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Synthesis and biological activity of naphthalene analogues of phenstatins: Naphthylphenstatins

Concepción Álvarez,[a] Raquel Álvarez,[a] Purificación Corchete,[b] Concepción Pérez-Melero,[a] Rafael Peláez[a,*] and Manuel Medarde[a,*]

[a]*Departament de Química Farmacéutica, Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain*
[b]*Departamento de Fisiología Vegetal, Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain*

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Abstract—Novel phenstatin analogues with a 2-naphthyl moiety combined with either a 2,3,4- or a 3,4,5-trimethoxyphenyl ring have been synthesized, and their tubulin polymerization inhibiting and cytotoxic activities have been evaluated. The 2-naphthyl ring is a better replacement for the 3-hydroxy-4-methoxyphenyl ring in the phenstatin series than in the combretastatin series. For the naphthylphenstatins, the carbonyl is required, and the preferred orientation of the trimethoxyphenyl ring is the one found in combretastatins.

Ligands binding at the colchicine site of tubulin, such as colchicine itself, podophyllotoxin, the combretastatins and the phenstatins, are receiving much attention due to their interesting antitumour, antiangiogenic and antiparasitic activities. Several such ligands are now undergoing clinical trials, for example, the combretastatin A4 phosphate prodrug.[1,2] Most of these ligands share common structural features, often summarized in the structure of combretastatin A4, such as two non-coplanar oxygenated aromatic rings (typically one trimethoxyphenyl and a 3-hydroxy-4-methoxyphenyl or related ring). Certain variability is allowed for the size of the bridge between the two phenyl rings, which ranges from zero to more than four atoms (Fig. 1).[3]

We have previously shown that the 3-hydroxy-4-methoxyphenyl ring of combretastatin A4 can be replaced by a 2-naphthyl ring (Fig. 2) with no substantial loss of potency. On the other hand, replacement of the 3,4,5-trimethoxyphenyl ring or the introduction of a 1-naphthyl ring is detrimental for the activity.[4–6] In an attempt to increase the aqueous solubility of the naphthyl analogues, we have also explored the consequences of the introduction of nitrogen atoms on the 2-naphthyl ring of naphthylcombretastatins and found that in quinoline and isoquinoline analogues of combretastatins the heteroatom has an effect on the activity which is dependent on its position.[7] The moderate solubility increase

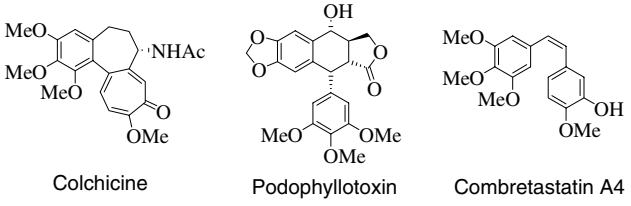

Figure 1. Representative colchicine site ligands with different bridge sizes.

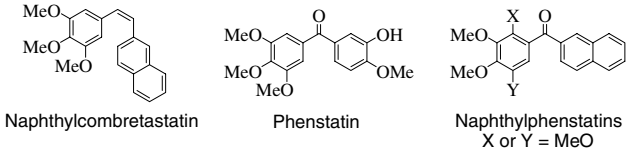

Figure 2. Structures of naphthylcombretastatin, phenstatin and designed naphthylphenstatins.

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* Corresponding authors. Tel.: +34 923 294528; fax: +34 923 294515 (R.P.); e-mail addresses: pelaez@usal.es; medarde@usal.es

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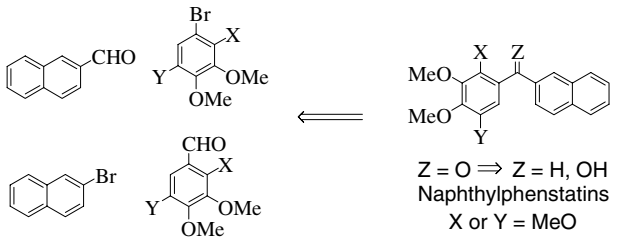
achieved and the more cumbersome handling make such a modification less attractive.

Recently, much synthetic effort has been devoted to the bisarylketones named phenstatins (Fig. 2) and related analogues, which show substantial differences in their structure–activity relationships when compared to the combretastatins.[8–10] In this paper, we disclose our preliminary results on the synthesis and biological activity of 2-naphthophenones (Fig. 2) bearing a 2-naphthyl ring (in substitution of the 3-hydroxy-4-methoxyphenyl or related ring) combined with either a 3,4,5-trimethoxyphenyl ring (such as the one present in combretastatins and podophyllotoxin) or a 2,3,4-trimethoxyphenyl ring (such as that found in colchicine). We have termed these compounds naphthylphenstatins, in analogy to the naphthylcombretastatins.

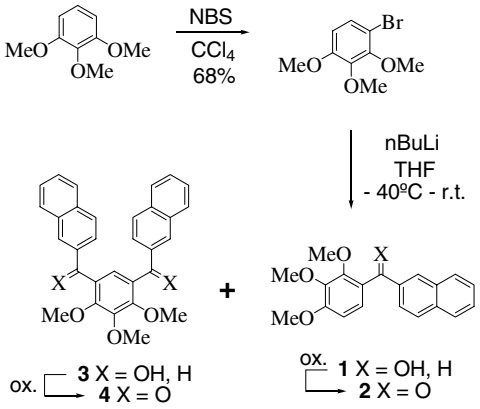
We planned the synthesis of the naphthylphenstatins based on the reaction of an organometallic (formed by transmetallation of the corresponding bromoderivative) with an aldehyde (Scheme 1). Of the two possible pairs of starting materials, we set out to start the synthesis with the halogenated trimethoxyphenyl rings and commercial 2-naphthaldehyde, a strategy we thought might be more convenient for the later preparation of related analogues, such as the quinolines, and which had previously shown itself successful.

Accordingly (Scheme 2), we brominated 1,2,3-trimethoxybenzene with NBS in carbon tetrachloride. The bromide was transmetallated with *n*-BuLi and treated with 2-naphthaldehyde. We obtained mixtures of mono- (**1**) and dialkylated (**3**) products even in the presence of excess of bromide. These byproducts could not be chromatographically resolved, not even after oxidation to the corresponding ketones (**2** and **4**), rendering this route inconvenient for this and related synthesis.

The alternative approach, starting from 2-bromonaphthalene and the isomeric trimethoxybenzaldehydes, was then attempted (Scheme 3). Initially, treatment of the 2-naphthyl bromide with Mg in THF was selected for the formation of the organometallic species, in order to avoid direct addition of the organolithic reagent to the aldehyde. However, even under inert atmosphere, mixtures of alcohols and ketones were formed. Therefore, we chose *n*-BuLi for the transmetallation reaction. With this reagent, the major byproducts (**9** and **10**) were formed by direct addition of *n*-BuLi to the aldehyde, but they were easily removed by chromatography.



Scheme 1. Retrosynthetic analysis for naphthylphenstatins.

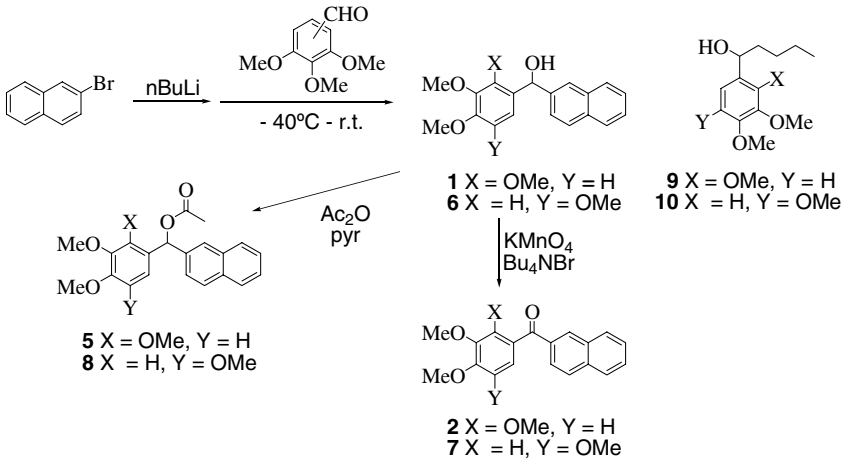


Scheme 2. Formation of mono- and dialkylated products.

Acetylation of the resulting alcohols with acetic anhydride and pyridine occurred in lower than usual yields (around 70%), probably due to the ease of formation of the corresponding bisbenzilic cation and its transformation into byproducts during chromatography. Oxidation of the alcohols was attempted with PCC, according to literature procedures, but low yields of the desired ketones were obtained. When the crude reaction mixture was treated with PCC in CH_2Cl_2, the only oxidized products were **9** and **10**. In our hands, the best conditions found for the oxidation were: $KMnO_4$ and a phase transfer catalyst (tetrabutylammonium bromide) with wet CH_2Cl_2 as solvent. Under these conditions, the ketones could be readily isolated after oxidation of the unpurified alcohols in 70% or better yields.

The synthesized compounds were assayed for tubulin polymerization inhibition[6] at a single concentration of 20 or 40 μM (Table 1). Compound **7** was the only active, showing an IC_{50} one order of magnitude lower than its naphthylcombretastatin analogue (Table 1) and three times lower than CA-4. Literature values for tubulin polymerization inhibition for phenstatin show that it is only one to two times more potent than CA-4.[8–10] Cross comparison of these data suggests that **7** is at least equipotent to phenstatin. In combretastatins, replacement of the 3-hydroxy-4-methoxyphenyl ring by the 2-naphthyl one decreases somewhat the potency. In phenstatins, no reduction is observed, indicating that the 2-naphthyl is an even better replacement in these structures. Neither the alcohols (**1** and **6**), nor the acetates (**5** and **8**) were active. The active orientation for the trimethoxyphenyl ring corresponds to that of combretastatins and podophyllotoxin. The lack of activity for the one-carbon bridged naphthylphenstatins with a 2,3,4-trimethoxyphenyl ring agrees with our earlier observation on the same ring combination (2-naphthyl and 2,3,4-trimethoxyphenyl) in zero-carbon bridged analogues[6] and indicates that it is not a good replacement in colchicine-like compounds.

The synthesized compounds were assayed following a described procedure[6] against several cancer cell lines, including human cervix epitheloid carcinoma HeLa, human lung carcinoma A-549 and human colon adeno-



Scheme 3. Synthesis of naphthylphenstatins.

Table 1. Tubulin polymerization inhibition assay results for compounds **1**, **2**, and **5**–**8**

Compound	% inhibition of tubulin polymerization (μM)[a]	IPT IC_{50}[b] (μM)
1	0 (20)	>20
2	4 (20)	>20
5	26 (20)	>20
6	0 (40)	>20
7	100 (40)	1.1
8	5 (20)	>20
CA-4	100 (20)	3 (1–3)
Naphthylcombretastatin	—	10
Phenstatin	—	(0.4–1.5)

[a] Values are means of at least three experiments (concentration assayed).

[b] Concentration inhibiting 50% of 1.0 mg/mL microtubular protein polymerization (literature values).

carcinoma HT-29, and compared with naphthylcombretastatin and CA-4 (Table 2). The most sensitive cell line was HeLa, in which all the compounds except **1** showed micromolar to submicromolar (compound **7**) potencies. The other two cell lines were more resistant to the compounds, again being **7** the only one with remarkable activity. Opposite to the tubulin polymerization inhibitory activity, **7** is less cytotoxic than CA-4 and even than naphthylcombretastatin. These observations agree well with previous work on phenstatins: they display higher tubulin inhibitory activity but lower cytotoxicity than their combretastatin counterparts.[11] This apparent contradiction might be explained by the different concentration ranges at which the two measured effects are produced and/or by differences in permeability. In such a context, the small difference in cytotoxicity observed between **7** and its naphthylcombretastatin analogue again suggests a more favourable substitution of the 3-hydroxy-4-methoxyphenyl ring by the 2-naphthyl in this family of compounds than in the combretastatins. As different trends of structure–activity relationships for both families have been previously found,[8–10] these results suggest that the 2-naphthyl ring represents a structural intersection between the structure activity relationships of both families of compounds.

Table 2. Cytotoxicity results (IC_{50}/μM) for compounds **1**, **2**, **5**, **7**, and **8**

Compound	HeLa[a]	A549[a]	HT29[a]
1	>10	>10	>10
2	1.7	>10	>10
5	2.7	>10	>10
7	0.042	0.25	—
8	3.8	>10	>10
CA-4	0.003	0.003	0.003
Naphthylcombretastatin	0.02	0.02	0.02

[a] Values are means of three experiments.

In summary, we have synthesized new naphthylphenstatins with two trimethoxyphenyl ring orientations. The most potent compound has a ketone on the bridge and a 3,4,5-trimethoxyphenyl, equivalent to that found in combretastatins and podophyllotoxin. On the other hand, a 2,3,4-trimethoxyphenyl, equivalent to that found in colchicine, turned out to be suboptimal. These results suggest that the 3D structure of podophyllotoxin in complex with tubulin might be a better model in which to perform docking experiments than the complex with colchicine. When compared to this family of compounds, the 2-naphthyl ring seems to be an even better substitution for the 3-hydroxy-4-methoxyphenyl ring than in the combretastatin family.

Acknowledgments

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