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Natural product leads for drug discovery: Isolation, synthesis and biological evaluation of 6-cyano-5-methoxyindolo[2,3-*a*]carbazole based ligands as antibacterial agents

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ABSTRACT

Indolo[2,3-*a*]carbazole based inhibitors were synthesized from readily available indigo via a seven-step linear synthetic sequence with a moderate overall yield. The inhibitors were selectively and readily functionalized at the nitrogen on the indole portion of the carbazole unit. The synthesized analogs displayed moderate inhibitory activities toward *Bacillus anthracis* and *Mycobacterium tuberculosis*, indicating that indolo[2,3-*a*]carbazoles could serve as promising leads in the development of new drugs to combat anthrax and tuberculosis infections.

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1. Introduction

The heterocyclic indolo[2,3-*a*]carbazole system is found in several biologically active natural products which are available from a variety of organisms. Examples of such natural products include the protein kinase inhibitor staurosporine (STS) from the bacterium *Streptomyces staurosporeus*,[1] anti-cancer agents such as the arcyriaflavins isolated from the slime mold *Arcyria denudate*,[2] and the topoisomerase I inhibitor rebeccamycin, which originates from the bacterium *Nocardia aerocoligenes* (Figs. 1 and 2).[3] Due to the wide spectrum of biological activities exhibited by the naturally occurring carbazoles, a variety of synthetic analogs based on the indolo[2,3-*a*]carbazole scaffold have garnered considerable attention in the development of medicines for psoriasis, hypertension, cancer, and HIV-infection.[4] During a screening effort for antibacterial agents from cyanobacteria, we identified a strong inhibitory activity towards *Bacillus anthracis* (*B. anthracis*) for the extract of *Nostoc muscorum* (UTEX 2301). Bioassay guided fractionation led to the isolation and identification of 6-cyano-5-methoxyindolo[2,3-*a*]carbazole (**1a**, Fig. 2) as the active principle.[5] Compound **1a** and 6-cyano-5-methoxy-12-methylindolo[2,3-*a*]carbazole (**1b**) were first isolated from the blue-green alga *Nostoc sphaericum* (strain EX-5-1) and identified by Moore and co-workers in 1990.[5] In their studies, compound **1b** showed modest antiviral activity against herpes simplex virus type 2; in infected mink lung cells, the virus titer is reduced 95% at 1 μg/mL, and weak, non-selective cytotoxicity (MIC 5 μg/mL) against KB and LoVo human carcinoma cell lines.[5]

As part of an ongoing, multi-faceted program aimed toward developing novel antiinfective chemotherapeutics against poten-

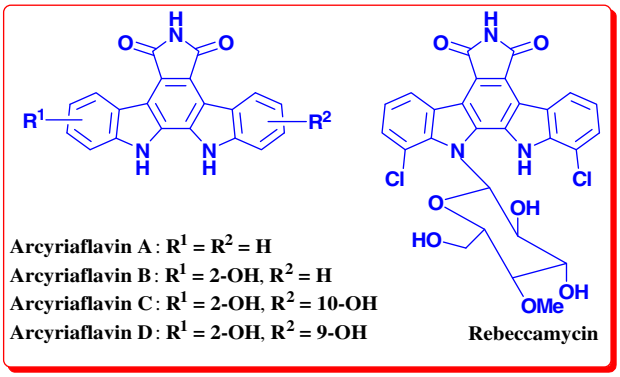

Figure 1. Indolo[2,3-*a*]carbazole based natural products: arcyriaflavins A–D and rebeccamycin.

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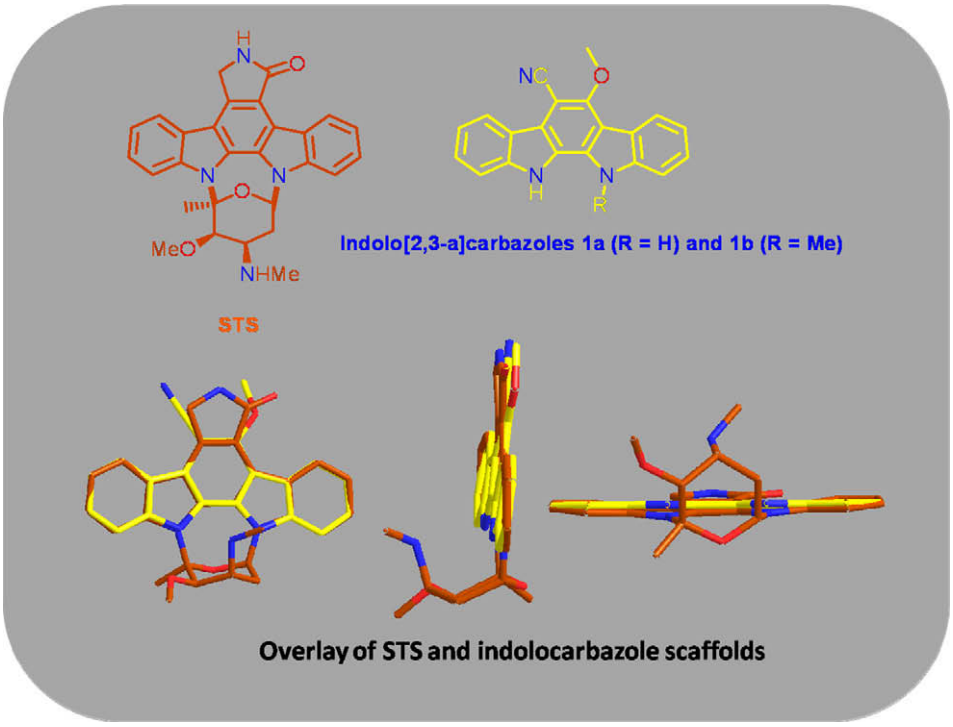


Figure 2. An overlay of chemical structures of STS and indolo[2,3-*a*]carbazoles **1a** and **1b**.

tial bio-warfare agents[6] and neglected diseases,[7] we synthesized and screened a set of compounds containing the indolo[2,3-*a*]carbazole system as a primary scaffold against *B. anthracis* and *Mycobacterium tuberculosis* (*M. tuberculosis*). Herein, we report the synthesis and biological activity of some of these new indolo[2,3-*a*]carbazoles.

We noted that the chemical scaffold of antiviral indolo[2,3-*a*]carbazoles **1a** and **1b** was similar to the aglycone portion of the protein kinase inhibitor STS (Fig. 2). It is well known that the substituents on the indole nitrogens play a significant role in determining the biological activity of the indolocarbazoles. Varying these substituents with various uncommon sugars via an α- or β-*N*-glycosidic linkage was a key element for modulating their cytotoxicity, anti-cancer activity, and topoisomerase inhibition.[8] For instance, rebeccamycin and its analogs with different sugar moieties were much more active than the corresponding aglycone.[9]

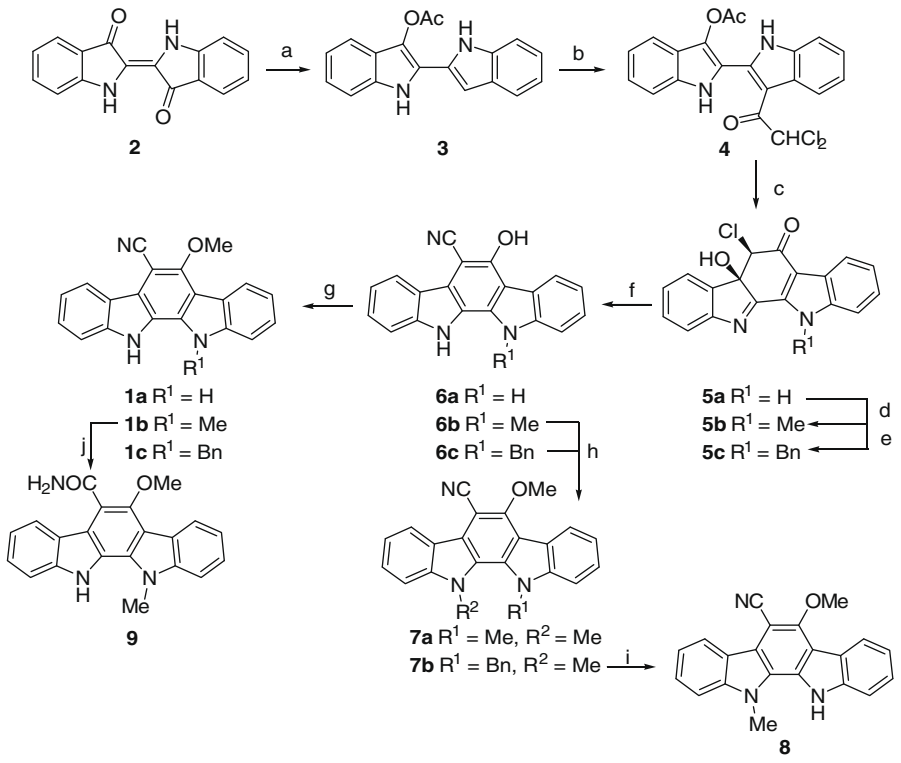
2. Chemistry

In view of the foregoing facts, we wished to evaluate the antibiotic activities of indolo[2,3-*a*]carbazoles (**1a**–**c**, **6a**–**c**, **7a**, **8** and **9**) with varying R^1 and R^2 substituents.

The advances in indolocarbazole chemistry were previously summarized in a comprehensive review focusing on carbazole and indolocarbazole synthesis.[10] A survey of available literature on the synthesis of indolocarbazoles indicated that these inhibitors are generally synthesized through strategies involving either direct formation of the indolocarbazole skeleton by indole ring synthesis, elaboration of bisindole precursors by the construction of the central carbocyclic ring, or through a combination of these two strategies.[11] The first synthesis of **1a** and **1b** was reported by Somei and co-workers in 1997[12c] and in the following years, they made extensive efforts toward optimizing those protocols.[12a,b] In addition, Snieckus and Cai reported an efficient route to indolo[2,3-*a*]carbazole **1b** via a metalation-cross-coupling reaction sequence.[13] This protocol provided a useful way to modify the substituents on the indole portions of the carbazole scaffold. However, this method relied heavily on a very substrate-selective Suzuki–Miyaura and Stille cross-coupling reactions of the two indole units that require elaborate synthesis themselves.

In our own efforts, we found that the syntheses described earlier were not readily amenable to modifications that enabled an easy build-up of a library of N-functionalized indolocarbazoles. For example, our attempts to modify Somei's synthesis by introducing a *tert*-butoxycarbonyl group as R^1 at the stage of intermediate **5a** failed (Scheme 1).

Alternatively, we were successful in functionalizing intermediate **5a** with an easily-cleavable benzyl group as R^1 and then selectively modifying R^2. The synthesis started from 3-acetoxy-2,2′-bi-1*H*-indole[14] (**3**), readily made from indigo (**2**). Intermediate **3** was reacted with dichloroacetyl chloride in refluxing anhydrous ethyl acetate to afford the 3-acetoxy-3′-dichloroacetyl-2,2′-biindolyl intermediate **4**.[12d,e] Subsequent cyclization of **4** using aqueous ammonia in MeOH/DMF at ambient temperature provided the tetrahydroindolo[2,3-*a*]carbazole **5a**.[12c] Methylation of **5a** with dimethyl sulfate in the presence of K_2CO_3 as a base resulted in the 12-methyl intermediate **5b** in quantitative yield, while benzylation of **5a** afforded **5c** under similar conditions in 83% yield. Reductive cyanation[12c] of **5a** using only 5 equiv of NaCN instead of the 30 equiv reported in the literature[12c] gave **6a** in 34% yield in comparison to the reported yield of 60%. However, using the lower amounts of NaCN, the reductive cyanation of **5b** and **5c** took place in higher yields of 60% and 90%, respectively. Subsequent methylation of intermediates **6a**–**c** with diazomethane provided **1a**–**c** in excellent yields. The free indole nitrogen as well as the phenolic hydroxyl group of intermediates **6b** and **6c** were methylated to give the corresponding compounds **7a** and **7b**. Compound **7b** was subsequently treated with $AlCl_3$ in anisole at 110 °C to remove the *N*-benzyl group to form **8** in quantitative yield.[15] The nitrile group at the 6-position of the indolo[2,3-*a*]carbazole **1b** was readily hydrolyzed in the presence of hydrogen peroxide to give the new carboxamide **9**.

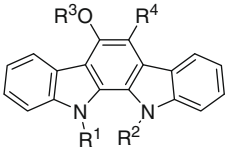


Scheme 1. Synthesis of indolocarbazoles **1a–c**, **6a–c**, **7a**, **8** and **9**. Reagents and conditions: (a) Sn, AcOH, Ac_2O, 64–66 °C; (b) $Cl_2CHCOCl$, EtOAc, reflux; (c) aq 1.3% NH_3, MeOH, DMF, rt; (d) Me_2SO_4, K_2CO_3, DMF, rt; (e) BnBr, K_2CO_3, DMF, rt; (f) NaCN, DMF, H_2O, 70 °C; (g) CH_2N_2, rt; (h) MeI, K_2CO_3, DMF, rt; (i) $AlCl_3$, anisole, 110 °C, 2 h; (j) 35% H_2O_2, 3 N NaOH, EtOH, 30 °C, 20 h.

3. Biological studies

The synthesized derivatives were tested for their inhibitory activity against the ΔANR strain of *B. anthracis* and H_{37}Rv strain of *M. tuberculosis*.[16] The results are summarized in Table 1. While there was no clear effect on the activity of indolocarbazoles stemming from the presence or absence of an alkyl group R^1 (for example, compare **1a** vs **1b** against **6a** vs **6c** or **7a** vs **8**), compounds with R^3 = H clearly displayed an improvement in their inhibitory activities (compare **1b** vs **6b** and **1a** vs **6a**). Significantly, it was interesting to note that the presence of an alkyl group as R^2 was detrimental to the activity of these indolocarbazoles (compare **1a** vs **8**). Replacement of the cyano functionality in **1b** with a carboxamide (compound **9**) did not alter activity for growth inhibition of *M. tuberculosis*. Interestingly, the protein kinase inhibitor STS displayed modest activity against *M. tuberculosis*, while it was inactive against *B. anthracis*. The newly synthesized analogs **6a–c** clearly had improved activities against both the pathogens compared to the known indolocarbazole **1b**. Benzyl protected compound **6c** was equipotent to **6a** in the inhibition of *B. anthracis*, while only fourfold less potent than **6a** in the inhibition of *M. tuberculosis*. Similarly, **1c** with a benzyl protected indole moiety displayed a moderate MIC (44.1 μM) against *M. tuberculosis*. Encouraged by these preliminary results, we are currently screening more N-functionalized indolo[2,3-*a*]carbazole derivatives to improve the activity of these promising leads.

Table 1
Inhibition of *B. anthracis* and *M. tuberculosis* by compounds **1a–c**, **6a–c**, **7a**, **8** and **9**



Compound	R^1	R^2	R^3	R^4	MIC[a] (*B. anthracis*) (μM)	MIC[b] (*M. tuberculosis*) (μM)
1a	H	H	Me	CN	6.3	>128
1b	Me	H	Me	CN	>200	>128
1c	Bn	H	Me	CN	>200	44.1
6a	H	H	H	CN	1.6	15.0
6b	Me	H	H	CN	6.3	56.6
6c	Bn	H	H	CN	1.6	60.6
7a	Me	Me	Me	CN	>200	>128
8	H	Me	Me	CN	>200	>128
9	Me	H	Me	$CONH_2$	NT[g]	>128
STS[c]					128	14.7
Cipro[d]					0.1	—
RMP[e]					—	0.1
INH[f]					—	0.6

[a] MIC = minimum inhibitory concentration against ΔANR *B. anthracis*.
[b] MIC against H_{37}Rv *M. tuberculosis*.
[c] Staurosporine.
[d] Ciprofloxacin hydrochloride.
[e] Rifampin.
[f] Isoniazid.
[g] Not tested.

4. Conclusions

In conclusion, a variety of indolo[2,3-*a*]carbazoles were synthesized from the commercially available and widely used indigo dye in seven linear steps. The described synthetic strategy allows selective functionalization of either of the indole nitrogens and is amenable to ready generation of a library of N-functionalized indolo[2,3-*a*]carbazole derivatives. Some of the synthesized compounds displayed reasonable activity against *B. anthracis* (compounds **1a**, **6a** and **6c**) and moderate activity against *M. tuberculosis* (compound **6a**). Further rounds of screening and

optimization of these new indolo[2,3-*a*]carbazoles are currently in progress.

5. Experimental

5.1. Biology methods

5.1.1. Isolation of 1a from *Nostoc muscorum*

Nostoc muscorum was acquired from the Culture Collection of Algae at the University of Texas at Austin (UTEX 2301). The cyanobacterium was grown in a 2.8 L Fernbach flask containing 1 L of inorganic media (Allen). Cultures were illuminated with fluorescent lamps at 1.93 klx with an 18/6 h light/dark cycle at 22 °C. After 6–8 weeks, the biomass of cyanobacteria was harvested by centrifugation and then freeze-dried. The freeze-dried biomass from a total of 7 L of culture (2.09 g) was extracted by repeated maceration with CH_2Cl_2:MeOH (1:1) to yield 246.8 mg of crude extract. The crude extract showed inhibitory activity towards *B. anthracis* (MIC 7.5 μg/mL) and was fractionated on silica gel using a gradient with increasing amount of MeOH in CH_2Cl_2 to afford 16 fractions. Fraction 5 (MIC 0.94 μg/mL) eluting with CH_2Cl_2:MeOH 60:1, was subjected to reversed-phase HPLC (Alltima C_{18}, 10 μm, 250 × 10 mm, 4 mL/min) with a solvent gradient of acetonitrile–H_2O (10:90) to acetonitrile–H_2O (90:10) over 35 min to afford one major compound (t_R = 29.8 min, 13.3 mg). The structure was identified by spectroscopic means (1D, 2D, NMR and MS) and the data were found to be identical to those previously reported for 6-cyano-5-methoxy-indolo[2,3-*a*]carbazole.

5.1.2. MIC against the ΔANR strain of *B. anthracis*

The test compound MIC values against the ΔANR (plasmid-cured Ames Strain) of *B. anthracis* were determined using an automated Microplate-based Alamar Blue assay. Assays were carried out using a TECAN Freedom Evo 200 liquid-handling robotics platform equipped with a TeMO96 multichannel pipette and TECAN GENios Pro spectrophotometer. Using the TeMO96, 50 μL of Cation-Adjusted Mueller-Hinton Broth (CAHB) was added to each well of a sterile, Falcon MICROTEST 96-well flat bottom tissue culture plate. A serial dilution of the desired inhibitors was created by adding 46 μL of the CAHB, using the Freedom Evo 200's 8 channel liquid-handling arm (LiHa), to each well of the first column which also contained 4 μL of inhibitor at 10 mM. Upon mixing, 50 μL of the first wells was transferred to the next column and mixed. This routine was repeated ten more times to generate the desired gradient. Sample and positive control wells were inoculated using the TeMo96 with 50 μL of a diluted *B. anthracis* ΔANR bacterial culture, whereas 50 μL of CAHB containing no bacteria was added to the negative control wells. Stock bacterial cultures were grown to mid-log phase (OD_{600} = 0.4–0.6), and diluted to the desired OD_{600} of 0.004 using fresh CAHB. After the final inoculation with the bacterial culture, the microplates were incubated at 37 °C overnight (~14 h) without agitation. After incubation, 5 μL of alamar blue dye (Serotec) was added to each well using the TeMo96. Plates were agitated for 15 min at 900 rpm on a TECAN TeShake, and the absorbance values at wavelengths of 570 nm and 600 nm were then measured using the TECAN GENios Pro. Individual wells were quantitatively assessed using the difference between the absorbance values at 600 nm and 570 nm. MICs for each compound and known antibiotic were assigned based on the first well that exhibited a negative absorbance difference, representative of no bacterial growth.

5.1.3. MIC against H_{37}Rv strain of *M. tuberculosis*

The compound MICs against replicating *M. tuberculosis* were assessed by the Microplate Alamar Blue assay[16] (MABA) using rifampin and isoniazid as positive controls. Compound stock solutions were prepared in DMSO at a concentration of 12.8 mM, and the final test concentrations ranged from 128 μM to 0.5 μM. Two fold dilutions of compounds were prepared in Middlebrook 7H12 medium (7H9 broth containing 0.1% w/v casitone, 5.6 μg/mL palmitic acid, 5 mg/mL bovine serum albumin, 4 mg/mL catalase, filter-sterilized) in a volume of 100 μL in 96-well microplates (black viewplates, manufacturer). *M. tuberculosis* H_{37}Rv (ATCC#27294) (100 μL inoculum of 1 × 105 cfu/mL) was added, yielding a final testing volume of 200 μL. The plates were incubated aerobically at 37 °C. On the 7 day of incubation 12.5 μL of 20% Tween 80, and 20 μL of Alamar Blue (Trek Diagnostic, Westlake, Ohio) were added to the test plate. After incubation at 37 °C for 16–24 h, fluorescence of the wells was measured (ex 530, em 590 nm). The MICs ware defined as the lowest concentration effecting a reduction in fluorescence of ⩾90% relative to the mean of replicate bacteria-only controls.

5.2. Chemistry methods

^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker spectrometer with TMS as an internal standard. Standard abbreviation indicating multiplicity was used as follows: s = singlet, d = doublet, t = triplet, q = quadruplet, quin = quintuplet, m = multiplet and br = broad. HRMS experiment was performed on Q–TOF–2TM (Micromass). The progress of all reactions was monitored by TLC on precoated *silica gel* plates (Merck *Silica Gel* 60 F254). Column chromatography was performed using Merck *silica gel* (230–400 mesh). All reactions were performed under inert atmosphere of nitrogen, unless otherwise mentioned. All spectral data of know compounds are identical to those reported.[5,12]

5.2.1. *Cis*-6-chloro-6a-hydroxy-5-oxo-12-benzyl-5,6,6s,12-tetrahydroindolo[2,3-*a*]carbazole 5c from 5a

K_2CO_3 (2500 mg, 18.1 mmol) and benzyl bromide (5500 mg, 32.2 mmol) were added to a solution of **5a** (1000 mg, 3.23 mmol) in DMF (30 mL), and the mixture was stirred at room temperature for 2 h. After addition of the H_2O under ice water bath, the whole solution was extracted with EtOAc. The extract was washed by brine, dried over Na_2SO_4, and evaporated under reduced pressure to leave a residue, which was column-chromatographed on SiO_2 with EtOAc–hexane (1:2, v/v) to give **5c** (1.1 g, 83%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 8.20 (d, J = 7.6 Hz, 1H), 7.87 (d, J = 7.6 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.66 (d, J = 8.0 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.50–7.35 (m, 5H), 7.35–7.15 (m, 3H), 6.96 (s, 1H), 6.19 (d, J = 15.6 Hz, 1H), 6.00 (d, J = 15.6 Hz, 1H), 5.45 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 185.1, 169.4, 154.9, 139.2, 138.9, 136.7, 136.5, 130.6, 128.8, 127.8, 127.4, 126.9, 126.3, 125.4, 124.3, 123.8, 122.2, 121.6, 115.5, 112.3, 87.5, 69.8, 47.8; HRMS (ESI) calculated for $C_{25}H_{17}ClN_2O_2$ $[M+H]^+$ 413.1051, found 413.1073.

5.2.2. 6-Cyano-5-hydroxy-12-benzylindolo[2,3-*a*]carbazole (6c) from 5c

NaCN (654 mg, 13.4 mmol) was added to a solution of **5c** (1100 mg, 2.7 mmol) in DMF (40 mL) and H_2O (20 mL), and the solution was stirred at 70 °C for 30 min. After addition of H_2O, the whole solution was extracted by EtOAc. The extract was washed by brine, dried over Na_2SO_4, and evaporated under reduced pressure to leave a residue, which was column-chromatographed on SiO_2 with EtOAc–hexane (1:2, v/v) to give **6c** (947 mg, 90%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 11.74 (s, 1H), 10.83 (s, 1H), 8.48 (d, J = 7.6 Hz, 1H), 8.41 (d, J = 8.0 Hz, 1H), 7.75–7.60 (m, 2H), 7.55–7.38 (m, 2H), 7.38–7.05 (m, 7H), 6.14 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 152.5, 140.4, 139.5, 137.9, 130.7, 128.8, 127.5, 126.7, 125.8, 125.2, 122.8, 122.3, 121.1, 120.8,

119.7, 119.6, 119.5, 119.4, 118.5, 112.3, 111.4, 110.3, 82.2, 47.5; HRMS (ESI) calculated for $C_{26}H_{17}N_3O$ $[M+H]^+$ 388.1444, found 388.1461.

5.2.3. 6-Cyano-5-methoxy-11-methyl-12-methylindolo[2,3-*a*]carbazole (7a) from 6b

K_2CO_3 (38 mg, 0.28 mmol) and MeI (197 mg, 1.4 mmol) were added to a solution of **6b** (43 mg, 0.14 mmol) in DMF (2 mL), and the mixture was stirred at rt for 2 h. After addition of the H_2O under ice water bath, the whole solution was extracted with EtOAc. The extract was washed by brine, dried over Na_2SO_4, and evaporated under reduced pressure to leave a residue, which was column-chromatographed on SiO_2 with EtOAc–hexane (1:2, v/v) to give **7a** (41 m g, 90%). ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.66 (d, J = 8.0 Hz, 1H), 8.31 (d, J = 7.6 Hz, 1H), 7.70–7.45 (m, 4H), 7.45–7.20 (m, 2H), 4.30 (s, 3H), 4.20 (s, 3H), 4.12 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 154.8, 143.9, 142.3, 132.4, 126.3, 125.7, 125.3, 122.5, 122.0, 121.9, 121.0, 120.8, 120.2, 117.3, 115.2, 110.0, 109.5, 87.5, 61.7, 36.5, 36.0; HRMS (ESI) calculated for $C_{22}H_{17}N_3O$ $[M+H]^+$ 340.1444, found 340.1457.

5.2.4. 6-Cyano-5-methoxy-11-methyl-12-benzylindolo[2,3-*a*]carbazole (7b) from 6c

Synthesized by method above by using **6c** as a starting material. Yield = 95%. ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.70 (d, J = 8.0 Hz, 1H), 8.38 (d, J = 7.2 Hz, 1H), 7.60–7.10 (m, 11H), 5.75 (s, 2H), 4.35 (s, 3H), 3.80 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 154.8, 144.0, 142.3, 136.5, 132.5, 128.9, 128.2, 127.3, 126.4, 125.8, 125.4, 122.7, 122.4, 122.3, 122.1, 121.5, 120.8, 120.2, 177.2, 115.8, 110.8, 110.0, 88.0, 61.8, 51.8, 36.2; HRMS (ESI) calculated for $C_{28}H_{21}N_3O$ $[M+H]^+$ 416.1757, found 416.1768.

5.2.5. 6-Cyano-5-methoxy-12-methylindolo[2,3-*a*]carbazole (8) from 7b

To a stirred suspension of $AlCl_3$ (143 mg, 1.1 mmol) in anisole (5 mL) in an ice bath was added a solution of **7b** (80 mg, 0.2 mmol) in anisole (5 mL). The mixture was stirred at 110 °C for 2 h. The reaction mixture was poured into water (20 mL) and extracted with EtOAc. The extracts were washed with 5% $NaHCO_3$ (10 mL), water (10 mL), and brine (10 mL) and dried over Na_2SO_4. The solvent was evaporated, and the residue column-chromatographed on SiO_2 with EtOAc–hexane (1:2, v/v) to give **8** (62 mg, 100%). ^1H NMR (DMSO-d_6, 400 MHz) δ (ppm): 12.16 (s, 1H), 8.46 (d, J = 7.6 Hz, 1H), 8.24 (d, J = 8.0 Hz, 1H), 7.90–7.70 (m, 2H), 7.63–7.45 (m, 2H), 7.45–7.25 (m, 2H), 4.36 (s, 3H), 4.22 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 154.4, 140.8, 139.8, 129.2, 126.1, 125.8, 123.9, 121.7, 121.0, 120.9, 120.8, 119.7, 119.6, 117.8, 117.7, 113.8, 112.1, 110.2, 86.1, 62.2, 31.9; HRMS (ESI) calculated for $C_{21}H_{15}N_3O$ $[M+H]^+$ 326.1288, found 326.1302.

5.2.6. 5-Methoxy-12-methylindolo[2,3-*a*]carbazole-6-carboxylic acid amide (9)

A mixture of the nitrile **1b** (44 mg, 0.14 mmol), 35% hydrogen peroxide (0.1 mL) and 3 N aqueous NaOH (0.03 mL) in ethanol (2 mL) was stirred at 30 °C for 20 h. The resulting mixture was acidified with 1 N sulphuric acid, the precipitate was filtered, dried, which was column-chromatographed on SiO_2 with MeOH–CH_2Cl_2 (1:19, v/v) to afford the compound **9** (33 mg, 70%). ^1H NMR (acetone-d_6, 400 MHz) δ (ppm): 11.10 (br s, 1H), 8.62 (d, J = 8.0 Hz, 1H), 8.35 (d, J = 7.8 Hz, 1H), 7.71 (t, J = 8.2 Hz, 2H), 7.57 (t, J = 7.2 Hz, 1H), 7.51 (t, J = 7.4 Hz, 1H), 7.37 (q, J = 7.8 Hz, 2H), 4.45 (s, 3H), 4.32 (s, 3H); ^{13}C NMR (acetone-d_6, 100 MHz) δ (ppm): 170.1, 154.6, 140.3, 140.2, 125.7, 125.3, 121.7, 121.4, 121.2, 120.3, 119.4, 116.9, 112.7, 111.5, 109.0, 86.5, 61.2, 41.7; HRMS (ESI) calculated for $C_{21}H_{17}N_3O_2$ $[M+H]^+$ 344.1395, found 344.1399.

Acknowledgments

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