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Synthesis and antineoplastic activity of combretastatin analogues: Heterocombretastatins

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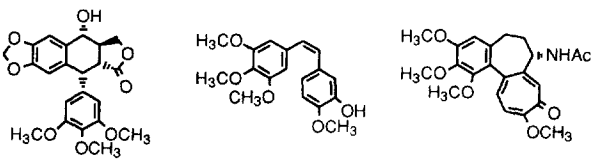
1. Introduction

Bridged biaryl represents a common structural feature for many antineoplastic agents based on active compounds of natural origin. Inhibitors of tubulin polymerization, such as podophyllotoxin [1, 2], colchicine [3–5] and combretastatin A-4 [6–11], are the best known models with this type of molecular arrangement (*figure 1*). All of them have two aromatic rings (which can be directly bonded) separated by one to four carbon atoms, in such a way that they are close in the space and maintained out of coplanarity, a structural requirement for activity which lack the plane and less active phenanthrene derivatives [12, 13].

Due to the simplicity of their structures, many synthetic analogues of combretastatins [13–15] have been obtained. Among others, the length of the alkyl bridge, the presence or absence and the configuration of the double bond, the substituents on the bridge chain and on the aromatic rings and additional aryl–aryl junctions (*figure 2*) have been studied to know their influence on the antineoplastic activity of this class of compounds.

In addition, several benzamides [15, 16], benzimines [17] and benzylanilines [15, 17] have been obtained as *aza*-bridged analogues of combretastatins. Replacement of benzene rings by other aromatic substructures has only been performed in the naphthylcombretastatins [18] and in several pyridocombretastatins [15] (*figure 2*). Other tubulin polymerization inhibitors, such as the imidazopyridazine derivatives described by Denyer et al. [19] and the highly active quinolones described by Lee et al. [20, 21], are also diarylethane derivatives with antineoplastic activity.

Although it has been deduced from SAR studies that the 3-hydroxy-4-methoxyphenyl moiety is necessary for a high antitumoral activity of combretastatin analogues [12–14, 22], many other analogues without this moiety are also active. In order to check the



Podophylloxotin | Combretastatin A-4 | Colchicine

Figure 1. Representative examples of antineoplastic bridged biaryls.

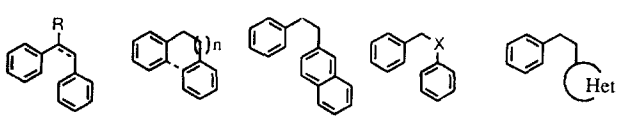


Figure 2. Types of structural variations of combretastatins and heterocombretastatins.

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effect of changing a benzene ring of combretastatins by π-excessive aromatic heterocyclic moieties, we have synthesized and assayed for antineoplastic activity, a number of furan and indole derivatives.

Two families of compounds (*figure 3*), which result from the formal replacement of either the trimethoxyphenyl (type I) or the 3-hydroxy-4-methoxyphenyl (type II) groups of combretastatin by heteroaromatic moieties have been prepared.

2. Chemistry

The synthesis of the designed heterocombretastatins of type I and II was achieved from suitable 2-heteroaryl-1,3-dithianes **5**, obtained from heteroarylcarbaldehydes, by alkylation with 3-benzyloxy-4-methoxybenzyl or 3,4,5-trimethoxybenzyl bromides (*figure 4*), following the methodology previously described by us for the synthesis of combretastatins [23] and naphthylcombretastatins [18]. The dithianes **6** and **7** were obtained in high yield, and used as versatile intermediates for the synthesis of combretastatin analogues with or without substitutions in the bridge between both aromatic rings. Hence, desulfurization (accompanied by debenzylation in type I compounds) with Raney-Nickel yielded heterocombretastatins **1** and **2**. The respective treatment with HgO followed by debenzylation of the protected phenolic group afforded the heterocombretastatones **3** and **4**. $NaBH_4$ reduction of series **4** produced representative compounds belonging to series **8**. Physical properties, MS and IR data for compounds **1–8** are shown in *table I* and their ^1H and ^{13}C-NMR data are shown in *table II*.

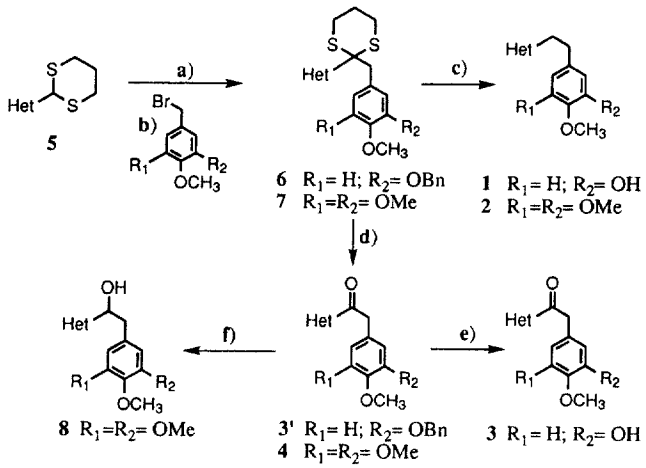

Figure 4. Reagents and conditions: (a) *n*BuLi, THF, –78 °C; (b) aryl bromide, THF, –40 °C, TMEDA; (c) Raney-Ni, Me_2CO; (d) HgO, $BF_3{\cdot}Et_2O$, THF, H_2O; (e) NaI, $BF_3{\cdot}Et_2O$, CH_3CN; (f) $NaBH_4$, MeOH.

3. Pharmacology

Combretastatin analogues synthesized by this methodology (**1–4** and **8**), and the dithiane intermediates (**6** and **7**) of the synthesis, were tested for anti-

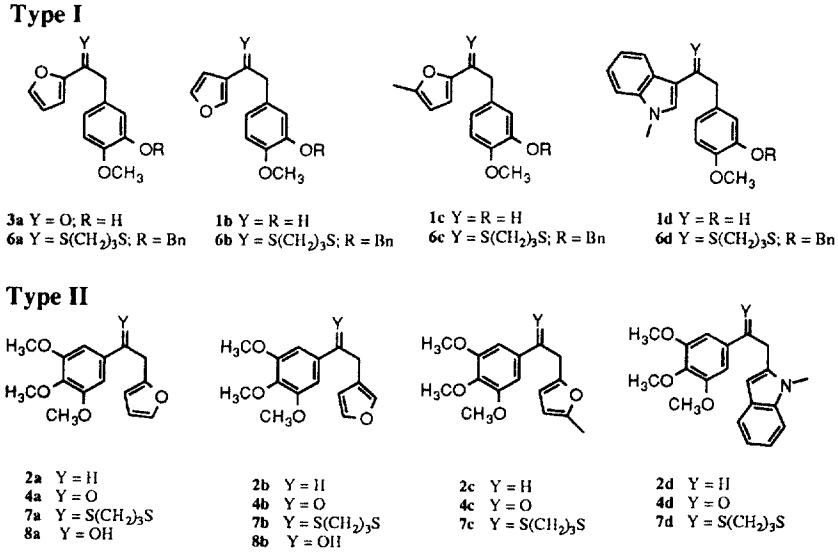

Figure 3. Types of heterocombretastatins by replacement of benzene rings of combretastatins. Structure of compounds obtained and assayed in this paper.

Table I. Physical properties, mass and IR spectra of compounds **1–8**.

Compound	Mp (°C)	Yield	Ms (*m/z*)	IR ν (cm^{-1})
1b	–	20%	218	3250 (OH), 1600, 1515, 750 (Ar)
1c	–	73%	232	3450 (OH),1600, 1510, 750 (Ar)
1d	–	38%	281	3500 (OH), 1600,1515, 740 (Ar)
2a	–	47%	262	1590, 1505, 810 (Ar)
2b	–	58%	262	1600, 1510, 780 (Ar)
2c	–	37%	276	1600, 1510, 830 (Ar)
2d	–	63%	325	1600, 1510, 750 (Ar)
3a	–	18%	232	3520 (OH), 1670 (C=O), 1610, 1515, 750 (Ar)
4a	68	98%	276	1650 (C=O), 1595, 1565, 1505, 800 (Ar)
4b	98	98%	276	1650 (C=O), 1580, 1565, 1500, 780 (Ar)
4c	92	92%	290	1660 (C=O), 1590, 1510, 780 (Ar)
4d	–	55%	339	1650 (C=O), 1600, 1540, 750 (Ar)
6a	122	54%	412	1590, 1515, 750 (Ar)
6b	94	39%	412	1600, 1520, 800 (Ar)
6c	72	35%	426	1600, 1520, 800 (Ar)
6d	–	30%	475	1600, 1520, 750 (Ar)
7a	134	70%	366	1590, 1500, 770 (Ar)
7b	78	75%	366	1610, 1560, 1515, 810 (Ar)
7c	154	50%	380	1605, 1520, 780 (Ar)
7d	130	43%	429	1600, 1520, 1500, 780 (Ar)
8a	–	97%	278	3500 (OH), 1600, 1510, 750 (Ar)
8b	–	81%	278	3460 (OH), 1590, 1505, 780 (Ar)

neoplastic activity against different cell lines, following the protocol described in previous papers [24]. The results are shown in *table III*, where it can be observed that almost all of these compounds retain some antineoplastic activity against the assayed cell lines.

These results are in agreement with the activity displayed by most of the combretastatin B (dihydrocombretastatin A) analogues described in the literature. The activities are not comparable with those of the most active compounds in *cis*-stilbene series (combretastatins A), even though derivatives of type I carry a 3-hydroxy-4-methoxyphenyl moiety in their structure. Among the tested compounds the most active is the *N*-methyl-3-indolyl derivative **4d**, of the heterocombretastatone series, and also the *N*-methyl-3-indolyl derivative **1d**, of the heterocombretastatin series with nonfunctionalized bridge.

A conclusion that can be deduced from these results is that the substitution pattern in the aromatic rings, displayed by the most active combretastatin-A analogues with a *cis*-ethylene bridge, not necessarily would be the most convenient arrangement for activity in other analogues [12, 13]. For example, the most active dihydrocombretastatin is the 4-methoxy derivative (two orders higher than the 3-hydroxy-4-methoxy derivative) [15]. In the case of heterocombretastatins it is noticeable that the most active compound is the ketoderivative **4d**, though the presence of this functionalization in the ethane bridge usually diminishes the antineoplastic potency and also the tubulin affinity of this type of biaryls [12, 13], as it happened with our previously reported naphthylcombretastatin and naphthylcombretastatone [18].

On the other hand, 2-furyl, 3-furyl and 5-methyl-2-furyl derivatives display lower activities than the *N*-methyl-3-indolyl derivatives in series **1** and **4**, and small differences in series **2**, **3** and **8**. The intermediates **6** and **7** have some activity, but not very high (see *table III*). These compounds have a cyclic substructure that changes their molecular shape in comparison with the other bridged biaryls included in our study of combretastatin analogues.

4. Conclusion

If we take into account our results with naphthyl and heteroaryl analogues of combretastatins, we can conclude that the substitution of benzene rings by other aromatic rings maintains the activity of these ethane-bridged diaryls if the system has a more extended π-system, such as naphthalene or indole. If the benzene rings are substituted by simple heteroaryl or other benzenic systems, the activity diminishes in several orders of magnitude or even disappears.

Table II. ^{1}H and ^{13}C NMR spectra of compounds **1–8**.

	^{1}H NMR ($CDCl_3$): δ (ppm), *J* (Hz)	^{13}C NMR ($CDCl_3$): δ (ppm), *J* (Hz)
1b	2.72–2.69 (m, 4H), 3.87 (s, 3H), 6.24 (d, 1H, *J* = 1.8 Hz), 6.66 (dd, 1H, *J* = 1.5; 8.1 Hz), 6.75 (d, 1H, *J* = 8.1 Hz), 6.78 (d, 1H, *J* = 1.5), 7.18 (s, 1H), 7.30 (d, 1H, *J* = 1.8 Hz)	26.9 (t), 35.8 (t), 56.1 (t), 110.6 (d), 111.1 (d), 114.7 (d), 119.8 (d), 124.6 (s), 135.2 (s), 139.0 (d), 142.7 (s), 142.7 (d), 145.5
1c	2.26 (s, 3H), 2.86 (s, 4H), 3.84 (s, 3H), 5.83 (s, 2H), 6.64 (dd, 1H, *J* = 2.1; 8.1 Hz), 6.75 (d, 1H, *J* = 8.1 Hz), 6.77 (d, 1H, *J* = 2.1 Hz)	13.4 (q), 33.9 (t), 34.0 (t), 56.1 (q), 105.7 (d), 105.9 (d), 110.9 (d), 114.8 (d), 119.7 (d), 135.6 (s), 145.1 (s), 145.7 (s), 150.3 (s), 153.8 (s)
1d	3.07–2.91 (m, 4H), 3.72 (s, 3H), 3.85 (s, 3H), 6.70 (dd, 1H, *J* = 1.2; 8.0 Hz),6.80 (d, 1H, *J* = 8.0 Hz), 6.81 (s, 1H), 6.86 (d, 1H, *J* = 1.2 Hz), 7.10 (ddd, 1H, *J* = 1.7; 6.0; 8.0 Hz), 7.22 (ddd, 1H, *J* = 1.7; 6.0; 7.4 Hz), 1H, 7.28 (dd, 1H, *J* = 1.7; 8.0), 7.60 (dd, *J* = 1.7; 7.4)	27.4 (t), 32.7 (q), 36.2 (t), 56.1 (q), 109.2 (d), 110.7 (d), 114.7 (d), 114.8 (s), 118.7 (d), 119.0 (d), 119.8 (d), 121.5 (d), 126.2 (d), 127.9 (s), 136.0 (s), 137.0 (s), 144.8 (s), 145.5 (s)
2a	2.91 (s, 4H), 3.92 (s, 9H), 5.98 (d, 1H, *J* = 3.2 Hz), 6.28 (dd, 1H, *J* = 2.1; 3.2 Hz), 6.36 (s, 2H), 7.15 (d, 1H, *J* = 2.1 Hz)	30.1 (t), 34.9 (t), 56.3 (q x 2), 60.9 (q), 105.4 (d), 106.1 (d x 2), 110.3 (d), 137.0 (s), 137.1 (s), 141.0 (d), 153.4 (s x 2), 153.5 (s)
2b	2.86–2.67 (m, 4H), 3.85 (s, 6H), 3.86 (s, 3H), 6.26 (dd, 1H, *J* = 0.8; 1.7 Hz), 6.39 (s, 2H), 7.20 (d, 1H, *J* = 0.8 Hz), 7.36 1H, *J* = 1.7 Hz)	26.9 (t), 36.9 (t), 56.3 (q x 2), 60.8 (q), 106.0 (s x 2), 111.0 (d), 125.9 (s), 137.3 (s x 2), 139.1 (d), 142.7 (d), 153.3 (s x 2)
2c	2.27 (s, 3H), 2.97 (s, 4H), 3.93 (s, 3H), 3.97 (s, 6H), 5.90 (s, 1H), 6.40 (s, 2H), 6.48 (s, 1H)	13.4 (q), 30.2 (t), 35.1 (t), 56.3 (q x 2), 60.4 (q), 106.0 (d x 2), 106.7 (d x 2), 137.2 (s x 2), 150.4 (s), 153.3 (s x 2), 153.6 (s)
2d	3.10–2.89 (m, 4H), 3.74 (s, 3H), 3.81 (s, 6H), 3.83 (s, 3H), 6.44 (s, 2H), 6.80 (s, 1H), 7.12 (ddd, 1H, *J* = 1.2; 7.2; 7.7 Hz), 7.22 (ddd, 1H, *J* = 1.2; 7.2; 7.2 Hz), 7.30 (dd, 1H, *J* = 1.2; 7.7 Hz), 7.58 (dd, 1H, *J* = 1.2; 7.2 Hz)	27.2 (t), 32.5 (q), 37.2 (t), 56.3 (q x 2), 60.9 (q), 106.2 (d x 2), 109.2 (d), 114.9 (s), 118.8 (d), 119.0 (d), 121.6 (d), 126.4 (d), 128.1 (s), 137.1 (s), 137.2 (s), 138.2 (s), 153.3 (s x 2)
3a	3.85 (s, 3H), 4.01 (s, 2H), 6.51 (dd, 1H, *J* = 1.8; 3.3 Hz), 6.79 (s, 2H), 6.88 (s, 1H), 7.20 (d, 1H, *J* = 3.3 Hz), 7.58 (d, 1H, *J* = 1.8 Hz)	44.9 (t), 56.1 (q), 111.1 (d), 112.3 (d), 116.0 (d), 117.6 (d), 121.1 (d), 133.8 (s), 145.9 (s), 146.4 (s), 146.4 (d), 152.0 (s), 186.7 (s)
4a	3.91 (s, 9H), 4.05 (s, 2H), 6.52 (dd, 1H, *J* = 1.8; 3.6 Hz), 6.54 (s, 2H), 7.25 (d, 1H, *J* = 3.6 Hz), 7.60 (d, 1H, *J* = 1.8 Hz)	45.0 (t), 55.7 (q x 2), 60.3 (q), 106.6 (d x 2), 112.1 (d), 117.6 (d), 129.3 (s), 137.9 (s), 146.4 (d), 152.0 (s), 152.9 (s x 2), 196.0 (s)
4b	3.80 (s, 6H), 3.81 (s, 3H), 3.96 (s, 2H), 6.49 (s, 2H), 6.76 (d, 1H, *J* = 2.0 Hz), 7.41 (t, 1H, *J* = 2.0 Hz), 8.07 (s, 1H)	47.3 (t), 55.9 (q x 2), 60.4 (q), 106.7 (d x 2), 108.7 (d), 127.2 (s), 129.6 (s), 137.8 (s), 144.0 (d), 147.5 (d), 153.2 (s x 2), 191.9 (s)
4c	2.39 (s, 3H), 3.93 (s, 9H), 4.00 (s, 2H), 6.17 (d, 1H, *J* = 3.5 Hz), 6.54 (s, 2H), 7.18 (d, 1H, *J* = 3.5 Hz)	13.8 (q), 45.1 (t), 56.0 (q x 2), 60.5 (q), 106.8 (d x 2), 109.1 (d), 119.8 (d), 130.0 (s), 137.8 (s), 151.0 (s), 153.2 (s x 2), 157.8 (s), 185.5 (s)
4d	3.80 (s, 3H), 3.85 (s, 3H), 3.86 (s, 6H), 3.92 (s, 2H), 6.74 (s, 2H), 7.29 (dd, 1H, *J* = 4.6; 6.2 Hz), 7.77 (d, 1H, *J* = 3.0 Hz), 7.84 (s, 1H), 8.34 (ddd, 1H, *J* = 1.8; 3.0; 4.6 Hz), 8.37 (dd, 1H, *J* = 1.8; 6.2 Hz)	33.7 (q), 49.7 (t), 56.6 (q x 2), 60.7 (q), 109.8 (d x 2), 110.7 (d), 116.2 (s), 123.1 (d), 123.3 (d), 124.1 (d), 127.0 (s), 135.7 (s), 136.3 (d), 138.1 (s), 140.9 (s), 154.6 (s x 2), 192.5 (s)

Table II. *Continued.*

6a	2.04–1.80 (m, 2H), 2.62 (dt, 1H, J = 3.0; 14.5 Hz), 2.81 (ddd, 1H, J = 3.0; 11.7; 14.5 Hz), 3.23 (s, 2H), 3.79 (s, 3H), 4.92 (s, 2H), 6.20 (d, 1H, J = 2.0 Hz), 6.31 (m, 2H), 6.53 (dd, 1H, J = 2.0; 8.1 Hz), 6.70 (d, 1H, J = 8.1 Hz), 7.40 (d, 1H, J = 1.2 Hz), 7.40–7.25 (m, 5H)	25.3 (t), 28.0 (t x 2), 48.3 (t), 53.3 (s), 56.1 (q), 71.1 (1), 110.8 (d), 111.3 (d), 112.2 (d), 116.4 (d), 123.5 (d), 127.1 (s), 127.4 (d), 127.8 (d x 2), 128.5 (d x 2), 137.5 (s), 142.2 (d), 147,9 (s), 149.1 (s), 153.5 (s)
6b	1.99–1.77 (m, 2H), 2.57 (dt, 2H, J = 3.3; 14.5 Hz), 2.76 (ddd, 2H, J = 3.3, 11.7; 14.5 Hz), 3.09 (s, 2H), 3.79 (s, 3H), 4.95 (s, 2H), 6.32 (d, 1H, J = 2.0 Hz), 6.42 (d, 1H, J = 2.0 Hz), 6.58 (dd, 1H, J = 2.0; 8.1 Hz), 6.70 (d, 1H, J = 8.1 Hz), 7.19 (d, 1H, J = 2.0 Hz), 7.41–7.19 (m, SH), 7.36 (s, 1H)	25.1 (t), 27.3 (1 x 2), 49.6 (1), 51.8 (s), 55.7 (q), 70.8 (t), 110.8 (d), 111.4 (d), 116.6 (d), 123.6 (d), 126.6 (s), 127.2 (d x 2), 127.5 (d), 127.9 (s), 128.2 (d x 2), 137.2 (s), 143.1 (d x 2), 147.1 (s), 148.9 (s)
6c	1.94–1.73 (m, 2H), 2.27 (s, 3H), 2.63–2.49 (m, 4H), 3.22 (s, 2H), 3.71 (s, 3H), 4.89 (s, 2H), 5.86 (d, 1H, J = 3.1 Hz), 6.17 (d, 1H, J = 3.1 Hz), 6.24 (s, 1H), 6.52 (d, 1H, J = 8.3 Hz), 6.66 (d, 1H, J = 8.3 Hz), 7.36–7.20 (m, 5H)	13.4 (q), 24.8 (t), 27.3 (t x 2), 47.5 (t), 52.6 (s), 55.4 (q), 70.3 (t), 106.1 (d), 110.7 (d), 112.3 (d), 115.9 (d), 123.0 (d), 126.7 (s) 126.7 (d x 2), 127.1 (d), 127.9 (d x 2), 136.9 (s), 147.0 (s), 148.4 (s), 150,8 (s), 151.1 (s)
6d	1.94–158 (m, 2H), 2.68–2.56 (m, 4H), 3.64 (s, 3H), 3.34 (s, 2H), 3.74 (s, 3H), 4.39 (s, 2H), 5.79 (d, 1H, J = 1.5 Hz), 6.50 (dd, 1H, J = 1.5; 8.1 Hz), 6.60 (d, 1H, J = 8.1 Hz), 6.88 (s, 1H), 7.37–7.03 (m, 8H), 8.13 (d, 1H, J = 8.3 Hz)	25.4 (t), 27.4 (t x 2), 32.4 (q), 48.2 (t), 54.6 (s), 55.6 (q), 70.0 (t), 109.0 (d), 110.2 (d), 114.3 (s), 116.1 (d), 118.8 (d), 121.4 (d), 122.0 (d), 123.2 (d), 126.1 (s), 126.9 (s) 127.0 (d x 2), 127.3 (d), 128.1 (d x 2), 131.7 (d), 137.0 (s), 137.9 (s), 146.6 (s), 148.2 (s)
7a	2.10–1.70 (m, 2H), 2.70 (dt, 2H, J = 3.6; 14.3 Hz), 2.86 (ddd, 2H, J = 2.8, 11.8; 14.3 Hz), 3.28 (s, 2H), 3.69 (s, 6H), 3.78 (s, 3H), 6.01 (s, 2H), 6.34 (dd, 1H, J = 1.3; 3.3 Hz), 6.38 (d, 1H, J = 3.3 Hz), 7.47 (d, 1H, J = 1.3 Hz)	25.3 (t), 27.9 (t x 2), 49.0 (t), 53.0 (s), 56.0 (q x 2), 60.7 (q), 107.6 (d x 2), 110.7 (d), 112.3 (d), 129.8 (s), 137.9 (s), 142.1 (d), 152.4 (s x 2), 153.2 (s)
7b	2.00–1.80 (m, 2H), 2.65 (dt, 2H, J = 3.6; 14.1 Hz), 2.83 (ddd, 2H, J = 2.5; 9.4; 14.1 Hz), 3.14 (s, 2H), 3.72 (s, 6H), 3.80 (s, 3H), 6.13 (s, 2H), 6.40 (d, 1H, J = 1.7 Hz), 7.25 (s, 1H), 7.42 (d, 1H, J = 1.7 Hz)	24.9 (t), 27.2 (t x 2), 50.2 (t), 51.4 (s), 55.5 (q x 2), 60.3 (q), 107.9 (d x 2), 111.3 (d), 127.7 (s), 129.5 (s), 137.8 (s), 142.8 (d), 143.0 (d), 151.8 (s x 2)
7c	2.10–1.72 (m, 2H), 2.32 (s, 3H), 2.78 (dt, 2H, J = 3.8; 14.3 Hz), 2.96 (ddd, 2H, J = 2.9, 12.0; 14.3 Hz), 3.27 (s, 2H), 3.71 (s, 3H), 3.79 (s, 6H), 5.92 (d, 1H, J = 3.1 Hz), 6.05 (s, 2H), 6.25 (d, 1H, J = 3.1 Hz)	13.8 (q), 25.4 (t), 27.9 (t x 2), 49.0 (t), 53.0 (s), 55.9 (s x 2), 60.8 (s), 106.7 (d), 107.8 (d x 2), 113.0 (d), 130.1 (s), 137.8 (s), 150.0 (s), 151.8 (s), 152.4 (s x 2)
7d	2.01–1.91 (m, 2H), 2.65 (dt, 2H, J = 3.6; 14.0 Hz), 2.90 (ddd, 2H, J = 3.9, 10.0; 14.0 Hz), 3.42 (s, 2H), 3.43 (s, 6H), 3.67 (s, 3H), 3.73 (s, 3H), 5.84 (s, 2H), 6.96 (s, 1H), 7.30–7.00 (m, 3H), 8.10 (d, 1H, J = 7.9 Hz)	25.4 (t), 27.6 (t x 2), 32.5 (t), 49.3 (t), 54.7 (s), 55.4 (q x 2), 60.5 (q), 107.9 (d x 2), 108.9 (d), 114.4 (s), 118.8 (d), 121.5 (d), 122.0 (d), 126.3 (s), 130.6 (s), 131.9 (d), 137.0 (s), 137.9 (s), 151.8 (s x 2)
8a	2.10 (dd, 2H, J = 2.5; 6.9 Hz), 3.80 (s, 6H), 3.81 (s, 3H), 4.33 (t, 1H, J = 6.9 Hz), 6.22 (d, 1H, J = 3.2 Hz), 6.31 (s, 2H), 6.33 (dd, 1H, J = 1.8; 3.2 Hz), 7.44 (d, J = 1H, 1.8 Hz)	41.2 (t), 56.2 (q x 2), 56.6 (q), 77.1 (d), 107.0 (d x 2), 108.5 (d), 110.1 (d), 133.8 (s), 137.2 (s), 142.2 (d), 153.1 (s x 2), 153.9 (s)
8b	2.90 (dd, 1H, J = 8.1; 13.9 Hz), 2.98 (dd, 1H, J = 5.1; 13.9 Hz), 3.83 (s, 9H), 4.87 (dd, 1H, J = 5.1; 8.1 Hz), 6.42 (s, 2H), 6.43 (dd, 1H, J = 1.8; 4.0 Hz), 7.37 (d, 1H, J = 4.0 Hz), 7.40 (d, 1H, J = 1.8 Hz)	45.2 (t), 56.3 (q x 2), 60.3 (q), 68.0 (d), 107.0 (d x 2), 108.7 (d), 128.6 (s), 133.3 (s), 137.4 (s), 139.3 (d), 143.3 (d), 153.4 (s x 2)

Table III. Antineoplastic activities for several combretastatin analogues **1–8** using Podophyllotoxin as reference compound in the same assays (IC_{50} μM inhibition of cell growth)[a].

	P-388	A-549	HT-29	MEL-28
1b	22.9	> 45.9	> 45.9	> 45.9
1c	21.5	21.5	43.1	43.1
1d	3.6	3.6	8.9	3.6
2a	19.1	19.1	19.1	19.1
2b	> 76.3	> 76.3	> 76.3	> 76.3
2c	7.2	7.2	7.2	7.2
2d	15.4	15.4	15.4	15.4
3a	10.8	21.5	21.5	21.5
4a	> 72.5	> 72.5	> 72.5	> 72.5
4b	7.2	7.2	7.2	7.2
4c	8.6	17.2	17.2	8.6
4d	0.6	0.6	0.6	0.3
6a	6.1	12.1	6.1	12.1
6b	6.1	12.1	2.9	12.1
6c	5.9	5.9	2.3	11.7
6d	5.3	10.5	21.1	10.5
7a	13.7	6.8	6.8	6.8
7b	13.7	6.8	6.8	6.8
7c	> 52.6	> 52.6	> 52.6	> 52.6
7d	5.8	5.8	11.6	11.6
8a	17.5	36.0	36.0	36.0
8d	35.9	> 71.9	> 71.9	> 71.9
Podophyllotoxin	0.05	0.05	0.05	0.06

[a]Antineoplastic activity of combretastatin A-4 [15]. $ED_{50} = 1.4 \times 10^{-5} - 3.0 \times 10^{-8}$ μM.

After these results, it seems very convenient to study the effect of other bridged biaryls, with fused benzene systems or with heteroaromatic rings, on the activity of this kind of compounds, in the same way as it has been done with the diphenyl analogues of combretastatins A and B.

5. Experimental protocols

5.1. Chemistry

Melting points were taken on a Buchi-510 apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 200 (^1H: 200.13 MHz; ^{13}C: 50.32 MHz). Chemical shifts are given in ppm downfield from tetramethylsilane as the internal standard and $CDCl_3$ as the solvent, unless otherwise stated. Splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), ddd (doublet of doublets of doublets), dt (doublet of triplets), dtt (doublet of triplets of triplets), m (multiplet). Coupling constants (*J* values) are listed in hertz (Hz). Carbon multiplicities were determined by DEPT experiments. IR spectra were obtained on a Beckman (Acculab VIII) spectrometer. Mass spectra were recorded on Gas Chromatograph-Mass spectrometer Hewlett-Packard 5890 Series II under standard conditions.

*5.1.1. Synthesis of 2-heteroaryl-1,3-dithianes **5**: typical procedure*

To a magnetically stirred solution of the appropriate arylcarbaldehyde (0.021 mol) in 21 mL of $CHCl_3$ cooled to 0 °C, were successively added 1,3-propanedithiol (2.1 mL; 0.023 mol) and trimethylsilyl chloride (0.5 mL; 4.6 mmol). The reaction was maintained at room temperature overnight, then quenched with saturated NaOH (100 mL) and extracted with $CHCl_3$ (2 x 20 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. Evaporation of the solvent left a brown solid that was purified by crystallization in EtOAc–hexane mixtures.

*5.1.2. Synthesis of dithiane derivatives **6** and **7** by alkylation of 2-heteroaryl-1,3-dithianes **5**: typical procedure*

To a solution of **5a–d** (3.3 mmol) in dry THF (22 mL) at –78 °C under argon, a 1.6 M solution of BuLi in hexanes (2.3 mL, 3.6 mmol) was added dropwise. After 1 h the mixture was warmed to –20 °C and 3,4,5-trimethoxybenzyl bromide or 3-benzyloxy-4-methoxybenzyl bromide (3.6 mmol) in dry THF (9 mL) and TMEDA (0.5 mL, 3.6 mmol) were slowly added consecutively. After 24 h at –20 °C, the reaction was quenched with a saturated NH_4Cl solution and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and the solvent evaporated in vacuum. The solid residue was purified by flash chromatography (33% EtOAc–hexane) to yield **6a–d** or **7a–d**.

*5.1.3. Synthesis of **1b–d** and **2a–d** by desulphurization of dithianes **6** and **7**: typical procedure*

To a slurry of Raney-Ni (950 mg), a solution of **6** or **7** (0.2 mmol) in ethanol (20 mL) was added. The mixture was gently refluxed during 1 h, cooled to room temperature, filtered over silica and the organic solvent evaporated in vacuum to afford **1b–d** or **2a–d** as an oily product.

*5.1.4. Synthesis of **3'a–c** and **4a–d** by deprotection of dithianes **6** and **7**: typical procedure*

To a magnetically stirred suspension of HgO (420 mg, 1.9 mmol) in H_2O–THF 15% (5 mL), BF_3•OEt_2 (0.2 mL, 1.9 mmol) was added dropwise under argon at 0 °C. After 5 min, a solution of **6** or **7** (0.97 mmol) in H_2O–THF 15% (19 mL) was slowly added and the mixture was allowed to react at room temperature overnight. The reaction was poured with CH_2Cl_2 (10 mL) and the precipitate was filtered, the organic layer was extracted with EtOAc, washed with brine and dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified by flash chromatography (EtOAc–hexane 50%) to yield **3'a–c** and **4a–d**.

*5.1.5. Synthesis of **3a** by debenzylation of **3'a**: typical procedure*

To a mixture of **3'a** (135 mg, 0.42 mmol) and NaI (150 mg, 1.26 mmol) in CH_3CN, BF_3•OEt_2 (0.05 mL, 0.46 mmol) was added dropwise under argon atmosphere at 0 °C. The reaction mixture was stirred overnight at room temperature and then quenched with water (10 mL), extracted with EtOAc (100 mL). The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under vacuum. The crude product was purified by flash chromatography to afford **3a**.

*5.1.6. Synthesis of **8a–b** by reduction of ketones **4**: typical procedure*

To a solution of **4a–b** (0.14 mmol) in anhydrous MeOH (15 mL), $NaBH_4$ (90 mg, 0.15 mmol) was slowly added with vigorous stirring, under argon at 0 °C. The reaction mixture was stirred at this temperature during 1.5 h and then quenched with water. The mixture was extracted with ether (3 x 25 mL) and the organic layer was washed with brine, dried over Na_2SO_4 and evaporated under vacuum. The crude product was purified by flash chromatography to yield **8a–b**.

5.2. Pharmacology

In vitro antitumor activity was screened using an adapted procedure of the method described by Bergeron [25] against the following cell lines: a suspension culture of a lymphoid neoplasm from DBA/2 mouse (P-388), a monolayer culture of the human lung carcinoma (A-549), a monolayer culture of the human colon carcinoma (HT-29) and a monolayer culture of the human melanoma (MEL-28). Cells were maintained in exponential phase growth in Eagle's minimum essential medium (EMEM), supplemented with 5% fetal calf serum (FCS), a 10^{-2} M solution of sodium bicarbonate, a mixture of 0.1 g/L penicillin G and 0.1 g/L streptomycin sulfate, Earle's balanced salts, and 2.0 mM L-glutamine.

Cells were seeded into 16 mm wells (multidishes NUNC 42001) at concentrations of 1 x 10^4 (P-388), 2 x 10^4 (A-549), (HT-29) and (MEL-28) cells/well in 1 mL aliquots of EMEM 5% FCS containing the compound to be evaluated at the concentrations tested. In each case, a separate set of control wells was incubated in the absence of sample to ensure that cells remained in the exponential phase growth. All determinations were carried out in duplicate. After 3 days of incubation at 37 °C, under a 10% CO_2, in a 98% humid atmosphere, P-388 cells were observed through an inverted microscopy and the degree of inhibition was determined by comparison with the controls, whereas A-549, HT-29 and MEL-28 were fixed with 0.4% formalin and stained with 0.1% crystal violet before examination. The results of these assays were used to obtain the dose–response curves from which IC_{50} (mM) values were determined. An IC_{50} value represents the concentration (mM) of the sample which produces a 50% cell growth inhibition.

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