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Original article

Structure–activity relationships of indole compounds derived from combretastatin A4: Synthesis and biological screening of 5-phenylpyrrolo[3,4-*a*] carbazole-1,3-diones as potential antivascular agents

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ABSTRACT

A series of 5-(3′,4′,5′-trimethoxyphenyl)pyrrolo[3,4-*a*]carbazole-1,3(2*H*,10*H*)-diones was designed as cis-restricted analogues of 3-aroylindoles, arylthioindoles and 3-benzylidoneindolin-2-ones derived from combretastatin A4 (CA-4). Starting from various indoles, compounds were synthesized by means of a convenient two-step procedure involving a one-pot multicomponent reaction as key step. Intermediate tetrahydro[3,4-*a*]carbazoles and their corresponding carbazoles were submitted to biological screening tests involved in antivascular action, including the cytotoxicity against murine B16 melanoma cells, the rounding up of endothelial cells (EA.hy 926) and the inhibition of tubulin polymerization. Of the 31 compounds screened, those bearing a methoxy group at the 8-position endowed significant biological activities. A carbazole compound **30** was identified as a promising candidate for further development of novel vascular targeting agents.

1. Introduction

During the two last decades, one of the most significant advances in cancer therapy has been likely the development of targeted therapies including small molecules and monoclonal antibodies, offering the theoretical advantages of limited adverse effects on normal cells and of their use in combination with other therapies without adversely affecting tolerability [1].

The tumor vasculature presents an attractive target in cancer therapy because a functional network of blood vessels is an essential requirement for tumor progression and metastasis, and also because tumor vessels are markedly distinct from functional normal vessels [2]. Furthermore, impairment of oxygen and nutrients supply by a single vessel can lead to the destruction of numerous tumor cells [2].

Two different approaches have emerged to target tumor vasculature: i) the antiangiogenic approach which aims to prevent the formation of new vessels in tumors, and ii) the antivascular approach that compromises established tumor vasculature, using 'vascular disrupting agents' (VDAs), which leads to hemorrhagic necrosis within the tumors [2,3].

Apart from flavone acetic acid analogues, small molecule VDAs [2,3c,4] also include antitubulin agents and particularly inhibitors of tubulin polymerization interacting at the colchicine (**1**) binding-site on tubulin such as combretastatin A4 (CA-4, 2), a stilbene initially isolated from the bark of *Combretum caffrum* Kuntz (Fig. 1) [5]. The corresponding disodium phosphate prodrug of CA-4 (fosbretabulin, **3**) is currently in advanced stage clinical development since it recently entered phase II/III studies in combination with carboplatin or paclitaxel in patients with anaplastic thyroid cancer. [4,6] Moreover, second-generation VDAs such as the CA-4P analogue OXi4503 (CA-1P, **4**) are under active development. The OXi4503 is a compound presenting a dual mechanism of action, i.e., in addition to its vascular disrupting properties, it also displays a direct cytotoxic activity against tumor cells, therefore offering an improved antitumor efficacy [5b,6,7].

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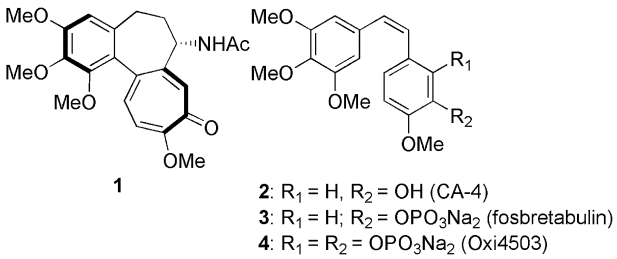


Fig. 1. Structures of colchicine, CA-4, fosbretabulin and Oxi4503.

CA-4 has attracted considerable research attention because of its potent biological properties and also because of its relatively simple chemical structure. Considerable efforts have been devoted to the synthesis of non-isomerizable analogues of CA-4, since its corresponding *E*-isomer (*trans*) is devoid of vascular disrupting activity. In this context, the indole nucleus, frequently found in natural tubulin polymerization inhibitors, has been advantageously employed as core structure of potent synthetic tubulin inhibitors [8]. Indeed, several recent studies have highlighted the potent activities of the structurally related arylthioindoles (ATIs) [9], 3-aroylindoles [10] and 1,3-dihydroindol-2-ones[11] (Fig. 2).

In these series, the most active compounds shared some common structural features, i.e., two non-coplanar aryl groups (including a 3,4,5-trimethoxyphenyl unit linked through a two-atom bridge), and a methoxy group either at the 5- or 6- position on the phenyl ring of indole nucleus. To our knowledge, there is no prior report on the synthesis and biological evaluation of *cis*-locked analogues of these indolic derivatives as potential antivascular agents. Therefore, as an extension of our previous work on 3-aroylindoles [10b] we focused our attention on the synthesis and biological activities of a new series of 5-phenylpyrrolo[3,4-*a*]carbazole-1,3-diones (**5**–**35**) [12]. In particular, we report here the effects on the biological activities of the following modifications: i) substitutions at the 7- and/or 8-positions in the A-ring (most of the synthesized compounds are disubstituted, bearing a methoxy group at the 7-position (R_2) and a substituent of various electronic character at the 8-position (R_3)); ii) aromaticity of the C-ring; and iii) pyrrole *N*-benzylation. The biological screening tests included cytotoxicity against B16 melanoma cells, the analysis of the morphological effects on immortalized HUVEC (EAhy 926), and the effect on the inhibition of tubulin polymerization (ITP).

2. Chemistry

The structures of target 5-phenylpyrrolo[3,4-*a*]carbazoles are depicted in Table 1. Tetrahydrocarbazoles (THCs) **5**–**10** and **12**–**17** were prepared by means of a convenient one-step, three component reaction involving a maleimide, the 3,4,5-trimethoxyphenylacetophenone, and various indoles (Scheme 1) [13]. Each THC is obtained as a unique racemic pair of diastereoisomers in a *cis* relative configuration [14] (Fig. 3). Reaction conditions, originally described by Noland et al. [13], were optimized to reduce the formation of both corresponding carbazoles and undesired by-products. However, in some cases, particularly when nitroindoles and/or maleimide were engaged in the one-pot reaction, carbazoles were obtained either concomitantly with tetrahydrocarbazoles (**26**, **28**, **31**, **32**, **34**) or as the sole reaction product (**23**). Finally, carbazoles **19**–**22**, **24**, **26**, **29**–**32** and **34** were synthesized, in good to excellent yield, by oxidation of the corresponding THCs with DDQ.

THCs **11**, **18** and carbazoles **27**, **35** (Scheme 2), bearing an amino group at the 8-position, were prepared by reduction with Zn/AcOH of respective 8-nitro derivatives **10**, **17** and **26**, **34**. Attempts to obtain 8-amino-7-methoxyTHCs from trifluoroacetamides **9** and **16** were unsuccessful, mainly due to the low stability of these compounds under basic conditions. In contrast, under the same conditions, carbazoles **24** and **32** could be easily deprotected to afford compounds **25** and **33**.

3. Biological evaluation

The biological activities of the 31 new 5-phenylpyrrolo[3,4-*a*]carbazoles are summarized in Tables 2–4. The compounds were tested for their potential vascular disrupting effects by means of

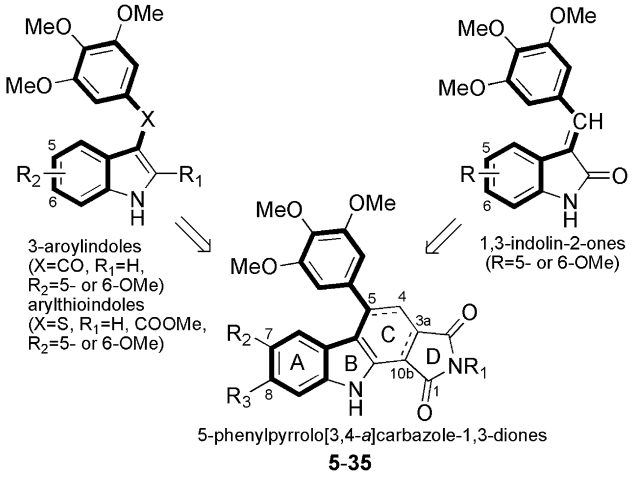
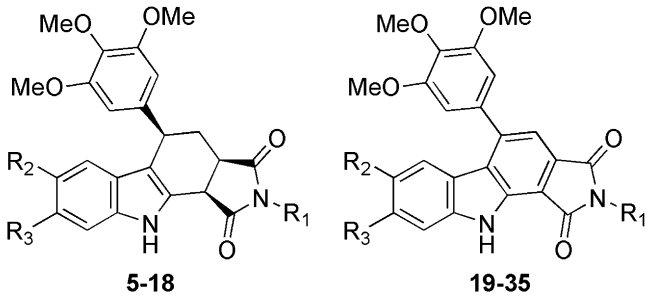


Fig. 2. General structures of selected 3-aroylindoles, 3-arylthioindoles, 1,3-indolin-2-ones, and of 5-phenylpyrrolo[3,4-*a*]carbazole-1,3-diones **5**–**35**.

Table 1
Structures of 5-phenylpyrrolo[3,4-*a*]carbazole-1,3-diones.



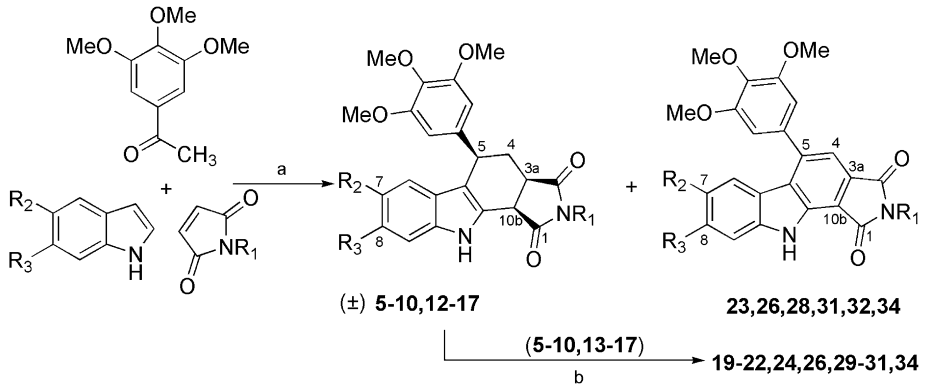
Compound[a]		R_1	R_2	R_3
THC	C			
5	**19**	Bn	H	H
6	**20**	Bn	OCH_3	H
7	**21**	Bn	H	OCH_3
8	**22**	Bn	OCH_3	Br
–	**23**	Bn	OCH_3	NO_2
9	**24**	Bn	OCH_3	$NHCOCF_3$
–	**25**	Bn	OCH_3	NH_2
10	**26**	Bn	NO_2	H
11	**27**	Bn	NH_2	H
12	**28**	H	H	H
13	**29**	H	OCH_3	H
14	**30**	H	H	OCH_3
15	**31**	H	OCH_3	Br
16	**32**	H	OCH_3	$NHCOCF_3$
–	**33**	H	OCH_3	NH_2
17	**34**	H	NO_2	H
18	**35**	H	NH_2	H

[a] THC = tetrahydrocarbazole; C = carbazole.

three screening tests that have previously been employed successfully to identify potent 3-aroylindoles [10b]. These tests include cytotoxicity against B16 melanoma cells, induction of a rapid morphological change of endothelial EA.hy 926 cells (rounding up), considered predictive of a potential in vivo anti-vascular activity [16], and the inhibition of tubulin polymerization (ITP). Colchicine (**1**) and CA-4 (**2**) were routinely included in the tests, because a good correlation is observed between their potent inhibitory activities on cancer cells, their marked effects on the morphology (rounding up) of endothelial cells, and their strong inhibition of tubulin polymerization.

The biological activities were examined for the following compounds: the non-substituted compounds (**5**, **12**, **19**, **28**); the 7-monosubstituted compounds bearing a methoxy (**6**, **13**, **20**, **29**), an amino (**11**, **18**, **27**, **35**) or a nitro (**10**, **17**, **26**, **34**) group; and the 7,8-disubstituted (**8**, **9**, **15**, **16**, **22**–**25**, **31**–**33**) compounds. As presented in Tables 2 and 3, the nature and the position of the A-ring substituents markedly influenced the biological effects.

Concerning the cytotoxic activity, compounds **5**, **14**, **19**, **21**, **27**, and **30** were the most active ones, with IC_{50} in the low micromolar range (<10 μM) (Tables 2 and 3). For comparison purposes, reference compounds colchicine **1** and CA-4 **2** exhibited cytotoxic activity in the nanomolar range, as expected. For the tetrahydrocarbazoles (Table 2), cytotoxic activity was best for the *N*-benzylated compound **5** bearing no substituent at positions R_2 and R_3; whereas for the *N*-H series compound **14** bearing a methoxyl at R_3



Scheme 1. Synthesis of Tetrahydrocarbazoles **5**–**10**, **12**–**17** and Carbazoles **19**–**24**, **26**, **28**–**32**, **34**. Reagents and conditions: a) 35% aq. HCl (0.3 or 1.3 equiv), EtOH, reflux; b) DDQ (2 equiv), 1,4-dioxane, reflux, 30 min.

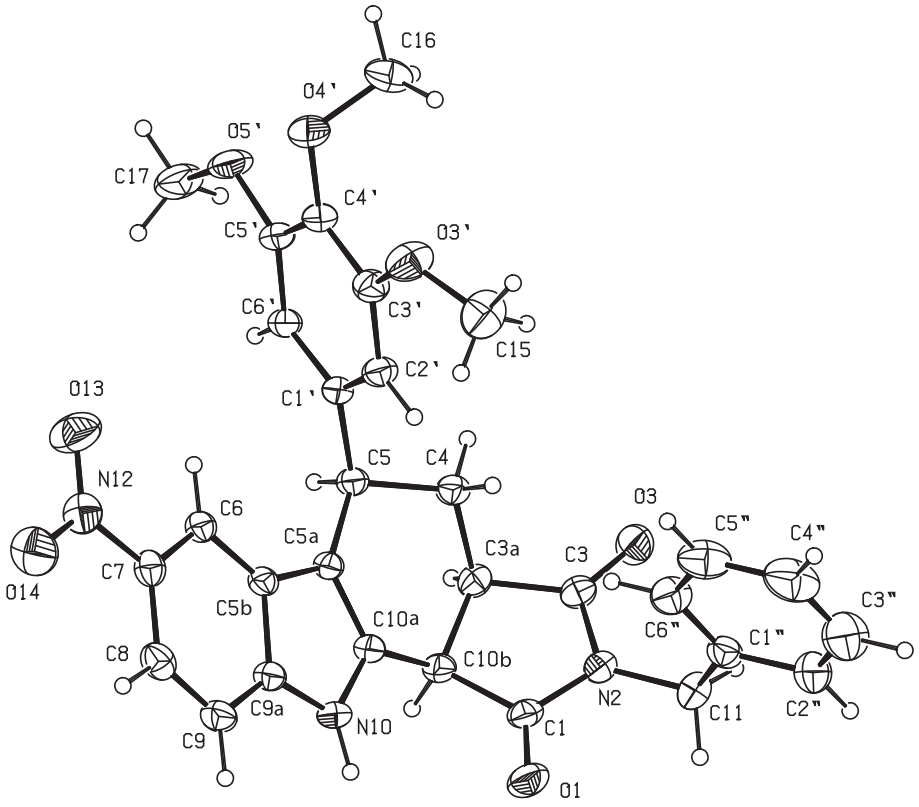
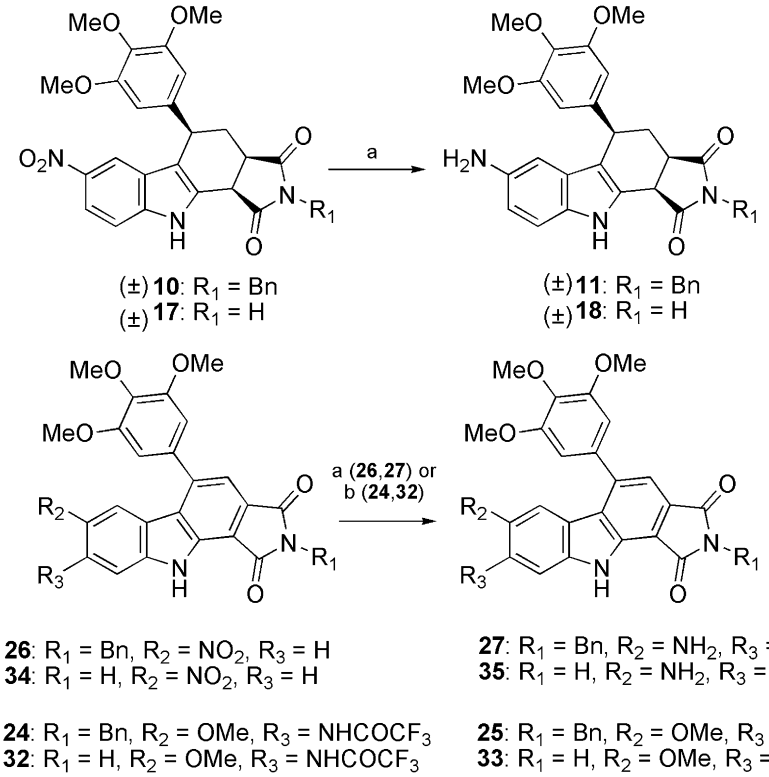
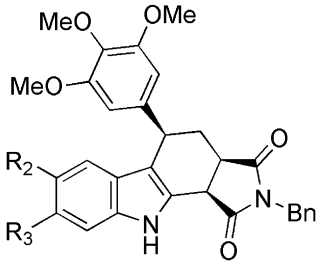


Fig. 3. Ortep drawing of the molecule (drawn with program *PLATON*) [15] giving the atomic labelling. Displacement ellipsoids are shown at the 30% probability level.

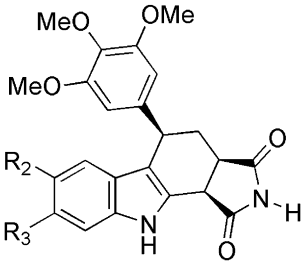


Scheme 2. Synthesis of aminotetrahydrocarbazoles **11**, **18** and aminocarbazoles **25**, **27**, **33**, **35**. Reagents and conditions: a) Zn (30 equiv), AcOH (20 equiv), THF, 20 °C; b) K_2CO_3 (2 equiv), 1,4-dioxane-H_2O, 20 °C, 17 h.

Table 2
Biological activities of tetrahydrocarbazoles in the *N*-Bn and the *N*-H series.



5-11



12-18

Compound	R_2	R_3	Cytotoxicity IC_{50} [μM][a]	Morphology[μM][b]
5	H	H	3.7 ± 0.1	50
6	OCH_3	H	ND[c] (>80)	NA[d]
7	H	OCH_3	ND (>80)	2.5
8	OCH_3	Br	ND (>40)	NA
9	OCH_3	$NHCOCF_3$	20.0 ± 5.3	20[e]
10	NO_2	H	ND (>40)	NA
11	NH_2	H	16.6 ± 2.8	10
12	H	H	ND (>40)	1.3
13	OCH_3	H	ND (>80)	NA
14	H	OCH_3	2.6 ± 1.5	0.3
15	OCH_3	Br	60.9 ± 4.3	50
16	OCH_3	$NHCOCF_3$	23.1 ± 4.2	NA
17	NO_2	H	ND (>50)	NA
18	NH_2	H	ND (>100)	100
Colchicine (**1**)	–	–	0.031 ± 0.003	0.003
CA-4 (**2**)	–	–	0.003 ± 0.001	0.008

[a] Cytotoxicity on murine B16 melanoma cells. Results are expressed as the mean ± SEM of the concentration that causes 50% cell kill (IC_{50}).
[b] The lowest active concentration that causes a rounding up of immortalized HUVEC (EA.hy 926) after a 2 h exposure time.
[c] ND: IC_{50} not precisely determined.
[d] NA: no rounding up observed at the maximum of solubility.
[e] Endothelial cells showed a stellate morphology.

was the most cytotoxic. In the carbazole series (Table 3), *N*-benzylated analogues bearing no substituent at R_2 and R_3 (**19**), or bearing a methoxyl at R_3 (**21**) was linked to a significant cytotoxic activity. Also of interest, compound **27** bearing a NH_2 on R_2 was also markedly cytotoxic. In the *N*-H carbazole series (Table 3), compound **30** bearing a methoxy group at R_3 was the most cytotoxic with an IC_{50} value in the nanomolar range.

With regard to the morphological effects on endothelial cells, only compounds **7**, **12**, **14**, and **30** presented a rounding up at low micromolar concentrations (<10 μM) (Tables 2 and 3). Fig. 4 shows representative photographs of these morphologically active compounds compared to control (1% DMSO) and CA-4 (**2**). Of these compounds, **30** was the most active with a rounding up of endothelial cells still significant in the nanomolar range (25 nM, Table 3). With the exception of **12**, this morphological activity on endothelial cells was linked to the presence of a methoxy group at R_3.

In terms of inhibition of tubulin polymerization (ITP) activity, only the 8-methoxylated compounds **7**, **14** and **30** presented significant activity with IC_{50} in the low micromolar to submicromolar concentrations (Table 4, Fig. 5). It is noteworthy that these IC_{50} values were rather close to the ones of the reference compounds colchicine (**1**) and CA-4 (**2**), especially regarding compound **30**.

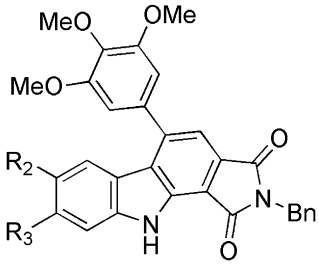
Taking into account the results of the three biological testing procedures employed in this study, **30** emerged as a particularly active compound. Indeed, in addition to its excellent inhibition of tubulin polymerization activity (close to reference compounds **1** and **2**), the concentration of 25 nM to cause the rounding up of endothelial cells was also remarkable. However, the cytotoxic activity of **30** was several-fold higher (200-fold) than CA-4 (2) indicating a selective antivascular activity with low intrinsic cytotoxicity. This may be a result of differences in cell permeability.

4. Discussion

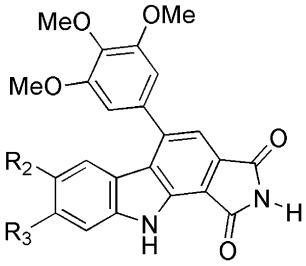
From the above results, some interesting structure–activity relationships can be disclosed. Several structural modifications led to a marked decrease in biological potency of **30**. Thus, the corresponding tetrahydrocarbazole **14**, which differs mainly in the spatial orientation of the 3,4,5-trimethoxyphenyl ring, was less active than **30** in the three biological tests (i.e. IC_{50} = 1.13 and 2.60 μM for tubulin inhibition and cytotoxicity, respectively, and a morphological effect observed at a 0.30 μM concentration). Furthermore, *N*-alkylation of compounds **30** and **14** at the 2-position on the maleimide ring, by a bulky benzyl group was clearly deleterious for biological activities. Indeed, carbazole **21** displayed weaker cytotoxicity (IC_{50} = 3.9 μM) and was almost inactive in the two other tests, whereas its corresponding tetrahydrocarbazole **7**, retained only poor activities in both the tubulin polymerization assay (IC_{50} = 3.49 μM) and the morphological test on endothelial cells (2.5 μM). Finally, and as already noted, shifting the methoxy group from the 8 to the 7 position (tetrahydrocarbazoles **6**, **13** and carbazoles **20**, **29**) led to a dramatic loss of activities.

These results clearly demonstrate that the planar pyrrolo[3,4-*a*] carbazole nucleus (designated herein as 'carbazole'), bearing a 3,4,5-trimethoxyphenyl ring at the 5-position, may represent a novel scaffold in the development of new potential antivascular agents. Beside the requirement of an aromatic C-ring to maintain an optimal geometry between the A-ring and the 3,4,5-trimethoxyphenyl ring, the main structural features that contribute to

Table 3
Biological activities of carbazoles in the *N*-Bn and the *N*-H series.



19-27



28-35

Compound	R_2	R_3	Cytotoxicity IC_{50} [μM][a]	Morphology [μM][b]
19	H	H	3.3 ± 2.0	NA[c]
20	OCH_3	H	12.8 ± 4.9	100
21	H	OCH_3	3.9 ± 1.3	100
22	OCH_3	Br	ND (>100)	NA
23	OCH_3	NO_2	ND (>50)	NA
24	OCH_3	$NHCOCF_3$	ND (>100)	NA
25	OCH_3	NH_2	ND (>50)	NA
26	NO_2	H	ND (>25)	NA
27	NH_2	H	4.1 ± 0.8	NA
28	H	H	ND (>100)	100
29	OCH_3	H	ND (>100)	100
30	H	OCH_3	0.6 ± 0.1	0.025
31	OCH_3	Br	13.5	100
32	OCH_3	$NHCOCF_3$	43.9 ± 4.0	NA
33	OCH_3	NH2	16.8 ± 0.2	100
34	NO_2	H	>50	NA
35	NH_2	H	12.8 ± 2.9	NA
Colchicine (**1**)	–	–	0.031 ± 0.003	0.003
CA-4 (**2**)	–	–	0.003 ± 0.001	0.008

[a] Cytotoxicity on murine B16 melanoma cells; ND: IC_{50} not precisely determined.
[b] The lowest active concentration that causes a rounding up of immortalized HUVEC (EA.hy 926) after a 2 h exposure time.
[c] NA, not active at maximum solubility in cell culture medium.

biological activity are the presence of a methoxy group at the 8-position, and a free NH at the pyrrole N2 position.

Interestingly, the lack of activity of the 7-methoxylated compounds may suggest that 5-phenylpyrrolo[3,4-*a*]carbazole-1,3-diones could rather be considered as *cis*-locked analogues of 3-aroylindoles and 1,3-indolin-2-ones, instead of analogues of ATIs. Indeed, in the first two series, and unlike ATIs, compounds bearing a methoxyl at a position equivalent to the 8-position on the carbazole scaffold (i.e., the 6-position, cf. Fig. 2) were found to be the most active ones [9,10,11].

5. Conclusion

By means of a systematic biological screening of 31 new 5-phenylpyrrolo[3,4-*a*]carbazole-1,3-diones, based on three simple tests, several cytoskeletal disrupting compounds were identified. Of particular interest, the carbazole **30**, bearing a methoxyl at the 8-position, emerged as new lead. This potent inhibitor of tubulin polymerization induces a significant rounding up of endothelial cells at nanomolar concentrations, and could be considered as a *cis*-restricted analogues of 3-aroylindoles and 1,3-indolin-2-ones derived from CA-4. Other substitution pattern on the A-ring led to decrease in biological activities. These results deserve further developments to fully ascertain the potential antivascular activity of **30** and to better define the SAR of the new pyrrolo[3,4-*a*] carbazole scaffold.

Table 4
Inhibition of tubulin polymerization of selected compounds.

Compound	IC_{50} [μM][a]
7	3.49
12	>30
14	1.13
30	0.82
Colchicine (**1**)	0.27
CA-4 (**2**)	0.37

[a] Above a threshold value of 30 μM, IC50 were not precisely determined.

6. Experimental section

All reagents were used as purchased without further treatment unless otherwise stated. All solvents were dried according to standard procedures. Melting points were determined on a LEICA VM microscope equipped with a heating stage and are uncorrected. Infrared spectra were obtained with a Nicolet 510 FT-IR apparatus. NMR spectra were recorded at 300 or 400 MHz (^1H) and at 75 or 100 MHz (^{13}C) with a AC 300 and an Advance 400 BRUKER spectrometers. Chemical shifts are given in parts per million (ppm, δ) relative to solvent peaks as internal standards (δ: $CDCl_3$: 7.27 ppm (^1H), 77.0 ppm (^{13}C); DMSO-*d6*: 2.50 ppm (^1H), 40.6 ppm (^{13}C); acetone-*d6*: 2.05 ppm (^1H), 29.8 and 206.0 ppm (^{13}C); coupling constants are given in Hertz (Hz, *J*)). Mass spectra (MS) were measured with a Nermag R10-10C mass spectrometer (CI/NH_3) or with a ZQ 2000 Waters mass spectrometer (ESI); High-resolution mass spectrometry (HRMS) spectra were performed at the Laboratoire de Spectrométrie de Masse (I.C.S.N./C.N.R.S., Gif sur Yvette,

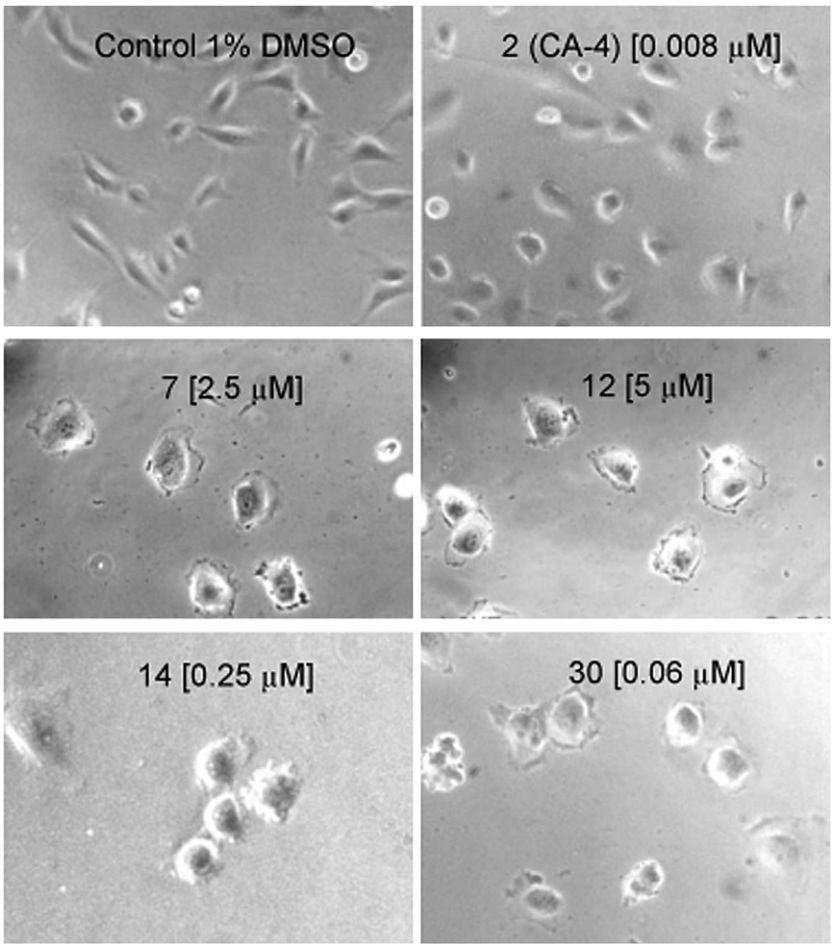


Fig. 4. Morphological effects of selected 5-phenylpyrrolo[3,4-*a*]carbazole-1,3-diones. Exponentially growing endothelial cells (EA.hy 926) were exposed to the test compounds at the indicated concentrations and incubated for 2 h (37 °C, 5% CO_2). Representative photographs shown were recorded at a magnification of 100× for Control and CA-4 (**2**), and at 200× for compounds **7**, **12**, **14** and **30**.

France). Elemental analysis were performed at the Laboratoire de Microanalyses (I.C.S.N./C.N.R.S., Gif sur Yvette, France). Analyses indicated by the symbols of the elements were within −0.4% of the theorical values. Flash column chromatography was performed with silica gel (SDS 60 ACC 35–70 μM). The reactions were monitored by thin layer chromatography (TLC) using Merck Kieselgel 60 F254 silica gel; zones were detected visually under ultraviolet irradiation (254 and 366 nm) and by spraying with sulfuric vanillin

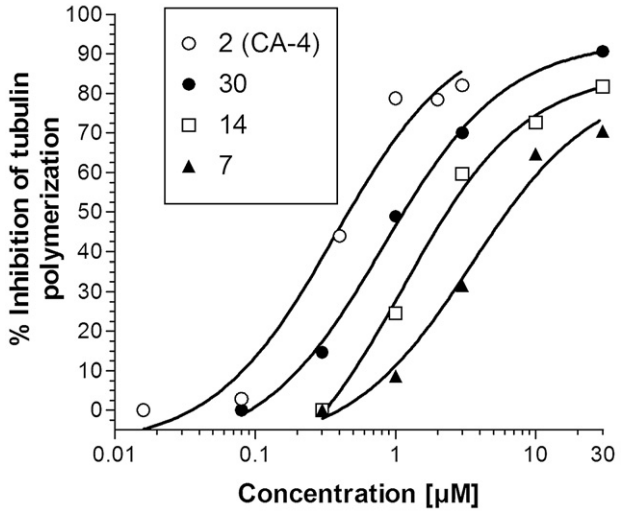


Fig. 5. Representative inhibition curves of tubulin polymerization with various concentrations of compounds **2** (CA-4, open circles), **30** (solid circles), **14** (open squares), and **7** (solid triangles).

followed by heating. OPLC (Optimum Performance Layer Chromatography) was performed with a OPLC 50™ Bionisis apparatus.

6.1. General procedure 1 for the synthesis of tetrahydrocarbazoles with N-benzylmaleimide: preparation of compounds **5**–**10**

To a mixture of indole, 3,4,5-trimethoxyacetophenone and *N*-benzylmaleimide in equimolar quantities (0.86 mmol) were successively added absolute ethanol (5 mL) and a 35% aqueous solution of hydrochloric acid (from 0.33 to 1.3 equivalents, depending on the case). The mixture was heated at reflux until the disappearance of the indole (usually noted after a reaction time of 16–20 h) and, after being cooled in an ice bath, was filtered. The precipitate was then washed with cold ethanol and dried over P_2O_5. A silica gel chromatography of the precipitate could be performed to isolate the desired tetrahydrocarbazole if needed.

6.1.1. (+/−)-(3aR,5R,10bR)-2-Benzyl-5-(3′,4′,5′-trimethoxyphenyl)-5,10,10 b-tetrahydropyrrolo[3,4-a]carbazole-1,3(2H,3aH)-dione (**5**)

According to general procedure 1 (volume of 35% HCl: 25 μL, 0.33 eq), compound **5** was obtained as a pale pink solid (242 mg, 57%). Mp (CH_2Cl_2/MeOH): 112–115 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.12 (m, 1H), 2.47 (m, 1H), 3.54 (m, 1H), 3.65 (s, 6H, $OCH_3 \times 2$), 3.72 (s, 3H, OCH_3), 4.26 (m, 1H), 4.41 (d, J = 7.5 Hz, 1H), 4.49 (d, J = 14.6 Hz, 1H), 4.57 (d, J = 14.6 Hz, 1H), 6.47 (s, 2H), 6.77 (d, J = 7.5 Hz, 1H), 6.82 (t, J = 7.5 Hz, 1H), 7.05 (td, J = 7.5 Hz, J = 1.0 Hz, 1H), 7.29–7.23 (m, 5H), 7.47 (d, J = 7.5 Hz, 1H), 10.35 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 36.4, 40.3, 40.7, 41.7, 43.2, 56.9 (x 2), 61.1, 107.2 (x 2), 113.0, 114.4, 120.0, 120.8, 122.7, 127.5, 129.5 (x 6), 137.9, 138.7 (x 2), 141.0, 154.8 (x 2), 177.1, 179.4; IR (NaCl): ν′ 3374, 3060, 2998, 2939, 2838, 1776, 1702, 1591, 1506, 1461, 1422 cm^{-1}; MS (ZQ2000/ESI+): m/z 519 [M + Na]$^+$, 535 [M + K]$^+$; Anal. $C_{30}H_{28}N_2O_5$ (C, H, N).

6.1.2. (+/−)-(3aR,5R,10bR)-2-Benzyl-7-methoxy-5-(3′,4′,5′-trimethoxyphenyl)-4,5,10,10b-tetrahydropyrrolo[3,4-a]carbazole-1,3(2H,3aH)-dione (**6**)

General procedure 1 was performed with 5-methoxyindole (0.86 mmol) and 100 μL (1.3 eq) of 35% HCl. After 22 h, tetrahydrocarbazole **6** was obtained pure as a white fluffy solid (171 mg, 38%). Mp (acetone/MeOH): 182–183 °C; ^{1}H NMR (300 MHz, acetone-*d6*): δ = 2.14 (ddd, J = 13.1 Hz, J = 9.6 Hz, J = 8.8 Hz, 1H), 2.47 (ddd, J = 13.1 Hz, J = 5.9 Hz, J = 4.5 Hz, 1H), 3.53 (m, 1H), 3.54 (s, 3H, OCH_3), 3.68 (s, 6H, $OCH_3 \times 2$), 3.73 (s, 3H, OCH_3), 4.23 (ddd, J = 8.8 Hz, J = 4.5 Hz, J = 1.4 Hz, 1H), 4.39 (dd, J = 8.4 Hz, J = 1.4 Hz, 1H), 4.49 (d, J = 14.6 Hz, 1H), 4.58 (d, J = 14.6 Hz, 1H), 6.22 (d, J = 2.5 Hz, 1H), 6.52 (s, 2H), 6.71 (dd, J = 8.8 Hz, J = 2.5 Hz, 1H), 7.31–7.22 (m, 5H), 7.36 (d, J = 8.8 Hz, 1H), 10.20 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, acetone-*d6*): δ = 34.8, 38.9, 39.3, 40.3, 41.8, 54.6, 55.5 (x 2), 59.6, 101.2, 105.8 (x 2), 111.3, 111.8, 112.9, 126.3, 127.5, 128.4–128.0 (x 4), 128.5, 132.3, 136.4, 137.1, 139.4, 153.3 (x 2), 153.5, 175.3, 178.0; IR (NaCl): ν′ 3380, 2929, 1778, 1702, 1590, 1459 cm^{-1}; MS (ZQ2000/ESI+): m/z 549 [M + Na]$^+$, 565 [M + K]$^+$; Anal. $C_{31}H_{30}N_2O_6$ (C, H, N).

6.1.3. (+/−)-(3aR,5R,10bR)-2-Benzyl-8-methoxy-5-(3′,4′,5′-trimethoxyphenyl)-4,5,10,10b-tetrahydropyrrolo[3,4-a]carbazole-1,3(2H,3aH)-dione (**7**)

General procedure 1 was performed with 6-methoxyindole (0.86 mmol) and 25 μL (0.33 eq) of 35% HCl. After 21 h, tetrahydrocarbazole **7** was obtained pure as a pink fluffy solid (108 mg, 24%). Mp (acetone/MeOH): 217–218 °C. ^{1}H NMR (300 MHz, acetone-*d6*): δ = 2.08 (m, 1H), 2.45 (ddd, J = 13.1 Hz, J = 5.9 Hz, J = 4.5 Hz, 1H), 3.52 (ddd, J = 9.7 Hz, J = 8.5 Hz, J = 5.9 Hz, 1H), 3.66 (s, 6H, $OCH_3 \times 2$), 3.73 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 4.21 (ddd, J = 8.5 Hz, J = 4.5 Hz, J = 1.5 Hz, 1H), 4.36 (dd, J = 8.4 Hz, J = 1.5 Hz, 1H), 4.50 (d, J = 14.6 Hz, 1H), 4.59 (d, J = 14.6 Hz, 1H), 6.48 (s, 2H), 6.51 (dd, J = 8.7 Hz, J = 2.2 Hz, 1H), 6.63 (d, J = 8.7 Hz, 1H), 7.02 (d, J = 2.2 Hz, 1H), 7.32–7.23 (m, 5H), 10.15 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, acetone-*d6*): δ = 35.1, 38.9, 39.2, 40.3, 41.7, 54.7, 55.5 (x 2), 59.7, 94.6, 105.6 (x 2), 108.6, 112.9, 119.9, 120.3, 126.6, 127.5, 128.0 (x 2), 128.4 (x 2), 136.4, 137.1, 138.1, 139.6, 153.3 (x 2), 156.2, 175.4, 178.0; IR (NaCl): ν′ 3372, 2930, 1774, 1702, 1590, 1455 cm^{-1}; MS (ZQ2000/ESI+): m/z 549 [M + Na]$^+$, 565 [M + K]$^+$; Anal. $C_{31}H_{30}N_2O_6$ (C, H, N).

6.1.4. (+/−)-(3aR,5R,10bR)-2-Benzyl-5-(3′,4′,5′-trimethoxyphenyl)-7-nitro-4,5,10,10b-tetrahydropyrrolo[3,4-a]carbazole-1,3(2H,3aH)-dione (**10**)

General procedure 1 was performed with 5-nitroindole (0.86 mmol) and 100 μL (1,3 eq) of 35% HCl. After 3 days, the crude precipitate (148 mg), consisting of carbazole **26** and tetrahydrocarbazole **10** in a 15:85 ratio (as estimated by ^{1}H NMR analysis; yields: 8 and 25%, respectively), was purified with difficulty by flash chromatography on silica gel (cyclohexane/EtOAc 95/5), providing pure compounds **26** and **10**.

Tetrahydrocarbazole **10** was obtained as a yellow fluffy solid. Mp (acetone/MeOH): 228–229 °C. ^{1}H NMR (300 MHz, acetone-*d6*): δ = 2.18 (ddd, J = 13.1 Hz, J = 10.1 Hz, J = 9.0 Hz, 1H), 2.55 (ddd, J = 13.1 Hz, J = 6.0 Hz, J = 4.3 Hz, 1H), 3.65 (ddd, J = 10.1 Hz, J = 8.5 Hz, J = 6.0 Hz, 1H), 3.68 (s, 6H, $OCH_3 \times 2$), 3.75 (s, 3H, OCH_3), 4.36 (ddd, J = 9.0 Hz, J = 4.3 Hz, J = 1.6 Hz, 1H), 4.53 (dd, J = 8.5 Hz, J = 1.6 Hz, 1H), 4.53 (d, J = 14.6 Hz, 1H), 4.61 (d, J = 14.6 Hz, 1H), 6.55 (s, 2H), 7.32–7.23 (m, 5H), 7.65 (d, J = 9.1 Hz, 1H), 7.68 (d, J = 2.2 Hz, 1H), 8.00 (dd, J = 9.1 Hz, J = 2.2 Hz, 1H), 11.12 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, acetone-*d6*): δ = 34.6, 38.5, 39.2, 40.2, 41.9, 55.6 (x 2), 59.8, 105.9 (x 2), 111.5, 115.8, 116.3, 116.7, 125.3, 127.6, 128.1 (x 2), 128.4 (x 2), 132.2, 136.3, 137.7, 138.5, 140.2, 141.1, 153.6 (x 2), 174.8, 177.6; IR (NaCl): ν′ 3330, 2924, 1778, 1704, 1587, 1458 cm^{-1}; MS (ZQ2000/ESI+): m/z 564 [M + Na]$^+$; Anal. $C_{30}H_{27}N_3O_7$ (C, H, N).

6.1.5. 2-Benzyl-5-(3′,4′,5′-trimethoxyphenyl)-7-nitropyrrolo[3,4-a] carba-zole-1,3(2H-10H)-dione (**26**)

Carbazole **26** was obtained as a bright yellow solid. Mp (acetone/*n*-hexane): 316–318 °C. ^{1}H NMR (300 MHz, DMSO-*d6*): δ = 3.82 (s, 3H, OCH_3), 3.83 (s, 6H, $OCH_3 \times 2$), 4.88 (s, 2H), 7.04 (s, 2H), 7.37–7.8 (m, 5H), 7.68 (s, 1H), 7.81 (d, J = 9.1 Hz, 1H), 8.39 (dd, J = 9.1 Hz, J = 2.3 Hz, 1H), 8.57 (d, J = 2.3 Hz, 1H), 13.06 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 41.3, 56.6 (x 2), 60.8, 106.7 (x 2), 113.1, 113.3, 116.2, 119.3, 121.0, 123.5, 126.5, 127.7 (x 2), 127.9, 129.1 (x 2), 130.9, 134.0, 135.6, 137.3, 138.6, 141.1, 144.1, 146.6, 153.9 (x 2), 167.8, 168.5; IR (NaCl): ν′ 3319, 2921, 2840, 1761, 1711, 1582, 1486, 1402 cm^{-1}; MS (ZQ2000/ESI+): m/z 560 [M + Na]$^+$, 576 [M + K]$^+$; Anal. $C_{30}H_{23}N_3O_7$ (C, H, N).

6.1.6. (+/−)-(3aR,5R,10bR)-2-Benzyl-8-bromo-7-methoxy-5-(3′,4′,5′-trimethoxyphenyl)-4,5,10,10b-tetrahydropyrrolo[3,4-a] carbazole-1,3(2H, 3aH)-dione (**8**)

General procedure 1 was performed with 6-bromo-5-methoxyindole (0.86 mmol) and 25 μL (0.33 eq) of 35% HCl. After 18.5 h, the crude product was purified by flash chromatography on silica gel (cyclohexane/EtOAc 96/4), providing tetrahydrocarbazole **9** as a light beige solid (270 mg, 52%). Mp (acetone/MeOH): 215–216 °C; ^{1}H NMR (300 MHz, acetone-*d6*): δ = 2.13 (ddd, J = 13.1 Hz, J = 10.1 Hz, J = 9.2 Hz, 1H), 2.49 (ddd, J = 13.1 Hz, J = 5.9 Hz, J = 4.3 Hz, 1H), 3.55 (s, 3H, OCH_3), 3.58 (ddd, J = 10.1 Hz, J = 8.5 Hz, J = 5.9 Hz, 1H), 3.69 (s, 6H, $OCH_3 \times 2$), 3.73 (s, 3H, OCH_3), 4.23 (ddd, J = 9.2 Hz, J = 4.3 Hz, J = 1.6 Hz, 1H), 4.41 (dd, J = 8.5 Hz, J = 1.6 Hz, 1H), 4.51 (d, J = 14.6 Hz, 1H), 4.60 (d, J = 14.6 Hz, 1H), 6.25 (s, 1H), 6.54 (s, 2H), 7.31–7.24 (m, 5H), 7.69 (s, 1H), 10.33 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, acetone-*d6*): δ = 34.7, 39.0, 39.2, 40.3, 41.8, 55.6 (x 3), 59.7, 101.7, 105.9 (x 3), 113.3, 115.5, 125.8, 127.5, 128.0 (x 2), 128.4 (x 2), 129.3, 132.4, 136.4, 137.2, 139.0, 149.3, 153.5 (x 2), 175.1, 177.9; IR (NaCl): ν′

3372, 2925, 1778, 1702, 1590, 1457, 1419 cm^{-1}; MS (ZQ2000/ESI+): *m/z* 629 $[M + Na]^+$, 645 $[M + K]^+$; Anal. $C_{31}H_{29}BrN_2O_6$ (C, H, N).

6.1.7. *N-[(+/−)-(3aR,5R,10bR)-2-Benzyl-1,2,3,3a,4,5,10,10b-octahydro-7-methoxy-5-(3′,4′,5′-trimethoxyphenyl)-1,3-dioxopyrrolo[3,4-a]carbazol-8-yl]-2,2,2-trifluoroacetamide (**9**)*

General procedure 1 was performed with 2,2,2-trifluoro-*N*-(5-methoxyindol-6-yl)acetamide (0.86 mmol) and 25 μL (0.33 eq) of 35% HCl. After 21 h, the crude product was purified by flash chromatography on silica gel (cyclohexane/EtOAc 95/5), providing tetrahydrocarbazole **9** as a white fluffy solid (229 mg, 42%). Mp (acetone/MeOH): 215–216 °C; ^{1}H NMR (300 MHz, acetone-*d6*): δ = 2.14 (m, 1H), 2.48 (ddd, *J* = 13.4 Hz, *J* = 5.8 Hz, *J* = 4.4 Hz, 1H), 3.57 (ddd, *J* = 9.9 Hz, *J* = 8.5 Hz, *J* = 5.8 Hz, 1H), 3.61 (s, 3H, OCH_3), 3.68 (s, 6H, $OCH_3 \times 2$), 3.72 (s, 3H, OCH_3), 4.24 (ddd, *J* = 9.1 Hz, *J* = 4.4 Hz, *J* = 1.3 Hz, 1H), 4.43 (dd, *J* = 8.5 Hz, *J* = 1.3 Hz, 1H), 4.50 (d, *J* = 14.6 Hz, 1H), 4.58 (d, *J* = 14.6 Hz, 1H), 6.28 (s, 1H), 6.53 (s, 2H), 7.29–7.23 (m, 5H), 8.30 (s, 1H), 9.19 (br. s, D_2O exch., 1H, NH), 10.41 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, acetone-*d6*): δ = 34.7, 39.0, 39.3, 40.3, 41.8, 55.4, 55.6 (x 2), 59.7, 100.3, 104.7, 105.9 (x 2), 113.4, 120.4, 123.3, 127.5, 128.0 (x 2), 128.4 (x 2), 129.1, 131.1, 136.4, 137.2, 139.6, 144.2, 153.4 (x 2), 175.2, 177.9; IR (NaCl): ν′ 3403, 2926, 1785, 1705, 1591, 1463, 1435 cm^{-1}; MS (ZQ2000/ESI+): *m/z* 660 $[M + Na]^+$, 676 $[M + K]^+$; Anal. $C_{33}H_{30}F_3N_3O_7$ (C, H, N).

6.1.8. *2-Benzyl-7-methoxy-5-(3′,4′,5′-trimethoxyphenyl)-8-nitropyrrolo[3,4-a]carbazole-1,3(2H,10H)-dione (**23**)*

General procedure 1 was performed with 5-methoxy-6-nitroindole (0.86 mmol) and 75 μL (1 eq) of 35% HCl. After 2 days, the crude product was purified by flash chromatography on silica gel (cyclohexane/EtOAc 75/25), providing carbazole **23** as a bright yellow solid (23 mg, 5%). Mp (acetone/*n*-hexane): 143–145 °C; ^{1}H NMR (300 MHz, DMSO-*d6*): δ = 3.63 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.82 (s, 6H, $OCH_3 \times 2$), 4.87 (s, 2H), 6.95 (s, 2H), 7.09 (s, 1H), 7.37–7.30 (m, 5H), 7.58 (s, 1H), 8.11 (s, 1H), 12.58 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 41.3, 56.6 (x 3), 60.7, 106.5 (x 3), 109.5, 112.5, 114.9, 125.0, 125.6, 127.7 (x 2), 128.8, 129.1 (x 2), 131.1, 134.1, 135.7, 136.0, 137.3, 138.4, 139.6, 144.7, 146.3, 153.8 (x 2), 167.9, 168.7; IR (NaCl): ν′ 3368, 2931, 2831, 1758, 1708, 1581, 1518, 1469, 1431, 1407 cm^{-1}; MS (ZQ2000/ESI+): *m/z* 567 $[M + H]^+$, 590 $[M + Na]^+$, 606 $[M + K]^+$; HRMS (ESI+): calcd for $C_{31}H_{25}N_3O_8Na$ $[M + Na]^+$ 590.1539, found 590.1564.

6.2. *General procedure 2 for the synthesis of tetrahydrocarbazoles with maleimide: preparation of compounds **12**–**17***

In these cases, the resulting ethanol solution of reactants was degassed prior to the heating stage.

6.2.1. *(+/−)-(3aR,5R,10bR)-5-(3′,4′,5′-Trimethoxyphenyl)-4,5,10,10b-tetrahydropyrrolo-[3,4-a]carbazole-1,3(2H,3aH)-dione (**12**)*

General procedure 2 was applied to 1*H*-indole (1.72 mmol) in presence of 50 μL (0.33 eq) of 35% HCl. After a reaction time of 2 days, the reaction mixture was filtered. The crude product was then washed with absolute ethanol, dried under vacuum and purified by flash chromatography on silica gel, using a gradient of EtOAc in CH_2Cl_2 (70/30 → 50/50) as eluent, in order to isolate carbazole **28** and tetrahydrocarbazole **12**.

Tetrahydrocarbazole **12** was obtained as an amorphous orange solid (272 mg, 39%). Mp (acetone/MeOH): 245–247 °C; ^{1}H NMR (300 MHz, acetone-*d6*): δ = 2.23 (ddd, *J* = 13.3 Hz, *J* = 8.7 Hz, *J* = 8.0 Hz, 1H), 2.46 (ddd, *J* = 13.3 Hz, *J* = 6.1 Hz, *J* = 4.8 Hz, 1H), 3.48 (td, *J* = 8.7 Hz, *J* = 6.1 Hz, 1H), 3.67 (s, 6H, $OCH_3 \times 2$), 3.71 (s, 3H, OCH_3), 4.27 (ddd, *J* = 8.0 Hz, *J* = 4.8 Hz, *J* = 1.5 Hz, 1H), 4.37 (dd, *J* = 8.7 Hz, *J* = 1.5 Hz, 1H), 6.51 (s, 2H), 6.79 (m, 1H), 6.84 (m, 1H), 7.06 (ddd, *J* = 8.1 Hz, *J* = 6.4 Hz, *J* = 1.8 Hz, 1H), 7.48 (d, *J* = 8.1 Hz, 1H), 10.06 (br. s, D_2O exch., 1H, NH), 10.30 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, acetone-*d6*): δ = 34.4, 38.8, 40.4, 41.4, 55.4 (x 2), 59.6, 105.7 (x 2), 111.2, 112.8, 118.6, 119.2, 121.3, 126.1, 128.3, 137.0, 137.3, 139.8, 153.3 (x 2), 176.2, 178.8; IR (KBr): ν′ 3470, 3345, 3186, 3073, 2942, 1784, 1708, 1592, 1508, 1459 cm^{-1}; MS (ZQ2000/ESI+): *m/z* 429 $[M + Na]^+$; Anal. $C_{23}H_{22}N_2O_5$ (C, H, N).

6.2.2. *5-(3′,4′,5′-Trimethoxyphenyl)pyrrolo[3,4-a]carbazole-1,3(2H,10H)-dione (**28**)*

Carbazole **28** was obtained as an orange amorphous solid (58 mg, 8%). Mp (acetone/*n*-hexane): 342–344 °C. ^{1}H NMR (300 MHz, acetone-*d6*): δ = 3.80 (s, 9H, $OCH_3 \times 3$), 6.92 (s, 2H), 7.10 (m, 1H), 7.42 (s, 1H), 7.47 (m, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 11.21 (br. s, D_2O exch., 1H, NH), 12.29 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, acetone-*d6*): δ = 56.5 (x 2), 60.7, 106.6 (x 2), 113.0 (x 2), 114.5, 120.4, 121.2, 122.6, 126.7, 128.1, 130.4, 134.1, 135.2, 138.1, 143.1 (x 2), 153.6 (x 2), 169.9, 170.5; IR (KBr): ν′ 3352, 3180, 3050, 2927, 2829, 2742, 1763, 1710, 1581, 1509, 1456 cm^{-1}; MS (ZQ2000/ESI+): *m/z* 425 $[M + Na]^+$, 441 $[M + K]^+$; Anal. $C_{23}H_{18}N_2O_5$ (C, H, N).

6.2.3. *(+/−)-(3aR,5R,10bR)-7-Methoxy-5-(3′,4′,5′-trimethoxyphenyl)-4,5,10,10b-tetrahydropyrrolo[3,4-a]carbazole-1,3(2H,3aH)-dione (**13**)*

General procedure 2 was performed with 5-methoxyindole (1.72 mmol) and 50 μL (0.33 eq) of 35% HCl. After a reaction time of 21 h, the crude product was purified by flash chromatography on silica gel (CH_2Cl_2/EtOAc 50/50), providing tetrahydrocarbazole **13** as a greenish fluffy solid (229 mg, 30%). Mp (acetone/MeOH): 253–255 °C; ^{1}H NMR (300 MHz, acetone-*d6*): δ = 2.23 (ddd, *J* = 13.2 Hz, *J* = 8.9 Hz, *J* = 8.3 Hz, 1H), 2.45 (ddd, *J* = 13.2 Hz, *J* = 6.0 Hz, *J* = 4.6 Hz, 1H), 3.47 (td, *J* = 8.9 Hz, *J* = 6.0 Hz, 1H), 3.54 (s, 3H, OCH_3), 3.69 (s, 6H, $OCH_3 \times 2$), 3.71 (s, 3H, OCH_3), 4.22 (ddd, *J* = 8.3 Hz, *J* = 4.6 Hz, *J* = 1.4 Hz, 1H), 4.35 (dd, *J* = 8.9 Hz, *J* = 1.4 Hz, 1H), 6.22 (d, *J* = 2.4 Hz, 1H), 6.55 (s, 2H), 6.70 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 1H), 7.36 (d, *J* = 8.8 Hz, 1H), 10.04 (br. s, D_2O exch., 1H, NH), 10.12 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, acetone-*d6*): δ = 34.3, 39.0, 40.4, 41.5, 54.6, 55.5 (x 2), 59.6, 101.2, 105.9 (x 2), 111.2, 111.8, 112.7, 126.4, 128.8, 132.3, 137.1, 139.6, 153.3 (x 2), 153.5, 176.2, 178.8; IR (KBr): ν′ 3408, 3335, 3199, 3076, 2946, 2833, 1783, 1709, 1589, 1463 cm^{-1}; MS (ZQ2000/ESI+): *m/z* 459 $[M + Na]^+$; Anal. $C_{24}H_{24}N_2O_6$ (C, H, N).

6.2.4. *(+/−)-(3aR,5R,10bR)-8-Methoxy-5-(3′,4′,5′-trimethoxyphenyl)-4,5,10,10b-tetrahydropyrrolo[3,4-a]carbazole-1,3(2H,3aH)-dione (**14**)*

General procedure 2 was performed with 6-methoxyindole (0.86 mmol) and 25 μL (0.33 eq) of 35% HCl. After 16 h, the reaction mixture was filtered and the mother liquors were thereafter purified by flash chromatography on silica gel (CH_2Cl_2/EtOAc 95/5) and then by OPLC (CH_2Cl_2/EtOAc 60/40, flash volume 750 μL, elution flow rate 750 μL/min), thus providing **14** as a beige amorphous solid (36 mg, 10%). Mp (acetone/*n*-hexane): 261–263 °C. ^{1}H NMR (300 MHz, acetone-*d6*): δ = 2.19 (ddd, *J* = 13.2 Hz, *J* = 8.8 Hz, *J* = 8.0 Hz, 1H), 2.43 (ddd, *J* = 13.2 Hz, *J* = 6.1 Hz, *J* = 4.8 Hz, 1H), 3.45 (td, *J* = 8.8 Hz, *J* = 6.1 Hz, 1H), 3.67 (s, 6H, $OCH_3 \times 2$), 3.71 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 4.21 (ddd, *J* = 8.0 Hz, *J* = 4.8 Hz, *J* = 1.7 Hz, 1H), 4.31 (dd, *J* = 8.8 Hz, *J* = 1.7 Hz, 1H), 6.50 (dd, *J* = 8.7 Hz, *J* = 2.2 Hz, 1H), 6.51 (s, 2H), 6.65 (d, *J* = 8.7 Hz, 1H), 7.02 (d, *J* = 2.2 Hz, 1H), 10.04 (br s., D_2O exch., 1H, NH), 10.09 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, acetone-*d6*): δ = 34.4, 38.9, 40.4, 41.4, 54.7, 55.4 (x 2), 59.7, 94.6, 105.6 (x 2), 108.6, 112.8, 119.8, 120.4, 126.8, 137.0, 138.1, 139.9, 153.3 (x 2), 156.2, 176.5, 178.9; IR (NaCl): ν′ 3406, 3166, 3068, 2938, 2829, 1756, 1710, 1628, 1591, 1459 cm^{-1}; MS (ZQ2000/ESI+): *m/z* 459 $[M + Na]^+$, 475 $[M + K]^+$; HRMS (ESI+): calcd for $C_{24}H_{24}N_2O_6Na$ $[M + Na]^+$ 459.1532, found 459.1526.

*6.2.5. (+/−)-(3aR,5R,10bR)-5-(3′,4′,5′-Trimethoxyphenyl)-7-nitro-4,5,10,10b-tetrahydropyrrolo[3,4-a]carbazole-1,3(2H,3aH)-dione (**17**)*

General procedure 2 was followed with 5-nitroindole (0.86 mmol) and 100 μL (1.3 eq) of 35% HCl. After 7 days, the crude precipitate (89 mg) consisting of an inseparable mixture of carbazole **34** and tetrahydrocarbazole **17** in a 15:85 ratio (as estimated by ^{1}H NMR analysis; yields: 4 and 25%, respectively). Purification of **17** was achieved by crystallization of the crude precipitate in a 1:1 mixture of DMSO/MeOH. In contrast, pure carbazole **34**, was obtained by oxidation of **17** (see below).

Tetrahydrocarbazole **17** is a bright yellow crystalline solid. Mp (DMSO/MeOH): 320–322 °C; ^{1}H NMR (300 MHz, DMSO-*d6*): δ = 2.16 (m, 1H), 2.31 (m, 1H), 3.40 (m, 1H), 3.62 (s, 6H, $OCH_3 \times 2$), 3.64 (s, 3H, OCH_3), 4.29 (m, 1H), 4.39 (d, J = 8.4 Hz, 1H), 6.43 (s, 2H), 7.53 (d, J = 9.0 Hz, 1H), 7.60 (d, J = 2.2 Hz, 1H), 7.93 (dd, J = 9.0 Hz, J = 2.2 Hz, 1H), 11.36 (s br. s, D_2O exch., 1H, NH), 12.08 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 33.5, 38.3, 40.5, 41.2, 56.2 (x 2), 60.5, 105.9 (x 2), 112.2, 115.0, 116.1, 116.9, 125.2, 133.1, 136.8, 139.3, 140.4, 140.5, 153.2 (x 2), 177.1, 180.2; MS (ZQ2000/ESI+): m/z 474 $[M + Na]^+$, 490 $[M + Na]^+$; HRMS (ESI+): calcd for $C_{23}H_{21}N_3O_7Na$ $[M + Na]^+$ 474.1277, found 474.1284.

*6.2.6. (+/−)-(3aR,5R,10bR)-8-Bromo-7-methoxy-5-(3′,4′,5′-trimethoxyphenyl)-4,5,10,10b-tetrahydropyrrolo[3,4-a]carbazole-1,3(2H,3aH)-dione (**15**)*

General procedure 2 was performed with 6-bromo-5-methoxyindole (0.86 mmol) and 25 μL (0.33 eq) of 35% HCl. After 24 h, the crude precipitate (181 mg) consisting of a mixture of carbazole **31** and tetrahydrocarbazole **15** in a 10:90 ratio (as estimated by ^{1}H NMR analysis; yields: 4 and 37% respectively) was purified with difficulty by OPLC (CH_2Cl_2/EtOAc 8/2, flash volume: 750 μL, elution flow rate: 750 μL), in order to obtain **31** (5 mg) and **15** (34 mg), respectively. Carbazole **31**, could also be obtained by oxidation of **15** (see below).

Tetrahydrocarbazole **15** is a beige amorphous solid. Mp (acetone/*n*-hexane): 263–265 °C; ^{1}H NMR (300 MHz, acetone-*d6*): δ = 2.20 (m, 1H), 2.46 (ddd, J = 13.2 Hz, J = 6.0 Hz, J = 4.4 Hz, 1H), 3.48 (ddd, J = 9.8 Hz, J = 8.6 Hz, J = 6.0 Hz, 1H), 3.54 (s, 3H, OCH_3), 3.69 (s, 6H, $OCH_3 \times 2$), 3.71 (s, 3H, OCH_3), 4.20 (ddd, J = 8.7 Hz, J = 4.4 Hz, J = 1.4 Hz, 1H), 4.34 (dd, J = 8.6 Hz, J = 1.4 Hz, 1H), 6.24 (s, 1H), 6.56 (s, 2H), 7.68 (s, 1H), 10.08 (br. s, D_2O exch., 1H, NH), 10.25 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, acetone-*d6*): δ = 34.4, 39.1, 40.3, 41.5, 55.5 (x 3), 59.6, 101.7, 105.9 (x 3), 113.2, 115.5, 125.8, 129.6, 132,5, 137.2, 139.2, 149.3, 153.4 (x 2), 176.0, 178.7; IR (KBr): ν′ 3290, 3199, 3079, 2939, 2851, 1786, 1700, 1594, 1467 cm^{-1}; MS (ZQ2000/ESI+): m/z 539 $[M + Na]^+$, 555 $[M + K]^+$; Anal. $C_{24}H_{23}BrN_2O_6$ (C, H, N).

*6.2.7. 8-Bromo-7-methoxy-5-(3′,4′,5′-trimethoxyphenyl)pyrrolo[3,4-a]carbazole-1,3(2H-10H)-dione (**31**)*

Carbazole **31** is a pale orange amorphous solid. Mp (acetone/MeOH): 358–360 °C; ^{1}H NMR (300 MHz, DMSO-*d6*): δ = 3.57 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.85 (s, 6H, $OCH_3 \times 2$), 6.91 (s, 3H), 7.43 (s, 1H), 7.84 (s, 1H), 11.24 (br. s, D_2O exch., 1H, NH), 12.21 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 56.2, 56.6 (x 2), 60.7, 104.7, 106.5 (x 2), 112.4, 113.4, 114.1, 116.8, 121.1, 126.2, 130.8, 134.4, 134.7, 138.0 (x 2), 143.2, 149.6, 153.6 (x 2), 169.8, 170.4; IR (NaCl): ν′ 3293, 3177, 3068, 2992, 2938, 2823, 2736, 1763, 1709, 1585, 1467, 1429 cm^{-1}; MS (ZQ2000/ESI-): m/z 511 $[M-H]^-$; HRMS (ESI-): calcd for $C_{24}H_{19}Br(79)N_2O_6$ $[M-H]^-$ 509.0348, found 509.0351 and calcd for $C_{24}H_{19}Br(81)N_2O_6$ $[M-H]^-$ 511.0328, found 511.0350.

*6.2.8. 2,2,2-Trifluoro-N-((+/−)-(3aR,5R,10bR)-1,2,3,3a,4,5,10,10b-octahydro-7-methoxy-5-(3′,4′,5′-trimethoxyphenyl)-1,3-dioxopyrrolo[3,4-a]carbazol-8-yl)acetamide (**16**)*

General procedure 2 was performed with 2,2,2-trifluoro-*N*-(5-methoxyindol-6-yl) acetamide (0.86 mmol) and 25 μL (0.33 eq) of 35% HCl. After 2.5 days, the reaction mixture was filtered to afford pure carbazole **32** as an amorphous orange solid (12 mg, 3%). Mother liquors were purified by chromatography on silica gel, using a gradient of EtOAc in CH_2Cl_2 (95/5 → 70/30) as eluent, in order to obtain further amounts of **32** (48 mg, 10%) and **16** (91 mg, 19%).

Tetrahydrocarbazole **16** is a tan amorphous solid. Mp (THF/*n*-hexane): 157–159 °C; ^{1}H NMR (300 MHz, acetone-*d6*): δ = 2.23 (ddd, J = 13.1 Hz, J = 9.6 Hz, J = 8.6 Hz, 1H), 2.47 (ddd, J = 13.1 Hz, J = 6.0 Hz, J = 4.4 Hz, 1H), 3.49 (ddd, J = 9.6 Hz, J = 8.6 Hz, J = 6.0 Hz, 1H), 3.62 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 3.70 (s, 6H, $OCH_3 \times 2$), 4.23 (ddd, J = 8.6 Hz, J = 4.4 Hz, J = 1.5 Hz, 1H), 4.38 (dd, J = 8.6 Hz, J = 1.5 Hz, 1H), 6.28 (s, 1H), 6.57 (s, 2H), 8.30 (s, 1H), 9.21 (br. s, D_2O exch., 1H, NH), 10.09 (br. s, D_2O exch., 1H, NH), 10.36 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, acetone-*d6*): δ = 34.3, 39.0, 40.4, 41.5, 55.4, 55.5 (x 2), 59.7, 100.3, 104.7, 105.9 (x 2), 113.2, 116.6 (q, 1*J*C-F = 286.1 Hz, CF3), 120.4, 123.3, 129.3, 131.0, 137.2, 139.1, 144.2, 153.4 (x 2), 153.9 (q, 2*J*C-F = 36.3 Hz, COCF3), 176.1, 178.8; IR (NaCl): ν′ 3404, 2938, 2834, 1713, 1592, 1462 cm^{-1}; MS (ZQ2000/ESI$^+$): m/z 548 $[M + H]^+$, 570 $[M + Na]^+$, 586 $[M + K]^+$; Anal. $C_{26}H_{24}F_3N_3O_7$ (C, H, N).

*6.2.9. 2,2,2-Trifluoro-N-(1,2,3,10-tetrahydro-7-methoxy-5-(3′,4′,5′-trimethoxyphenyl)-1,3-dioxopyrrolo[3,4-a]carbazol-8-yl)-acetamide (**32**)*

Carbazole **32** is an orange amorphous solid (60 mg, 13%). Mp (acetone/*n*-hexane): 331–333 °C; ^{1}H NMR (300 MHz, acetone-*d6*): δ = 3.57 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.81 (s, 6H, $OCH_3 \times 2$), 6.93 (s, 3H), 7.43 (s, 1H), 7.87 (s, 1H), 10.75 (br. s, D_2O exch., 1H, NH), 11.22 (br. s, D_2O exch., 1H, NH), 12.25 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, acetone-*d6*): δ = 55.9, 56.6 (x 2), 60.7, 104.2, 106.6 (x 2), 109.6, 113.2, 114.0, 119.6, 125.0, 126.4, 130.4, 134.5, 134.9, 137.1, 138.1, 142.9, 147.0, 153.6 (x 2), 169.9, 170.5, $COCF_3$ and CF_3 not visible; IR (NaCl): ν′ 3469, 3397, 3298, 2949, 2834, 1768, 1726, 1581, 1470 cm^{-1}; MS (ZQ2000/ESI+): m/z 544 $[M + H]^+$, 566 $[M + Na]^+$, 582 $[M + K]^+$; Anal. $C_{26}H_{20}F_3N_3O_7$ (C, H, N).

*6.3. General procedure 3 for the oxidation of tetrahydrocarbazoles into their corresponding carbazoles: preparation of compounds **19**–**22**, **24**, **26**, **29**–**31**, **34***

To a solution of tetrahydrocarbazole in 1,4-dioxane (concentration of about 0.02 M) was added 2,3-dichloro-5,6-dicyanoquinone (2 eq). The reaction mixture was heated under reflux with stirring during generally 30 min, cooled at room temperature and filtered on Büchner. The precipitate was washed with CH_2Cl_2 and the combined organic phases were successively washed with saturated aqueous $NaHCO_3$ and water until neutral, dried over $MgSO_4$, filtered and concentrated under reduced pressure, in order to obtain the generally pure corresponding carbazole. If necessary, further purification involved flash chromatography on silica gel.

*6.3.1. 2-Benzyl-5-(3′,4′,5′-trimethoxyphenyl)pyrrolo[3,4-a]carbazole-1,3(2H,10H)-dione (**19**)*

According to general procedure 3, carbazole **19** was obtained from **5** (34 mg, 0.069 mmol) as a yellow amorphous solid (34 mg, quantitative). Mp (CH_2Cl_2/*n*-hexane): 303–305 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.87 (s, 6H, $OCH_3 \times 2$), 4.00 (s, 3H, OCH_3), 4.92 (s, 2H, CH_2), 6.82 (s, 2H), 7.11 (td, J = 7,8 Hz, J = 1,2 Hz, 1H), 7.28 (m, 1H), 7.34 (t, J = 7,2 Hz, 2H), 7.49 (m, 3H), 7.53 (d, J = 7,8 Hz, 1H), 7.60 (s, 1H), 7.63 (d, J = 7,8 Hz, 1H), 9.27 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 41.5, 56.2 ($OCH_3 \times 2$), 61.1 (OCH_3), 105.8 (x 2), 111.4, 111.7, 115.8, 120.7, 121.7, 123.2, 127.1, 127.7, 128.1, 128.4 (x 2), 128.7 (x 2), 129.1, 134.3, 134.9, 136.6, 138.2, 141.7, 143.4, 153.5 (x 2), 168.8 (x 2); IR (NaCl): ν′ 3374, 2932, 2823, 1756, 1694, 1581, 1494, 1456, 1429 cm^{-1}; MS (ZQ2000/ESI+): m/z 515 $[M + Na]^+$, 531 $[M + K]^+$; Anal. $C_{30}H_{24}N_2O_5$ (C, H, N).

*6.3.2. 2-Benzyl-7-methoxy-5-(3′,4′,5′-trimethoxyphenyl)pyrrolo [3,4-a]carbazole-1,3(2H-10H)-dione (**20**)*

According to general procedure 3, carbazole **20** was obtained from **6** (36 mg, 0.069 mmol) as a yellow amorphous solid (34 mg, 95%). Mp (acetone/*n*-hexane): 324–326 °C; ^{1}H NMR (300 MHz, DMSO-*d6*): δ = 3.57 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.81 (s, 6H, $OCH_3 \times 2$), 4.85 (s, 2H), 6.87 (d, J = 2.5 Hz, 1H), 6.92 (s, 2H), 7.15 (dd, J = 9.0 Hz, J = 2.5 Hz, 1H), 7.36–7.25 (m, 5H), 7.47 (s, 1H), 7.58 (d, J = 9.0 Hz, 1H), 12.17 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 41.2, 55.5, 56.5 (x 2), 60.7, 105.1, 106.6 (x 2), 111.8, 113.7, 114.2, 117.7, 121.6, 126.9, 127.7 (x 2), 127.8, 129.1 (x 3), 134.6, 134.9, 137.5, 138.1 (x 2), 143.2, 153.6 (x 2), 153.8, 168.3, 169.0; IR (NaCl): ν′ 3368, 2927, 2829, 1756, 1694, 1582, 1490, 1464, 1431 cm^{-1}; MS (ZQ2000/ESI+): m/z 545 $[M + Na]^+$, 561 $[M + K]^+$; Anal. $C_{31}H_{26}N_2O_6$ (C, H, N).

*6.3.3. 2-Benzyl-8-methoxy-5-(3′,4′,5′-trimethoxyphenyl)pyrrolo [3,4-a]carbazole-1,3(2H,10H)-dione (**21**)*

Tetrahydrocarbazole **7** (35 mg, 0.066 mmol) was oxidized according to general procedure 3. The crude product was purified by flash chromatography (CH_2Cl_2/EtOAc 98/2) to isolate the desired carbazole **21** as a bright yellow amorphous solid (24 mg, 70%). Mp (acetone/*n*-hexane): 275–277 °C; ^{1}H NMR (300 MHz, DMSO-*d6*): δ = 3.79 (s, 3H, OCH_3), 3.80 (s, 6H, $OCH_3 \times 2$), 3.83 (s, 3H, OCH_3), 4.84 (s, 2H), 6.75 (dd, J = 9.0 Hz, J = 2.3 Hz, 1H), 6.92 (s, 2H), 7.14 (d, J = 2.3 Hz, 1H), 7.36–7.26 (m, 5H), 7.39 (d, J = 9.0 Hz, 1H), 7.46 (s, 1H), 12.20 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 41.1, 55.8, 56.5 (x 2), 60.7, 95.9, 106.5 (x 2), 110.0, 111.4, 114.9, 115.1, 123.7, 127.3, 127.7 (x 3), 127.8, 129.1 (x 2), 134.4, 135.1, 137.5, 138.0, 141.6, 145.1, 153.6 (x 2), 160.4, 168.3, 168.9; IR (NaCl): ν′ 3368, 2938, 2840, 1695, 1624, 1583, 1456, 1412 cm^{-1}; MS (ZQ2000/ESI+): m/z 545 $[M + Na]^+$, 561 $[M + K]^+$; HRMS (ESI+): calcd for $C_{31}H_{26}N_2O_6Na$ $[M + Na]^+$ 545.1689, found 545.1673.

*6.3.4. 2-Benzyl-5-(3′,4′,5′-trimethoxyphenyl)-7-nitropyrrolo[3,4-a] carbazole-1,3(2H-10H)-dione (**26**)*

According to general procedure 3, carbazole **26** was obtained from **10** (40 mg, 0.074 mmol) as a bright yellow solid (40 mg, quantitative).

*6.3.5. 2-Benzyl-8-bromo-7-methoxy-5-(3′,4′,5′-trimethoxyphenyl) pyrrolo[3,4-a]carbazole-1,3(2H-10H)-dione (**22**)*

According to procedure 3, carbazole **22** was obtained from **8** (42 mg, 0.069 mmol) as a bright yellow amorphous solid (40 mg, 95%). Mp (acetone/*n*-hexane): 250–252 °C; ^{1}H NMR (300 MHz, DMSO-*d6*): δ = 3.58 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.81 (s, 6H, $OCH_3 \times 2$), 4.85 (s, 2H), 6.93 (s, 2H), 6.94 (s, 1H), 7.36–7.27 (m, 5H), 7.51 (s, 1H), 7.84 (s, 1H), 12.26 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 41.2, 56.2, 56.6 (x 2), 60.7, 104.8, 106.5 (x 2), 112.2, 112.6, 114.6, 116.7, 121.1, 126.5, 127.7 (x 2), 127.9, 129.1 (x 2), 129.6, 134.6 (x 2), 137.4, 138.2 (x 2), 143.4, 149.7, 153.7 (x 2), 168.2, 168.9; IR (NaCl): ν′ 3330, 2927, 2823, 1756, 1701, 1582, 1466, 1427 cm^{-1}; MS (ZQ2000/ESI+): m/z 625 $[M + Na]^+$; Anal. $C_{31}H_{25}BrN_2O_6$ (C, H, N).

*6.3.6. N-(2-Benzyl-1,2,3,10-tetrahydro-7-methoxy-5-(3′,4′,5′-trimethoxyphenyl)-1,3-dioxopyrrolo[3,4-a]carbazol-8-yl)-2,2,2-trifluoroacetamide (**24**)*

According to procedure 3, carbazole **24** was obtained from **9** (62 mg, 0.097 mmol) as a bright yellow amorphous solid (61 mg, quantitative). Mp (acetone/*n*-hexane): 236–238 °C; ^{1}H NMR (300 MHz, DMSO-*d6*): δ = 3.59 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.82 (s, 6H, $OCH_3 \times 2$), 4.85 (s, 2H), 6.94 (s, 2H), 6.96 (s, 1H), 7.36–7.27 (m, 5H), 7.51 (s, 1H), 7.90 (s, 1H), 10.74 (br. s, D_2O exch., 1H, NH), 12.30 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 41.2, 55.9, 56.6 (x 2), 60.7, 104.2, 106.6 (x 2), 109.6, 112.0, 114.4, 119.6, 125.2, 126.7, 127.7 (x 2), 127.8, 129.1 (x 2), 129.2, 134.6, 134.7, 137.2, 137.5, 138.1, 143.1, 147.1, 153.6 (x 2), 168.2, 168.9, $COCF_3$ and CF_3 not visible; IR (NaCl): ν′ 3401, 2932, 2840, 1759, 1704, 1581, 1535, 1472, 1400 cm^{-1}; MS (ZQ2000/ESI+): m/z 656 $[M + Na]^+$, 672 $[M + K]^+$; Anal. $C_{33}H_{26}F_3N_3O_7$ (C, H, N).

*6.3.7. 7-Methoxy-5-(3′,4′,5′-trimethoxyphenyl)pyrrolo[3,4-a] carbazole-1,3(2H-10H)-dione (**29**)*

According to procedure 3, carbazole **29** was obtained from **13** (30 mg, 0.069 mmol) as a bright yellow amorphous solid (29 mg, 96%). Mp (acetone/*n*-hexane): 317–319 °C; ^{1}H NMR (300 MHz, DMSO-*d6*): δ = 3,57 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.81 (s, 6H, $OCH_3 \times 2$), 6.85 (d, J = 2.5 Hz, 1H), 6.91 (s, 2H), 7.14 (dd, J = 8.9 Hz, J = 2.5 Hz, 1H), 7.39 (s, 1H), 7.57 (d, J = 8.9 Hz, 1H), 11.17 (br. s, D_2O exch., 1H, NH), 12.12 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 55.5, 56.5 (x 2), 60.7, 105.0, 106.6 (x 2), 113.0, 113.6, 113.7, 117.5, 121.6, 126.6, 130.3, 134.5, 135.1, 138.0, 138.1, 143.1, 153.6 (x 2), 153.7, 170.0, 170.5; IR (KBr): ν′ 3315, 3179, 3058, 2991, 2829, 1760, 1708, 1582, 1508, 1489, 1463, 1417 cm^{-1}; MS (ZQ2000/ESI+): m/z 455 $[M + Na]^+$, 471 $[M + K]^+$; Anal. $C_{24}H_{20}N_2O_6$ (C, H, N).

*6.3.8. 8-Methoxy-5-(3′,4′,5′-trimethoxyphenyl)pyrrolo[3,4-a] carbazole-1,3(2H-10H)-dione (**30**)*

General procedure 3 was applied to tetrahydrocarbazole **14** (30 mg, 0.069 mmol). The crude product was purified by flash chromatography (CH_2Cl_2/EtOAc 80/20) to isolate the desired carbazole **30** as a bright yellow amorphous solid (25 mg, 85%). Mp (acetone/*n*-hexane): 344–346 °C; ^{1}H NMR (300 MHz, DMSO-*d6*): δ = 3.77 (s, 6H, $OCH_3 \times 3$), 3.80 (s, 3H, OCH_3), 6.72 (dd, J = 8.6 Hz, J = 1.8 Hz, 1H), 6.88 (s, 2H), 7.11 (d, J = 1.8 Hz, 1H), 7.35 (d, J = 8.6 Hz, 1H), 7.36 (s, 1H), 11.11 (br. s, D_2O exch., 1H, NH), 12.12 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 55.8, 56.4 (x 2), 60.7, 95.9, 106.5 (x 2), 109.8, 112.6, 114.6, 114.9, 123.5, 126.9, 129.0, 134.2, 135.2, 137.9, 141.4, 144.9, 153.5 (x 2), 168.2, 169.9, 170.5; IR (NaCl): ν′ 3396, 3229, 2918, 2849, 1758, 1701, 1626, 1580, 1468 cm^{-1}; MS (ZQ2000/ESI-): m/z 431 $[M-H]^-$; HRMS (ESI-): calcd for $C_{24}H_{19}N_2O_6$ $[M-H]^-$ 431.1243, found 431.1226.

*6.3.9. 5-(3′,4′,5′-Trimethoxyphenyl)-7-nitropyrrolo[3,4-a] carbazole-1,3(2H-10H)-dione (**34**)*

General procedure 3 was applied to tetrahydrocarbazole **17** (128 mg, 0.28 mmol) with a reaction time of 16 h. Carbazole **34** precipitated upon cooling as a yellow amorphous solid and was collected by filtration, washed several times with saturated aqueous $NaHCO_3$ and water, dried under vacuum. A further crop was obtained from mother liquors and was treated as above mentioned (total: 125 mg, quantitative). Because of its poor solubility, NMR analyses were performed at 50 °C. ^{1}H NMR (300 MHz, DMSO-*d6*): δ = 3.85 (s, 6H, $OCH_3 \times 2$), 3.86 (s, 3H, OCH_3), 7.00 (s, 2H), 7.58 (s, 1H), 7.82 (d, J = 9.0 Hz, 1H), 8.35 (dd, J = 9.0 Hz, J = 2.0 Hz, 1H), 8.49 (d, J = 2.0 Hz, 1H), 11.20 (br. s, D_2O exch., 1H, NH), 12.72 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 56.9 (x 2), 60.8, 107.4 (x 2), 113.3, 114.4, 115.7, 119.2, 121.2, 123.2, 126.5, 132.2, 134.1, 135.6, 139.4, 141.3, 144.0, 146.6, 154.0 (x 2), 169.2, 169.9; IR (KBr): ν′ 3277, 2938, 2840, 1764, 1735, 1618, 1582, 1490, 1459 cm^{-1}; MS (ZQ2000/ESI+): m/z 470 $[M + Na]^+$, 486 $[M + K]^+$; HRMS (ESI-): calcd for $C_{23}H_{16}N_3O_7$ $[M-H]^-$ 446.0988, found 446.1006.

*6.3.10. 8-Bromo-7-methoxy-5-(3′,4′,5′-trimethoxyphenyl)pyrrolo [3,4-a]carbazole-1,3(2H-10H)-dione (**31**)*

According to procedure 3, carbazole **31** was obtained from **15** (66 mg, 0.13 mmol) as a pale orange amorphous solid (59 mg, 90%).

*6.3.11. 2,2,2-Trifluoro-N-(1,2,3,10-tetrahydro-7-methoxy-5-(3′,4′,5′-trimethoxyphenyl)-1,3-dioxopyrrolo[3,4-a]carbazol-8-yl) acetamide (**32**)*

General procedure was applied to tetrahydrocarbazole **16** (40 mg, 0.073 mmol). Purification of the resulting crude product by

flash chromatography on silica gel (CH_2Cl_2/EtOAc 87/13) provided carbazole **32** as a bright yellow solid (29 mg, 74%).

6.4. General procedure 4 for the reduction of nitro groups: preparation of compounds *11*, *18*, *25*, *27*, *33* and *35*

To a solution of nitrotetrahydrocarbazole or nitrocarbazole (1 eq) in THF (C = 0.2 M) were successively added activated zinc powder (30 eq) and acetic acid (20 eq). The reaction mixture was stirring 2 h at room temperature until disappearance of starting material then, diluted with EtOAc and filtered on a celite pad. The organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried over MgSO4, filtered and concentrated under vacuo. Crude product was purified by flash chromatography on silica gel in order to afford the desired amino compound.

6.4.1. (+/−)-(3aR,5R,10bR)-7-Amino-2-benzyl-5-(3′,4′,5′-trimethoxyphenyl) tetrahydropyrrolo[3,4-a]carbazole-1,3(2H-10H)-dione (*11*)

General procedure 4 was applied to nitrotetrahydrocarbazole **10** (62 mg, 0.114 mmol) with a reaction time of 2 h. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/EtOAc 70/30) to provide the corresponding aminotetrahydrocarbazole **11** as a pink lacquer (51 mg, 87%). Mp (THF/*n*-hexane): 271–273 °C; ^{1}H NMR (300 MHz, acetone-*d6*): δ = 2.07 (m, 1H), 2.48 (ddd, J = 13.1 Hz, J = 5.9 Hz, J = 4.4 Hz, 1H), 2.97 (br. s, D_2O exch., 2H, NH_2), 3.55 (ddd, J = 10.3 Hz, J = 8.4 Hz, J = 5.9 Hz, 1H), 3.66 (s, 6H, OCH_3 × 2), 3.72 (s, 3H, OCH_3), 4.19 (ddd, J = 8.9 Hz, J = 4.4 Hz, J = 1.5 Hz, 1H), 4,40 (dd, J = 8.4 Hz, J = 1.5 Hz, 1H), 4,53 (d, J = 14.6 Hz, 1H), 4,60 (d, J = 14.6 Hz, 1H), 5.94 (d, J = 2.0 Hz, 1H), 6.47 (dd, J = 8.5 Hz, J = 2.0 Hz, 1H), 6.49 (s, 2H), 7.31–7.22 (m, 5H), 7.41 (dd, J = 8.5 Hz, J = 0.6 Hz, 1H), 10.23 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, acetone-*d6*): δ = 35.4, 39.1, 39.3, 40.4, 41.8, 55.4 (x 2), 59.6, 105.6 (x 2), 109.0, 111.2, 112.8, 115.1, 126.2, 127.5, 128.0 (x 2), 128.4 (x 3), 133.9, 136.4, 137.0, 139.6, 144.0, 153.3 (x 2), 175.3, 178.0; IR (NaCl): ν′ 3372, 2916, 2845, 1783, 1701, 1589, 1460 cm^{-1}; MS (ZQ2000/ESI+): m/z 534 $[M + Na]^+$, 550 $[M + K]^+$; Anal. $C_{30}H_{29}N_3O_5$ (C, H, N).

6.4.2. (+/−)-(3aR,5R,10bR)-7-Amino-5-(3′,4′,5′-trimethoxyphenyl) tetrahydropyrrolo[3,4-a]carbazole-1,3(2H-10H)-dione (*18*)

General procedure 4 was applied to nitrotetrahydrocarbazole **17** (30 mg, 0.066 mmol) with a reaction time of 7 h. The crude product was purified by flash chromatography on silica gel using a gradient of EtOAc in CH_2Cl_2/EtOAc (50/50 → 30/70) to provide the corresponding aminotetrahydrocarbazole **18** as a pale orange amorphous solid (23 mg, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.04 (m, 1H), 2.57 (m, 1H), 3.43 (m, 1H), 3.69 (s, 6H, OCH_3 × 2), 3.82 (s, 3H, OCH_3), 4.15 (m, 2H), 6.01 (s, 1H), 6.34 (s, 2H), 6.59 (d, J = 8.3 Hz, 1H), 7.29 (d, J = 8.3 Hz, 1H), 8.46 (br. s, D_2O exch., 1H, NH), 8.63 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 34.7, 38.9, 40.3, 41.3, 56.0 (x 2), 60.9, 104.9, 105.0 (x 2), 111.6, 113.0, 113.1, 126.7, 127.0, 131.6, 136.6, 138.7, 139.2, 153.1 (x 2), 176.1, 178.6; IR (NaCl): ν′ 3387, 3332, 3250, 2936, 2837, 1775, 1714, 1590, 1504, 1456 cm^{-1}; MS (ZQ2000/ESI+): m/z 422 $[M + H]^+$, 444 $[M + Na]^+$, 460 $[M + K]^+$; HRMS (ESI+): calcd for $C_{23}H_{24}N_3O_5$ $[M + H]^+$ 422.1716, found 422.1728.

6.4.3. 7-Amino-2-benzyl-5-(3′,4′,5′-trimethoxyphenyl)pyrrolo[3,4-a]carbazole-1,3(2H-10H)-dione (*27*)

General procedure 4 was applied to nitrocarbazole **26** (45 mg, 0.084 mmol) with a reaction time of 2 h. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/EtOAc 70/30) to provide the corresponding aminocarbazole **27** as a red amorphous solid (47 mg, quantitative). Mp (acetone/*n*-hexane): 274–276 °C; ^{1}H NMR (300 MHz, DMSO-*d6*): δ = 3.81 (s, 9H, OCH_3 × 2), 4.83 (br. s, D_2O exch.*, 4H, CH_2, NH_2^* overlapped), 6.83 (m, 1H), 6.85 (dd, J = 8.6 Hz, J = 2.2 Hz, 1H), 6.90 (s, 2H), 7.35 (s, 1H), 7.36–7.26 (m, 5H), 7.37 (m, 1H), 11.87 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 41.1, 56.5 (x 2), 60.7, 106.0, 106.7 (x 2), 111.0, 113.1, 114.2, 118.2, 122.0, 126.6, 127.6 (x 2), 127.8, 128.4, 129.1 (x 2), 134.5, 135.1, 136.1, 137.6, 138.1, 142.8, 143.0, 153.5 (x 2), 168.4, 169.1; IR (NaCl): ν′ 3352, 2931, 2836, 1750, 1698, 1581, 1494, 1461, 1428, 1403 cm^{-1}; MS (ZQ2000/ESI+): m/z 530 $[M + Na]^+$, 546 $[M + K]^+$; Anal. $C_{30}H_{25}N_3O_5$ (C, H, N).

6.4.4. 7-Amino-5-(3′,4′,5′-trimethoxyphenyl)pyrrolo[3,4-a] carbazole-1,3(2H-10H)-dione (*35*)

General procedure 4 was applied to nitrocarbazole **34** (79 mg, 0.176 mmol) with a reaction time of 3 h. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/EtOAc 50/50) to provide the corresponding aminocarbazole **35** as a red amorphous solid (30 mg, 41%). ^{1}H NMR (300 MHz, DMSO-*d6*): δ = 3.81 (s, 9H, OCH_3 × 3), 4.77 (br. s, D_2O exch., 2H, NH_2), 6.81 (m, 1H), 6.84 (dd, J = 8.6 Hz, J = 2.2 Hz, 1H), 6.89 (s, 2H), 7.28 (s, 1H), 7.37 (d, J = 8.6 Hz, 1H), 11.06 (br. s, D_2O exch., 1H, NH), 11.80 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 56.5 (x 2), 60.7, 106.0, 106.6 (x 2), 112.3, 113.0, 113.7, 118.0, 122.0, 126.2, 129.7, 134.4, 135.3, 136.0, 138.1, 142.7, 142.9, 153.4 (x 2), 170.1, 170.7; IR (NaCl): ν′ 3428, 3388, 3357, 3221, 2924, 2852, 1753, 1702, 1580, 1473 cm^{-1}; MS (ZQ2000/ESI+): m/z 418 $[M + H]^+$; HRMS (ESI+): calcd for $C_{23}H_{20}N_3O_5$ $[M + H]^+$ 418.1403, found 418.1409.

6.4.5. 8-Amino-2-benzyl-7-methoxy-5-(3′,4′,5′-trimethoxyphenyl) pyrrolo[3,4-a]carbazole-1,3(2H-10H)-dione (*25*)

To a solution of **24** (40 mg, 0.063 mmol) in 1,4-dioxane (4 mL) was added a 0.05 M aqueous solution of K_2CO_3 (2.5 mL, 0.125 mmol). The mixture was stirring at room temperature during 17 h until the disappearance of the starting trifluoroacetamide. The reaction was then quenched by addition of 250 of water and extracted by EtOAc. The combined organic layers were washed with water and brine, dried over MgSO4, filtered and concentrated under vacuo. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/EtOAc 88/12) in order to obtain carbazole **25** as a bright red lacquer (26 mg, 76%). Mp (acetone/*n*-hexane): 225–227 °C; ^{1}H NMR (300 MHz, DMSO-*d6*): δ = 3,62 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.90 (s, 6H, OCH_3 × 2), 4.81 (s, 2H), 5.05 (br. s, D_2O exch., 2H, NH_2), 6.83 (s, 1H), 6.93 (s, 2H), 6,99 (s, 1H), 7.25 (t, J = 7.5 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2H), 7.38 (s, 1H), 7.40 (d, J = 7.5 Hz, 1H), 10.73 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 40.7, 54.9, 55.8 (x 2), 59.9, 95.1, 103.1, 106.4 (x 2), 111.0, 114.1, 125.9, 127.3, 127.7 (x 2), 128.5 (x 3), 133.7, 135.5, 137.7, 138.3, 139.4, 139.6, 139.8, 140.4, 143.0, 153.7 (x 2), 168.2, 168.7; IR (NaCl): ν′ 3484, 3368, 3001, 2935, 2827, 1753, 1694, 1632, 1577, 1489 cm^{-1}; MS (ZQ2000/ESI+): m/z 560 $[M + Na]^+$, 576 $[M + K]^+$; Anal. $C_{31}H_{27}N_3O_6$ (C, H, N).

6.4.6. 8-Amino-7-methoxy-5-(3′,4′,5′-trimethoxyphenyl)pyrrolo [3,4-a]carbazole-1,3(2H-10H)-dione (*33*)

Carbazole **32** (40 mg, 0.063 mmol) was treated in the same conditions than **24**. The crude product was purified by flash chromatography (CH_2Cl_2/EtOAc 50/50) in order to obtain the corresponding aminocarbazole **33** as a red lacquer (24 mg, quantitative). ^{1}H NMR (400 MHz, DMSO-*d6*): δ = 3.52 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.81 (s, 6H, OCH_3 × 2), 5.40 (br. s, D_2O exch., 2H, NH_2), 6.66 (s, 1H), 6.85 (s, 1H), 6.88 (s, 2H), 7.29 (s, 1H), 10.96 (br. s, D_2O exch., 1H, NH), 11.72 (br. s, D_2O exch., 1H, NH); ^{13}C NMR (75 MHz, DMSO-*d6*): δ = 55.4, 56.4 (x 2), 60.6, 95.2, 103.0, 106.5 (x 2), 110.1, 112.0, 113.8, 126.8, 128.2, 133.5, 135.6, 137.7, 139.3, 140.1, 140.9, 142.7, 153.4 (x 2), 170.2, 170.8; IR (NaCl): ν′ 3482, 3364, 3002, 2940, 2831, 1756, 1692, 1635, 1580, 1490 cm^{-1}; MS (ZQ2000/

ESI-): *m/z* 446 $[M-H]^-$; HRMS (ESI+): calcd for $C_{24}H_{21}N_3O_6$ $[M + H]^+$ 447.1430, found 418.1425.

7. Biological evaluation

7.1. Cytotoxicity

Evaluation of cytotoxicity in murine B16 melanoma cells. Murine B16 melanoma cells were grown in DMEM medium containing 2 mM L-glutamine, 10% foetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin (3 °C, 5% CO_2). All compounds were initially dissolved in DMSO at a stock concentration of 2.5 mg/mL and were further diluted in cell culture medium. For comparative purposes, CA-4 and colchicine were routinely included in the experiments as reference compounds. Exponentially growing B16 cells were plated onto 96 well plates at 5000 cells per well in 100 μl of culture medium. Twenty-four hour after plating, 100 μl of medium containing the compound of interest at final concentrations ranging from 0.01 to 100 μM were added to the wells (in triplicate) containing the cells, and incubated for 48 h at 37 °C and 5% CO_2. After the 48 h exposure period to the test compounds, cell viability was assayed using the MTT test [17] and absorbance was read at 56 m in a microplate reader (Bio-Kinetics Reader, EL340). Appropriate controls with DMEM only and MTT were run to substract background absorbance. Results are presented as percent of controls containing 1% DMSO, which was not cytotoxic at this concentration. The concentration of compound that inhibited cell viability by 50% (inhibitory concentration for 50% of cells, or IC_{50}) was determined using the GraphPad Prism software. Results are presented as the mean ± SEM of 3–7 independent experiments each run in triplicate.

7.2. Effect on the morphology of EA hy 926 cells

Effect on the morphology of transformed HUVEC cells (EA·hy 926 cells). To assess the effects of the compounds on the morphology of endothelial cells, we used the EA·hy 926 endothelial cell line which is derived from the fusion of human umbilical vein endothelial cells (HUVEC) with the permanent human cell line A549 [18]. The EA·hy 926 cell line is considered as one of the best immortalized HUVEC cell lines because these cells express most of the biochemical markers of parental HUVEC EA·hy 926 cells [19], originally obtained from Dr Cora-Jean S. Edgell (Pathology Department, University of North Carolina, Chapel Hill, NC 27599-7525, USA) were used with her permission, and were grown in DMEM containing 2 mM L-glutamine, 10% foetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin (37 °C, 5% CO_2). Exponentially growing EA·hy 926 cells were plated onto 96 well plates at 5000 cells/100 μl/well. Twenty-four hour after plating, the medium was aspirated, and 100 μl of medium containing the test compound was added to the well containing the cells (in triplicate) in 10-fold dilutions, and incubated for 2 h. After the 2 h-incubation period, digital photographs were taken of representative centre areas of each well at a magnification of 100× and 200×. Combretastatin A4 (CA-4) and colchicine were routinely included in the experiments as internal standards.

7.3. Inhibition of tubulin polymerization

Inhibition of tubulin polymerization. Tubulin microtubule assembly in microtubules was carried out using the fluorescent dye DAPI (4′,6-diamidino-2-phenylindole) [20] in a 96-well plate format as described by Barron et al. [21] and Bane et al. [22]. The standard assay was performed as follows: wells were charged with tubulin (Cytoskeleton, 97% pure, final concentration 1 mg/ml) in PME buffer (100 mM PIPES (1,4-piperazinebis(ethanesulfonic acid)); 1 mM $MgSO_4$; 2 mM EGTA) with 10 μM DAPI and varying concentrations of the test compounds using colchicine as an internal control. After a preincubation of 45 min at room temperature, 5 μl of 1 mM GTP was added to each well to initiate tubulin polymerization, and the plate was then transferred to a thermostated Victor plate reader at 37 °C for an additional 2 h. Fluorescence was then read at the excitation wavelength of 360 nm and emission of 450 nm. The percent inhibition was determined as follows: $1 - (\Delta F(\text{sample})/\Delta F(\text{control}) \times 100)$, where ΔF control = F (no inhibition) − F(complete inhibition), and ΔF sample = F (sample) − F(complete inhibition with colchicine). The IC50 for compound-induced inhibition of tubulin polymerization is the concentration of compound at which the extent of inhibition of polymerization is 50% of the maximum value as determined from the semi-logarithmic plot of percent inhibition as a function of the drug concentration using the nonlinear regression software SigmaPlot (Jandel Scientific).

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Appendix. Supplementary data

Supplementary data associated with article can be found in online version at doi:10.1016/j.ejmech.2010.05.022.

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