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Conformationally restricted macrocyclic analogues of combretastatins

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Abstract—New analogues of combretastatins have been evaluated as inhibitors of tubulin polymerization. These compounds present a macrocyclic structure, in which the *para* positions of the aromatic moieties have been linked by a 5- or 6-atoms chain, in order to produce a conformational restriction. This could contribute to determine the active conformation for these ligands. Such a conformational restriction and/or the steric hindrance makes them less potent inhibitors than the model compound CA-4.

Microtubules are structures formed by polymerization of αβ-tubulin dimers. They are essential to many cellular processes, such as maintenance of cellular shape, intracellular transport, or mitotic spindle assembly during cell division.[1] Inhibition of microtubule formation leads to mitotic arrest and promotes vascular disruption in angiogenic vessels through activation of the RhoA and/or the VE-cadherin/β-catenin pathways.[2] Tubulin-binding agents such as colchicine or vinblastine display both effects, but in these two cases the vascular shutdown occurs at concentrations close to the maximum tolerated dose.[3]

Combretastatin A-4 (CA-4, Fig. 1) is one of the most potent inhibitors of tubulin polymerization, binding at the colchicine site on the protein. It is currently in phase I/II clinical trials as its phosphate prodrug[4] due to its antimitotic and antiangiogenic properties. Other members of the combretastatin family also inhibit tubulin polymerization, such as deoxycombretastatin,[5] combretastatin,[6] and related dihydroxy and dioxolane derivatives (Fig. 1),[7] among others. In the two latter, the configuration of the stereogenic carbons has proved to be essential for antitubulin activity.

The structural requirements for a potent antitubulin activity are those present in CA-4.[8] A 3,4,5-trimethoxyphenyl and a 4-methoxy-3-X-phenyl (being X = H, OH, NH_2 and their aminoacyl, phosphate, or other solubilizing derivatives) separated by a 2-atoms bridge with *cis* disposition are the structural features for the most potent compounds. The aromatic rings show a non-coplanar disposition. However, the conformation of CA-4 bound to tubulin is not known. Colchicine and podophyllotoxin show different spatial arrangement of the aromatic rings when bound to tubulin[9] and, despite this, both of them are potent inhibitors of polymerization (IC_{50} = 0.8–3.3 and 0.3–3 μM, respectively). This fact has been attributed to the flexibility of the binding site, which can accommodate ligands bearing different structural scaffolds and spatial dispositions of the aromatic rings.

Many combretastatin analogues have been prepared in which the phenyl rings are locked in the *cis* orientation by the formation of small cycles on the bridge, in an attempt to prevent them from isomerizing.[8,10]

As part of our research directed at the synthesis and evaluation of new antimitotic agents, we have designed new analogues of combretastatins in which a macrocycle is formed by linking the *para* positions of both aromatic rings, through the corresponding oxygen or nitrogen atoms. This restriction generates new *cis*-locked analogues, irrespective of the bridge structure. Conformational restriction by macrocycle formation on bioactive

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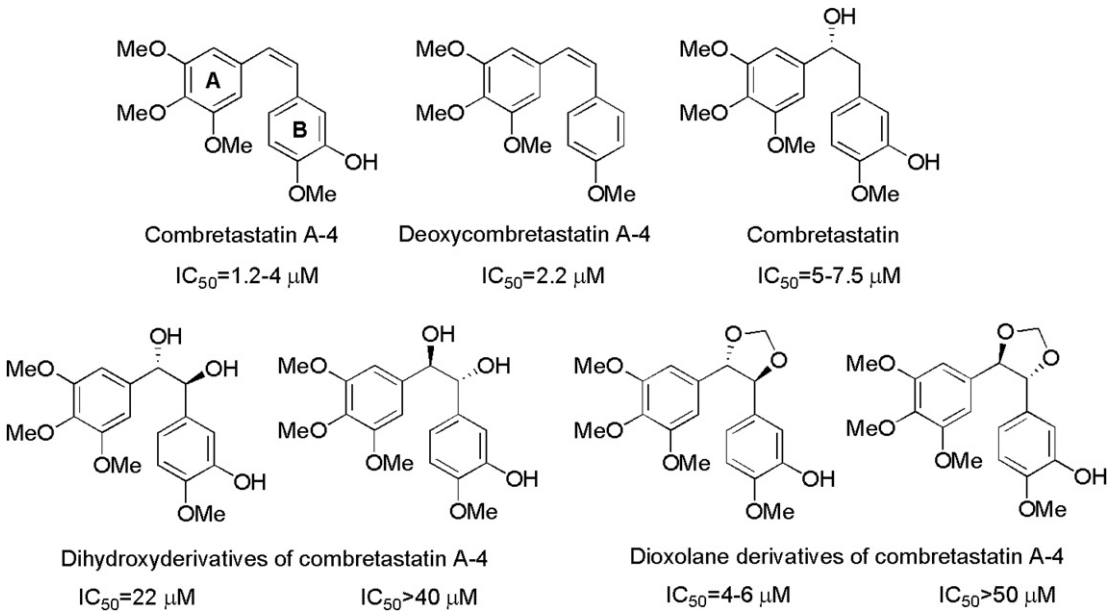

Figure 1. Structure and potency as inhibitors of tubulin polymerization of combretastatin A-4, deoxycombretastatin A-4, combretastatin and related diols and dioxolanes.

compounds has been used to increase binding affinity, selectivity, and metabolic stability. Recently, the effect of macrocyclization and stereochemistry on the biological activity of small molecules has been quantitatively measured.[11]

Two linkers with different length (5 or 6 atoms) were chosen to afford a different degree of conformational restriction by varying the macrocycle size. Thus, 3-oxapentamethylene or hexamethylene linkers were employed, giving rise to Families **I** and **II**. This modification involves a minor increment of molecular size (CH_2–O–CH_2 or $(CH_2)_4$, see Fig. 2), restricted to the space between the aromatic rings and thus without affecting the more external surface.

The substitution pattern of both aromatic rings was selected so to mimic the structural features that confer high potency to combretastatins. Thus, 3,5-dimethoxy groups were attached to phenyl ring A (the 4-methoxy group being part of the linker). For ring B, three possibilities were envisaged. First, keep it without substitution, as in deoxycombretastatin A-4. Second, attach a hydroxyl group at position 3, as in combretastatin A-4 (the 4-methoxy group also being part of the linker in both cases). And third, replace the phenyl ring by a more rigid 5-indolyl system, which had produced potent compounds as surrogate for ring B.[12]

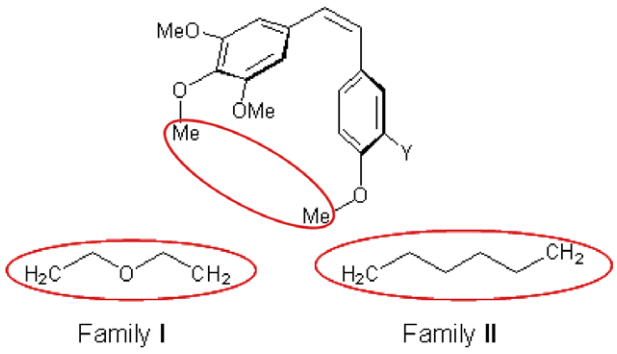

Figure 2. Structure of the linkers.

As the bridge between the rings, the analogues bear the double bond present in combretastatin A-4, the corresponding glycol (*cis* or *trans*) or its derivatives (acetate or dioxolane). We expected that the conformational restriction imposed on these compounds by the formation of the macrocycle (also influenced by the substitution pattern on the rings[13] and on the bridge[14]) could make them to adopt optimal binding geometries, so they could show an increase in tubulin binding potency. The results will then shed light on which is the active conformation of combretastatins when bound to tubulin.

The synthesis of this type of compounds has been reported previously by us.[13,14] Briefly, olefins and glycols were prepared by means of an intramolecular McMurry reaction of the dialdehydes. These are in turn synthesized by double Mitsunobu reaction starting from the corresponding aromatic aldehydes and the appropriate linker diol (diethyleneglycol for Family **I** or 1,6-hexanediol for Family **II**) (Fig. 3). The synthesized compounds are shown in Figure 4.

These macrocyclic combretastatin analogues were evaluated as tubulin polymerization inhibitors. The assay conditions are as previously reported.[12] The compounds were usually assayed at 20 or 40 μM (some analogues were assayed at lower concentration due to scarce aqueous solubility) and the results expressed as percentage of polymerization of the control (Table 1). It should be noted that, in order to compare the potencies of the analogues, some values of ITP must be extrapolated due to the different concentrations assayed.