



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

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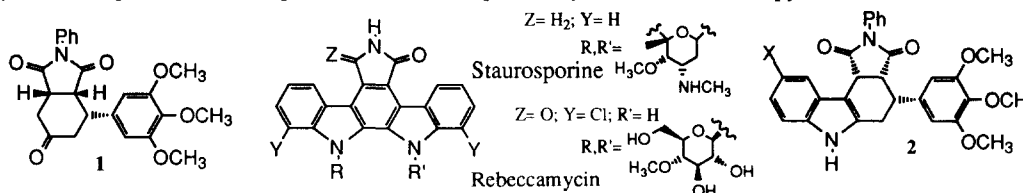
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Abstract.- A synthetic methodology for the preparation of fused carbazole derivatives has been developed. The presence of a trimethoxyphenyl substituent and the hexahydropyrrolo[3,4-*c*]carbazole system produced a new family of compounds, that display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

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Polycyclic fused aromatic-heteroaromatic systems are present in many antitumoral agents and other active compounds of natural and synthetic origin.¹ The indole, pyridindole, 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 pyridindoles 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 podophyllotoxin⁸ and combretastatins⁹ or synthetic analogues¹⁰ 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 Lamellarines,¹² but we focused on the transformation of **1** into new analogues **2** of the structurally related indolo-carbazoles. Rebeccamycin¹³ and Staurosporine¹⁴ 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

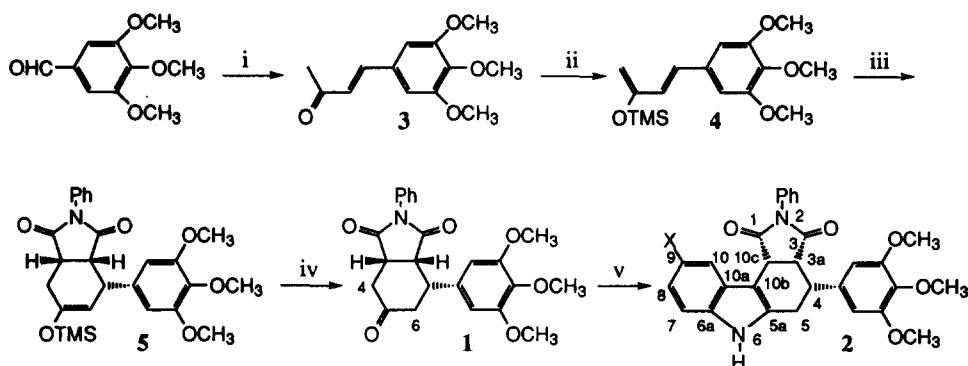


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In this initial paper we describe the synthetic methodology and the evaluation of the cytotoxic activity of three compounds with new structures, that can be considered as open analogues of the well known indolo-carbazoles.

Chemistry

The synthesis has been carried out as depicted in Scheme 1, starting from acetone and 3,4,5-trimethoxybenzaldehyde. The product of condensation **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/H₂O, 45 min., r.t., 79% ii) Et₃N, TMSOTf, 1h., r.t., 95%
 iii) *N*-phenylmaleimide, C₆H₆, 48h, r.t., iv) HOAc/H₂O/THF 6:2:3, 45 min., r.t., 55% two steps
 v) EtOH/H₂O 1:1, H₂NNH-C₆H₄-X, 90 min, reflux, **2a**: X=OCH₃ 82%, **2b**: X=CH₃ 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 hydrolyzed without isolation to the aforementioned 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 exhaustive 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**. (IC₅₀ μ M inhibition of cell growth¹⁹)

The results of the NCI confirmed the activity of **2a** in many of the assayed cell lines, displaying Log₁₀GI₅₀ 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 (Log₁₀GI₅₀= -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 GI ₅₀ 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 GI ₅₀ 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.

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- 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-2H,6H-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). ¹H-RMN (DMSO-d₆): 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). ¹³C-NMR (DMSO-d₆): 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).
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