Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2303–2308

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF DIARYLINDOLE DERIVATIVES. CYTOTOXIC AGENTS BASED ON COMBRETASTATINS

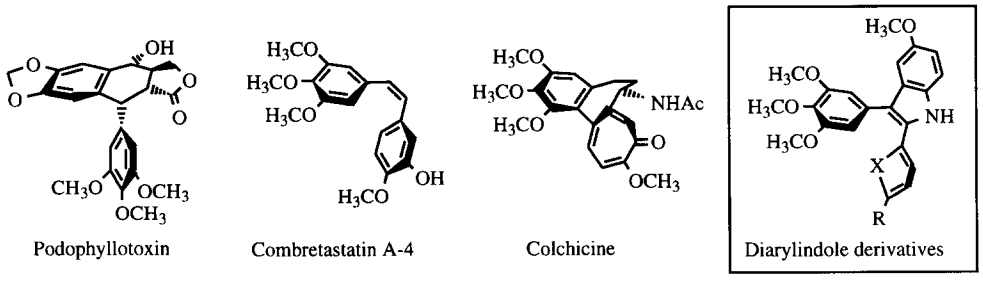
Manuel Medarde*, Angel C. Ramos, Esther Caballero, Rafael Peláez-Lamamié de Clairac, Jose Luis López, Dolores G[a] Grávalos[#] and Arturo San Feliciano

Lab. de Química Orgánica y Farmacéutica. Fac. Farmacia. Av. Campo Charro s/n. 37007. Salamanca. SPAIN.
[#]Pharma Mar S.A. c/ La Calera n° 3. 28780 Tres Cantos (Madrid). SPAIN.

Received 12 April 1999; accepted 25 June 1999

Abstract.- Taking into account the structure of Combretastatins, we have synthesized and assayed for cytotoxic activity of new indole derivatives. Two aryl groups are maintained in the *cis* orientation required for activity by means of an indole moiety built up on less active ketoderivatives used as starting materials.

Interest in new cytotoxic agents displaying selectivity towards neoplastic cells, that might be used in the treatment of cancer, has led to the synthesis or isolation from natural sources of a great number of new molecular entities. One of the most simple structures among these compound is that of the diarylethenes, which comprise most of the natural combretastatins[1] and many synthetic derivatives and analogues.[2] The easy synthesis and high cytotoxic activity of this type of compounds makes it very attractive to prepare a great number of structuraly related compounds.[3,4] The most representative of diarylethenes is the natural Combretastatin A-4, one of the most cytotoxic agents yet described, which also shows a very interesting antiangiogenic activity.[5] Combretastatin A-4 is a representative of bridged biaryl, a common structural feature for many antineoplastic agents with inhibitory activity on tubulin polymerization, as podophyllotoxin[6] and colchicine.[7] All of them have two spatially close non-coplanar aromatic rings separated by one to four carbon atoms, a structural requirement for activity which is missing in less active derivatives[2] with a similar substitution pattern.



Podophyllotoxin | Combretastatin A-4 | Colchicine | Diarylindole derivatives

Figure 1. Representative examples of antineoplastic bridged biaryls.

Dedicated to the memory of Professor Joaquín de Pascual Teresa.

E-mail: medarde@gugu.usal.es

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00370-4

Other derivatives with N atoms replacing C atoms on the bridge have been described, as soluble *aza*-analogues of combretastatins.[4,8,9] Replacement of a phenyl ring by other aromatic and heteroaromatic systems has only been occasionally achieved.[10] The highly active quinolones described by Lee et al.[11] can also be viewed as diarylethane derivatives displaying high citotoxicity and tubulin polymerization inhibition.

During our research, directed at the synthesis of different types of Combretastatin analogues, we have prepared naphtyl[12] and heterocyclic[13] derivatives by means of the dithiane approach[14] used to synthesize this type of compounds. These analogues were designed by replacing one of the benzene rings of the combretastatins by different heterocyclic or extended aromatic systems. All of them display lower activities than the model combretastatins, but they lack the double bond which is present in the most active molecules. After these results, we planned to build up rigid structures on the flexible ethane bridge by the construction of heterocyclic systems, to check the effect on cytotoxic activity.[15] The rationale for this approach is that the inhibition of tubulin polymerization, produced by combretastatins and podophyllotoxin derivatives, is a consequence of the binding to the colchicine site, which can accomodate these compounds in different spatial orientations and three different domains can be involved in the binding of these molecules.[16] The presence of a new residue on the ethane bridge could have two benneficial effects for the activity: produce a rigid bridge and to introduce an indole moiety, which is present in many antitumoral agents (Rebeccamicins, Elipticins, Azaeliptitoxins, Azatoxin), and could be accomodated in the colchicine binding site. Similar considerations have prompted the synthesis and evaluation of other combretastatin-like antitumor agents, as the recently described dioxole derivatives produced from combretastatins by hydroxylation followed by ring formation.[17] The construction of heterocyclic moieties on the ethane bridge of combretastatins has also been recently reported.[18]

In this paper we present the synthesis and evaluation of cytotoxicity of several indole bridged combretastatin analogues, prepared from previously described ketones, belonging to the naphthyl-[12] and hetero-combretastatin[13] families.

Chemistry

The synthetic approach employed for the preparation of title compounds exploits our previous work on combretastatin derivatives and analogues. Using dithiane derivatives **1**, that were also reduced to dihidrocombretastatins **3**, we had obtained diaryl ketoderivatives **2**. Fischer indolization reaction of ketones **2a-e** with *p*-methoxyphenylhydrazine in refluxing AcOH-EtOH during 1.5 hours gave us the target molecules **4a-e**, in medium yields (40-60%, overall yield from starting dithianes 15-40%)[19]. Longer periods of reflux produce a lower yield and/or degradation of the reaction products. By reduction of **2** with $NaBH_4$ combretastatin analogues **5** were prepared. All the synthesized products carry the 3,4,5-trimethoxyphenyl moiety (Ar^2= 3,4,5-triOMe-Ph) at position C-3, whereas in position C-2 furyl, benzodioxole and naphthyl moities are present.

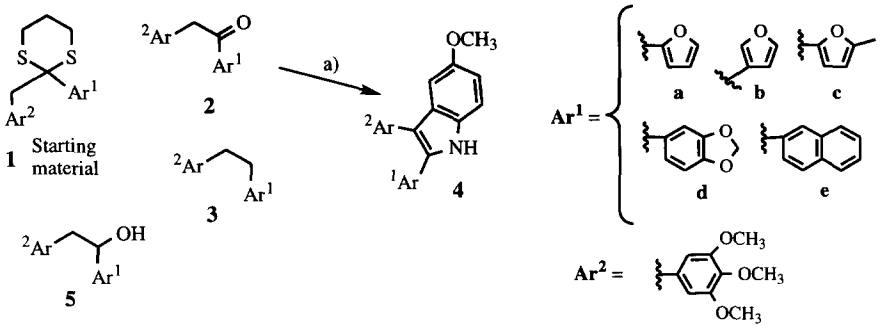


Figure 2. Synthesis of **1-3** and **5** according to Refs. 12, 13, 14; a) p-CH_3O-C_6H_4-NH-NH_2 , AcOH, EtOH, 1.5 h, reflux

Pharmacology

Following the procedure described in previous papers[20] we have checked the cytostatic activity of these compounds against different cancer cell lines. In table 1 the results of the indole derivatives **4a-e** are presented in comparison with the previously prepared "non rigid" combretastatones **2a-e**, dihydrocombretastatin **3a-e** and hydroxycombretastatins **5a-e** analogues.

	P-388	A-549	HT-29	MEL-28		P-388	A-549	HT-29	MEL-28
2a	> -4.0	> -4.0	> -4.0	> -4.0	**3c**	-5.14	-5.14	-5.14	-5.14
3a	-4.72	-4.72	-4.72	-4.72	**4c**	-5.29	-5.29	-5.19	-5.29
4a	-6.15	-5.88	-5.88	-5.88	**3d**	-5.20	-5.20	-5.20	-5.20
5a	-4.74	-4.44	-4.44	-4.44	**4d**	-5.64	-5.20	-5.20	-5.20
2b	-5.14	-5.14	-5.14	-5.14	**5d**	> -4.0	> -4.0	> -4.0	> -4.0
3b	> -4.0	> -4.0	> -4.0	> -4.0	**2e**	-4.82	-4.82	-4.52	-4.82
4b	-5.49	-5.49	-5.49	-5.49	**3e**	-6.52	-6.52	-6.52	-6.52
5b	-4.44	-4.14	-4.14	-4.14	**4e**	-5.95	-5.95	-5.65	-5.95
2c	-5.06	-4.76	-4.76	-5.06	**5e**	-5.53	-5.53	-5.53	-5.53

Table 1. Antineoplastic activities for synthesized 2,3-diarylindoles **4a-e** compared with that of combretastatin analogues **2a-e**, **3a-e** and **5a-e**. (Log_{10} IC_{50} M inhibition of cell growth[21])

From the above studies several conclusions can be drawn for the (hetero-)combretastatins and indolobridged (hetero-)combretastatins obtained in this and previous research on combretastatin analogues. The first one is the activity displayed by nearly all these derivatives of varied structures, that only have in common the presence of a trimethoxyphenyl moiety. Most of these compounds have moderate to medium potency in comparison with the tubulin polymerization inhibitor podophyllotoxin, taken as reference in these assays (Log_{10} IC_{50} = -7.22M). The cytotoxicity observed for a wide variety of bridged biaryls seems to be a property of these structures, which reach a maximun effect when the relative geometry and structure of aryl moieties are combined

in a proper fashion. This combination is produced in the tubulin inhibitor colchicine, podophyllotoxin lignans, synthetic quinolones or combretastatins, that bind to the same receptor.[16,22]

According to the assumption that fixation of the diaryl system by means of an indole moiety would produce an increase in the activity, the higher citotoxic effect among the tested compounds was observed for derivatives of this type (**4**) in series **a-d**. From a moderate 1.5 fold increase when comparing **3c** with **4c**, passing by the three fold increase between **3d** and **4d**, to a 10 or higher (>10^2) fold increase in the rest of the compounds in these series.

These results are in agreement with the high cytotoxicities of the heterocyclic bridged biaryls described in the literature.[18] In spite of the larger size of the indole bridge presented in this paper, compared to those five membered heterocycles reported in the above mentioned papers, the activity is noticeable, thus suggesting that large aromatic moieties can replace the ethene bridge of combretastatins.

In the naphthalene substituted family **e**, the indole (**4e**) produces only a moderate increase of the activity compared to **2e** and **5e** or even a decrease with respect to **3e**. This fact is in consonance with the already commented[13] differences that can exist with respect of the SAR deduced for combretastatins when an heterocycle or extended benzene system replace one of the phenyl groups of the combretastatins.

Among the indole derivatives, the most potent **4a** was assayed on the sixty tumoral cell lines panel at the NCI. The results demonstrated that this compound displays a noticeable cytostatic activity against all the tested lines (Log_{10} GI50 ranging from -4.78 for T-47D breast cancer to -7.64 for SNB-19 CNS cancer) with highest effect against leukemia, non-small cell lung cancer and CNS cancer. The cytotoxicity displayed by **4a** against some of these lines is noticeable for two CNS lines and two colon cancer lines. A selection of the Log10 GI50 and Log10 LC50 values for the most sensitive cell lines is presented in table 2.

	Leukemia		Non-samall cell lung cancer	Colon cancer	
	HL-60(TB)	**RPMI-8226**	**NCI-H522**	**COLO 205**	**HTC-116**
Log_{10} GI50	-7.29	-7.04	-7.12	-6.73	-6.86
Log_{10} LC50	> -4.0	-4.12	-5.47	-6.17	-5.60
	CNS cancer			Melanoma	Ovarian cancer
	SF-295	**SF-539**	**SNB-19**	**SK-MEL-5**	**OVCAR-3**
Log_{10} GI50	-6.86	-6.93	-7.64	-6.61	-6.77
Log_{10} LC50	-4.63	-6.03	-6.01	-4.54	-5.14

Table 2. Selected values from the NCI sixty tumoral cell lines panel test of diarylindole **4a**. Cytostatic LG_{10} GI50 and cytotoxic Log_{10} LC50 effects in M.[23]

In conclusion, we have prepared several 2,3-diarylindoles as analogues of *cis*-combretastatins in order to compare their actvities with the previously synthesized dihydrocombretastatins and combretastatones, which have a non rigid bridge between both aryl systems. The introduction of the indole moiety in the bridge produces the expected increase in their cytostatic effect, altough this effect is not observed for the naphthyl derivative.

Acknowledgments: Financial support came from the "Junta de Castilla y León" (SA-66/12/92) and Spanish DGICYT (SAF98-0103). A.C.R. thanks the University of Salamanca for a predoctoral grant and R.P.L. de C. thank the Spanish MEC his contract of reincorporation.

References and Notes:

1. Pettit, G.R.; Cragg, G.M.; Herald, D.L.; Schmidt, J.M.; Lohavanijaya, P. *Can. J. Chem.* **1982**, *60*, 1374-1376. Pettit, G.R.; Singh, B.S. *Can. J. Chem.* **1987**, *65*, 2390-2396. Pettit, G.R.; Singh, B.S.; Niven, M.L.; Hamel, E.; Schmidt, J.M. *J. Nat. Prod.* **1987**, *50*, 119-131. Pettit, G.R.; Cragg, G.M.; Singh, S.B. *J. Nat. Prod.* **1987**, *50*, 386-391. Pettit, G.R.; Singh, B.S.; Schmidt, J.M.; Niven, M.L.; Hamel, E.; Lin, Ch.M. *J. Nat. Prod.* **1988**, *51*, 517-527. Pettit, G.R.; Singh, S.B.; Hamel, E.; Lin, Ch.M.; Alberts, D.S.; Garcia-Kendall, D. *Experientia* **1989**, *45*, 209-211.

2. Lin, Ch.M.; Singh, S.B.; Chu, P.S.; Dempcey, R.O.; Schmidt, J.M.; Pettit, G.R.; Hamel, E. *Mol. Pharmacol.* **1988**, *34*, 200-208. Cushman, M.; Nagarathnam, D.; Gopal, D.; He, H.M.; Lin, Ch.M.; Hamel, E. *J. Med. Chem.* **1992**, *35*, 2293-2306.

3. Getahun, Z.; Jurd, L.; Chu, P.S.; Lin, Ch.M.; Hamel, E. *J. Med. Chem.* **1992**, *35*, 1058-1067. Cushman, M.; Nagarathnam, D.; Gopal, D.; He, H-M.; Lin, Ch.M.; Hamel, E. *J. Med. Chem.* **1992**, *35*, 2293-2306.

4. Cushman, M.; Nagarathnam, D.; Gopal, D.; Chakraborti, A.K.; Lin, Ch.M.; Hamel, E. *J. Med. Chem.* **1991**, *34*, 2579-2588.

5. Dark, G.G.; Hill, S.A.; Prise, V.E.; Toze, G.M.; Pettit, G.R.; Chaplin, D.J. *Cancer Res.* **1997**, *57*, 1829-1834.

6. Jardine, I. "Podophyllotoxins" in "*Anticancer Agents Based on Natural Products Models*" Casady, J.M.; Douros, J. Ed.; Academic Press: New York, 1980; 319-351. Ayres, D.C.; Loike, J.D. "Lignans. Chemical, Biological and Clinical Properties" Chap. 3. Cambridge University Press, Cambridge, U.K. 1990.

7. Capraro, H.G.; Brossi, A. "Tropolonic *Colchicum* Alkaloids" in "*The Alkaloids*" Vol. 23. Brossi, A. Ed.; Academic Press, New York, 1984; 1-70. Boye, O..; Brossi, A. "*The Alkaloids*" Vol. 41. Brossi, A. Ed.; Academic Press, New York, 1992; 125-150. Muzaffar, A.; Brossi, A.; Lin, C.M.; Hamel, E. *J. Med. Chem.* **1990**, *33*, 567-571.

8. Shirai, R.; Tokuda, K.; Koiso, Y.; Iwasaki, S. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 699-704.

9. Cushman, M.; He, H.H.; Lin, Ch.M.; Hamel, E. *J. Med. Chem.* **1993**, *36*, 2817-2821.

10. Denyer, C.V.; Reddy, M.T.; Jenkins, D.C.; Rapson, E.;Watts, S.D.M. *Arch. Pharm. (Weinheim)* **1994**, *327*, 95-98.

11. Kuo, S.C.; Lee, H.Z.; Juang, J.P.; Lin, Y.T.; Wu, T.S.; Chang, J.J.; Lednicer, D.; Paull, K.D.; Lin, M.C.; Hamel, E.; Lee, K.H. *J. Med. Chem.* **1993**, *36*, 1146-1156. Li, L.; Wang, H.K.; Kuo, S.Ch.; Wu, T.S.; Mauger, A.; Lin, Ch.M.; Hamel, E.; Lee, K.H. *J. Med. Chem.* **1994**, *37*, 3400-3407.

12. Medarde, M.; Peláez Lamamié de Clairac, R.; Ramos, A.C.; Caballero, E.; López, J.L.; Grávalos, Mª D.G.; San Feliciano, A. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 229-232.

13. Nandy, P.; Banerjee, S.; Gao, H.; Hui, M.B.V.; Lien, E.J. *Pharm. Research.* **1991**, *8*, 776-781.

14. Medarde, M.; Peláez-Lamamié de Clairac, R.; López, J.L.; San Feliciano, A. *J. Nat. Products* **1994**, *57*, 1136-1144.

15. Ramos, A.C. Ph.D. dissertation. University of Salamanca, June 1997. Caballero, E.; Medarde, M. ; Ramos, A.C.; Peláez, R.; Rey, B., San Feliciano, A. XVth EFMC International Symposium on Medicinal Chemistry. Book of Abstracts p. 115. Edinburgh, 1998.

16. Haar, E.; Rosenkranz, H.S.; Hamel, E.; Day, B.W. *Bioorg. Med. Chem.* **1996**, *4*, 1659-1671.

17. Shirai, R.; Takayama, H.; Nishikawa, A.; Koiso, Y.; Hashimoto, Y. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1997-2000.

18. Ohsumi, K.; Hatanaka, T.; Fujita, K.; Nakagawa, R.; Fukuda, Y.; Nihei, Y.; Suga, Y.; Morinaga, Y.; Akiyama, Y.; Tsuji, T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3153-3158. Shirai, R.; Ukabe, T.; Iwasaki, S. *Heterocycles* **1997**, *46*, 1457-1466.

19. All the obtained indole derivatives **4** were purified by CC (hexane/ether 2:1) and checked by CG/MS. Elemental analysis and spectroscopic data (IR, ^{1}H and ^{13}C-NMR) are consistent with proposed structures **4a-e**. Purity of all tested compounds is higher than 95%.

20. San Feliciano, A.; Medarde, M.; Pelaez Lamamié de Clairac, R.; García, M.D.; Ruiz, P.; García de Quesada, M.T *Arch. Pharm. (Weinheim)* **1993**, *326*, 421-426.

21. 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).

22. Shi, Q.; Chen, K.; Morris-Natschke, S.L.; Lee, K-H. *Current Pharm. Design* **1998**, *4*, 219- 248.

23. Log_{10} GI50 = Log concentrations which reduced cell growth to 50% of level at the onset of the experiment. Log10 LC50 = Log concentrations which reduces the number of cells to 50% at the start of the experiment. Grever, M.R.; Schepartz, S.A.; Chabner, B.A. *Seminars Oncol.* **1992**, *19*, 622-638. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P., Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J. and Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757-766.