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SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF COMBRETASTATIN ANALOGUES. NAPHTHYLCOMBRETASTATINS AND RELATED COMPOUNDS

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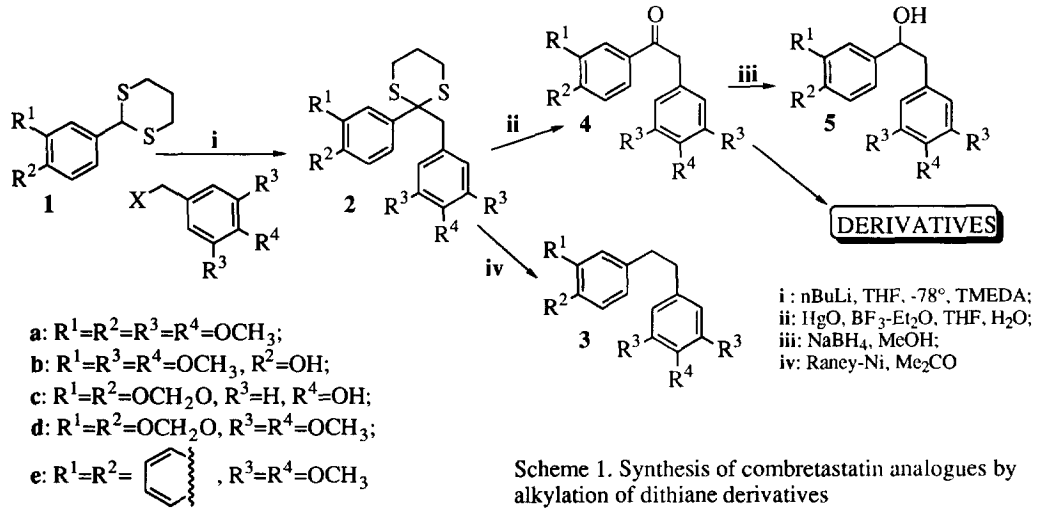
Abstract.- Through a versatile synthesis of combretastatins we have prepared and assayed for activity some new compounds of this kind of antitumoral agents. Ketoderivatives (combretastatones) and other diphenylethane derivatives display low activity, but naphthylcombretastatin **3e** shows activity comparable to that of the most potent combretastatins-B (dihydrocombretastatins), in spite of the lack of the 3-hydroxy-4-methoxyphenyl substructure considered to be neccesary for high antineoplastic potency.

Among the antitumoral compounds of natural origin that have been used as a model for the synthesis of new families of active compounds combretastatins constitute a very interesting type of diphenyl derivatives. Only a few natural combretastatins have been described[1], but many other derivatives have been recently synthesized and evaluated for antimitotic and antitumoral activity.[2] The knowledge of their mechanism of action[3] and the structural simplicity of these compounds are very attractive for the development of new analogues[4]. Iwasaki *et. al.* have summarized these ideas in a paper dealing with the synthesis of *aza*-combretastatins[5]. Other papers devoted to SAR of combretastatins and other diphenyl alkanes/alkenes have also been published in the last few years[6].

To prepare these compounds, we have used a current methodology[7] based on the alkylation of dithianes of type **1** (Scheme 1) which is suitable for the synthesis of combretastatin like compounds in yields higher than those previously described and based on Wittig or Grignard reactions[1]. Along with ethylene derivatives **3** (combretastatins-B or dihydrocombretastatins-A), it is easy to obtain combretastatones **4**[8] which could be used to prepare hydroxycombretastatins **5** (as combretastatin) and other functionalized derivatives. Combretastatones and the intermediate dithianes **2** contain the C-C bridge present in the most active compounds. Furthermore, the preferred conformations of compounds of the type **2**, **3** (combretastatins-B) and **4** (combretastatones)[9] around the central C-C bond display the two phenyl groups in an almost *syn*-parallel arrangement closer to that of the more active *cis*-olefins (combretastatins-A) rather than to that of the less active *trans*-isomers.

In this communication we present the first results obtained in the assays for antitumoral and antiviral activity[10] of representative compounds prepared by this methodology. All the synthesized derivatives have a trimethoxyphenyl moiety bounded to the differently substituted ethane bridge. Although structure-activity studies about the influence of the structure of the carbon bridge, the *cis-trans* configuration of the ethylenic double bond, substitution pattern of the rings, cyclized compounds, etc... the optimal structure has not yet been found. A *cis*-ethylenic bridge and one 3-hydroxy-4-methoxyphenyl moiety have been found to produce good activity. In a first stage we prepared and checked for antineoplastic activity the aforementioned derivatives in order to determine the influence of differently substituted aromatic rings on the antitumoral activity.



a: R^1=R^2=R^3=R^4=OCH_3;
b: R^1=R^3=R^4=OCH_3, R^2=OH;
c: R^1=R^2=OCH_2O, R^3=H, R^4=OH;
d: R^1=R^2=OCH_2O, R^3=R^4=OCH_3;
e: R^1=R^2= , R^3=R^4=OCH_3

Scheme 1. Synthesis of combretastatin analogues by alkylation of dithiane derivatives

Following the previously described protocol[11,12]: dithiane **2**, ketone **4** and ethylenic derivatives **3** of diphenylethanes have been tested and compared with podophyllotoxin (**6**), which is a well known tubulin polymerization inhibitor acting through the reversible binding to the colchicine site (Table 1). This is the same mechanism that has been demonstrated for combretastatins. The assayed diphenylethanes **2-5a**, **4b**, **3c** and **2-3d** lack noticeable activity. This result is in accordance with the SAR studies[2,6] of combretastatins in relation with the substitution of aromatic rings. Trimethoxy (**a**) and methylenedioxyphenyl (**c**) groups are not adequate for antitumoral activity (tubulin binding) of this kind of compound if the other aromatic residue has not suitable substituents.

After these results, we carried out molecular modeling studies to compare the most active dihydrocombretastatins carrying a trimethoxyphenyl moiety (those with 4-methoxyphenyl **7** and 3-hydroxy-4-methoxyphenyl **8** moieties) with compounds **2-5 a-d** and other diarylethanes. Among the studied compounds we found a noticeable fitting between the trimethoxyphenyl-naphthylethane **3e** and compound **8**. When the trimethoxyphenyl moieties of dihydrocombretastatin A-4 (**8**) and **3e** are held together, the 3-hydroxy-4-methoxyphenyl moiety of **8** and the naphthyl moiety of **3e** are located in the same position (Figure 1). So, we synthesized naphthylcombretastatin **3e** by desulfurization of dithiane derivative **2e**, and naphthylcombretastatone **4e** by deprotection of the same intermediate. Activity results of these compounds are also included in table 1.

Table 1. Antineoplastic activities for several combretastatin analogues. (IC_{50} μM inhibition of cell growth[12,13])

	P-388	A-549	HT-29	MEL-28
2a: R^1=R^2=R^3=R^4= OCH_3	22.9	22.9	22.9	>22.9
3a: R^1=R^2=R^3=R^4= OCH_3	15.0	15.0	15.0	15.0
4a: R^1=R^2=R^3=R^4= OCH_3	>28.9	>28.9	>28.9	>28.9
5a: R^1=R^2=R^3=R^4= OCH_3	28.7	>28.7	>28.7	>28.7
4b: R^1=R^3=R^4= OCH_3, R^2= OH	30.0	>30.0	>30.0	>30.0
3c: R^1=R^2= OCH_2O, R^3= H; R^4= OH	41.2	>41.2	>41.2	>41.2
2d: R^1,R^2=O-CH2-O, R^3=R^4=OCH_3	5.9	5.9	5.9	4.7
3d: R^1,R^2=O-CH2-O, R^3=R^4=OCH_3	6.3	6.3	6.3	6.3
2e: R^1=R^2=$(CH{=}CH\text{-})_2$, R^3=R^4= OCH_3	5.8	5.8	5.8	11.7
3e: R^1=R^2=$(CH{=}CH\text{-})_2$, R^3=R^4= OCH_3	0.3	0.3	0.3	0.3
4e: R^1=R^2=$(CH{=}CH\text{-})_2$, R^3=R^4= OCH_3	14.8	14.8	29.7	14.8
6: Podophyllotoxin	0.05	0.05	0.05	0.06

Naphthylcombretastatin **3e** shows cytotoxicity against all the assayed neoplastic cell lines of the same order of magnitude (5-6 times less active) as the reference compound **6**. This finding is rather interesting because this compound maintains the trimethoxyphenyl moiety but not the recommended 3-hydroxy-4-methoxyphenyl grouping, which has been replaced by a naphthyl residue. The other naphthyl derivatives **2e** and **4e** display some cytotoxic activity in comparison with the inactive **2a** and **4a,b**, although modifications of the two carbon bridge are not convenient for good activity as it has also been found in other SAR studies of combretastatins.[6] The requirement of two aromatic rings with the appropriate spatial disposition for activity of antitumoral agents acting by bonding to the colchicine site is thus fulfilled in naphthocombretastatin.

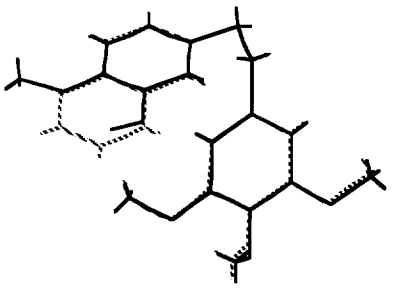


Figure.1 Superposition of the more stable conformations of naphthylcombretastatin (**3e**) and dihydrocombretastatin A-4 (**8**).

In conclusion, further modification of diphenyl moieties can lead to more potent combretastatin analogues different from the recently assayed replacement of the two carbon bridge by heteroatoms[5]. The effect of 3-hydroxy-4-methoxyphenyl, 3,4,5-trimethoxyphenyl and other aromatic groupings on the activity of this kind of new naphthylethanes are now under investigation.

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8. We have named combretastatones the phenylacetophenone analogues of combretastatins.

9. Molecular modelling was done with Macromodel 3.5 software and minimization carried out with MM2 energy minimization program. Preferred conformations of compounds type **2**, **3** and **4** have a value of ~55° for the C_{Ar}-C_1-C_2-C_{Ar} dihedral angle .

10. None of the tested compounds showed significant antiHIV activity.

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12. Compound **3a** was useful to compare our assays with bibliographic data. The reported value (Reference 1e) of cell grow inhibition IC_{50}= 6.3 μg/mL (20μM) on L1210 leukemia cells is comparable to that observed in this work IC_{50}= 5 μg/mL (15μM) (Table 1) against those tumoral cell lines used by us.

13. IC_{50} values were obtained by cell counting, after three days of incubation in presence of different concentrations of the compounds. Separate sets of cell cultures were counted daily to ensure that the cells remained in exponential growth. IC_{50} for compounds displaying low inhibition of cell growth at the assayed concentrations were not determined and are indicated >(highest tested concentration).

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