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A new family of quinoline and quinoxaline analogues of combretastatins

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Abstract—The 3-hydroxy-4-methoxyphenyl ring of combretastatin A-4 can be replaced by a 2-naphthyl moiety without significant loss of cytotoxicity and inhibition of tubulin polymerization potency. In this paper we show that the 6- or 7-quinolyl systems can in turn replace both cyclic moieties, keeping in the first case most of the potency as cytotoxic agent and in the second case as inhibitor of tubulin polymerization, related to the activities displayed by model compounds.

Microtubules generated by polymerization of tubulin α,β-dimers are the main constituents of the mitotic spindle, whose formation and activity are required for chromosome segregation during mitosis and for other cell processes.[1] Natural and synthetic compounds of varied structures have been shown to inhibit tubulin polymerization through interaction with the protein at different binding sites; the colchicine, taxol and *Vinca* alkaloids sites are the best known of them.[2] Among the ligands of the colchicine binding site, combretastatins[3] are highlighted as strongly cytotoxic[4] and antiangiogenic[5] agents, combretastatin A-4 (CS A-4) being the most potent member of this family[6] (Fig. 1). The main problem associated with this class of compounds is their low aqueous solubility. Therefore, efforts have been directed at obtaining soluble derivatives, leading to combretastatin A-4 phosphate prodrug,[7] which currently is in phase II clinical trials.

From different SAR studies on combretastatins[8,9] it was deduced that a 3,4,5-trimethoxyphenyl ring and a 4-methoxy-3-X-substituted phenyl ring systems, separated by a two atoms bridge in a *cis* disposition, is the common structural characteristic for these compounds to be active (X = H, OH, NH_2 and their aminoacyl, phosphate or other derivatives for solubilizing purposes). It has been reported that lipophilicity is an important feature for ligand binding, showing a good correlation between log*P* and binding to tubulin,[8] although there are also contradictory reports.[9] We have recently shown that one of the oxygenated **A** or **B** phenyl rings of CS A-4 can be replaced by a naphthalene moiety keeping high potency,[10] whereas substitution of both **A** and **B** rings leads to an inactive compound. Following these results, we proceeded further in order to determine which one of the CS A-4 rings is actually best replaced by the naphthalene in the CS A-4 analogues. We then prepared different families of compounds that were tested for cytotoxicity and inhibition of tubulin polymerization,[11] leading to the conclusion that the naphthalene system is a good surrogate for the 3-hydroxy-4-methoxyphenyl ring **B**, producing much less potent derivatives when the naphthalene replaces the trimethoxyphenyl ring **A** (Fig. 1).

Even though the introduction of a naphthalene system keeps most of the potency of combretastatins and increases their lipophilicity, their low aqueous solubility is a main drawback in order to assay these compounds in vivo. To extend our studies to other bicyclic systems as surrogates of the **B** ring of CS A-4 and to increase their poor solubility, we have designed a new family of analogues replacing the naphthalene moiety by a quinoline or quinoxaline system and keeping the

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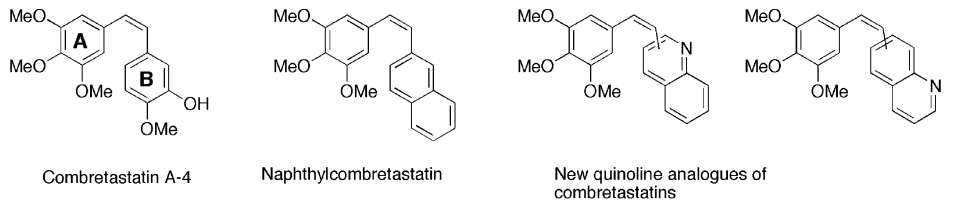


Figure 1. Structure of combretastatin A-4, naphthylcombretastatin and the new quinoline analogues of combretastatins.

3,4,5-trimethoxyphenyl moiety as ring **A** (Fig. 1). These heterocycles maintain the same size as the original naphthalene, so steric factors would not affect the binding to the protein. However, the presence of one or two nitrogen atoms allows these compounds or their salts to be more water soluble, so they could be assayed in aqueous solution. The effect of the position of the nitrogen atoms on the activity and/or potency of the analogues would also contribute to enlarge the SAR for these compounds.

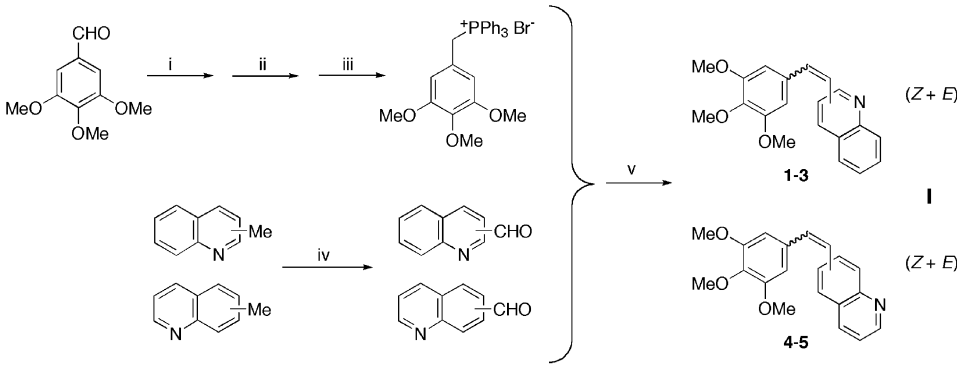
The subfamily of quinoline derivatives (**I**) was prepared following the methodology described for the naphthylcombretastatins[10,11] (Scheme 1), by Wittig reactions of the corresponding quinoline carbaldehydes with 3,4,5-trimethoxybenzyltriphenylphosphorane, obtained in turn by treatment of the phosphonium bromide with *n*-BuLi. The quinoline carbaldehydes were prepared from the corresponding methylquinolines by oxidation with selenium dioxide in refluxing xylene.[12] The Wittig reaction afforded the desired compounds as a mixture of *Z* and *E* isomers (variable ratios from 1:2 to 2:1), which were separated by chromatography on silica gel. The stereochemistry of the isomers was determined as previously reported.[10]

For the synthesis of the quinoxaline subfamily (**II**, Scheme 2), 3,4-diaminobenzoic acid was used as starting material. It was condensed with glyoxal (for the plain quinoxaline derivatives) or butanedione (for the 2,3-dimethyl analogues) in refluxing ethanol and acetic acid.[13] The resulting quinoxalinecarboxylic acid was then reduced to the primary alcohol with $LiAlH_4$ and subsequently oxidized to the aldehyde with pyridinium chlorochromate in CH_2Cl_2. The Wittig methodology aforementioned was employed for synthesizing the quinoxaline analogues of combretastatins.

The aqueous solubility of all the *Z*-quinolines, **1a**–**5a**, is 3- to 7-fold higher than that of the model naphthylcombretastatin, whereas the solubility of the quinoxaline derivative **7a** is about the same.[14] The most water-soluble compound **3a** has even a higher solubility than CS A-4.

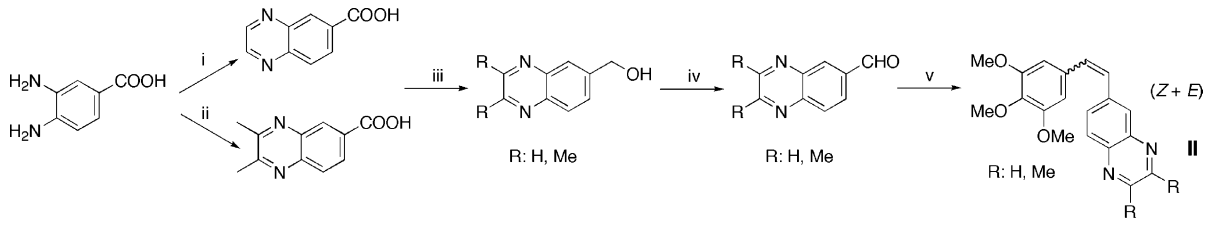
The cytotoxic activity of all these compounds was assayed against different tumour cell lines as described.[15] The results are presented in Table 1. The *cis* isomers are more potent than the corresponding *trans* isomers, as usually observed for all the combretastatins and their analogues, even though the difference in potency is lower than in the case of the most potent combretastatins.

Among quinoline-substituted compounds, those bearing the nitrogen atom on the nonattached ring (**4** and **5**) show higher cytotoxic potency than the ones with the nitrogen atom on the ring directly attached to the ethylene (**1**, **2** and **3**). This fact is in agreement with the requirement of a benzene ring directly attached to the ethylene bridge for high potency, supporting our previ-



Compound **I**	Substituent / double bond	Compound **I**	Substituent / double bond
1a	2-quinolyl / *Z*	**4a**	6-quinolyl / *Z*
1b	2-quinolyl / *E*	**4b**	6-quinolyl / *E*
2a	3-quinolyl / *Z*	**5a**	7-quinolyl / *Z*
2b	3-quinolyl / *E*	**5b**	7-quinolyl / *E*
3a	4-quinolyl / *Z*		
3b	4-quinolyl / *E*		

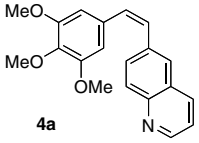
Scheme 1. Reagents and conditions: (i) $NaBH_4$, MeOH (>95%); (ii) PBr_3, Et_2O, −40 °C (97%); (iii) PPh_3, toluene (98%); (iv) SeO_2, xylene, reflux (50–80%); (v) *n*-BuLi, THF, −78 °C, then aldehyde, THF, −20 °C (60–85%).



Compound **II**	R	Double bond
6a	H	*Z*
6b	H	*E*
7a	Me	*Z*
7b	Me	*E*

Scheme 2. Reagents and conditions: (i) glyoxal, AcOH, EtOH, reflux (31%); (ii) butanedione, AcOH, EtOH, reflux (52%); (iii) $LiAlH_4$, THF (75%); (iv) CCP, DCM (30%); (v) 3,4,5-trimethoxybenzyl bromide, THF, *n*-BuLi, −78 °C, then aldehyde, THF, −20 °C (55–60%).

Table 1. Cytotoxic activity of the compounds assayed expressed as the −log (IC_{50}) M



4a

Compound	P-388	A-549	HT-29	MEL-28	H116
Combretastatin A-4	8.5	8.5	7.5	8.5	8.5
Naphthylcombretastatin	7.8	7.8	7.8	7.8	7.8
I					
1a	6.5	6.5	6.5	6.5	6.5
1b	6.4	6.4	6.4	6.4	6.4
2a	6.4	6.4	6.4	6.4	6.4
2b	<5.5	<5.5	<5.5	<5.5	<5.5
3a	5.1	5.1	4.8	5.1	5.1
4a	7.5	7.5	7.5	7.5	7.5
5a	6.8	6.8	6.8	6.8	6.8
5b	5.8	5.8	5.8	5.8	5.8
II					
6a	5.1	5.1	5.1	5.1	5.1
6b	<4.8	<4.8	<4.8	<4.8	<4.8
7a	<4.8	<4.8	<4.8	<4.8	<4.8
7b	<4.8	<4.8	<4.8	<4.8	<4.8

ous results with other analogues, for example, 3- and 5-indolyl derivatives.[11] Quinoxaline analogues display lower potency than their quinoline counterparts, indicating that an additional nitrogen atom is detrimental for activity.

The more potent *cis* isomers were also assayed for inhibition of tubulin polymerization, following the methodology described in the literature,[11,16] in order to know the correlation between their cytotoxic activity and their interaction with the microtubule system. The results are shown in Figure 2.

The 6-quinolyl derivative **4a**, that showed the highest cytotoxic potency, unexpectedly displays a very weak potency as inhibitor of tubulin polymerization. This would mean that the actual mechanism of action for this compound is not the inhibition of microtubule formation through binding to tubulin, but a different one leading nonetheless to a highly cytotoxic effect. Thus, compound **4a** is a good candidate to study the mechanism of action of the analogues of combretastatins that have shown unexpected low potency as inhibitors of tubulin polymerization related to their cytotoxicity.

However, the second most potent analogue, 7-quinolyl derivative **5a**, inhibits tubulin polymerization with an IC_{50} 20 μM, close to the 12 μM measured for naphthylcombretastatin. In this respect, the decrease in cytotoxicity parallels that in polymerization inhibition, as usually observed for the most potent antimitotic agents of this type. Compound **5a** mimics the structural rigidity of the naphthalene moiety and also includes a nitrogen atom at the same relative position as the highly potent 3′-amine analogue of CS A-4.[17]

The other compounds tested for inhibition of tubulin polymerization did not reveal any significant activity, in accordance with their lower cytotoxicity. None of them inhibited more than 20% of the polymerization activity of the control at 40 μM. Compounds **6a** and **7a** did not display any polymerization inhibitory activity at even 60 μM.

In summary, we have synthesized a new family of analogues of combretastatin A-4 bearing a quinoline or quinoxaline system replacing the 3-hydroxy-4-methoxyphenyl ring of combretastatin A-4 or the naphthyl moiety of our previously described naphthylcombretastatin. The quinoline derivatives showed an increased aqueous solubility related to the parent naphthylcombretastatin. Only the quinoline derivatives in which the nitrogen atom is on the nonattached ring elicited relevant cytotoxic activity. Such cytotoxicity correlated with an inhibitory effect on tubulin polymerization only in the case of **5a**. Thus, the introduction of quinoline moieties bonded through the non-nitrogenated ring seems to be a convenient structural modification for antimitotic agents acting at the colchicine binding site, as it is the case with the related naphthyl system. Compound **4a**, highly cytotoxic but very weak inhibitor of tubulin polymerization, could be very useful for the elucidation of the mechanism of action of combretastatin

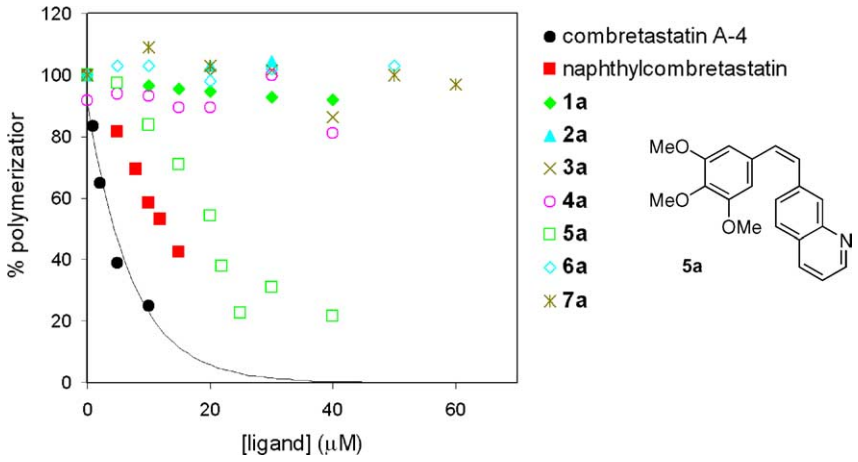


Figure 2. Inhibition of tubulin polymerization (as microtubule protein, MTP) by compounds **1a**–**7a**, CS A-4 and naphthylcombretastatin, expressed as percentage of polymerization for noninhibited protein. MTP concentration is 1.5 mg/mL.

analogues with activities that do not fit into the SAR for these type of compounds.

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