Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 20, pp. 2459-2462, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00454-4

SYNTHESIS AND CYTOTOXIC ACTIVITY OF PHENYL-HEXAHYDROPYRROLO[3,4-*c*]CARBAZOLES

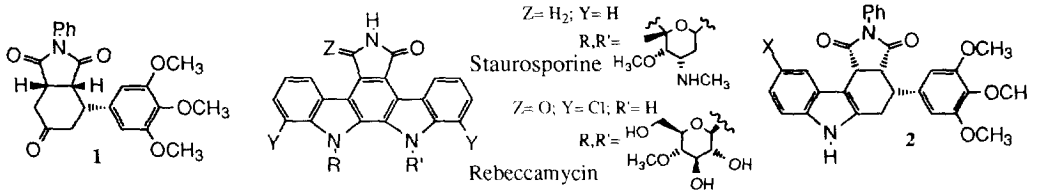
Esther Caballero, Francine García, Dolores Gª Grávalos#, Manuel Medarde*, Heidi Sahagún and Fernando Tomé*

Dept. Química Orgánica . Fac. Farmacia. Av. Campo Charro 37007, Salamanca. SPAIN
#Pharma Mar S.A. La Calera nº 3. 28780 Tres Cantos (Madrid). SPAIN.

Abstract.- A synthetic methodology for the preparation of fused carbazole derivatives has been developed. The presence of a trimethoxyphenyl substituent and the hexahydropyrrolo[3,4-*c*]carbazole system produced a new family of compounds, that display cytotoxic activity.

Polycyclic fused aromatic-heteroaromatic systems are present in many antitumoral agents and other active compounds of natural and synthetic origin.[1] The indole, pyridoindole, carbazole and related moieties are common substructures present in representative members of several families of these active compounds. Among these families, Ellipticines,[2] *Vinca* alkaloids,[3] Camptothecins,[4] substituted indoles,[5] mitomycins,[6] pyridoindoles of manzamine type,[7] etc... are good representatives of polycyclic heteroaromatic antitumoral agents.

During our research directed at the synthesis of interesting natural products as podophyllotoxin[8] and combretastatins[9] or synthetic analogues[10] containing the trimethoxyphenyl subunit, which is present in many antineoplastic compounds and seems to be implicated in the binding at the tubuline avoiding the cell division,[11] we have prepared the phenyl-isoindole derivative **1**. This synthetic intermediate suggested the possibility of synthesizing several phenyl substituted polycyclic systems in order to check their antitumoral properties. A model for such a kind of compound could be the marine-origin antitumoral Lamelarines,[12] but we focused on the transformation of **1** into new analogues **2** of the structurally related indolo-carbazoles. Rebeccamycin[13] and Staurosporine[14] are known representatives of this kind of natural products, displaying different types of pharmacological activities. The protein kinase C inhibition displayed by these compounds has been pointed out as a new possibility for the cancer therapy.[15]

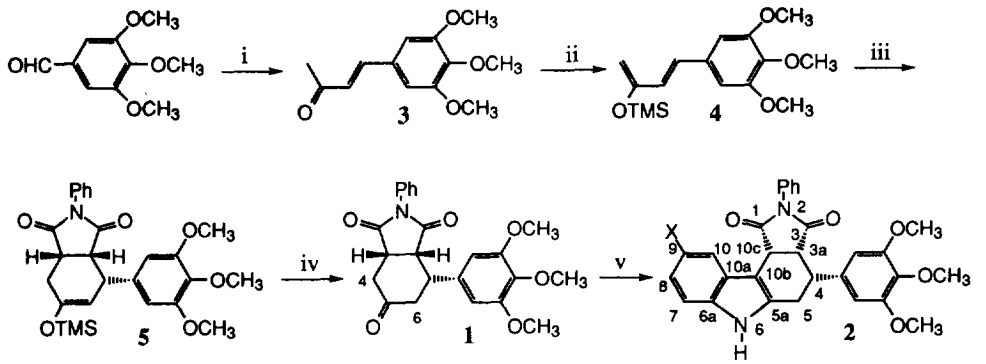


* Adress for correspondence. Fax: 34-23-294515. E-mail: frena@gugu.usal.es ; medarde@gugu.usal.es

In this initial paper we describe the synthetic methodology and the evaluation of the cytotoxic activity of three compounds with new structures, that can be considered as open analogues of the well known indolo-carbazoles.

Chemistry

The synthesis has been carried out as depicted in Scheme 1, starting from acetone and 3,4,5-trimethoxybenzaldehyde. The product of condesation **3**, was silylated to produce the diene **4** in 75% overall yield from starting aldehyde. There are few references of this type of phenyldienes,[16] which are very interesting building blocks for the synthesis of active compounds related to natural products. A great number of dienes carrying different substituents in the diene and phenyl moieties can be readily prepared by this methodology.



i) Acetone, NaOH 10%, EtOH/H_2O, 45 min., r.t., 79% ii) Et_3N, TMSOTf, 1h., r.t., 95%
iii) *N*-phenylmaleimide, C_6H_6, 48h, r.t., iv) HOAc/H_2O/THF 6:2:3, 45 min., r.t., 55% two steps
v) EtOH/H_2O 1:1, H_2NNH-C_6H_4-X, 90 min, reflux, **2a:** X=OCH_3 82%, **2b:** X=CH_3 40%, **2c:** X=Br 39%.

Diels-Alder reaction of diene **4** with *N*-phenylmaleimide at room temperature without catalyst, afforded adduct **5**, which was directly hydrolized without isolation to the aforecited ketone **1**, obtained as a crystalline product in 55% yield from **4**. Finally, Fischer indolization with *p*-methoxyphenylhydrazine produced the compound **2a** in high yield. The same treatment of **1** with *p*-tolylhydrazine and *p*-bromophenylhydrazine afforded respectively **2b** and **2c**, in moderate yields.[17] Only one regioisomer with structure **2**, by formation of the new C-C bond of the indole system to position 4 of the starting cyclohexanone, was isolated by crystallization in the three cases. The other regioisomer, from the C-C bond formation to position 6, was detected in the crude of the reaction (5-30%), in agreement with the regioselectivity observed in the Fischer reaction with cyclohexanones.[18] This preference can be explained by the greater stability (due to its less rigid structure) of the tautomer of the intermediate enehydrazine which produces the final product **2**.

Cytotoxic activity

In this preliminary study, the cytotoxic activities of final products with structure of pyrrolo-carbazoles **2a-c** were tested against several cell lines, representative of solid tumors and leukemias (Table 1). The most active of these compounds (**2a**), was then checked for activity in a more exahustive analysis at the National Cancer Institute (Bethesda, Maryland. NCI's in vitro disease-oriented antitumor screen), and the results are shown in table 2.

As it can be seen in table 1, compound **2a** displayed a noticeable activity against the assayed tumoral cell lines, while the methyl substitution at position 9 of the pyrrolocarbazole system (**2b**) produced a noticeable decrease of this activity and the bromo substitution (**2c**) a complete lack of cytotoxic activity at the assayed concentrations.

	X	P-388	A-549	HT-29	MEL-28
2a	H	0.5	2.1	2.1	2.1
2b	CH_3	20.1	20.1	20.1	20.1
2c	Br	>17.8	>17.8	>17.8	>17.8

Table 1. Cytotoxic activities for phenyl-hexahydropyrrolo[3,4-*c*]carbazoles **2a-c**. (IC_{50} μM inhibition of cell growth[19])

The results of the NCI confirmed the activity of **2a** in many of the assayed cell lines, displaying $Log_{10}GI50$ between -4.0 and -5.0 (M). A selection of these results is presented in table 2, showing the highest activity of compound **2a** against non small cell lung cancer lines and a noticeable activity for all the tested breast cancer cell lines (only four shown in table 2). Although not as active as the most potent antitumoral agents ($Log_{10}GI50$= -5 to -8), this hexahydro-pyrrolo[3,4-*c*]carbazole exhibits a promising activity for the development of this and other families of related new compounds.

2a	HL-60(TB)	K-562	HOP-62	HOP-92	SNB-19	SNB-75	U251	OVCAR-4
$Log_{10}GI50$[20]	-4.55	-4.45	-4.79	-4.88	-4.59	-4.57	-4.46	-4.57
2a	SK-MEL-2	UACC-62	A498	RXF393	MDA-MB-435	MDA-N	RT-549	T-47D
$Log_{10}GI50$[20]	-4.56	-4.62	-4.48	-4.69	-4.66	-4.52	-4.60	-4.60

Table 2. Cytotoxic activity data obtained from NCI's in vitro human tumor cells screen for the phenyl-hexahydropyrrolo[3,4-*c*]carbazole **2a**. HL-60(TB) and K-562, Leukemia cell lines; HOP-62 and HOP-92, non small cell lung cancer cell lines; SNB-19, SNB-75 and U251, CNS tumor cell lines; OVCAR-4, ovarian cancer cell line; SK-MEL-2 and UACC-62, melanoma; A498 and RXF393, renal cancer cell lines; MDA-MB-435, MDA-N, RT-549 and T-47D, breast cancer cell lines.

In conclusion, the synthesis of a new family of polycyclic compounds with trimethoxyphenyl and fused heterocyclic moieties has been developed. This methodology is very versatile and there are a lot of possibilities for the structural variations among this family by using different starting aldehydes, substituted maleimides and phenylhydrazines. A promising cytotoxic activity has been demonstrated by one of the synthesized products. We are now preparing other analogues and derivatives, searching for better activities and in order to study the mechanism of action of this class of compounds.

Acknowledgements: We thank Dr. B. Macías (Dep. Química Inorgánica. Salamanca) for E.A. Financial support came from Junta de Castilla y León (SA 18/95) and DGICYT (SAF 95-1566). H.S. thanks the University of Salamanca a predoctoral fellowship position and F.G. (ENSSPICAM. Marseille. France) thanks the EC an Erasmus Grant.

References and Notes:

1. Pettit, G.R.; Pierson, F.H. and Herald, C.L. "*Anticancer Drugs from Animals, Plants and Microorganisms*" Wiley-Interscience: New York, 1994. Cheng C.C. "*Structural Aspects of Antineoplastic Agents - A New Approach*" in *Progress in Medicinal Chemistry* v. 25 [Ellis, G.P. and West, .B. Eds.] Chap. 2. Elsevier: Amsterdam, 1988.
2. Gribble, G.W. "*Synthesis and Antitumor Activity of Ellipticine Alkaloids and Related Compounds*" in *The Alkaloids* v.39 [Brossi, A; Ed.]. Academic Press : San Diego, 1990. Kansal, V.K. and Potier, P. *Tetrahedron* **1986**, *42*, 2389. Honda, T.; Kato, M.; Inoue, M.; Shimamoto, T.; Shima, K.; Nakanishi, T. ; Yoshida, T. and Noguchi, T. *J. Med. Chem.* **1988**, *31*, 1295.
3. Gerzon, K. "*Dimeric Catharanthus Alkaloids*" in *Medicinal Chemistry a Series of Monographs* v.16 [Cassady, J.M. and Douros, J.D.; Eds.]. Academic Press, New York, 1980. Taylor, W.T. and Farnsworth, N.R.: Eds. "*The Catharanthus Alkaloids*" Marcel Dekker: New York, 1975.
4. Shen, W.; Coburn, C.A.; Bornmann, W.G. and Danishefsky, S.J. *J. Org. Chem.* **1993**, *58*, 611. Hisiang, Y.H.; Hertzberg, R.; Hecht, S. and Liu, L.F. *J. Biol. Chem.* **1985**, *260*, 14873. Hisiang, Y.H.; Liu, L.F.; Wall, M.E.; Wani, M.C.; Nicholas, A.W.; Manikumar, G.; Kirshenbaum, S.; Silber, R. and Potmesil, M. *Cancer. Res.* **1989**, *49*, 4385.
5. Fattorusso, E.; Lanzotti, V.; Magno, S. and Novellino, E. *J. Nat. Products* **1985**, *48*, 924. Lidgren, G.; Bohlin, L. and Bergman, J. *Tetrahedron Lett.* **1986**, *27*, 3283.
6. Tomasz, M.; Lipman, R.; McGuinness, B.F. and Nakanishi, K. *J. Am. Chem. Soc.* **1988**, *110*, 5892. Carter, S.K. and Crooke, S.T. "*Mitomycin C. Current Status and New Developments*" Academic Press: New York, 1979.
7. Ichiba, T.; Sakai, R.; Kohmoto, S.; Saucy, G. and Higa, T. *Tetrahedron Lett.* **1988**, *29*, 3083. Nakamura, H.; Deng, S.; Kobayashi, J.; Ohizumi, Y.; Tomotake, Y.; Matsuzaki, T. and Hirata, Y. *Tetrahedron Lett.* **1987**, *28*, 621. Sakai, R.; Kohmoto, S.; Higa, T.; Jefford, C.W. and Bernardinelli, G. *Tetrahedron Lett.* **1987**, *28*, 5493.
8. Medarde, M.; Ramos, A.C.; Caballero, E.; López, J.L.; Peláez-Lamamié de Clairac, R. and San Feliciano, A. *Tetrahedron Lett.* **1996**, *37*, 2663.
9. Medarde, M.; Peláez-Lamamié de Clairac, R.; López, J.L. and San Feliciano, A. *J. Nat. Products* **1994**, *57*, 1136.
10. Medarde, M.; Peláez Lamamié de Clairac, R.; Ramos, A.C.; Caballero, E.; López, J.L.; G[a] Grávalos, M[a] D. and San Feliciano, A. *BioMed. Chem. Letters* **1995**, *5*, 229. Medarde, M.; Peláez-Lamamié de Clairac, R.; Tomé, F.; López, J.L.; San Feliciano, A. and Grávalos, D.G[a]. *Arch. Pharm* (Weinheim) **1995**, *328*, 403.
11. Hodgson, S.T. "*Discovery of 1069C. A Novel Synthetic Antitumor Agent with Low Cross-Resistence Potential*" in "Medicinal Chemistry. Principles and Practice" [King, F.D. Ed.] Royal Society of Chemistry,: Cambridge, 1994.
12. Lindquist, N.; Fenical, W.; Van Duyne, G.D. and Clardy, J. *J. Org. Chem.* **1988**, *53*, 4570. Andersen, R.J.; Faulkner, D.J.; He, C.; Van Duyne, G.D. and Clardy, J. *J. Am. Chem. Soc.* **1985**, *107*, 5492.
13. Bush, J.A.; Long, B.H.; Catino, J.J.; Bradner, W.T. and Tomika, K. *J. Antibiotics* **1987**, *40*, 668. Nettleton, D.E.; Doyle, T.W.; Krishnan, B.; Matsumoto, G.K. and Clardy, J. *Tetrahedron Lett.* **1985**, *26*, 4011.
14. Tamaoki, T.; Nomato, H.; Takahashi, I.; Kato, Y.; Morimoto, M. and Tomita, F. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 397. Furusaki, A.; Hashiba, N.; Matsumoto, T.; Hirano, A.; Iwai, Y. and Omura, S. *J. Chem. Soc. Chem. Commun.* **1978**, 800.
15. Kleinschroth, J.; Hartenstein, J.; Rudolph, C. and Schächtele, C. *BioMed. Chem. Lett.* **1995**, *5*, 55. Wender, P.A. and Maryanoff, C. "Computer Assisted Molecular Design related to the Protein Kinase C Receptor" in "Advances in Medicinal Chemistry" v.1 [Maryanoff, B.E. and Maryanoff, C.A., Eds.] Jai Press Inc.: Greenwich (CO. USA), 1992.
16. Pilli, R.A.; Dias, L.C. and Maldaner, A. *J. Org. Chem.* **1995**, *60*, 717.
17. The spectroscopic properties of these compounds are in agreement with structures: 9-methoxy-2-phenyl-4-(3,4,5-trimethoxyphenyl)-1,3,3a,4,5,10c-hexahydro-2*H*,6*H*-pyrrolo[3,4-*c*]carbazole-1,3-dione (**2a**) and the 9-methyl- (**2b**) and 9-bromo- (**2c**) derivatives. H/C long-range correlations denoted the proximity between 10-10a-10b-10c positions. (**2a**: Mp: 188°C. EM(m/z): 512(M^+). EA: Calc: C(70.4), H(5.30), N(5.10). Found: C(69.61), H(5.40), N(4.91). ^{1}H-RMN (DMSO-d_6): 3.07(1H,dd,*16.2,7.2*), 3.22(1H,dd,*16.2,4.4*), 3.38(6H,s), 3.62(3H,s), 3.65-3.74(1H,m), 3.75(3H,s), 4.03(1H,dd,*7.6,4.8*), 4.57(1H,d,*7.6*), 6.58(2H,s), 6.72(1H,dd,*9.0,2.0*), 6.76-6.81(2H,m), 7.23(1H,d,*9.0*), 7.30(1H,d,*2.0*), 7.32-7.38(3H,m). ^{13}C-NMR (DMSO-d_6): 26.2(t), 39.2(d), 40.4(d), 45.4(d), 55.4(q), 55.7(qx2), 59.9(q), 102.2(d), 102.6(s), 106.4(dx2), 110.4(d), 111.4(d), 126.6(dx2), 127.0(s), 128.0(d), 128.5(dx2), 131.2(s), 132.3(s), 135.7(s), 136.8(s), 136.9(s), 152.3(sx2), 153.3(s), 175.7(s), 176.1(s).
18. Reed, G.W.B.; Cheng, P.T.W. and McLean, S. *Can. J. Chem.* **1982**, *60*, 419. Zhao, D.; Hughes, D.L.; Bender, D.R.; DeMarco, A.M. and Reider, P.J. *J. Org. Chem.* **1991**, *56*, 3001.
19. 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). For experimental details see: Medarde, M.; Peláez-Lamamié de Clairac, R.; López, J.L.; Grávalos, D.G[a]. and San Feliciano, A. *Arch. Pharm* (Weinheim) **1995**, *328*, 640.
20. Log concentrations which reduced cell growth to 50% of level at start of experiment. Grever, M.R.; Schepartz, S.A.; Chabner, B.A. *Seminars Oncol.* **1992**, *19*, 622. 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.

(*Received in Belgium* 31 *July* 1996; *accepted* 23 *September* 1996)