



Design, Synthesis and Cytotoxic Activities of Naphthyl Analogues of Combretastatin A-4

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Abstract—The 3,4,5-trimethoxyphenyl and 3-hydroxy-4-methoxyphenyl rings of combretastatin A-4 are deemed optimal for its activity as antimetabolic agent. The replacement of either one by a naphthalene ring results in compounds with a potency comparable to that of the parent compound. These results show that the naphthalene ring is a good surrogate for the 3,4,5-trimethoxyphenyl or the 3-hydroxy-4-methoxyphenyl rings of combretastatin A-4 and that neither of them is essential for the antitumor activity. © 2000 Elsevier Science Ltd. All rights reserved.

The microtubule system of eukaryotic cells is an appealing target for the design of potential anticancer agents. A variety of compounds, including colchicinoids, lignans and combretastatins inhibit tubulin polymerization by binding to a common site (Fig. 1). Combretastatin A-4 is reported to be one of the most potent antimetabolic agents so far described and to strongly inhibit the polymerization of tubulin.¹

The structure–activity analysis of the combretastatins has shown that the optimal bridge between the two phenyl rings is a *cis* ethylene. The 3,4,5-trimethoxyphenyl and the 3-hydroxy-4-methoxyphenyl rings or others structurally close are present in almost all of the more potent combretastatin analogues and have thus been considered as responsible for the activity.² The rationale for the substitution of the polyoxygenated rings by the nonpolar naphthalene is a previous QSAR for such compounds, which indicated a good correlation between the cytotoxic activity and the hydrophobicity values of the functional groups on ring B of combretastatins,³ and our previous experience: compounds with the naphthalene substitution and either a dihydrocombretastatin skeleton or conformationally restricted analogues bearing an indole bridge (Fig. 2) display activities close to those of the traditional substitutions of combretastatins.^{4,5} The SARs have also indicated

that the trends in potency for the substituents on the aromatic rings are maintained when going from the less potent dihydrocombretastatins to the *cis*-stilbenes.⁶ In this paper we describe the synthesis and some preliminary biological data for some new compounds in this family displaying a high potency.

The naphthyl combretastatins were synthesized following the classical Wittig methodology. The 3,4,5-trimethoxybenzaldehyde and 2-naphthalenecarbaldehyde were converted in good overall yields into the corresponding phosphonium salts by sequential reduction with NaBH₄, substitution of the resulting benzylic alcohol with PBr₃ and subsequent displacement of the bromide with PPh₃. The corresponding ylides obtained by treatment with *n*BuLi were reacted with the required aldehydes (the phenolic hydroxyls protected when necessary as the ethoxymethyl ethers) to yield roughly 1:1 mixtures of the *E* and *Z* isomers, which were separated by chromatography. The stereochemistries of the isomers were unambiguously established by means of NOE and the value of the *J* coupling between the olefinic protons when available. These data are in agreement with the mutual shielding observed in the ¹H NMR spectra for several aromatic hydrogens on both rings when the stereochemistry holds them in a spatial disposition required for such an effect to be seen (Scheme 1).⁷

The cytotoxic activities against four different tumor cell lines were measured as previously described⁸ and are

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summarized in Table 1. The *cis* isomers are more potent than the corresponding *trans* compounds, as usually observed for the combretastatins. The activities of the dihydro-compounds, previously obtained and taken as models for the synthesis of these more potent analogues, are close to or higher than those displayed by the *trans* isomers and two orders of magnitude lower than those of the *cis* analogues. Such an observation is in agreement with previous results on the activity of similar series of compounds and with the lack of 'pre-organization' of the hydrogenated products, when compared

to the *cis*-stilbenes, and further supports our initial hypothesis that the trends of the activities reported for the different substituents on both series of compounds are parallel. The indole-bridged compound **3**, despite its conformational rigidity did not improve the potency, suggesting that the combination of three extended aromatic rings might be too bulky in order to achieve a strong interaction with its target.

The naphthyl-combretastatins **4a** and **5a** display a cytotoxic potency similar to each other and to that of

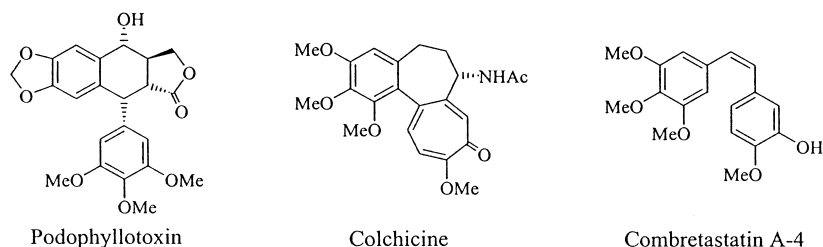


Figure 1. Colchicine-site ligands.

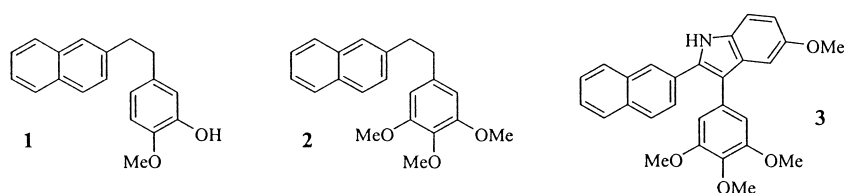
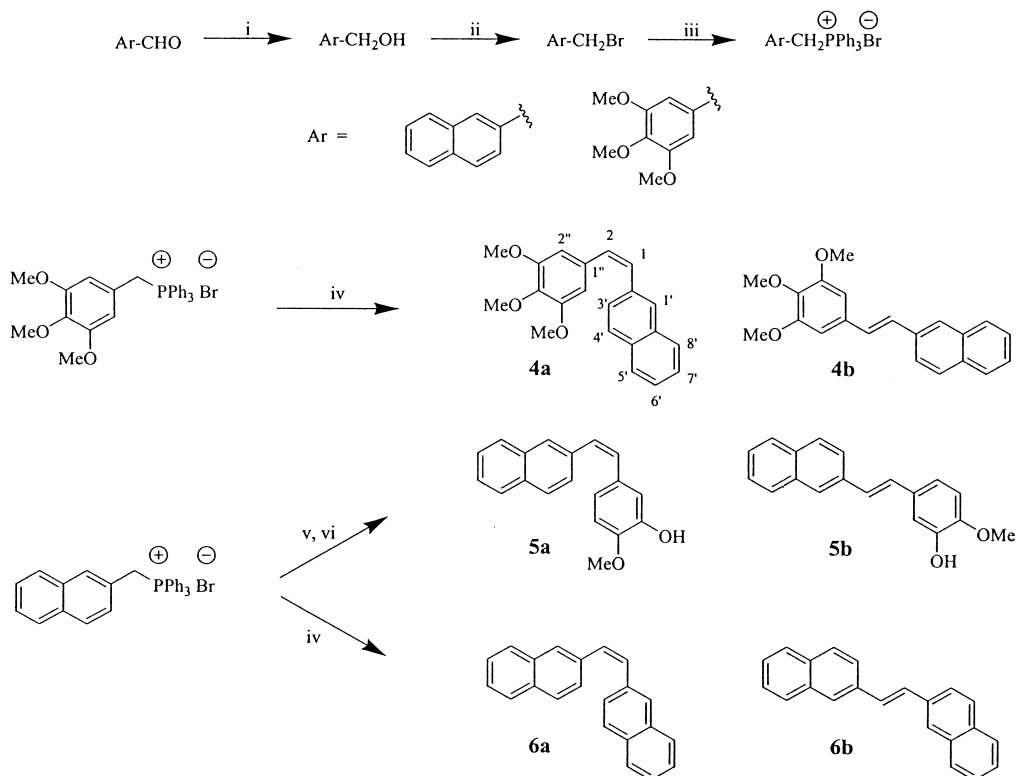


Figure 2. Described naphthyl analogues.



Scheme 1. (i) NaBH₄/MeOH (> 95%); (ii) PBr₃/Et₂O (65–85%); (iii) PPh₃/toluene (> 95%); (iv) *n*BuLi, –78 °C and then 2-naphthalenecarbaldehyde (> 90%); (v) *n*BuLi, –78 °C and then 3-ethoxymethoxy-4-methoxybenzaldehyde (90%); (vi) HCl/MeOH (70%).

Table 1. Cytotoxic potencies of compounds synthesized expressed as the log(IC₅₀) M

Compound	P388	A-549	HT29	MEL-28
Combretastatin A-4	-8.5	-8.5	-7.5	-8.5
1 ⁴	-5.3	-5.3	-5.0	-5.3
2 ⁴	-6.5	-6.5	-6.5	-6.5
3 ⁵	-5.9	-5.9	-5.9	-5.9
4a	-7.8	-7.8	-7.8	-7.8
4b	-6.4	-6.4	-6.4	-6.4
5a	-7.4	-7.4	-7.4	-7.4
5b	-5.4	-5.4	-5.4	-5.4
6a	-5.4	-5.4	-5.4	-5.4

combretastatin A-4. This result implies that the naphthalene ring is a good surrogate for either the trimethoxyphenyl or the 3-hydroxy-4-methoxyphenyl rings of combretastatin A-4 and that neither one is required for the activity. The capability of the naphthalene ring for substituting for either ring suggests that the interaction with tubulin is hydrophobic in nature and that some polar interactions can be lost at the expense of lower entropic loss upon binding. The lower potency of bis-naphthyl analogue **6a** indicates that the whole substitution by hydrophobic naphthalene moieties is detrimental for the activity.

In order to confirm that the activities of these compounds are due to interactions with the microtubule system, in vitro studies on their effect on cellular microtubules of leukemic cells have been performed.⁹ The preliminary results show that the tested compounds disrupt the microtubule system, **4a** being roughly 40 times more potent than **4b** but 5 times less than model combretastatin A-4.

The new compounds and cytotoxicity results are of interest for establishing the SAR of the combretastatins and the design of new antimitotic agents based on naphthalene systems that could inhibit tubulin polymerization. Further studies are under way to better characterize the interactions of these naphthylcombretastatins with tubulin.

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- For example, for compound **4a** $J_{1,2} = 12$ Hz; NOE: {1}: 2, 1' and 3'; {2}: 1 and 2''/6''; {2''/6''}: 2, C3''/C5''-OMe and 3'. δ (¹H): 7.78 (1H, sa, H1'); 7.73–7.78 (2H, m, H6'/H7'); 7.68 (1H, d, $J = 8.8$, H4'); 7.42–7.46 (3H, m, H3'/H5'/H8'); 6.74 (1H, d, $J = 12.0$, H1); 6.59 (1H, d, $J = 12.0$, H2); 6.53 (2H, s, H2''/H6''); 3.84 (3H, s, 4''-OMe); 3.59 (6H, s, 3''/5''-OMe). δ (¹³C): 153.5 (C, C3''/C5''); 137.5 (C, C4''); 135.0 (C, C2'); 133.6 (C, C8a'); 132.6 (C, C4a'); 132.6 (C, C1''); 130.5 (CH, C2); 129.9 (CH, C1); 128.2 (CH, C7'); 127.9 (CH, C1'); 127.7 (CH, C6'); 127.5 (CH, C3'); 127.1 (CH, C4'); 126.2 (CH, C8'); 126.1 (CH, C5'); 106.3 (CH, C2''/C6''); 61.0 (CH₃-O-C4''); 56.2 (CH₃-O-C3''/5'').
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