechanism could not be applied to the intermediate **4**, formed in the case of using primary amines, probably due to the possible hydrogen bonding between the NH and the C=O of the side chain moiety, and consequently the C=O group becomes far from the carbazole C-2 atom. Thus no nucleophilic attack of the carbazole C-2 anion on the C=O carbon could occur, but deprotection of the ethoxycarbonyl guanidine moiety leading to the guanidines **5a**–**g** (Scheme 4).

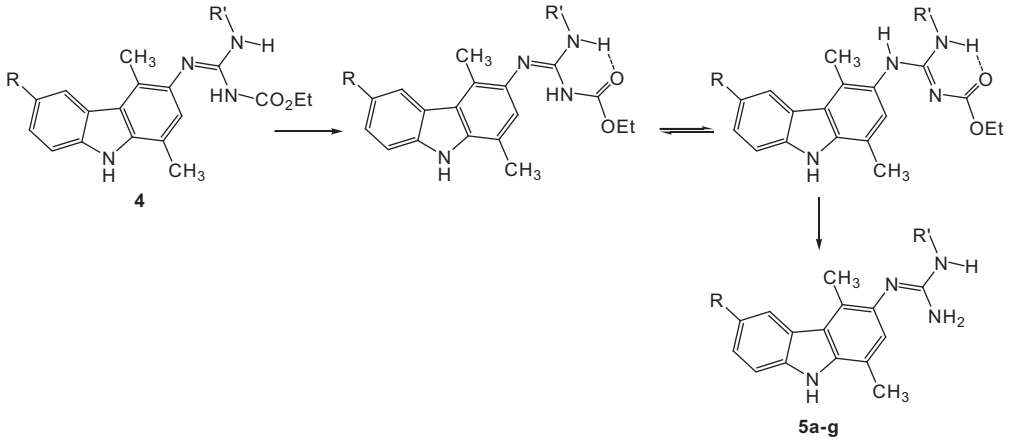
Keeping in mind the pharmaceutical interest of guanidine-containing heterocycles,[15,16] it can be expected that the presence of a guanidine group as a cationic side chain in the carbazole nucleus might improve its intercalation in the DNA and its topoisomerase II inhibitory activity.

Accordingly, the synthesized guanidines were screened for their cytotoxic activity against KB cell lines. The results obtained are shown in Table 2. Then, the molecules that showed the highest inhibition values against KB cell lines were tested against the leukemic HL60 cell line and cancer and non-cancer MCF7, HCT116, PC3 and MRC5 cell lines for the best compound.[17]

In particular, the prepared guanidine derivatives **5a**–**f** were evaluated for their anticancer activity against KB cell lines at

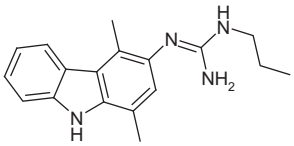
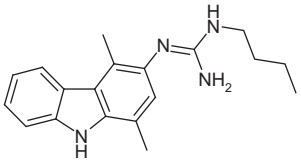
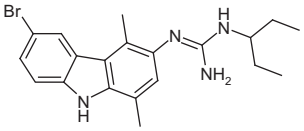
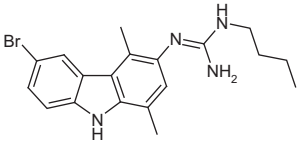
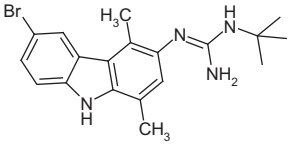
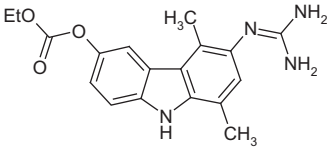


Scheme 3. Pyrimidocarbazole formation.



Scheme 4. Guanidine formation.

Table 2
Antiproliferative activity of guanidinocarbazoles **5a–f** against KB human cell lines

Compound	Structure	10^{-5} M Inhibition (%) $n = 3$	10^{-6} M Inhibition (%) $n = 3$
5a		91 ± 1	18 ± 3
5b		98 ± 1	5 ± 1
5c		100 ± 1	15 ± 2
5d		100 ± 1	26 ± 1
5e		100 ± 1	16 ± 1
5f		73 ± 1	0 ± 1

10^{-6} and 10^{-5} molar concentrations (Table 2). All the compounds tested showed no inhibition at a concentration of 10^{-6} M with the exception of the four compounds **5a**, **5c**, **5d** and **5e** which showed very low inhibition. However, at 10^{-5} molar concentration, some of the tested compounds showed inhibitory activity.

Out of the synthesized compounds, the three derivatives **5c**, **5d** and **5e** showed the relative higher inhibition values at a concentration of 10^{-5} M. Thus, these three derivatives were also tested against HL60 cell line and their IC_{50} values were found to be 3.1, 3.5 and 4 μM, respectively. Since the relatively highest activity was shown by the derivative **5c**, accordingly this derivative was subjected to a further testing against other cell lines namely MCF7, HCT116, PC3 and MRC5. The results obtained indicated that **5c** showed a high cytotoxicity at 10^{-5} M against the all aforementioned cell lines; however, a low activity was shown at 10^{-6} M. This is in accordance with previous results obtained with KB and HL60 cell lines.

Moreover, the predicted pK_a values of compounds **5a-f** were calculated using Marvin software (v. 6.1.3 from ChemAxon), which is a reference software in the field of prediction.[19] The results obtained showed that compounds **5a–f** are positively charged at physiological pH (7.4) as shown in Table 3.

Finally, the DNA binding properties of compounds **5a–e** has been evaluated. In particular, the DNA binding properties were studied using two widely used methods for investigating complex formation between small organic molecules and DNA: fluorescence and absorption spectrophotometric titration.[20,21]

The fluorescence characteristics of compounds **5a–e** were measured at 37 °C in Tris buffer (0.01 M, pH = 7.4) in absence or in presence of increasing concentration of CT-DNA. The results obtained are presented in Figures S1–S5 (Supplementary data).

The results showed a strong decrease in the fluorescence of all compounds in presence of CT-DNA. This decrease is due to the increase of CT-DNA concentration. Accordingly, these results prove the binding between compounds **5a–e** and CT-DNA.

Then, the absorption spectral pattern of compound **5c** was measured at 37 °C in Tris buffer (0.01 M, pH = 7.4) in absence or in presence of a fixed concentration of CT-DNA (100 μg/mL). The results obtained are shown in Figure S6 (Supplementary data).

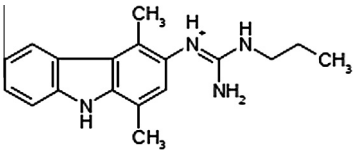
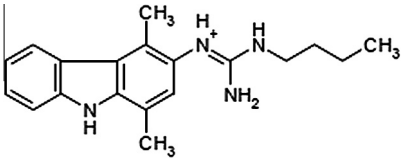
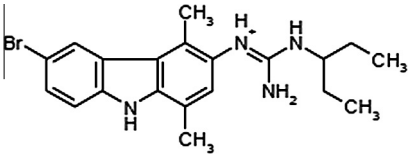
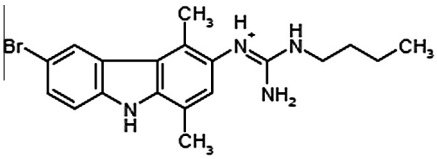
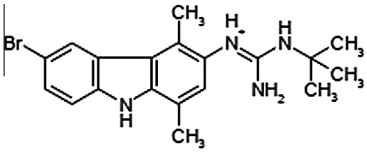
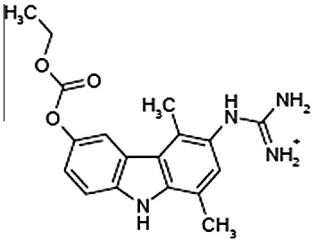
This result confirms the hypothesis that compound **5c** has some DNA binding properties.

The results of the fluorescence measurements of compounds **5a–e** were confirmed by the absorption spectrum measurement of compound **5c**. We have carried out this test for the derivative **5c** only which showed the best biological activity.

In conclusion, we have reported a simple and facile one-pot reaction for the synthesis of novel carbazolylguanidines starting from 3-aminocarbazole derivatives. The novel compounds prepared were tested for their anticancer activity against several cell lines. Among all the molecules tested, three derivatives (**5c–e**) were particularly active against HL60 cell line at 10^{-5} M. Compound **5c** showed also a potent antiproliferative activity against MCF7, HCT116, PC3, MRC5 cell lines at 10^{-5} M.

Notes: The authors declare no competing financial interest.

Table 3
Predicted pK_a values and the corresponding predicted forms at physiological pH

Compound number	Predicted pK_a value	Predicted form at physiological pH (7.4)	Percent of predicted form
5a	10.45		99.91
5b	10.48		99.92
5c	10.13		99.82
5d	10.09		99.80
5e	10.04		99.77
5f	8.58		93.78

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.12.047.

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14. *Experimental section:* Commercial reagents were purchased from Aldrich, Acros Organics and Alfa Aesar and used without additional purification. Melting points were determined on a Kofler melting point apparatus. Elemental analyses were performed at the 'Institut de Recherche en Chimie Organique Fine' (Rouen). IR spectra were taken with a Perkin Elmer BX FT-IR. Mass spectra were taken on a JEOL JMS GCMate spectrometer at ionising potential of 70 eV (EI) or were performed using a spectrometer LC–MS Waters alliance 2695 (ESI+). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JEOL Lambda 400 spectrometer. Chemical shifts are expressed in parts per million downfield from tetramethylsilane as an internal standard. Thin layer chromatography (TLC) was performed on silica gel 60F-264 (Merck).
*General procedure for the preparation N-(1,4-dimethyl-9H-carbazol-3-yl)-N′-alkylguanidines (**5a**–**g**):* To a solution of the amino carbazoles **1** (10.98 mmol) in DMF (150 mL), ethoxycarbonylisothiocyanate (10.98 mmol) was added and the mixture was stirred at room temperature for 4 h. The reaction mixture was cooled to 0 °C, then appropriate alkylamine (32.66 mmol), in case of the

derivatives **5a**–**e**, **5g**, was added or $NH_{3(g)}$ was bobbled during 5 minutes in case of the derivative **5f**, followed by the addition of $HgCl_2$ (10.98 mmol) and the resulting mixture was stirred at rt for overnight. The reaction mixture was then heated under reflux at 160 °C for 2 h, cooled, filtered through a celite pad and the filtrate was concentrated under vacuum. The solid obtained was crystallized from acetonitrile.

N-(1,4-Dimethyl-9H-carbazol-3-yl)-N′-propylguanidine (***5a***): Cream powder (65% yield). Mp = 208 °C. (KBr) (cm^{-1}): 3171, 2964, 1659, 1457, 1298, 1093, 661. ^{1}H NMR (DMSO-d_6): δ 0.89–0.93 (t, 3H, CH_3), 1.47–1.49 (m, 2H, CH_2CH_3), 2.53 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 3.28–3.30 (m, 2H, NCH_2), 7.02 (s, 1H, *Ar*), 7.17–7.21 (m, 2H, *Ar*), 7.25 (br, 2H, NH_2), 7.39–7.43 (t, 1H, *Ar*), 8.16 (d, 1H, *J* = 8.04 Hz, *Ar*), 9.31 (s, 1H, N*H*), 11.37 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 162.31, 156.17, 140.32, 138.52, 127.98, 125.97, 125.17, 123.41, 122.20, 121.13, 118.90, 118.61, 111.23, 42.54, 22.07, 16.66, 14.57, 11.04. MS (EI) *m*/*z* (%): 294 (M^+,77), 235 (52) (M^+−NH−$CH_2CH_2CH_3$). Anal. Calcd for $C_{18}H_{22}N_4$: C, 73.44; H, 7.53; N, 19.03. Found: C, 73.47; H, 7.50; N, 19.00.

N-(1,4-Dimethyl-9H-carbazol-3-yl)-N′-n-butylguanidine (***5b***): Cream powder (35% yield). Mp = 176 °C. (KBr) (cm^{-1}): 3176, 2929, 1666, 1635, 1373, 1261, 663. ^{1}H NMR (DMSO-d_6): δ 0.90–1.49 (m, 5H, CH_2CH_3), 1.96–1.98 (m, 2H, $CH_2CH_2CH_3$), 2.55 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 3.88–4.10 (m, 2H, NCH_2), 7.02 (s, 1H, *Ar*), 7.19–7.41 (m, 4H, 2*Ar*, NH_2), 7.55–7,57 (m, 1H, *Ar*), 8.14–8.16 (m, 1H, *Ar*), 9.33 (br, 1H, N*H*), 11.43 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 162.98, 155.18, 141.33, 136.50, 127.99, 126.91, 124.18, 123.48, 122.18, 121.15, 119.90, 118.54, 111.23, 42.32, 32.58, 22.10, 16.76, 14.60, 11.10. MS (EI) *m*/*z* (%): 308 (M^+,100), 210 (100) (M^+−H_2N–C–NH–C_4H_9). Anal. Calcd for $C_{19}H_{24}N_4$: C, 73.99; H, 7.84; N, 18.17. Found: C, 73.96; H, 7.80; N, 18.12.

N-(6-Bromo-1,4-dimethyl-9H-carbazol-3-yl)-N′-(pent-3-yl)-guanidine (***5c***). White powder (30% yield). Mp >270 °C. (KBr) (cm^{-1}): 3411, 3178, 1655, 1633, 1443, 1298, 1147, 806. ^{1}H NMR (DMSO-d_6): δ 0.91–0.94 (m, 6H, CH_3), 1.491–1.56 (m, 5H, CH_2CHCH_2), 2.53 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 7.06 (s, 1H, *Ar*), 7.07 (br, 2H, NH_2), 7.53–7.55 (m, 2H, *Ar*), 8.25 (s, 1H, *Ar*), 9.32 (br, 1H, N*H*), 11.63 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 156.21, 139.01, 128.47, 127.67, 126.87, 125.08, 124.23, 123.88, 120.22, 119.01, 113.18, 110.95, 40.12, 26.92, 16.58, 14.48, 10.07. MS (EI) *m*/*z* (%): 401 (M^+,5), 288 (100). Anal. Calcd for $C_{20}H_{25}BrN_4$: C, 59.85; H, 6.28; N, 13.96. Found: C, 59.87; H, 6.31; N, 14.00.

N-(6-Bromo-1,4-dimethyl-9H-carbazol-3-yl)-N′-n-butylguanidine (***5d***): Yellow powder (68%). Mp = 230 °C. (KBr) (cm^{-1}): 3191, 2959, 1648, 1445, 1300, 1147, 804. ^{1}H NMR (DMSO-d_6): δ 0.81–0.91 (m, 3H, CH_3), 1.29–1.31 (m, 2H, CH_2CH_3), 1.47–1.49 (m, 2H, $CH_2CH_2CH_3$), 2.56 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 3.28–3.31 (m, 2H, $CH_2CH_2CH_2CH_3$), 7.06 (s, 1H, *Ar*), 7.42 (br, 2H, NH_2), 7.48–7.54 (m, 2H, *Ar*), 8.22 (s, 1H, *Ar*), 9.42 (s, 1H, N*H*), 11.69 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 156.01, 138.99, 128.37, 127.67, 126.82, 125.08, 124.23, 123.85, 120.22, 118.99, 113.17, 110.95, 40.70, 30.72, 19.29, 16.58, 14.49, 13.62. MS (EI) *m*/*z* (%): 387 (M^+,18), 288 (100). Anal. Calcd for $C_{19}H_{23}BrN_4$: C, 58.92; H, 5.99; N, 14.47. Found: C, 58.96; H, 5.96; N, 14.50.

N-(6-Bromo-1,4-dimethyl-9H-carbazol-3-yl)-N′-tert-butylguanidine (***5e***): Green powder (55%). Mp >270 °C. (KBr) (cm^{-1}): 3410, 2977, 1656, 1632, 1375, 1297, 1156, 932. ^{1}H NMR (DMSO-d_6): δ 1.38 (s, 9H, CH_3), 2.49 (s, 3H, CH_3), 2.56 (s, 3H, CH_3), 6.96–7. 04 (m, 2H, *Ar*), 7.50 (s, 1H, *Ar*), 7.96 (s, 1H, N*H*), 8.23 (s, 1H, *Ar*), 9.13 (s, 1H, N*H*), 11.72 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6): 155.04, 139.01, 128.40, 127.66, 126.87, 125.07, 124.22, 124.03, 120.19, 118.99, 113.19, 110.95, 51.47, 40.12, 39.91, 28.83, 16.57, 16.54. MS (EI) *m*/*z* (%): 387 (M^+,66), 315 (58) (M^+−$HN(CH_3)_3$). Anal. Calcd for $C_{19}H_{23}BrN_4$: C, 58.92; H, 5.99; N, 14.47. Found: C, 58.89; H, 6.01; N, 14.49.

N-(6-Ethoxycarbonyloxy-1,4-dimethyl-9H-carbazol-3-yl)-N′-guanidine (***5f***): White powder (40% yield). Mp = 221 °C. IR (KBr) (cm^{-1}): 3392, 2110, 1739, 1262, 1005, 784, 554. ^{1}H NMR (DMSO-d_6): 1.35–1.39 (t, 3H, CH_3), 2.55 (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 4.30–4.35 (q, 2H, CH_2), 7.30–7.32 (m, 5H, 1*Ar*, $2NH_2$), 7.35 (dd, J_1 = 1.92 Hz, J_2 = 8.80 Hz, 1H, *Ar*), 7.61 (d, *J* = 8.80 Hz, 1H, *Ar*), 8.01 (s, 1H, *Ar*), 11.64 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 158.04, 152.60, 139.09, 128.43, 127.69, 126.92, 125.15, 124.16, 124.09, 120.20, 118.89, 113.73, 111.50, 65.40, 19.50, 13.80, 13.10. MS (ESI^+): 339 (M^+−1). Anal. Calcd for $C_{18}H_{20}N_4O_3$: C, 63.52; H, 5.92; N, 16.46. Found: C, 63.54; H, 5.95; N, 16.50.

N-(6-Ethoxycarbonyloxy-1,4-dimethyl-9H-carbazol-3-yl)-N′-n-butylguanidine (***5g***): Green powder (30%). Mp = 226 °C. IR (KBr) (cm^{-1}): 3408, 3193, 2971, 1768, 1632, 1463, 1245, 1056, 807, 658. ^{1}H NMR (DMSO-d_6): δ 0.87–0.91 (t, 3H, CH_3), 1.28–1.32 (t, 3H, CH_3), 1.41–1.58 (m, 2H, $CH_2CH_2CH_3$), 2.52 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 3.16–3.22 (m, 4H, NCH_2CH_2), 4.22–4.28 (q, 2H, CH_2CH_3), 7.05 (s, 1H, *Ar*), 7.25 (d, *J* = 8.04 Hz, 1H, *Ar*), 7.55 (d, *J* = 8.04 Hz, 1H, *Ar*), 7.56 (br, 2H, NH_2), 7.96 (s, 1H, *Ar*), 9.33 (br, 1H, N*H*), 11.58 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 158.19, 152.68, 139.13, 128.46, 127.76, 126.95, 125.65, 124.34, 124.12, 120.27, 117.89, 113.79, 111.70, 65.40, 37.08, 32.61, 19.50, 13.80, 13.10. MS (EI) m/z (%): 396 (M^+,15), 251 (100) (M^+−HNC_4H_9, $CO_2C_2H_5$). Anal. Calcd for $C_{22}H_{28}N_4O_3$: C, 66.65; H, 7.12; N, 14.13. Found: C, 66.61; H, 7.10; N, 14.16.

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17. *Cell culture and cell proliferation assay:* The human cell lines KB (nasopharyngeal epidermoid carcinoma) obtained from NCI and MRC5-SV2 (human fetal lung) obtained from ECACC were grown in D-MEM medium supplemented with 10% fetal calf serum (Invitrogen), in the presence of penicillin, streptomycin and fungizone in 75 cm^2 flask under 5% CO_2. HL60 cells (acute promyelocytic leukemia) were similarly grown in supplemented RPMI medium, as well as MCF7 (breast adenocarcinoma),[18] HCT116 (colon adenocarcinoma) and PC3 (prostate adenocarcinoma).
Cells were seeded in 96-well tissue culture microplates at a density of 650–2000 cells/well depending on cell line, in 200 μL medium and treated 24 h later with compounds dissolved in DMSO using a Biomek 3000 automate (Beckman–Coulter). Controls received the same volume of DMSO (1% final volume). After 72 h exposure, MTS reagent (Promega) was added and incubated for 3 h at 37 °C: the absorbance was monitored at 490 nm and results expressed as the inhibition of cell proliferation calculated as the ratio [(1-(OD490 treated/OD490 control)) × 100]. For IC_{50} determinations (50% inhibition of cell proliferation) experiments were performed in separate duplicate with compound concentrations ranged 0.5 nM to 10 μM.
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21. *Fluorescence and UV–Vis absorption measurements:* All spectra were measured on a TECAN Infinite M200 UV–Vis spectrophotometer. All measurements were performed in Tris buffer (0.01 M, pH = 7.4) at 37 °C.
Stock solutions of compounds **5a**–**e** were prepared in DMSO at 10^{-2} M, and then the working solutions were prepared by dilution with 0.01 M Tris buffer to a final concentration of 10^{-4} M. Compound **5f** was not studied because of its insolubility in the buffer solution.
Calf Thymus DNA (CT-DNA) was purchased from Sigma Aldrich Co. A stock solution of CT-DNA was prepared in 0.01 M Tris, 1 mM EDTA buffer, then the working solutions were prepared within a concentration range from 25 to 100 μg/mL.
Fluorescence spectra were recorded in the region of 330–390 nm using an excitation wavelength of 290 nm. These conditions were determined in preliminary experiments.
UV–Vis absorption spectra were recorded within the range 230–500 nm.