New Trimethoxybenzamides and Trimethoxyphenylureas Derived from Dimethylcarbazole as Cytotoxic Agents. Part I

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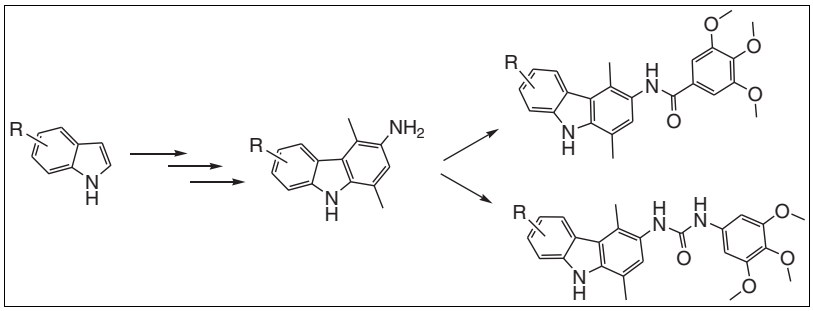

A convenient synthesis of novel functionalized 1,4-dimethylcarbazole derivatives containing 3,4,5-trimethoxybenzamido-ureido or *N*-(3,4,5-trimethoxyphenyl)ureido group starting from their corresponding indole derivatives is reported. Three derivatives prepared (**5g**, **6c**, and **6g**) were active against leukemia cell lines HL60. Both **5g** and **6g** showed potent antiproliferative activity against KB cell lines, likely associated with the inhibition of tubulin polymerization.

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INTRODUCTION

Many of carbazole derivatives show antioxidative and biological activities, such as antitumor, psychotropic, anti-inflammatory, antihistaminic, and antibiotic activities [1].

The carbazole-based naturally occurring heterocycles, Mahanine (**I**) [2] and Ellipticine (**II**) [3] (Fig. 1), are among the anti-cancer drugs acting selectively on tumor cells by inducing apoptosis or pseudo-apoptosis. Mahanine has been shown to exhibit a wide range of pharmacological effects: antimutagenicity against heterocyclic amines; antimicrobial activity against Gram positive bacteria; anti-inflammatory effect; and acts as a potent apoptosis-inducing agent in HL-60 cells [4–7]. Ellipticine, a DNA intercalator, is used for the treatment of osteolytic metastases of breast cancer [8].

On the other hand, the important naturally occurring antimitotic tubulin-binding agents Combretastatin A-4 (**III**) and Colchicine (**IV**) involve a common trimethoxybenzene nucleus in their structures, which plays a crucial role in their biological activity (Fig. 2).

Thus, it deemed of interest to incorporate this trimethoxybenzene moiety in a carbazole nucleus with the aim that such a combination may result in an enhanced anticancer activity.

RESULTS AND DISCUSSION

Numerous derivatives of DMC are potent cytostatic agents [9], and probably their action is due to intercalating mechanism into DNA. They may interact directly or indirectly with nuclear DNA leading to severe chromosome aberrations. However, experimental evidences showed that these compounds may bind to DNA not only through intercalation but also by covalent binding or generation of strand breaks [10] and exert their cytotoxic effects by enhancing enzyme-mediated DNA breaking within the genome [11–14]. Moreover, they can target topoisomerases, the enzymes that modulate DNA topology *in vivo*, and involved in virtually every aspect of DNA metabolism [15].

Rescifina *et al*. showed that in order to achieve an effective intercalation in the DNA, it is necessary that, in addition to the presence of fused polyaromatic systems, these systems may carry amino and/or amide groups. These groups favor a good interaction through the formation of hydrogen bonding with DNA bases [16].

Currently, anticancer therapy, on the basis of microtubule-targeting agents, is receiving growing attention [17]. Tubulin heterodimers, the major component of microtubules, are the molecular target of these agents. Inhibition

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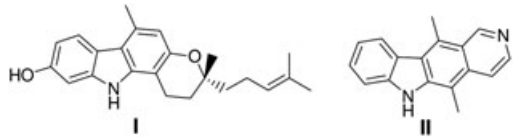


Figure 1. Naturally occurring heterocycles Mahanine (**I**) and Ellipticine (**II**).

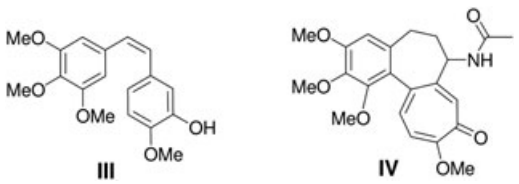


Figure 2. Combretastatin A-4 (**III**) and Colchicine (**IV**).

of tubulin polymerization or interfering with microtubule disassembly disrupts several cellular functions, including cell motility and mitosis [18–20].

De Martino *et al*. [17] have shown that 3-(3,4,5-trimethoxyphenylthio)indoles have potencies comparable with those of the reference compounds Colchicine and Combretastatin A-4 in both tubulin assembly and cell growth inhibition assays.

Yue-Ming Wang [21] has synthesized a new tubulin ligand *N*-(2,6-dimethoxypyridine-3-yl)-9-methylcarbazole-3-sulfonamide (IG-105) (**V**) (Fig. 3), which showed a potent activity against human leukemia and solid tumors in breast, liver, prostate, lung, skin, colon, and pancreas with

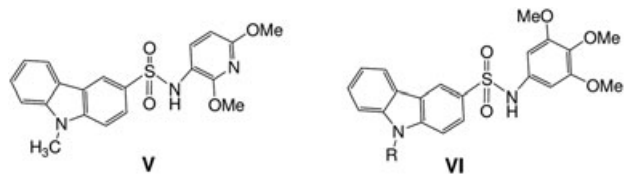


Figure 3. Carbazole-3-sulfonamides. IG-105 (**V**).

IC_{50} values between 0.012 and 0.298 μmol/L. Also, some carbazole-3-sulfonamides (**VI**) (Fig. 3) related to Combretastatin A-4 (**III**) (Fig. 2) have been prepared and evaluated for their antiproliferative activity [22]. The biological activities of urea-containing compounds have been also reported [23].

In this context, we wish to report herein the synthesis novel functionalized DMC derivatives containing 3,4,5-trimethoxybenzamido- and *N*(3,4,5-trimethoxyphenyl) ureido group **5a–h** and **6a–h**, respectively as potential anticancer agents (Scheme 1). These compounds were prepared starting from known 3-aminocarbazole derivatives **4a–h** that were synthesized previously by us [24] starting from the indole derivatives **1a–h** (Scheme 1).

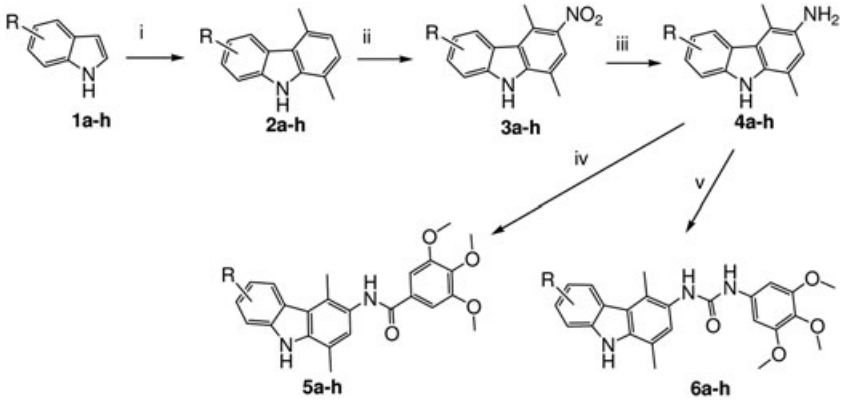
Thus, the reaction of 3-aminocarbazoles **4a–h** with stoichiometric amounts of 3,4,5-trimethoxybenzoic acid (**7**) in chloroform at room temperature in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and 4-dimethylaminopyridine gave the corresponding 3-(3,4,5-trimethoxybenzamido)carbazoles **5a–h** in 41–70 % yield (Scheme 2, Table 1).

On the other hand, when **4a–h** were reacted with 3,4,5-trimethoxybenzoyl azide **8** in refluxing anhydrous acetonitrile, the *N*-(3,4,5-trimethoxyphenyl)ureas **6a–h** were obtained in good yield (58–74 %) (Scheme 3, Table 2). The acid azide **8** has been prepared earlier by converting the 3,4,5-trimethoxybenzoic acid into the corresponding acid chloride followed by treatment with sodium azide under phase-transfer catalysis [25]. However, we have prepared this azide following a more convenient one-pot reaction through the formation of its mixed acid anhydride, as shown in the Experimental part.

BIOLOGICAL EVALUATION

All the carbazole derivatives prepared **5a–h** and **6a–h** were evaluated for their anticancer activity against KB cell lines

Scheme 1. Novel functionalized 1,4-dimethylcarbazole derivatives. Reaction conditions: (i) acetonylacetone, *p*-TSA, reflux EtOH; (ii) HNO_3, $(CH_3CO)_2O$, rt; (iii) $SnCl_2$, HCL, reflux or Pd/C, H_2, EtOH; (iv) 3,4,5-trimethoxybenzoic acid, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), 4-dimethylaminopyridine (DMAP), $CHCl_3$ rt; and (v) 3,4,5-trimethoxybenzoyl azide, reflux CH_3CN.



Scheme 2

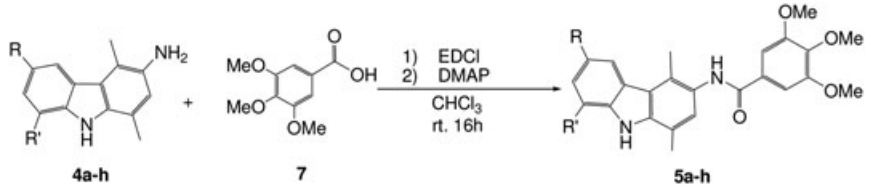
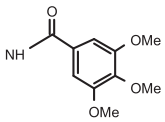
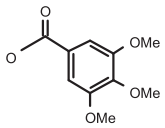


Table 1

Mini-library of synthesized amidocarbazoles **5a**-**h**.

Starting compound	R	R′	Product	R	R′	Yield (%)
4a	Cl	H	**5a**	Cl	H	70
4b	F	H	**5b**	F	H	60
4c	Br	H	**5c**	Br	H	43
					H	56
4d	NH_2	H	**5d**			
4e	H	CH_2CH_3	**5e**	H	CH_2CH_3	43
					H	41
4f	OH	H	**5f**			
4g	CH_3	H	**5g**	CH_3	H	42
4h	$OCO_2C_2H_5$	H	**5h**	$OCO_2C_2H_5$	H	51

at 10^{-6} and 10^{-5} M concentrations (Table 3). All the compounds tested showed no inhibition at a concentration of 10^{-6} M with the exception of the two compounds **6a** and **6c** that showed very low inhibition. However, at 10^{-5} M concentration, some of the tested compounds showed inhibitory activity. In the trimethoxyureido series **6a–h**, compound **6g** that possesses a methyl group at position 6 displayed the highest inhibition. The substitution of this methyl group by halogens led to a decrease of activity in the order Br › F › Cl **6c**, **6b**, **6a** [24,26]. However, the activity is abolished when the methyl group was substituted by trimethoxybenzoylamino or trimethoxybenzoyloxy substituent. Similarly, in the trimethoxyphenylcarbamido series **5a–h**, compound **5g** having also a methyl group at position 6 showed the highest activity. The substitution of this methyl group by bromine atom led to a decrease of activity, whereas its substitution with fluorine atom strongly decreased the activity. Complete abolishing of activity was observed when the methyl group was substituted with chlorine, trimethoxybenzamido, trimethoxybenzoyloxy, or ethoxycarbonyloxy substituent.

Within the two series compounds, **5g**, **6h**, and **6g** showed the highest inhibition values of 81, 91, and 100, respectively at a concentration of 10^{-5} M.

Scheme 3

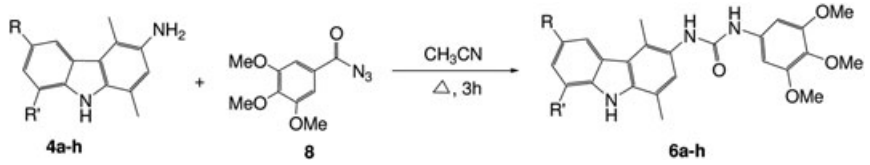
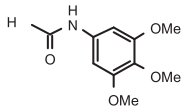


Table 2

Mini-library of synthesized ureidocarbazoles **6a-h**.

Starting compd.	R	R′	Product	R	R′	Yield (%)
4a	Cl	H	**6a**	Cl	H	70
4b	F	H	**6b**	F	H	60
4c	Br	H	**6c**	Br	H	74
					H	60
4d	NH_2	H	**6d**			
4e	H	CH_2CH_3	**6e**	H	CH_2CH_3	62
4f	OH	H	**6f**	OH	H	58
4g	CH_3	H	**6g**	CH_3	H	68
4h	$OCO_2C_2H_5$	H	**6h**	$OCO_2C_2H_5$	H	67

These three latter derivatives were also screened against HL60 cell lines (Table 4) and for their inhibition activity of tubulin polymerization (Table 5).

As shown in Table 3, compounds **5g** and **6g** proved to be potent inhibitors of cell proliferation, whereas the replacement of the methyl group of **6g** by a bromine atom **6c** resulted in a reduction of its antiproliferative activity (62% at 10^{-4} M). These three molecules showed comparable activities when tested against the leukemia cell lines HL60 (Table 4).

In an attempt to elucidate the mode of action of carbazole derivatives, the inhibition of tubulin polymerization was investigated. As expected, compounds are weak inhibitors of tubulin assembly (Table 5).

CONCLUSION

Several novel functionalized DMC derivatives containing 3,4,5-trimethoxybenzamido- or *N*-(3,4,5-trimethoxyphenyl) ureido group have been synthesized starting from their corresponding indole derivatives. Out of all the carbazoles prepared, three derivatives (**5g**, **6c**, and **6g**) were active against leukemia cell lines HL60. Both **5g** and **6g** showed potent antiproliferative activity against KB cell lines, likely associated with the inhibition of tubulin polymerization; however, the presence of a bromine atom in the structure of **6c** diminishes the antiproliferative activity.

EXPERIMENTAL

Chemistry. Commercial reagents were purchased from Aldrich (Saint-Quentin Fallavier, France), Acros Organics (Geel, Belgium), and Alfa Aesar (Schiltigheim, France) and used without additional purification. Melting points were determined on a Kofler melting point apparatus. IR spectra were recorded on a Perkin Elmer BX FT-IR. Mass spectra were obtained by using a JEOL JMS GCMate spectrometer at ionizing potential of 70 eV (EI) or by using a spectrometer LC-MS Waters alliance 2695 (ESI^+). ^1H 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 was performed on silica gel 60F-264 (Merck).

Preparation of 1,4-dimethyl-8-ethyl-9H-carbazole derivatives (2e, 3e, 4e). Compound **2e** has been prepared by the reaction of the indole derivative **1e** (10 g, 0.069 mol) with hexane-2,5-dione (11.77 g, 0.103 mol) in the presence of p-toluenesulfonic acid (13.09 g, 0.069 mol) in boiling ethanol (100 mL) as reported by Cranwell and Saxton reaction [27].

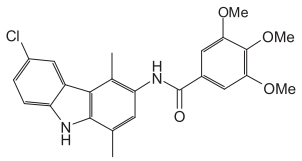
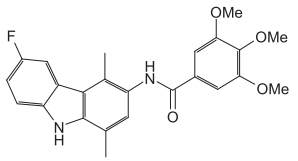
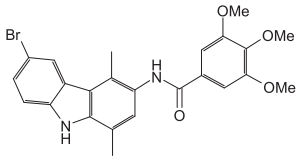
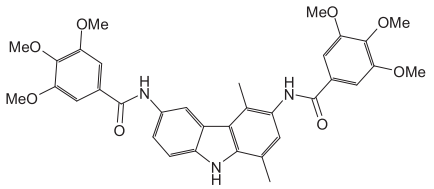
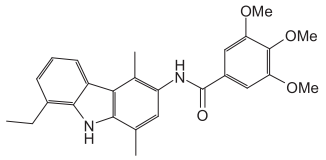
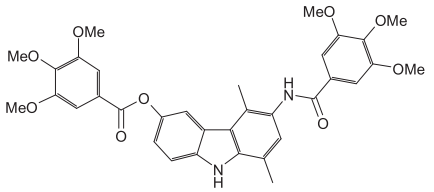
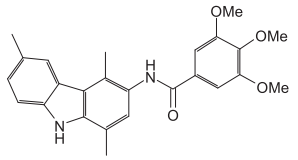
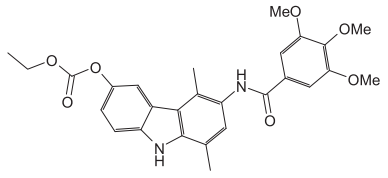
The 1,4-dimethylcarbazole **2e** (2 g, 0.009 mol) obtained has been treated with concentrated HNO_3 (0.56 g, 0.009 mol) in acetic anhydride (30 mL) at room temperature to give the 3-nitro carbazole **3e** (2 g, 0.007 mol), which was in turn transformed into the amino derivative **4e** by the reduction of corresponding nitro group of **3e** with stannous chloride (10 g, 0.045 mol), in hydrochloric acid 20 mL.

1,4-Dimethyl-8-ethyl-9H-carbazole (2e). Gray powder (yield 85%). Mp 118°C. IR (KBr): $\bar{\nu}$ = 3470 (NH) cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.36 (t, J = 7.3 Hz, 3H, CH_2CH_3); 2.61 (s, 3H, CH_3); 2.78 (s, 3H, CH_3); 3.05–3.11 (q, J = 7.3 Hz, 2H, CH_2CH_3); 6.87 (d, J = 6.8 Hz, 1H, *Ar*); 7.10 (d, J = 6.8 Hz, 1H, *Ar*); 7.15 (t, J = 7.8 Hz, 1H, *Ar*); 7.25 (d, J = 6.8 Hz, 1H, *Ar*); 7.98 (d, J = 7.8 Hz, 1H, *Ar*); 10.72 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 14.62; 17.28; 20.35; 23.88; 117.84; 118.96; 119.59; 120.11; 121.08; 123.43; 123.61; 125.80; 126.55; 129.43; 138.35; 139.09. MS (ESI^+): 224 $(M+H)^+$. *Anal.* Calcd for $C_{16}H_{17}N$: C, 86.05; H, 7.67; N, 6.27. Found: C, 86.09; H, 7.70; N, 6.31.

1,4-Dimethyl-8-ethyl-3-nitro-9H-carbazole (3e). Yellow powder (yield 75%). Mp 232°C. IR (KBr): $\bar{\nu}$ = 3354 (NH); 1304 (NO_2) cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.30 (t, J = 7.3 Hz, 3H, CH_2CH_3); 2.59 (s, 3H, CH_3); 2.90 (s, 3H, CH_3); 2.99–3.03 (q, J = 7.3 Hz, 2H, CH_2CH_3); 7.17–7.21 (m, 1H, *Ar*); 7.30 (d, J = 6.8 Hz, 1H, *Ar*); 7.80 (s, 1H, *Ar*); 8.03 (d, J = 7.8 Hz, 1H, *Ar*); 11.28 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 13.58; 17.38; 20.32; 24.00; 114.20; 117.17; 118.11; 120.09;122.93; 123.98; 124.53; 127.00; 128.80; 130.43; 145.70; 146.28. MS (ESI^+): 269 $(M+H)^+$. *Anal.* Calcd for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.58; H, 6.04; N, 10.48.

Table 3

Antiproliferative activity of carbazoles **5a–h** and **6a–h** against human cell lines KB.

Compound number	Structure	10^{-5} M (%)	10^{-6} M (%)
5a		**0**	**0**
5b		**2**	**0**
5c		**35**	**0**
5d		**0**	**0**
5e		**7**	**0**
5f		**0**	**0**
5g		**81**	**0**
5h		**0**	**0**

(*Continued*)

Table 3
(Continued)

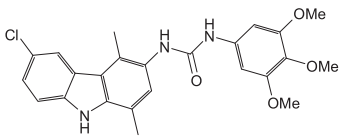
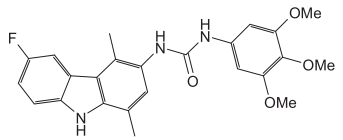
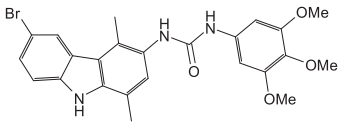
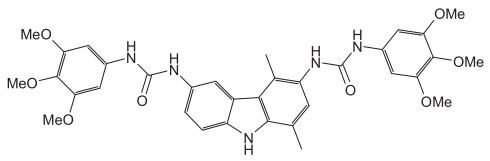
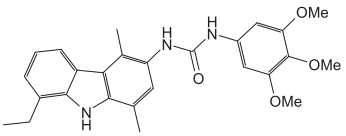
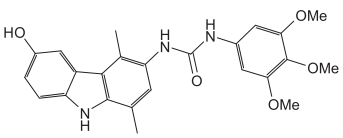
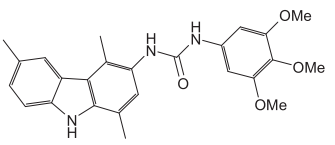
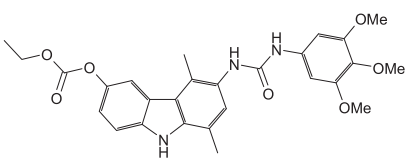
Compound number	Structure	10^{-5} M (%)	10^{-6} M (%)
6a		**51**	**3**
6b		**33**	**0**
6c		**25**	**5**
6d		**0**	**0**
6e		**50**	**0**
6f		**0**	**0**
6g		**100**	**0**
6h		**91**	**0**

Table 4

Antiproliferative activity of selected carbazoles against KB and HL60 cell lines.

Compound number	KB	HL60	
	IC_{50} (μM)	10^{-5} M/10^{-6} M	IC_{50} (μM)
6c	>10	52 ± 5/7 ± 11	9.1 ± 0.3
6g	6.7 ± 3.0	97 ± 1/5 ± 8	5.3 ± 0.6
5g	9.6 ± 0.1	26 ± 3/7 ± 3	>10

Table 5

Tubulin polymerization inhibitory activity of selected carbazoles.

Compound number	Inhibition of tubulin polymerization
6c	8% at 6.7×10^{-5} M
6g	50% at 6.7×10^{-5} M[a]
	3% at 3.3×10^{-5} M
5g	20% at 6.7×10^{-5} M

[a]The concentration was reduced to 3.3×10^{-5} M because of the optical interference at 350 nm.

3-Amino-1,4-dimethyl-8-ethyl-9H-carbazole (4e). Yellow powder (yield 45%). Mp 182°C. IR (KBr): $\bar{\nu}$ = 3410, 3344 (NH_2); 3254 (NH) cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.29 (t, J = 7.8 Hz, 3H, CH_2CH_3); 2.45 (s, 3H, CH_3); 2.48 (s, 3H, CH_3); 2.93–3.00 (q, J = 7.8 Hz, 2H, CH_2CH_3); 4.35 (bs, 2H, NH_2); 6.63(s, 1H, *Ar*); 6.98 (t, J = 7.8 Hz, 1H, *Ar*); 7.09 (d, J = 6.8 Hz, 1H, *Ar*); 7.92 (d, J = 7.7 Hz, 1H, *Ar*); 10.16 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 13.75; 14.65; 17.42; 23.95; 111.89; 116.74; 117.72; 118.16; 119.82; 122.11; 123.04; 123.64; 126.37; 132.83; 138.68; 138.99. MS (EI) *m/z* (%): 238 ($M^{+\cdot}$ 100); 208 (74). *Anal*. Calcd for $C_{16}H_{18}N_2$: C, 80.63; H, 7.61; N, 11.75. Found: C, 80.59; H, 7.65; N, 11.79.

General procedure for the preparation of 3,4,5-trimethoxybenzamides (5a–h). To a stirred solution of aminocarbazole **4a–h** (2 g, 0.009 mol) in $CHCl_3$ (80 mL), 3,4,5-trimethoxybenzoic acid (2,4 g 0.012 mol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (2.2 g 0.012 mol), and 4-dimethylaminopyridine have been added successively. Stirring was continued at rt for 16 h. The reaction mixture was then diluted with chloroform, and the organic layer was separated, washed with saturated aqueous solution of $NaHCO_3$ and then with water. It was then dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The solid precipitate obtained was filtered and recrystallized from acetonitrile.

N-(6-Chloro-1,4-dimethyl-9H-carbazol-3-yl)-3,4,5-trimethoxybenzamide (5a). White powder (yield 40%). Mp > 260°C. IR (KBr): $\bar{\nu}$ = 3243 (NH); 2939 (OCH_3); 1700–1623 (C=O amide); 1131 (C–N); 1580; 1493; 814; 766 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.52 (s, 3H, CH_3); 2.60 (s, 3H, CH_3); 3.73 (s, 3H, OCH_3); 3.86 (s, 6H, OCH_3); 7.13 (s, 1H, *Ar*); 7.37–743 (m, 3H, *Ar*); 7.55 (d, J = 8.7 Hz, 1H, *Ar*); 8.12 (s, 1H, *Ar*); 9.98 (s, 1H, N*H*C=O); 11.44 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 15.19; 16.57; 56.07; 60.16; 105.16; 112.51; 117.73; 120.01; 121.33; 122.88; 124.60; 124.71; 126.69; 126.74; 127.53; 129.78; 138.25; 138.71; 140.14; 152.71; 165.18. MS (ESI^+): 438 $(M+H)^+$. *Anal*. Calcd for $C_{24}H_{23}ClN_2O_4$: C, 65.68; H, 5.28; N, 6.38. Found: C, 65.64; H, 5.31; N, 6.42.

N-(1,4-Dimethyl-6-fluoro-9H-carbazol-3-yl)-3,4,5-trimethoxybenzamide (5b). Yellow powder (yield 30%). Mp > 260°C. IR (KBr): $\bar{\nu}$ = 3240 (NH); 1622 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.46 (s, 3H, CH_3); 2.56 (s, 3H, CH_3); 3.70 (s, 3H, OCH_3); 3.83 (s, 6H, OCH_3); 7.08 (s, 1H, *Ar*); 7.22 (t, J = 8.7 Hz, 1H, *Ar*); 7.35 (s, 2H, *Ar*); 7.47-7.51 (m, 1H, *Ar*); 7.86 (d, J = 7.8 Hz, 1H, *Ar*); 9.95 (s, 1H, N*H*C=O); 11.28 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 15.08; 16.57; 56.06; 60.15; 105.12; 107.52; 111.81; 112.54; 117.66; 120.55; 123.59; 126.51; 126.62; 127.11; 129.81; 136.76; 138.69; 140.10; 152.70; 156.32; 165.14. MS (ESI^+): 423 $(M+H)^+$. *Anal*. Calcd for $C_{24}H_{23}FN_2O_4$: C, 68.23; H, 5.49; N, 6.63. Found: C, 68.27; H, 5.46; N, 6.59.

N-(6-Bromo-1,4-dimethyl-9H-carbazol-3-yl)-3,4,5-trimethoxybenzamide (5c). White powder (yield 43%). Mp > 260°C. IR (KBr): $\bar{\nu}$ = 3242 (NH); 2939 (OCH_3); 1644 (C=O amide); 1130 (CN); 1580; 1491; 1005; 737 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.52 (s, 3H, CH_3); 2.60 (s, 3H, CH_3); 3.73 (s, 3H, OCH_3); 3.86 (s, 6H, OCH_3); 7.13 (s, 1H, *Ar*); 7.38 (s, 2H, *Ar*); 7.51 (s, 2H, *Ar*); 8.25 (s, 1H, *Ar*); 9.98 (s, 1H, N*H*C=O); 11.46 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 15.20; 16.57; 56.07; 60.15; 105.17; 110.70; 113.01; 117.73; 119.89; 124.20; 125.29; 126.69; 126.77; 127.32; 127.57; 129.76; 138.06; 138.96; 140.14; 152.71; 165.18. MS (ESI^+): 483 and 485 $(M+H)^+$. *Anal*. Calcd for. $C_{24}H_{23}BrN_2O_4$: C, 59.64; H, 4.80; N, 5.80. Found: C, 59.67; H, 4.78; N, 5.77.

N-(1,4-Dimethyl-6-(3,4,5-trimethoxybenzamido)-9H-carbazol-3-yl)-3,4,5-trimethoxy benzamide (5d). White powder (yield 36%). Mp > 260°C. IR (KBr): $\bar{\nu}$ = 3367–3215 (NH); 1631 (C=O amide); cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.53 (s, 3H, CH_3); 2.64 (s, 3H, CH_3); 3.73 (s, 6H, OCH_3); 3.87 (s, 12H, OCH_3); 7.08 (s, 1H, *Ar*); 7.34 (s, 2H, *Ar*); 7.39 (s, 2H, *Ar*); 7.52 (d, J = 8.7 Hz, 1H, *Ar*); 7.71 (d, J = 8.7 Hz, 1H, *Ar*); 8.58 (s, 1H, *Ar*); 9.96 (s, 1H, N*H*C=O); 10.18 (s, 1H, N*H*C=O); 11.22 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 15.10; 16.64; 56.09; 56.14; 60.18; 105.17; 105.22; 110.73; 114.98; 117.55; 119.90; 120.84; 123.24; 126.06; 126.32; 127.13; 129.86; 130.45; 130.66; 137.28; 138.20; 140.10; 140,14; 152.67; 152.73; 164.69; 165.19. MS (ESI^+): 614 $(M+H)^+$. *Anal*. Calcd for $C_{34}H_{35}N_3O_8$: C, 66.55; H, 5.75; N, 6.85. Found: C, 66.52; H, 5.72; N, 6.88.

N-(1,4-Dimethyl-8-ethyl-9H-carbazol-3-yl)-3,4,5-trimethoxybenzamide (5e). Yellow powder (yield 23%). Mp 172°C. IR (KBr): $\bar{\nu}$ = 3283 (NH); 1645 (C=O amide) cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.33 (t, J = 7.3 Hz, 3H, CH_3); 2.58 (s, 3H, CH_3); 2.60 (s, 3H, CH_3); 3.04 (q, J = 6.8 Hz, 2H, CH_2CH_3); 3.73 (s, 3H, OCH_3); 3.86 (s, 6H, OCH_3); 7.07 (s, 1H, *Ar*); 7.12 (t, 1H, *Ar*); 7.22 (d, 1H, *Ar*); 7.38 (s, 2H, *Ar*); 7.99 (d, 1H, *Ar*); 9.96 (s, 1H, N*H*C=O); 10.73 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 14.58; 15.29; 17.09; 23.79; 56.07; 60.16; 105.13; 117.75; 119.09; 119.71; 121.36; 123.52; 123.73; 125.91; 126.22; 126.75; 127.24; 129.89; 137.57; 138.76; 140.09; 152.72; 165.13. MS (ESI^+): 433 $(M+H)^+$. *Anal*. Calcd for $C_{26}H_{28}N_2O_4$: C, 72.20; H, 6.53; N, 6.48. Found: C, 72.23; H, 6.50; N, 6.51.

5,8-Dimethyl-6-(3,4,5-trimethoxybenzamide)-9H-carbazol-3-yl-3,4,5-trimethoxybenzoate (5f). White powder (yield 41%). Mp 150°C. IR (KBr): $\bar{\nu}$ = 3361 (NH); 1715 (C=O ester); 1646 (C=O amide) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.55 (s, 3H, CH_3); 2.59 (s, 3H, CH_3); 3.73 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 3.86 (s, 6H, OCH_3); 3.88 (s, 6H, OCH_3); 7.12 (s, 1H, *Ar*); 7.28 (d, J = 8.7 Hz, 1H, *Ar*); 7.38 (s, 2H, *Ar*); 7.46 (s, 2H, *Ar*); 7.59 (d, J = 11.1 Hz,

1H, *Ar*); 7.99 (s, 1H, *Ar*) 9.97 (s, 1H, N*H*C=O); 11.38 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 15.14; 16.64; 56.08; 56.17; 60.16; 60.32; 105.17; 107.16; 111.38; 114.83; 117.69; 119.19; 120.66; 123.56; 124.42; 126.46; 126.56; 127.30; 129.82; 138.10; 138.45; 140,14; 142.27; 143.34; 152.72; 152.94; 165.13; 165.18. MS (ESI$^+$): 615 $(M+H)^+$. *Anal*. Calcd for $C_{34}H_{34}N_2O_9$: C, 66.44; H, 5.58; N, 4.56. Found: C, 66.41; H, 5.61; N, 4.59.

3,4,5-Trimethoxy-N-(1,4,6-trimethyl-9H-carbazol-3-yl) benzamide (5g). White powder (yield 22%). Mp > 260°C. IR (KBr): $\bar{\nu}$ = 3249 (NH); 1644 (C=O amide) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 2.49 (s, 6H, CH_3); 2.61 (s, 3H, CH_3); 3.73 (s, 3H, OCH_3); 3.86 (s, 6H, OCH_3); 7.04 (s, 1H, *Ar*); 7.21 (d, *J* = 7.8 Hz, 1H, *Ar*); 7.38 (s, 2H, *Ar*); 7.42 (d, *J* = 7.8 Hz, 1H, *Ar*); 7.95 (s, 1H, *Ar*) 9.94 (s, 1H, N*H*C=O); 11.08 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 15.27; 16.62; 21.39; 56.06; 60.14; 105.12; 110.78; 117.21; 120.58; 122.00; 123.72; 125.72; 126.17; 126.37; 126.92; 127.12; 129.87; 137.82; 138.51; 140.06; 152.68; 165.09. MS (ESI$^+$): 419 $(M+H)^+$. *Anal*. Calcd for $C_{25}H_{26}N_2O_4$: C, 71.75; H, 6.26; N, 6.69. Found: C, 71.78; H, 6.23; N, 6.72.

Ethyl (1,4-dimethyl-3-(3,4,5-trimethoxybenzamido)-9H-carbazol-6-yl)carbonate (5h). White powder (yield 31%). Mp 206°C. IR (KBr): $\bar{\nu}$ = 3366–3265 (NH); 2935 (OCH$_3$); 1733 (C=O ester); 1645 (C=O amide); 1129 (CN); 1583; 1492; 1266; 1003; 762 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.30 (t, *J* = 6.83 Hz, 3H, CH_3); 2.53 (s, 3H, CH_3); 2.58 (s, 3H, CH_3); 3.73 (s, 3H, OCH_3); 3.86 (s, 6H, OCH_3); 4.26 (q, *J* = 6.8 Hz, 2H, CH_2CH$_3$); 7.11 (s, 1H, *Ar*); 7.25 (d, *J* = 8.7 Hz, 1H, *Ar*); 7.38 (s, 2H, *Ar*); 7.54 (d, *J* = 8.7 Hz, 1H, *Ar*); 7.95 (s, 1H, *Ar*) 9.97 (s, 1H, N*H*C=O); 11.36 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 14.13; 15.12; 16.59; 56.07; 60.15; 64.43; 105.14; 111.29; 114.32; 117.65; 118.61; 120.58; 123.37; 126.44; 126.54; 127.29; 129.80; 138.04; 138.46; 140.10; 143.60; 152.70; 154.03; 165.10. MS (ESI$^+$): 493 $(M+H)^+$. *Anal*. Calcd for $C_{27}H_{28}N_2O_7$: C, 65.84; H, 5.73; N, 5.69. Found: C, 65.87; H, 5.70; N, 5.72.

General procedure for the preparation of *N*-(carbazol-3-yl)-*N′*-(trimethoxyphenyl) ureas (6a–h). To a stirred solution of the aminocarbazole **4a–h** (2 g, 0.009 mol) in anhydrous acetonitrile (50 mL) was added 3,4,5-trimethoxybenzoyl azide, and the reaction mixture was heated at reflux for 3 h. The solid precipitate obtained was filtered and purified by washing with diethyl ether.

N-(6-Chloro-1,4-dimethyl-9H-carbazol-3-yl)-N′-(3,4,5-trimethoxyphenyl)urea (6a). Brown powder (yield 70%). Mp 210°C. IR (KBr): $\bar{\nu}$ = 3254 (NH); 1642 (C=O amide) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3); 2.63 (s, 3H, CH_3); 3.59 (s, 3H, OCH_3); 3.72 (s, 6H, OCH_3); 6.81 (s, 2H, *Ar*); 7.32 (s, 1H, *Ar*); 7.35–7.38 (m, 1H, *Ar*); 7.51 (d, *J* = 8.79 Hz, 1H, *Ar*); 7.96 (s, 1H, *Ar*); 8.10 (s, 1H, N*H*C=O); 8.70 (s, 1H, N*H*C=O); 11.33 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 14.61; 16.69; 55.63; 60.15; 95.66; 112.40; 117.42; 119.98; 121.31; 122.66; 124.07; 124.54; 124.59; 125.51; 128.00; 132.08; 136.57; 137.33; 138.67; 152.86; 154.00. MS (ESI$^+$): 454 $(M+H)^+$. *Anal*. Calcd for $C_{24}H_{24}ClN_3O_4$: C, 63.50; H, 5.33; N, 9.26. Found: C, 63.53; H, 5.37; N, 9.23.

N-(1,4-Dimethyl-6-fluoro-9H-carbazol-3-yl)-N′-(3,4,5-trimethoxyphenyl)urea (6b). Brown powder (yield 60%). Mp 208°C. IR (KBr): $\bar{\nu}$ = 3331 (NH); 1644 (C=O amide) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 2.54 (s, 3H, CH_3); 2.63 (s, 3H, CH_3); 3.59 (s, 3H, OCH_3); 3.73 (s, 6H, OCH_3); 6.82 (s, 2H, *Ar*); 7.21 (t, *J* = 8.7 Hz, 1H, *Ar*); 7.29 (s, 1H, *Ar*); 7.47–7.51 (m, 1H, *Ar*); 7.87 (d, *J* = 7.7 Hz, 1H, *Ar*); 7.95 (s, 1H, N*H*C=O); 8.68 (s, 1H, N*H*C=O); 11.19 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 14.51; 16.69; 55.64; 60.15; 95.67; 107.48; 111.70; 112.37; 117.40; 120.46; 123.58; 124.19; 125.45; 127.57; 132.09; 136.58; 136.76; 137.86; 152.87; 154.05; 156.23. MS (ESI$^+$): 438 $(M+H)^+$. *Anal*. Calcd for $C_{24}H_{24}FN_3O_4$: C, 65.89; H, 5.53; N, 9.61. Found: C, 65.91; H, 5.50; N, 9.58.

N-(6-Bromo-1,4-dimethyl-9H-carbazol-3-yl)-N′-(3,4,5-trimethoxyphenyl)urea (6c). Silver powder (yield 74%). Mp 212°C. IR (KBr): $\bar{\nu}$ = 3345–3251 (NH); 1644 (C=O amide) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3); 2.63 (s, 3H, CH_3); 3.59 (s, 3H, OCH_3); 3.72 (s, 6H, OCH_3); 6.81 (s, 2H, *Ar*); 7.33 (s, 1H, *Ar*); 7.48 (s, 2H, *Ar*); 7.94 (s, 1H, *Ar*); 8.23 (s, 1H, N*H*C=O); 8.68 (s, 1H, N*H*C=O); 11.34 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 14.62; 16.69; 55.65; 60.16; 95.69; 110.49; 112.93; 117.44; 119.78; 124.05; 124.20; 125.29; 125.52; 127.16; 128.04; 132.11; 136.54; 137.16; 138.94; 152.88; 153.99. MS (ESI$^+$): 498, 500 $(M+H)^+$. *Anal*. Calcd for $C_{24}H_{24}BrN_3O_4$: C, 57.84; H, 4.85; N, 8.43. Found: C, 57.81; H, 4.83; N, 8.47.

N-(1,4-Dimethyl-6-(3,4,5-trimethoxyureido)-9H-carbazol-3-yl)-N′-(3,4,5-trimethoxyphenyl)urea (6d). Pink powder (yield 60%). Mp > 260°C. IR (KBr): $\bar{\nu}$ = 3289 (NH); 1640 (C=O amide) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3); 2.65 (s, 3H, CH_3); 3.60 (s, 3H, OCH_3); 3.62 (s, 3H, OCH_3); 3.73 (s, 6H, OCH_3); 3.75 (s, 6H, OCH_3); 6.81 (s, 2H, *Ar*); 6.83 (s, 2H, *Ar*); 7.24 (s, 1H, *Ar*); 7.38–7.46 (m, 2H, *Ar*); 7.91 (s, 1H, N*H*C=O); 8.24 (s, 1H, N*H*C=O); 8.52 (s, 1H, N*H*C=O); 8.54 (s, 1H, N*H*C=O); 8.67 (s, 1H, *Ar*); 11.00 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 14.39; 16.43; 55.66; 55.76; 60.00; 96.12; 96.50; 110.64; 112.89; 116.96; 118.25; 120.59; 123.40; 123.94; 124.68; 127.29; 130.91; 132.43; 132.68; 136.02; 136.36; 136.46; 137.29; 152.76; 152.80; 153.16; 153.94. MS (ESI$^+$): 644 $(M+H)^+$. *Anal*. Calcd for $C_{34}H_{37}N_5O_8$: C, 63.44; H, 5.79; N, 10.88. Found: C, 63.41; H, 5.81; N, 10.84.

N-(1,4-Dimethyl-8-ethyl-9H-carbazol-3-yl)-N'-(3,4,5-trimethoxyphenyl)urea (6e). Yellow powder (yield 62%). Mp 240°C. IR (KBr): $\bar{\nu}$ = 3307 (NH); 1640 (C=O amide) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.32 (t, *J* = 7.3 Hz, 3H, CH_3); 2.56 (s, 3H, CH_3); 2.64 (s, 3H, CH_3); 3.03 (q, *J* = 6.8 Hz, 2H, CH_2CH$_3$); 3.60 (s, 3H, OCH_3); 3.74 (s, 6H, OCH_3); 6.82 (s, 2H, *Ar*); 7.06–7.11 (m, 1H, *Ar*); 7.18–7.22 (m, 1H, *Ar*); 7.25 (s, 1H, *Ar*); 7.92 (s, 1H, N*H*C=O); 7.98 (d, *J* = 7.8 Hz, 1H, *Ar*); 8.67 (s, 1H, N*H*C=O); 10.62 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 14.20; 14.45; 16.89; 23.49; 56.66; 60.00; 96.15; 117.33; 118.72; 119.45; 121.22; 123.34; 123.44; 123.79; 124.68; 126.47; 127.58; 132.45; 136.38; 136.68; 138.67; 152.77; 153.96. MS (ESI$^+$): 448 $(M+H)^+$. *Anal*. Calcd for $C_{26}H_{29}N_3O_4$: C, 69.78; H, 6.53; N, 9.39. Found: C, 69.81; H, 6.55; N, 9.41.

N-(1,4-Dimethyl-6-hydroxy-9H-carbazol-3-yl)-N′-(3,4,5-trimethoxyphenyl)urea (6f). Silver powder (yield 58%). Mp 210°C. IR (KBr): $\bar{\nu}$ = 3479–3291 (OH, NH); 1631 (C=O amide) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 2.46 (s, 3H, CH_3); 2.60 (s, 3H, CH_3); 3.59 (s, 3H, OCH_3); 3.73 (s, 6H, OCH_3); 6.81 (s, 2H, *Ar*); 6.88 (d, *J* = 7.8 Hz, 1H, *Ar*); 7.17 (s, 1H, *Ar*); 7.30 (d, *J* = 7.8 Hz, 1H, *Ar*); 7.50 (s, 1H, *Ar*); 7.86 (s, 1H, N*H*C=O); 8.63 (s, 1H, O*H*); 8.84 (s, 1H, N*H*C=O); 10.76 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 14.53; 16.69; 55.60; 60.12; 95.59; 107.05; 111.26; 114.07; 116.90; 120.50; 124.06; 124.10; 124.68; 126.80; 131.98; 134.32; 136.61; 137.53; 150.15; 152.81; 154.08. MS (ESI$^+$): 436 $(M+H)^+$. *Anal*. Calcd for $C_{24}H_{25}N_3O_5$: C, 66.19; H, 5.79; N, 9.65. Found: C, 66.22; H, 5.76; N, 9.62.

N-(1,4,6-Trimethyl-9H-carbazol-3-yl)-N′-(3,4,5-trimethoxyphenyl) urea (6g). Brown powder (yield 68%). Mp 244°C. IR (KBr): $\bar{\nu}$ = 3298 (NH); 2934 (OCH$_3$); 1641 (C=O amide); 1131 (CN); 1506; 1228; 999; 797 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 2.49 (s,

6H, CH_3); 2.67 (s, 3H, CH_3); 3.60 (s, 3H, OCH_3); 3.73 (s, 6H, OCH_3); 6.83 (s, 2H, *Ar*); 7.19 (d, $J = 7.8$ Hz, 1H, *Ar*); 7.24 (s, 1H, *Ar*); 7.40 (d, $J = 7.8$ Hz, 1H, *Ar*); 7.90 (s, 1H, NHC=O); 7.95 (s, 1H, *Ar*); 8.66 (s, 1H, NHC=O); 10.60 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 14.75; 16.76; 21.39; 55.64; 60.16; 95.67; 110.73; 116.98; 120.52; 122.03; 123.74; 124.03; 124.67; 126.05; 126.94; 127.36; 132.08; 136.64; 137.03; 138.54; 152.88; 145.10. MS (ESI$^+$): 434 $(M+H)^+$. *Anal.* Calcd for $C_{25}H_{27}N_3O_4$: C, 69.27; H, 6.28; N, 9.69. Found: C, 69.30; H, 6.26; N, 9.66.

Ethyl (1,4-dimethyl-3-(3,4,5-thrimethoxyphenylureido)-9H-carbazol-6-yl)carbonate (6h). Yellow powder (yield 67%). Mp 218°C IR (KBr): $\bar{\nu} = 3428$–3247 (NH); 2932 (OCH_3); 1740 (C=O ester); 1646 (C=O amide); 1129 (CN); 1507; 1006; 782 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 1.30 (t, $J = 6.8$ Hz, 3H, CH_3); 2.50 (s, 3H, CH_3); 2.62 (s, 3H, CH_3); 3.59 (s, 3H, OCH_3); 3.73 (s, 6H, OCH_3); 4.26 (q, $J = 6.8$ Hz, 2H, CH_2CH$_3$); 6.82 (s, 2H, *Ar*); 7.22 (d, $J = 10.7$ Hz, 1H, *Ar*); 7.31 (s, 1H, *Ar*); 7.50 (d, $J = 8.7$ Hz, 1H, *Ar*); 7.94 (s, 1H, *Ar*); 7.96 (s, 1H, NHC=O); 8.71 (s, 1H, NHC=O); 11.26 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6): 14.13; 14.58; 16.71; 55.64; 60.16; 64.43; 95.70; 111.20; 114.34; 117.40; 118.43; 120.52; 123.40; 124.00; 125.28; 127.78; 132.12; 136.60; 137.60; 138.06; 143.50; 152.89; 154.04; 154.08. MS (ESI$^+$): 508 $(M+H)^+$. *Anal.* Calcd for $C_{27}H_{29}N_3O_7$: C, 63.89; H, 5.76; N, 8.28. Found: C, 63.90; H, 5.79; N, 8.31.

Preparation of 3,4,5-trimethoxybenzoyl azide (8). A mixture of triethylamine (2.47 g, 0.235 mol) in acetone (10 mL) was added to a solution of the acid **7** (5 g, 0.235 mol) in acetone–water mixture (50/5 mL) at 0°C. After stirring for 30 min, ethyl chloroformate (2.55 g, 0.235 mol) was added with stirring for 1 h. Finally, a solution sodium azide (1.68 g, 0.258 mol) in cold water (10 mL) was added and stirring was further continued for 1.5 h, keeping the temperature at 0°C. The reaction mixture was then diluted with water (70 mL), and the solid formed was collected by filtration. The azide formed was used without any further purification. White powder (yield 75%). Mp 96°C. IR (KBr): $\bar{\nu} = 2145$ (CON_3); 1674 (C=O) cm^{-1}.

Biology. ***Cell culture and cell proliferation assay.*** The human cell lines KB (month epidermoid carcinoma) were obtained from ECACC (Salisbury, UK) and grown in DMEM 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.

Cells were plated in 96-well tissue culture microplates at a density of 650 or 2000 cells/well, respectively, for KB and HL60 in 200 µL medium and treated 24 h later with compounds dissolved in DMSO with concentrations that ranged from 0.5 nM to 10 µM with the use of 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 the results were 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.

Tubulin polymerization inhibition. Sheep brain microtubule proteins were incubated at 37°C for 10 min and at 0°C for 5 min with the tested compounds at a concentration of $6.7 \cdot 10^{-5}$ M. The tubulin polymerization rate was measured by turbidimetry at 350 nm using deoxypodophyllotoxin as positive control [28]. Because of the optical interference of **6g** at 350 nm, the concentration was reduced to $3.3 \cdot 10^{-5}$ M.

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