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Montmorillonite K-10 catalyzed cyclization of *N*-ethoxycarbonyl-*N*′-arylguanidines: Access to pyrimido[4,5-*c*]carbazole and pyrimido[5,4-*b*]indole derivatives

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ABSTRACT

Two new heterocycles, pyrimido[4,5-*c*]carbazole and pyrimido[5,4-*b*]indole, were prepared in three steps from 3-aminocarbazole and 3-aminoindole, respectively. The key Friedel–Crafts intramolecular cyclization was realized under microwave irradiation using montmorillonite K-10 clay as a catalyst. The pyrimido[4,5-*c*]carbazole derivative shows significant micromolar IC_{50} against cancer cell lines. Unlike similar carbazole and indolocarbazole compounds, the molecule does not interfere with topoisomerase activity.

Our group is involved in the synthesis of new heterocycles and their biological evaluation as antitumor agents. Recently,[1] we reported a short and efficient route to quinazoline-4-one derivatives containing a variety of fused heterocycles (Fig. 1). Tetra- and penta-cyclic molecules were thus prepared from simple aminoacridines and evaluated as quadruplex binders.[2,3] Considering the importance of carbazole[4,5] in drug design, it appeared valuable to apply our methodology to prepare new carbazole analogues containing fused pyrimidine ring (see Fig. 1). A few examples of carbazole derivatives containing pyrimidine or pyridine fused heterocycles are shown in Figure 2. The pyrido[4,3-*b*]carbazole family, with ellipticine and olivacine as the lead compounds, displays broad antitumor activities and has been extensively studied. These molecules and their derivatives bind DNA by intercalation and interfere with topoisomerase II activity. The presence of cationic side-chains greatly increases both DNA affinity and biological efficacy. For example, compound S16020-2 (**I** in Fig. 1) was proven to be much more efficient than the parent ellipticine and was selected for clinical trials.[6]

Amongst the analogues of S16020-2 that have been prepared, the pyrimido[4,5-*b*]carbazole analogue (**II** in Fig. 2) displayed similar activity to the parent compound.[7] Ditercalinium, containing two pyrido[4,3-*c*]carbazoles, represents the only example of angular structure. Due to its dimeric structure ditercalinium displays very high affinity for DNA and behaves as a bis-intercalator.[8] Modification of one phenyl ring of carbazole was also explored in the search for bioactive molecules. If α- or β-carbolines (pyrido[2,3-*b*] or [3,4-*b*]indole, respectively) have been largely developed, there are only few examples of pyrimido analogues (**IV** and **V** in Fig. 2).[9] Recent papers point to the diversity of their biological activities (antibacterial agents, by acting on the bacterial topoisomerase IA,[10] or ligands for the α1-adrenergic receptors).[11] To our best knowledge, their interaction with DNA has not been evaluated so far.

We report here the synthesis by an original methodology, of pyrimido[4,5-*c*]carbazole **3** and pyrimido[5,4-*b*]indole **6**. The two

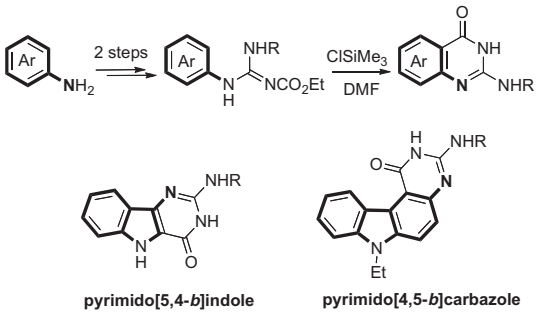

Figure 1. Preparation of quinazoline derivatives from aromatic or heterocyclic amines.

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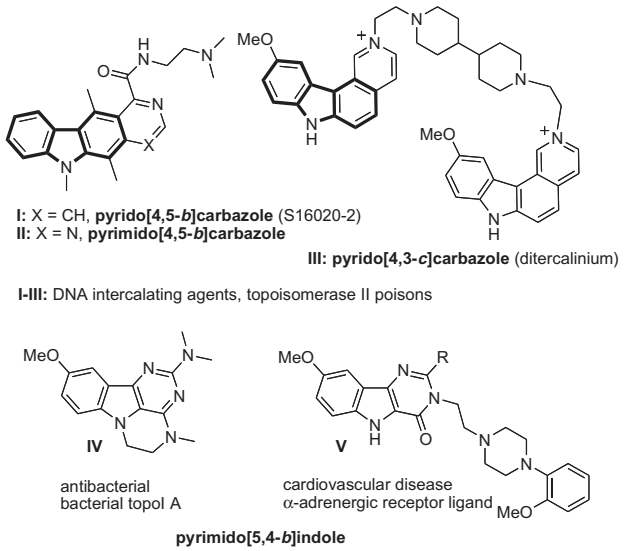


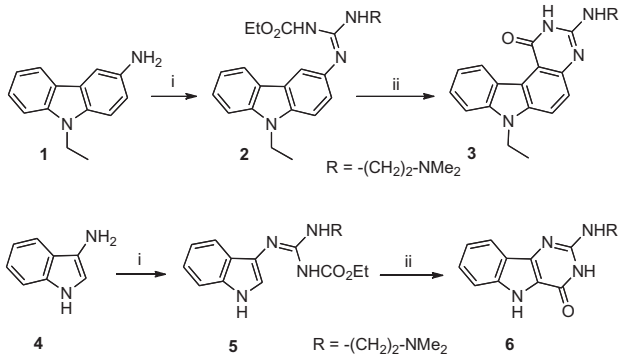
Figure 2. Representative molecules derived from carbazole.

molecules were evaluated as antitumor agents. The data obtained for DNA binding, topoisomerase inhibition and cytostatic effect against two cancer cell lines are discussed.

The key step of the quinazolinone synthesis involved the Friedel–Craft type cyclization of ethoxycarbonyl protected guanidine intermediates as depicted in Figure 1.[1] The cyclization, catalyzed by chlorotrimethylsilane or trichloromethylsilane in DMF, works well, however, the efficiency of the work-up, which includes the extraction of the desired molecule from water, is very sensitive to the nature of the R group. In particular, we noticed lower yields for molecules containing polar amino groups.[2] We therefore adapted our methodology to prepare two new analogues of carbazole containing polar dimethylaminopropylamine substituants, the tetracyclic pyrimido[4,5-*c*]carbazole **3** and the tricyclic pyrimido[5,4-*b*]indole **6**. The [3-(dimethylamino)propyl]amino substituent was chosen by analogy with compounds such as S16020-2, and to increase both the solubility in water, required for biological evaluation, and DNA affinity.

In an effort to ease isolation and purification, and improve yields, we replaced the Lewis acid previously used (halosilanes or $AlCl_3$) by the acidic montmorillonite K-10 clay.

As depicted in Scheme 1, the ethoxycarbonyl protected guanidine intermediates **2** and **5** were prepared in two steps realized in one-pot and in nearly quantitative yields from the corresponding 3-aminocarbazole **1** and 3-aminoindole **4**. The ethoxycarbonyl



Scheme 1. Syntheses. Regents and conditions: (i) EtOCONCS, CH_2Cl_2 then EDCl, RNH_2, NEt_3, (ii) K-10 clay, C_2H_2Cl, MW 80 °C, 1 h.

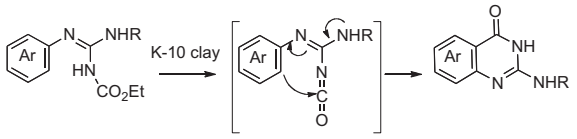


Figure 3. Postulated mechanism of cyclization.

guanidines were then heated in dichloroethane (80 °C) in the presence of K-10 clay. In these conditions, the reaction required prolonged heating (24 h) to reach completion. However, microwave irradiation efficiently shortened the reaction time. We tried several conditions, with and without solvent to optimize the process. Indeed, the reaction can be performed solventless, however adding a minimum of solvent eased stirring during irradiation and therefore facilitated homogenization.

We finally ended up with the following process: A mixture of the guanidine, **2** or **5**, and montmorillonite K-10 clay (5 equiv w/w) was irradiated in a CEM microwave oven.[15] A minimum amount of solvent (dichloroethane, 0.5–1 ml) was added to facilitate magnetic stirring during irradiation. The reaction course was monitored by hplc by taking samples at regular intervals. After 1 h of irradiation at 80 °C, the starting guanidines, **2** or **5**, had fully reacted. The reaction mixtures were filtered to remove the clay, which can be recycled, and the products were purified by standard methods after evaporation of the solvent. Tetracyclic pyrimido[4,5-*c*]carbazole **3** and tricyclic pyrimido[5,4-*b*]indole **6** were thus obtained in 77% and 73% yield, respectively after purification.[15]

Concerning the mechanism of formation of the pyrimidine ring, we know from previous attempts that the cyclization does not occur in methanesulfonic, trifluoroacetic or hydrochloric acids, thus indicating that the reaction does not proceed by direct acid catalyzed Friedel–Crafts reaction.[12] We therefore propose the mechanism shown in Figure 3, with intermediate formation of electrophilic isocyanate intermediate. The mechanism is based on the recent work of Fernandez and co-workers[13] who reported the formation of isocyanates from carbamates using K-10 clay catalyst.

The two new molecules were evaluated as potential antitumor agents. Since carbazole derivatives are known to target DNA and to interfere with topoisomerase activities, the cytostatic effects of

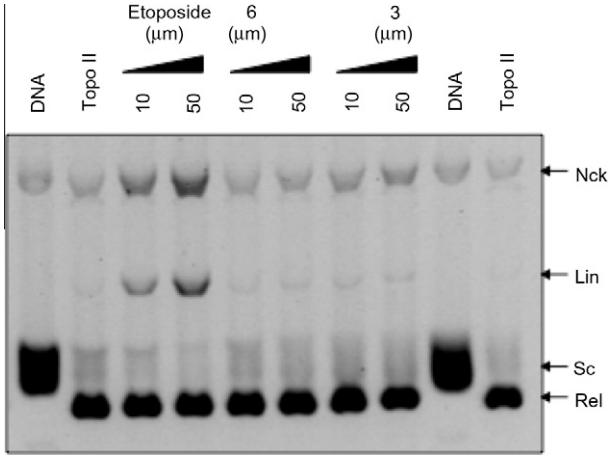


Figure 4. Effects of novel derivatives on the relaxation of plasmid DNA by human topoisomerase II. Native supercoiled pUC18 (750 ng, lane DNA) was incubated with 4 units topoisomerase II in the absence (lane Topo II) or presence of tested compounds at the indicated concentration. Etoposide was used at the same concentrations. DNA samples were separated by electrophoresis on a 1% agarose gel containing 1 μg/mL ethidium bromide. Gels were photographed under UV light. Nck, nicked; Sc, supercoiled; Lin, linear; Rel, relaxed.

Table 1
Biological evaluation

	IC_{50} (μM)		ΔT_m[a]			
	HL60 N	HL60 MX2	CT-DNA		Poly(dAdT)$_2$	
			$R = 1$	$R = 0.5$	$R = 1$	$R = 0.5$
Etoposide	1.3	11.9	0	0	0	0
6	>100	>100	0.4	0	4.4	3
3	17	17	3	2.3	15.9	15.4

[a] Variations in melting temperature ($\Delta T_m = T_m^{complex} - T_m^{DNA}$). T_m measurements were performed in BPE buffer, pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA), using 10 or 20 μM (for $R = 0.5$ or 1 respectively) drug and 20 μM of DNA calf thymus (CT-DNA) or poly(dAdT)$_2$ at 260 nm with a heating rate of 1 °C/min. T_m values were measured from the first derivative plots of the melting profiles. Absorption spectra from novel derivatives were performed using a Uvikon XL spectrophotometer with Thermosystem (Peltier).

compounds **3** and **6** were tested against human leukemic topoisomerase II sensitive (HL60N) and resistant (HL60 MX2) cell lines. Etoposide was used as reference compound. Unlike the tricyclic pyrimido[5,4-*b*]indole **6** that did not show any effect (IC_{50} >100 μM in both cell lines), the tetracyclic pyrimidocarbazole **3** displayed significant micromolar IC_{50} values (17 μM in both cell lines). Etoposide, tested in the same conditions, gave a 10 time higher activity on HL60 N cell line than **3** (IC_{50} = 1.3 μM). The lower efficiency of etoposide observed against resistant HL60 MX2 cell line (IC_{50} = 11.9 μM) is characteristic of the cellular mechanism underlying, that is, topoisomerase inhibition. Despite the lower efficiency against HL60N cell line compared to etoposide, the IC_{50} values of the tetracyclic pyrimidocarbazole **3** were encouraging. The absence of resistance index between the two cell lines (compared to a resistance index of 10 for etoposide) suggests that the topoisomerase II is probably not the main cellular target.

The activity on topoisomerase II was therefore assessed by plasmid relaxation assay in the presence of the enzyme (Fig. 4). For compound **3**, a very weak activity was observed (10% of nicked DNA formed after treatment at the highest concentration of **3**, compared to 5% residual nicked DNA present in the reference lanes, see Supplementary data). No significant activity was found for **6**. We also checked that molecules **3** and **6** did not interfere with topoisomerase I activity (see Supplementary data).

We then studied the interaction of the two molecules with DNA. To evaluate the relative affinities of the two molecules for DNA, melting temperature (T_m) measurements were performed with calf thymus DNA (42% GC bp) and the alternating polynucleotide poly(dAdT)$_2$. The difference in T_m values between the drug–DNA complexes and free DNA or polynucleotide in solution provides a useful mean to assess the strength of the interaction of the molecules with double stranded DNA. The ΔT_m values ($\Delta T_m = T_m^{complex} - T_m^{DNA}$) measured with each compound at drug/DNA–phosphate (D/P) ratios of 0.5 and 1, are collected in Table 1.

The tetracyclic molecule **3** showed stronger binding to the macromolecule than the tricyclic compound **6**, both on CT-DNA and poly(dAdT)$_2$. However the stabilization effect remains weaker than what could be expected for intercalating agents (e.g., a tetracyclic pyrido[4,3,2-*kl*]acridine[14] containing the same dimethylaminoethylamino side-chain shows ΔT_m values of 13.3 and 16.9 for $R = 0.5$ and 1, respectively with CT-DNA). The pyrimidocarbazole **3** displayed a better interaction with poly(dAdT)$_2$ than with CT-DNA as reflected by a five times higher value of ΔT_m. This preference for poly(dAdT)$_2$ suggests a possible minor groove recognition for **3**, possibly due to the presence of the amino substituted pyrimidone ring and its strong H-bond pattern (Fig. 5).

As a conclusion, we have designed a new strategy to prepare polar pyrimido fused heterocycles (quinazoline derivatives) in three steps from simple aromatic amines, and exemplified its efficiency by preparing two carbazoles analogues: a tricyclic molecule in which one benzene ring was replaced by a 2-alkylamino-pyrimidin-4-one heterocycle, and a new tetracyclic compound with a 2-alkylamino-pyrimidin-4-one ring fused to the 3–4 bond of the carbazole unit. The key-step involved the K-10 clay catalyzed intramolecular cyclization of ethoxycarbonyl protected guanidine intermediates. This method appears particularly suitable for preparing highly polar basic molecules, for which extraction from water is time and solvent consuming. These conditions also represent an interesting alternative for compounds bearing substituents sensitive to halosilane reagents. The potential interest of the two molecules as antitumor agents was evaluated. The pyrimido-[4,5-*c*]carbazole **3** displays significant IC_{50} in HL60 cell lines. The molecules were also tested on the known cellular targets of carbazole derivatives, DNA and topoisomerase II. The very weak effect on topoisomerase II activity and the weak interaction with CT-DNA suggest that, despite its planar structure and structural similarity to pyrido- and pyrimido-carbazoles **I–III** shown in Figures 2 and 4 does not bind to DNA by intercalation, and that minor groove binding may be favoured. These data point to an original mechanism of action for this molecule **3** that remains to be elucidated.

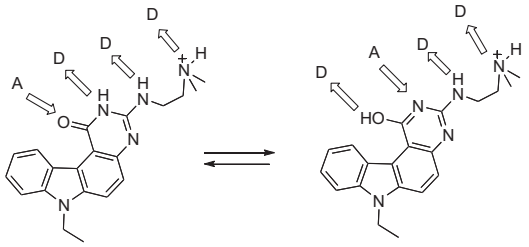


Figure 5. H-bond acceptor/donor pattern of compound **3**.

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Supplementary data

Copies of ^1H and ^{13}C nmr spectra of compounds **3** and **6** are given, along with the gels showing the effects of the two molecules on the activity of topoisomerases **I** and **II**. The experimental procedure used for IC_{50} determination is detailed. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.028.

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15. *Cyclization reaction*. A mixture of the protected guanidine (40 mg) and K-10 clay (400 mg) in $C_2H_4Cl_2$ (0.5 ml) was irradiated in a microwave oven CEM Discover Synthesis Unit, equipped with a continuous focused microwave power delivery system with selectable power output. The experiments were conducted under magnetic stirring. The mixture was irradiated in a sealed tube at 80 °C for 1 h (ramp time 30 s, T_{max} = 80 °C, power max = 200 W). The clay was then filtered off, washed with CH_2Cl_2 and the organic phase was evaporated to dryness. Molecule, **3** or **6**, was purified by silica gel column chromatography and obtained in good yield (70–75%).
Compound ***3***. Mp: 127–129 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.66 (d, 1H, J = 8 Hz, Har), 7.60–7.42 (m, 1H, Har), 7.40–7.15 (m, 4H, Har), 4.31 (q, 2H, J = 7.2 Hz, CH_2), 3.55–3.52 (m, 2H, CH_2), 2.73 (t, 2H, J = 7.2 Hz, CH_2), 2.39 (s, 6H, 2× CH_3), 1.33 (t, 3H, J = 7.2 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.7, 149.6, 139.6, 135.2, 127.6, 125.5, 123.4, 121.9, 117.9, 116.6, 112.1, 108.7, 57.7, 44.6, 37.5, 36.7, 13.9; MS (ESI, CH_3CN): m/z calcd 351 $[M+H]^+$; found 352.
Compound ***6***. Mp: 99–100 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.37 (br, NH), 7.82 (d, 1H, J = 8 Hz, Har), 7.39–7.31 (m, 2H, Har), 7.09–7.05 (m, 1H, Har), 6.26 (br, NH), 3.52 (q, 2H, J = 5.6 Hz, CH_2), 2.70 (t, 2H, J = 5.6 Hz, CH_2), 2.40 (s, 6H, 2× CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 155.2, 150.3, 140.0, 138.9, 126.4, 120.5, 120.3, 120.2, 118.7, 116.3, 112.4, 57.5, 44.4, 37.7. MS (ESI, CH_3CN): m/z calcd 271 $[M+H]^+$; found 272.