European Journal of Medicinal Chemistry 46 (2011) 95–100

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Domino approach to 2-aroyltrimethoxyindoles as novel heterocyclic combretastatin A4 analogues

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ARTICLE INFO

Article history:
Received 13 July 2010
Received in revised form
15 October 2010
Accepted 18 October 2010
Available online 10 November 2010

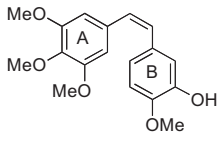
Keywords:
Combretastatin
Phenstatin
Aroylindole
Tubulin
Cytotoxicity

ABSTRACT

Two series of 2-aroyltrimethoxyindoles were designed to investigate the effects of the replacement of the trimethoxyphenyl ring of phenstatin with a trimethoxyindole moiety. These compounds were efficiently prepared through a domino palladium-catalyzed sequence from 2-*gem*-dibromovinylanilines substituted by three methoxy groups and arylboronic acids under carbon monoxide atmosphere. These novel heterocyclic combretastatin A4 analogues were evaluated for their cell growth inhibitory properties and their ability to inhibit the tubulin polymerization.

1. Introduction

Combretastatin A4 (CA4), a natural product isolated by Pettit et al. from the South African willow tree *Combretum caffrum*, strongly inhibits tubulin polymerization and the proliferation of murine and human cancer cell lines [1]. By interfering with microtubule dynamics, CA4 was shown to perturb several cell signalling pathways involved in regulating and maintaining the cytoskeleton of proliferating endothelial cells in tumour vasculature [2]. As a consequence of this cytoskeleton disruption, CA4 causes a rapid tumour vasculature shutdown leading to central tumour necrosis, while leaving normal vasculature intact [3].



Combretastatin A-4 (CA4).

The encouraging antivascular and antitumour profile of CA4 has greatly contributed to the current interest in the design and synthesis of several CA4 analogues [4]. Through SAR studies, it has been established that the *cis* orientation of both phenyl groups, and the 3,4,5-trimethoxy system on the A-ring, are essential requirements for the interaction of combretastatin-type analogues with the tubulin colchicine site and the inhibition of its assembly into microtubules. Therefore, the olefinic linker and the B-ring of CA4 have received greater attention from medicinal chemists. The *cis* double bond in the stilbene framework of CA4 was, for example, efficiently replaced by a carbonyl group leading to benzophenone derivatives, such as in phenstatin **1** or as in the 1,2 or 3-aroylindoles **2**, which demonstrated strong antiproliferative and antitubulin activities [5–7] (Fig. 1).

However, it is interesting to note that replacement of the trimethoxyphenyl ring by benzoheterocyclic structures has received little attention so far [8]. We thus decided to explore the possibility to replace the trimethoxyphenyl ring of phenstatin with a trimethoxyindole skeleton, by synthesizing 2-aroylindole derivatives with general structures **3** and **4** (Fig. 1). Our goal was to determine, for the benzophenone-type CA4 analogues, the influence of a more hindered A-ring bearing three methoxy groups in different positions on the antiproliferative and antitubulin activities.

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doi:10.1016/j.ejmech.2010.10.018

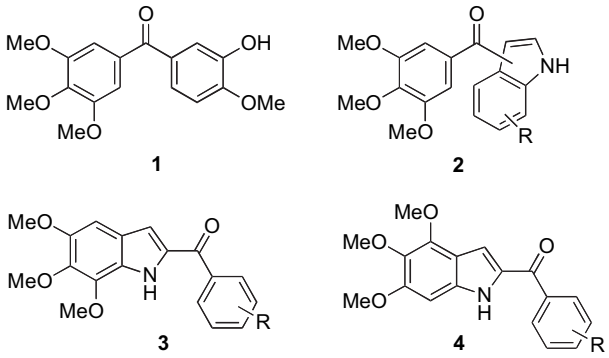
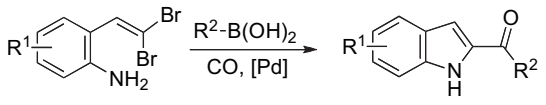


Fig. 1. Structures of phenstatin, representative and designed aroylindole derivatives.

2. Chemistry

Relatively few methods for the synthesis of the 2-aroylindole frameworks have been reported. The most common synthetic route involves addition of a variety of acyl electrophiles on a N-protected-2-lithioindole species [9]. However, this method is limited because it requires a "*de novo*" construction of conveniently N-protected indoles [10,11]. Alternatively, a 5-exo-*dig* iodocyclization of conveniently tethered dimethylanilines afforded 2-aroyl-N-methylindoles [12], and some 2-acylindoles have been synthesized using a Suzuki-type coupling reaction between the indole-2-carboxylic acid chloride and boronic acids [13]. For our part, the synthesis of the target 2-aroylindoles **3** and **4** was investigated according to our reported procedure (Scheme 1), through a domino palladium-catalyzed reaction involving a 2-*gem*-dibromovinylaniline and a boronic acid under a pressure of carbon monoxide [14].

The required trimethoxyanilines **5** [14] and **6** were easily prepared in a two-step sequence (Ramirez olefination followed by reduction of the nitro group) from the *o*-nitrobenzaldehyde derivatives **7** and **8** [15]. The domino reaction was first conducted with the dibromoolefine **5** and phenylboronic acid under the previously optimized conditions [$Pd(PPh_3)_4$, K_2CO_3, CO (12 bar), dioxane]. By heating at 85 °C for 24 h, the desired 2-aroylindole **3a** could be isolated in satisfactory yield (65%), opening the possibility to get a novel series of CA4 analogues (Scheme 2).



Scheme 1. Palladium-catalyzed 2-aroylindoles synthesis.

Coupling reactions were next performed with arylboronic acids bearing substituents known to favour interactions with tubulin: a *para*-methoxy group [16] and, in place of the hydroxyl function of CA4, a fluorine [16] or an amino group [17,18]. In agreement with the pharmacophoric model established by Nguyen et al. [19], the influence of a methyl group in the *para*-position was also examined, as the hydrophobic center is considered as an essential feature for activity. Thus, the target ketones **3b**–**3d** were obtained in 59, 73, 55 and 45% yields, respectively. It is noteworthy that the domino reaction may be carried out with a boronic acid bearing an amino reactive group giving aniline **3e** in 45% yield. Introduction of a pyridine ring, which might generate additional interactions with tubulin amino acids, was also effective, albeit in modest yield, and after an increased reaction time of 60 h (compound **3f**, 45% yield).

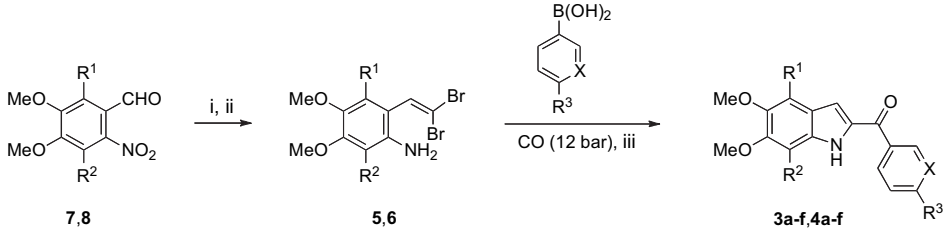
To explore the influence of the substitution pattern of the indole nucleus, domino reactions were performed with the regioisomeric 4,5,6-trimethoxyaniline **6**. Under the same conditions, coupling with phenylboronic acid generated the ketone **4a** albeit in modest yield (47%). Significant yield improvement was obtained by increasing the reaction time to 60 h (61% yield). Therefore, reactions of the aniline **6** with the five boronic partners previously used, were conducted for 60 h, affording the desired aroylindoles **4b**–**4f** in yields ranging from 46 to 63%.

Phenylvinylboronic acid also proved to be an efficient partner of the domino reaction with anilines **5** and **6**, affording the enones **9** and **10** in satisfactory yields (61 and 49%, respectively).

3. Biological results and discussion

The synthesized aroylindoles were evaluated (Table 1) for their *in vitro* cytotoxicity against the murine B16 melanoma cell line (MTT assay), and also for their ability to inhibit tubulin polymerization (fluorometric assay) [20], using CA4 as the reference compound. Within the 2-aroyl-4,5,6-trimethoxyindole series, compounds **3c** and **3d** presented a modest cytotoxic effect (IC_{50}: 16 and 10 μM, respectively). With regard to the tubulin polymerization inhibition, only compound **3d**, bearing a methyl group at the 4-position of the B-ring, displayed a fair activity (IC_{50}: 4 μM) but less than that of CA4. Within the subset of 5,6,7-trimethoxyindole analogues, cytotoxic activity was nearly abolished except for the *p*-toluyl derivative **4d** (IC_{50}: 10 μM), for which cytotoxicity and antitubulin activity were not correlated, contrary to its isomeric analogue **3d**. The enones **9** and **10**, designed as analogues of a diarylbutadienic derivative that we have previously reported as a potent tubulin polymerization inhibitor [21], were devoid of any appreciable activity.

Selected compounds were next evaluated for their cell growth inhibitory potency against three human colon adenocarcinoma cell lines, HCT-116, the P-glycoprotein (P-gp)-expressing HCT-15 and

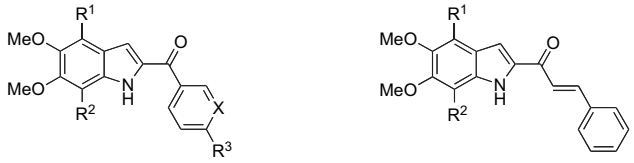


7, **5**, **3a-f:** R^1 = OMe, R^2 = H
8, **6**, **4a-f**: R^1 = H , R^2 = OMe

X and R^3 exemplified in Table 1

Scheme 2. Reagents and conditions: (i) CBr_4 (1.5 equiv.), PPh_3 (3 equiv.), CH_2Cl_2, 0 °C to rt, 1 h; (ii) $SnCl_2.2H_2O$ (5.5 equiv.), EtOH, reflux, 1.5 h; (iii) $Pd(PPh_3)_4$ (5 mol%), K_2CO_3 (5 equiv.), dioxane, 85 °C, 24 h for adducts **3a**–**3e**, 60 h for adducts **3f**, **4a**–**4f**.

Table 1
Cytotoxicity and tubulin polymerization inhibition of synthesized aroylindole derivatives.



3a-f,**4a-f**

9, 10

3a-f, **9:** R^1 = OMe, R^2 = H
4a-f, **10**: R^1 = H , R^2 = OMe

Compd	X	R^3	IC_{50}(μM)		Compd	X	R^3	IC_{50}(μM)	
			B16[a]	ITP[b]				B16[a]	ITP[b]
3a	CH	H	45	>20	**4a**	CH	H	>50	9
3b	CH	OMe	>50	9	**4b**	CH	OMe	>50	12
3c	C–F	OMe	16	>20	**4c**	C–F	OMe	>50	20
3d	CH	Me	10	4	**4d**	CH	Me	10	>20
3e	C–NH_2	Me	36	10	**4e**	C–NH_2	Me	nt [c]	nt [c]
3f	N	OMe	>50	15	**4f**	N	OMe	>50	18
9	–	–	16	>20	**10**	–	–	33	18
CA4			0.005	0.27					

[a] Compound concentration required to reduce murine B16 melanoma cell viability by 50% (48 h exposure time).
[b] Compound concentration required to inhibit tubulin polymerization (ITP) by 50%. *In vitro* microtubule assembly was monitored using a fluorescent probe (DAPI) [20].
[c] nt, not tested.

the CA4-refractory HT-29 [22] cells (Table 2). The 4,5,6-trimethoxyindole derivatives **3b**, **3d** and **3e** showed similar activity against HCT-116 and HT-29 cells (IC_{50}: 1–4 μM), whereas CA4 was ten-fold less potent on the HT-29 cell line (0.01 *vs* 0.001 μM). Surprisingly, these compounds presented significant activity on the HCT-15 cell line with IC_{50} in the submicromolar range. Nevertheless, the antiproliferative effects for these three compounds were not in good agreement with their antitubulin activities, suggesting that tubulin may not be their single target. In this series, compound **3e** with an amino group displayed the most potent antiproliferative activity (IC_{50}: 0.3–2.0 μM). As previously observed, the 5,6,7-trimethoxyindole derivatives **4a** and **4d** were found inactive on the three colon cancer cell lines (IC_{50} > 50 μM).

Table 2
Antiproliferative effects of selected trimethoxyaroylindoles against human colon carcinoma cell lines.

Compd	IC_{50} (μM)[a]		
	HCT-116	HCT-15	HT-29
3b	4.2	0.45	1.24
3d	2.8	0.61	1.09
3e	2.0	0.32	0.99
4a	>50	>50	>50
4d	>50	>50	>50
CA4	0.001	0.001	0.013

[a] Compound concentration required to inhibit human tumoral cell proliferation by 50% after 72 h incubation.

4. Conclusions

The present study was undertaken to explore the possibility to replace the usual trimethoxyphenyl ring present in a large majority of CA4 analogues with a more bulky moiety, a trimethoxyindole ring. Thus, several 2-aroyltrimethoxyindoles have been prepared through a palladium-catalyzed domino reaction which proved to be an efficient and convergent synthetic route to highly substituted 2-aroylindoles.

With regard to the interaction with tubulin, this study indicates that the 4,5,6-trimethoxy substitution pattern on the indole ring and a small hydrophobic group in the *para*-position of the B-ring are appropriate structural features, and therefore allows the identification of compound **3d** as a frame for further investigations in the search of novel tubulin polymerization inhibitors. This specific trimethoxy motif is also critical for cytotoxicity, while some flexibility was tolerated for the B-ring.

It is noteworthy that the A-ring modulation studied here, has also proven beneficial in a series of derivatives with a methylene bridge, the 1-benzyl-4,5,6-trimethoxyindoles, recently reported as a novel class of potent antimitotic agents [23]. Therefore, we are presently taking these data and our own results into account to design novel and more potent CA4 analogues.

5. Experimental

5.1. Chemistry

Dichloromethane was dried over P_2O_5, dioxane over Na/benzophenone. Unless otherwise noted, all the materials were obtained from commercial sources and were used without purification. Aniline **5** and compound **3a** were prepared as previously reported [14]. ^{1}H NMR spectra were recorded with a Bruker ACP 300 spectrometer at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR spectroscopy. Chemical shifts are given as δ values in ppm relative to the residual solvent peak ($CHCl_3$) as the internal reference, coupling constants are given in Hertz. All ^{13}C NMR spectra were recorded with complete proton decoupling. Peak assignment was unambiguously performed using HMQC, HMBC and NOESY techniques. IR spectra were obtained using an FT-IR Perkin–Elmer spectrometer. Melting points were determined by the capillary method using an Electrothermal 9200 apparatus and are uncorrected. Mass spectra were recorded on a Waters ZQ 2000 system using electrospray ionization (ESI). High-resolution mass spectra (ESI) were acquired from the "imaGIF" service (CNRS-ICSN, 91198 Gif sur Yvette, France) on a Waters LCT spectrometer. Reactions were followed with Merck TLC silica gel 60 F_{254}. Flash chromatographies were carried out on Merck silica gel (320–400 mesh).

5.1.1. 2-(2,2-Dibromovinyl)-4,5,6-trimethoxyaniline (*6*)

Aniline **6**, prepared from 2-nitro-3,4,5-trimethoxybenzaldehyde [24] (12 g, 50 mmol) following the procedure reported by Fang and Lautens [15], was obtained after purification by flash chromatography (cyclohexane/ethyl acetate 9/1) as a beige solid (70% over the 2 steps): mp 87–88 °C, $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3452, 3376, 3052, 2939, 1588, 1490, 1464, 1355, 1198, 1128, 1077; δ_H (300 MHz; $CDCl_3$) 7.33 (1H, s), 6.76 (1H, s), 3.90 (3H, s), 3.89 (3H, s), 3.80 (3H, s), 3.75 (2H, br s); δ_C (75 MHz; $CDCl_3$) 145.4, 143.1, 133.3, 132.4, 116.2, 107.8, 91.5, 60.9, 60.5, 56.6; *m*/*z* (ESI) 388 (50%), 389 (100), 392 (40): $[M + Na]^+$; HRMS (ESI) *m*/*z* 365.9358 $[M + H]^+$, $C_{11}H_{14}NO_3^{79}Br_2$ requires 365.9340.

5.1.2. General procedure for the domino reactions

The autoclave and the magnetic stirring bar were dried in an oven and then cool to room temperature under an argon atmosphere. Boronic acid (1.1 mmol), K_2CO_3 (5 mmol, flame dried prior to use) and $Pd(PPh_3)_4$ (0.05 mmol) were introduced then the autoclave was flushed with argon for 5 min. A degassed solution (argon bubbling for 10 min) of aniline **5** or **6** (1 mmol) in dry dioxane (10 mL) was added and the autoclave was flushed three times with CO and pressurized to 12 bar.

After heating at 85 °C in an oil bath for the appropriate time (24 h for adducts **3a**–**3e**, 60 h for adducts **3f**, **4a**–**4f**, **9** and **10**), the autoclave was cooled to room temperature and then cautionary discharged of the gas excess. Reaction mixture was diluted in ethyl acetate (10 mL) and washed with water (10 mL), saturated aqueous NH_4Cl (10 mL) and brine (10 mL). The aqueous layers were combined, saturated with NaCl, acidified (by adding HCl 1 M until pH = 2) and extracted with ethyl acetate (2 × 20 mL). Organic layers were combined, dried over $MgSO_4$, filtered and concentrated under reduce pressure. The crude residue was purified by flash chromatography and crystallized in the indicated solvents to give the attempted compounds.

Caution: CO is a highly toxic odorless and colorless gas. Reactions involving Carbon Monoxide must be performed in a well-ventilated hood with a Carbon Monoxide detector nearby.

*5.1.2.1. (4,5,6-Trimethoxy-1H-indol-2-yl)(4-methoxyphenyl)methanone (**3b**).* Compound **3b** was isolated after chromatography (toluene/ethyl acetate 9/1) and recrystallization (dichloromethane/hexane) as a yellow crystalline powder in 55% yield: mp 162–163 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3443, 3056, 2935, 1619, 1570, 1500, 1466, 1375, 1272, 1264, 1256, 1170, 1143, 1108, 1032; δ_H (300 MHz; $CDCl_3$) 9.40 (1H, br s), 8.02 (2H, d, *J* 6.8), 7.18 (1H, dd, *J* 2.2 and 0.8, 3-H), 7.02 (2H, d, *J* 6.8), 6.64 (1H, d, $J_{3,7}$ 0.8, 7-H), 4.11 (3H, s), 3.91 (6H, s), 3.87 (3H, s); δ_C (75 MHz; $CDCl_3$) 184.9 (CO), 163.0, 154.9, 147.0, 136.2, 134.9, 133.2, 131.3, 130.8, 116.1, 113.7, 110.2 (CH-3), 88.9 (CH-7), 61.5, 61.0, 56.2, 55.5; *m*/*z* (ESI) 342 $[M + H]^+$; HRMS (ESI) *m*/*z* 342.1352 $[M + H]^+$, $C_{19}H_{20}NO_5$ requires 342.1341.

*5.1.2.2. (3-Fluoro-4-methoxyphenyl)(4,5,6-trimethoxy-1H-indol-2-yl)methanone (**3c**).* Compound **3c** was isolated after chromatography (toluene/ethyl acetate 8/2 to 7/3) and recrystallization (ethanol/heptane) as yellow crystals in 73% yield: mp 211–212 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3443, 3054, 2986, 1621, 1574, 1513, 1501, 1276, 1260, 1121; δ_H (300 MHz; $CDCl_3$) 9.26 (1H, br s), 7.82 (1H, ddd, *J* 8.4, 2.0 and 1.2), 7.75 (1H, dd, *J* 11.6 and 2.0), 7.19 (1H, dd, *J* 2.2 and 0.8), 7.07 (1H, t, *J* 8.4), 6.62 (1H, d, *J* 0.8), 4.11 (3H, s), 3.99 (3H, s), 3.93 (3H, s), 3.87 (3H, s); δ_C (75 MHz; $CDCl_3$) 183.7, 155.3, 151.9 (*J* 246), 151.3 (*J* 10), 147.1, 136.0, 135.1, 132.8, 131.0 (*J* 5.3), 126.3 (*J* 3.6), 116.9 (*J* 19.3), 116.3, 112,5, 110.5, 88.9, 61.5, 61.0, 56.3, 56.2; *m*/*z* (ESI) 360 $[M + H]^+$; HRMS (ESI) *m*/*z* 360.1256 $[M + H]^+$, $C_{19}H_{19}FNO_5$ requires 360.1247.

*5.1.2.3. (4,5,6-Trimethoxy-1H-indol-2-yl)(p-tolyl)methanone (**3d**).* Compound **3d** was isolated after chromatography (toluene/ethyl acetate 95/5) and recrystallization (dichloromethane/hexane) as bright yellow needles in 55% yield: mp 190–191 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3443, 3063, 2961, 2931, 2860, 2359, 2341, 1722, 1619, 1519, 1499, 1466, 1376, 1272, 1141, 1108; δ_H (300 MHz; $CDCl_3$) 9.41 (1H, br s), 7.89 (2H, d, *J* 8.1), 7.32 (2H, d, *J* 8.1), 7.19 (1H, dd, *J* 2.2 and 0.9), 6.64 (1H, d, *J* 0.8), 4.10 (3H, s), 3.91 (3H, s), 3.86 (3H, s), 2.46 (3H, s); δ_C (75 MHz; $CDCl_3$) 185.1, 155.1, 147.1, 142.8, 136.3, 135.5, 135.1, 133.2, 129.2, 129.1, 116.2, 110.8, 88.9, 61.5, 61.0, 56.1, 21.6; *m*/*z* (ESI) 326 $[M + H]^+$; HRMS (ESI) *m*/*z* 326.1403 $[M + H]^+$, $C_{19}H_{20}NO_4$ requires 326.1392.

*5.1.2.4. (3-Amino-4-methylphenyl)(4,5,6-trimethoxy-1H-indol-2-yl)methanone (**3e**).* Compound **3e** was isolated after chromatography (toluene/ethyl acetate 8/2 to 1/1) and recrystallization (dichloromethane/hexane) as a yellow crystalline powder in 45% yield: mp 204–205 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3687, 3444, 3059, 2936, 2834, 1618, 1568, 1519, 1500, 1466, 1374, 1285, 1253, 1187, 1136, 1107, 1040; δ_H (300 MHz; $CDCl_3$) 9.30 (1H, br s), 7.33 (1H, dd, *J* 7.7 and 1.7), 7.26 (1H, d, *J* 1.7), 7.21 (1H, dd, *J* 2.2 and 0.8), 7.18 (1H, d, *J* 7.7), 6.61 (1H, s), 4.09 (3H, s), 3.91 (3H, s), 3.86 (3H, s), 3.81 (2H, br s), 2.26 (3H, s); δ_C (75 MHz; $CDCl_3$) 186.1, 154.9, 147.1, 144.7, 137.1, 136.3, 135.0, 133.3, 130.4, 127.0, 119.8, 116,1, 115.0, 110.7, 89.0, 61.5, 61.0, 56.1, 17.6; *m*/*z* (ESI) 341 $[M + H]^+$; HRMS (ESI) *m*/*z* 363.1329 $[M + Na]^+$, $C_{19}H_{20}N_2O_4Na$ requires 363.1321.

*5.1.2.5. (4,5,6-Trimethoxy-1H-indol-2-yl)(6-methoxypyridin-3-yl)methanone (**3f**).* Compound **3f** was isolated after chromatography (cyclohexane/ethyl acetate 7/3) and recrystallization (dichloromethane/hexane) as yellow crystals in 48% yield: mp 161–162 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3442, 3307, 3058, 2939, 2834, 2571, 2359, 1617, 1601, 1519, 1492, 1466, 1372, 1272, 1257, 1147, 1108, 1016; δ_H (300 MHz; $CDCl_3$) 9.35 (1H, br s), 8.87 (1H, dd, *J* 2.4 and 0.6), 8.17 (1H, dd, *J* 8.6 and 2.4), 7.22 (1H, dd, *J* 2.2 and 0.8), 6.86 (1H, dd, *J* 8.6 and 0.6), 6.62 (1H, d, *J* 0.8), 4.11 (3H, s), 4.05 (3H, s), 3.92 (3H, s), 3.87 (3H, s); δ_C (75 MHz; $CDCl_3$) 183.2, 166.3, 155.4, 149.0, 147.1, 139.3, 136.4, 135.2, 132.9, 127.6, 116.3, 111.1, 110.8, 88.8, 61.5, 61.0, 56.2, 54.1; *m*/*z* (ESI) 343 $[M + H]^+$; HRMS (ESI) *m*/*z* 343.1300 $[M + H]^+$, $C_{18}H_{19}N_2O_5$ requires 343.1294.

*5.1.2.6. (5,6,7-Trimethoxy-1H-indol-2-yl)(phenyl)methanone (**4a**).* Compound **4a** was isolated after chromatography (toluene/ethyl acetate 9/1) and recrystallization (dichloromethane/hexane) as yellow crystals in 61% yield: mp 179–180 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3436, 2940, 2839, 1626, 1530, 1493, 1302, 1253, 1230, 1124, 1107; δ_H (300 MHz; $CDCl_3$) 9.30 (1H, br s), 7.95 (2H, dt, *J* 7.1 and 1.4), 7.59 (1H, tt, *J* 7.5 and 1.4), 7.51 (2H, ddt, *J* 7.5, 7.1 and 1.4), 7.03 (1H, d, *J* 2.3), 6.82 (1H, s), 4.08 (3H, s), 3.95 (3H, s), 3.89 (3H, s); δ_C (75 MHz; $CDCl_3$) 186.5, 150.4, 141.6, 139.0, 138.2, 134.3, 132.2, 129.1, 128.4, 127.6, 123.4, 112.8, 98.0, 61.5, 61.2, 56.2; *m*/*z* (ESI) 312 $[M + H]^+$; HRMS (ESI) *m*/*z* 334.1067 $[M + Na]^+$, $C_{18}H_{17}NO_4Na$ requires 334.1055.

*5.1.2.7. (5,6,7-Trimethoxy-1H-indol-2-yl)(4-methoxyphenyl)methanone (**4b**).* Procedure on 5 mmol scale. Compound **4b** was isolated after flash chromatography (cyclohexane/ethyl acetate 8/2) and recrystallization (dichloromethane/heptane) as yellow crystals (1.0 g) in 59% yield: mp 154–155 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3684, 3436, 2939, 2840, 1623, 1602, 1529, 1491, 1464, 1302, 1254, 1170, 1124, 1108; δ_H (300 MHz; $CDCl_3$) 9.28 (1H, br s), 7.99 (2H, dt, *J* 8.8 and 2.7), 7.02 (1H, s, 3-H), 7.00 (2H, dt, *J* 8.9 and 2.1), 6.84 (1H, s, 4-H), 4.08 (3H, s), 3,94 (3H, s), 3.91 (3H, s), 3.90 (3H, s); δ_C (75 MHz; $CDCl_3$) 185.1 (CO), 163.1, 150.3, 141.3, 139.0, 134.4, 131.4, 130.8, 127.2, 123.4, 113.7, 111.9 (CH-3), 98.0 (CH-4), 61.5, 61.2, 56.3, 55.5; *m*/*z* (ESI) 342 $[M + H]^+$; HRMS (ESI) *m*/*z* 342.1353 $[M + H]^+$, $C_{19}H_{20}NO_5$ requires 342.1341.

*5.1.2.8. (3-Fluoro-4-methoxyphenyl)-(5,6,7-trimethoxy-1H-indol-2-yl)-methanone (**4c**).* Compound **4c** was isolated after chromatography (toluene/ethyl acetate 9/1) and recrystallization (chloroform/heptane) as a yellow crystalline powder in 46% yield: mp 192–193 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3540, 3006, 2939, 2842, 1624, 1610, 1578, 1530, 1517, 1530, 1517, 1490, 1464, 1428, 1302, 1279, 1229, 1132, 1103; δ_H (300 MHz; $CDCl_3$) 9.26 (1H, br s), 7.80 (1H, ddd, *J* 8.4, 2.0 and 0.9), 7.76 (1H, dd, *J* 11.6 and 2.0), 7.07 (1H, t, *J* 8.2), 7.04 (1H, d, *J* 2.1), 6.84 (1H, s), 4.08 (3H, s), 3.99 (3H, s), 3.95 (3H, s), 3.90 (3H, s); δ_C (75 MHz; $CDCl_3$) 183.9 (*J* 2), 151.9 (*J* 246), 151.3 (*J* 10.3), 150.4, 141.5, 139.0, 133.9, 131.0 (*J* 5.4), 127.5, 126.3 (*J* 3.3), 123.4, 117.1 (*J* 19.9), 112.5 (*J* 3.3), 112.1, 97.9, 61.5, 61.2, 56.4, 56.3; *m*/*z* (ESI) 360 $[M + H]^+$; HRMS (ESI) *m*/*z* 360.1258 $[M + H]^+$, $C_{19}H_{19}FNO_5$ requires 360.1247.

*5.1.2.9. (5,6,7-Trimethoxy-1H-indol-2-yl)(p-tolyl)methanone (**4d**).* Compound **4d** was isolated after chromatography (cyclohexane/ethyl acetate 85/15) and recrystallization (chloroform/heptane) as pale yellow crystals in 46% yield: mp 232–233 °C;

$\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3452, 2939, 2838, 1622, 1606, 1529, 1490, 1464, 1404, 1301, 1259, 1180, 1124, 1108; δ_H (300 MHz; $CDCl_3$) 9.24 (1H, br s), 7.88 (2H, d, *J* 8.1), 7.32 (2H, d, *J* 8.1), 7.04 (1H, d, *J* 2.3), 6.83 (1H, s), 4.09 (3H, s), 3.95 (3H, s), 3.90 (3H, s), 2.46 (3H, s); δ_C (75 MHz; $CDCl_3$) 186.2, 150.3, 142.9, 141.4, 139.0, 135.5, 134.4, 129.3, 129.1, 127.4, 123.4, 112.3, 98.0, 61.5, 61.2, 56.3, 21.7; *m/z* (ESI) 348 $[M + Na]^+$; HRMS (ESI) *m/z* 326.1400 $[M + H]^+$, $C_{19}H_{20}NO_4$ requires 326.1392.

*5.1.2.10. (3-Amino-4-methylphenyl)(5,6,7-trimethoxy-1H-indol-2-yl) methanone (**4e**).* Compound **4e** was isolated after chromatography (cyclohexane/ethyl acetate 7/3) and recrystallization (chloroform/heptane) as an orange amorphous solid in 54% yield: $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3690, 3451, 2939, 2838, 1619, 1571, 1530, 1491, 1464, 1302, 1227, 1122, 1107; δ_H (300 MHz; $CDCl_3$) 9.23 (1H, br s), 7.33 (1H, dd, *J* 7.7 and 1.5), 7.18 (1H, d, *J* 7.7), 7.07 (1H, d, *J* 2.2), 6.83 (1H, s), 4.08 (3H, s), 3.96 (3H, s), 3.89 (3H, s), 3.83 (2H, br s), 2.27 (3H, s); δ_C (75 MHz; $CDCl_3$) 186.4, 150.3, 144.7, 141.4, 139.0, 137.1, 134.5, 130.3, 127.3, 127.2, 123.4, 119.9, 98.0, 61.5, 61.2, 56.3, 17.6; *m/z* (ESI) 341 $[M + H]^+$; HRMS (ESI) *m/z* 341.1496 $[M + H]^+$, $C_{19}H_{21}N_2O_4$ requires 341.1501.

*5.1.2.11. (5,6,7-Trimethoxy-1H-indol-2-yl)(6-methoxypyridin-3-yl) methanone (**4f**).* Compound **4f** was isolated after chromatography (cyclohexane/ethyl acetate 7/3) and recrystallization (chloroform/heptane) as beige crystals in 63% yield: mp172-173 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3450, 2940, 2839, 1620, 1601, 1530, 1487, 1464, 1372, 1301, 1292, 1258, 1126, 1108; δ_H (300 MHz; $CDCl_3$) 9.24 (1H, br s), 8.86 (1H, dd, *J* 2.2 and 0.5), 8.17 (1H, dd, *J* 8.7 and 2.3), 7.06 (1H, d, *J* 2.2), 6.87 (1H, dd, *J* 8.7 and 0.5), 6.83 (1H, s), 4.09 (3H, s), 4.05 (3H, s), 3.95 (3H, s); 3.91 (3H, s); δ_C (75 MHz; $CDCl_3$) 183.6, 166.4, 150.5, 149.2, 141.6, 139.3, 138.9, 134.1, 127.7, 127.6, 123.4, 112.2, 111.1, 98.0, 61.5, 61.2, 56.7, 54.1; *m/z* (ESI) 343 $[M + H]^+$; HRMS (ESI) *m/z* 343.1309 $[M + H]^+$, $C_{18}H_{19}N_2O_5$ requires 343.1294.

*5.1.2.12. (E)-1-(4,5,6-Trimethoxy-1H-indol-2-yl)-3-phenylprop-2-en-1-one (**9**).* Compound **9** was isolated after chromatography (toluene/ethyl acetate 95/5) and recrystallization (dichloromethane/hexane) as yellow crystals in 61% yield: mp 219–220 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3452, 2961, 2932, 1720, 1645, 1629, 1519, 1281, 1170; δ_H (300 MHz; $CDCl_3$) 9.25 (1H, br s), 7.88 (1H, d, *J* 15.7), 7.69–7.66 (2H, m), 7.50 (1H, d, *J* 15.7), 7.46–7.41 (4H, m), 6.62 (1H, s), 4.16 (3H, s), 3.93 (3H, s), 3.88 (3H, s); δ_C (75 MHz; $CDCl_3$) 179.9, 155.3, 147.1, 142.6, 136.4, 135.3, 134.9, 130.4, 129.0, 128.4, 121.5, 116.2, 108.0, 88.9, 61.5, 61.0, 56.2; *m/z* (ESI) 338 $[M + H]^+$; HRMS (ESI) *m/z* 338.1403 $[M + H]^+$, $C_{20}H_{20}NO_4$ requires 338.1392.

*5.1.2.13. (E)-1-(5,6,7-Trimethoxy-1H-indol-2-yl)-3-phenylprop-2-en-1-one (**10**).* Compound **10** was isolated after chromatography (cyclohexane/ethyl acetate 85/15) and recrystallization (chloroform/heptane) as yellow crystals in 49% yield: mp 154–155 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3451, 3004, 2939, 2838, 1648, 1594, 1530, 1493, 1311, 1220, 1195, 1170, 1111; δ_H (300 MHz; $CDCl_3$) 9.28 (1H, br s,), 7.90 (1H, d, *J* 15.6), 7.69–7.66 (2H, m), 7.47 (1H, d, *J* 15.6), 7.45–7.42 (3H, m), 7.25 (1H, d, *J* 2.3), 6.86 (1H, s), 4.08 (3H, s), 3.95 (3H, s), 3.92 (3H, s); δ_C (75 MHz; $CDCl_3$) 180.4, 150.4, 143.0, 141.6, 139.0, 136.4, 134.9, 130.5, 129.0, 128.5, 127.7, 123.4, 121.6, 109.5, 98.0, 61.5, 61.2, 56.3; *m/z* (ESI) 338 $[M + H]^+$; HRMS (ESI) *m/z* 338.1405 $[M + H]^+$, $C_{20}H_{20}NO_4$ requires 338.1392.

5.2. Biological evaluation procedures

5.2.1. Evaluation of cytotoxicity in murine B16 melanoma cells

Murine B16 melanoma cells were grown in DMEM medium containing 2 mM L-glutamine, 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin (37 °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, CA4 was routinely included in the experiments as reference compound. Exponentially growing B16 cells were plated onto 96-well plates at 5000 cells per well in 100 μl of culture medium. Twenty-four h after plating, 100 μl of medium containing the compound of interest at final concentrations ranging from 0.01 to 30 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 [25] and absorbance was read at 562 nm in a microplate reader (BioKinetics Reader, EL340). Appropriate controls with DMEM only and MTT were run to subtract background absorbance. 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 of three independent experiments each run in triplicate.

5.2.2. Antiproliferative assays on human colon carcinoma cell lines

These assays were monitored at the laboratory "Ciblothèque Cellulaire", ICSN-CNRS, Gif sur Yvette, France. Human colon carcinoma cells (HCT-116, HCT-15 and HT-29) were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, streptomycin and fungizone, and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Cells (500–800/well) were seeded in 96-well microplates containing 200 μL growth medium. After 24 h of culture, the cells were exposed to varying concentrations (0.5 nM–10 μM) of the tested compound dissolved in DMSO (less than 1% in each preparation). After 72 h of incubation, 40 μL of the MTS reagent (Promega) was added for 2 h before the absorbance at 490 nM was recorded. The IC_{50} corresponds to the concentration of the tested compound eliciting a 50% inhibition of cell growth.

5.2.3. Inhibition of tubulin polymerization

Tubulin microtubule assembly in microtubules was carried out using the fluorescent dye DAPI (4′,6-diamidino-2-phenylindole) [26] in a 96-well plate format as described by Barron et al. [20b] and Bane et al. [27] 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 IC_{50} 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.

Acknowledgments

This work was financially supported by the Centre National de la Recherche Scientifique (CNRS), the Institut Curie, the Institut National de la Santé et de la Recherche Médicale (Inserm), and by a grant from the Institut National du Cancer (INCa, Boulogne Billancourt, France). We acknowledge Dr Thierry Cresteil (ICSN, CNRS-UPR 2301, Gif sur Yvette, France) for accurate *in vitro* cytotoxicity evaluation on human cells. M.A. is grateful to the Ministère

de l'Enseignement Supérieur et de la Recherche for a Ph.D. Fellowship Grant. We also thank Thomas Perseille for expert technical assistance.

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