Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2589–2591

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Antiproliferative Activity of Benzocyclobutacarbazol Derivatives. A New Class of Potential Antitumor Agents

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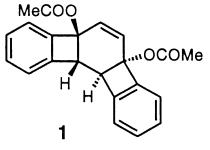
Received 15 May 2000; revised 4 September 2000; accepted 5 September 2000

Abstract—Several benzocyclobutacarbazol derivatives were synthesized and evaluated for their potential cytotoxic properties. A number of these compounds exhibited significant antiproliferative activity with concomitant interaction with the cell cycle and represent a new class of potential anticancer agents.

Numerous anticancer agents are planar or partially planar π-electron-containing molecules.[1] Accordingly we thought that benzocyclobutene derivatives, easily obtained by arynic reaction[2] could own cytotoxic properties. Actually we found[3] that **1** and **2** strongly interacted with phagic double stranded DNA but not with single stranded DNA.[4] These results could be indicative of some intercalating properties and/or DNA interaction with the strained reactive unsaturation of the central rings.

Although these preliminary results were promising, structural alterations soon appeared to be necessary. Indeed, **1** and **2** are devoid of in vitro cytotoxicity against human lymphoblasts[3] and L1210 murine leukemia cell line (present work). These drawbacks were attributed to a lack of hydrophilicity as well as to badly suited structures. Taking into account that indole rings, also easily obtained by arynic reactions,[5] are part of numerous anticancer agents,[1] we felt that joining indole and benzocyclobutene units, could lead to a new class of antitumor agents.



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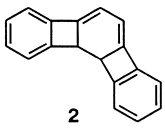

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Here we describe the synthesis and antiproliferative activity of benzocyclobutacarbazoles **4**, **5**, **7**, **8**, **10** (Scheme 1). Hydroxyl groups were unprotected in order to maintain hydrophilicity. For the sake of comparison, activities of **1** and of two non-hydroxylated derivatives are also mentioned.

The synthesis of **1** was previously reported.[6] The other compounds were prepared following Scheme 1.[7] Tetrahydrobiphenylenones **3a–d** and tetrahydrocarbazolones **6a–e**, **9a–c** were respectively obtained by arynic reactions from appropriate starting materials according to procedures previously published.[5,8] Benzocyclobutacarbazoles **4a–e** and **5** were synthesized with about 20% yields from the corresponding starting material **3a–d**. Compounds **7a–h** were obtained with yields varying from 15 to 40%. The main by-products of the arynic condensations were the corresponding aryl tetrahydrocarbazolones. Finally, yields of **10a–c** varied from 20 to 57%. All products were obtained by flash chromatography. Spectroscopic properties and elemental analysis are in agreement with the structures.

The derivatives were evaluated in vitro for their antiproliferative activity using the murine L1210 leukemia cell line.[10] The results expressed as IC_{50} are reported in Table 1 as well as the cell cycle perturbations induced by the most active compounds ($IC_{50} \leq 15$ μM).

The structure–activity relationships were first established within the benzo[3,4]cyclobuta[1,2-*c*]carbazol-3a-ol series.

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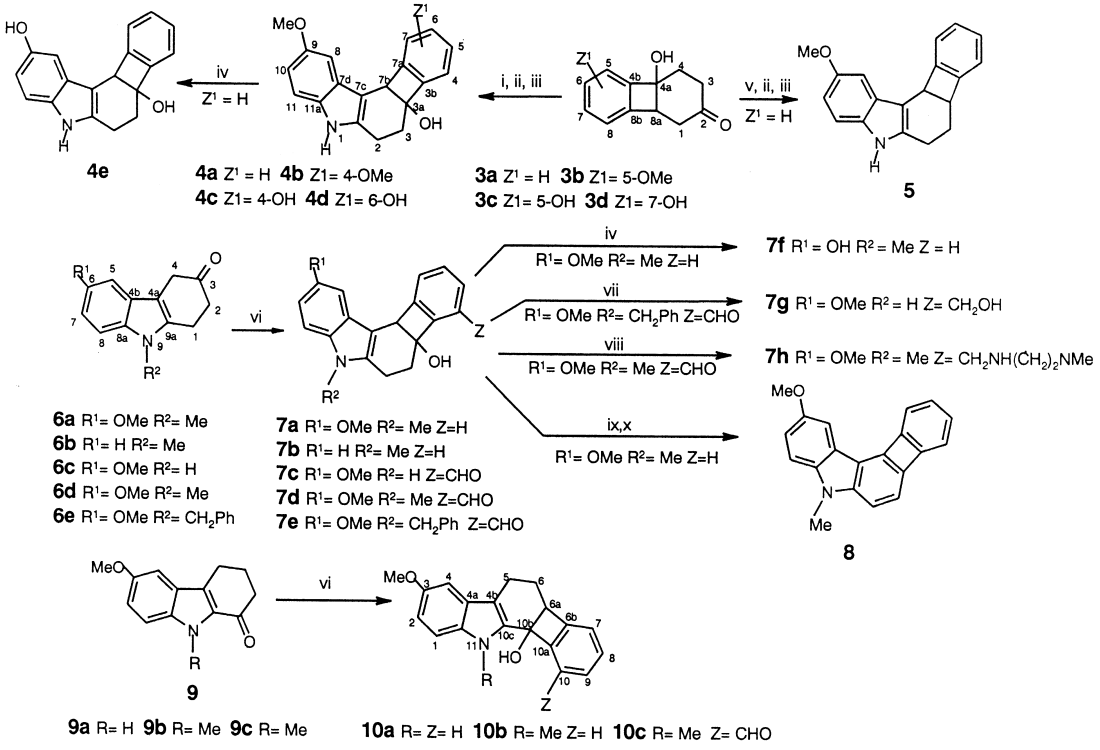
0960-894X/00/$ - see front matter
PII: S0960-894X(00)00532-1

It soon appeared that *N*-methylated compounds (**7f** $R^1 = OH$, $IC_{50} = 45.8$ μM; **7a** $R^1 = OMe$, $IC_{50} > 50$ μM) were significantly less active than their unsubstituted counterpart **4e** ($IC_{50} = 0.6$ μM) and **4a** ($Z^1 = H$, $IC_{50} = 9.7$ μM). Removal of the 9-methoxy or hydroxy substituent (**7b** $R^1 = H$, $R^2 = Me$, $IC_{50} = 40.4$ μM) had no clear effect on the cytotoxicity.The important part played by the 3a-hydroxy group was illustrated by the inactivity of **5** and **8** devoid of such a substituent.

Surprisingly, **4e**, despite its good cytotoxicity, possessed no specific interaction with the cell cycle whilst **4a** induced a clear accumulation of the cell in the $G_2 + M$ phase of the cycle (68% at 100 μM versus 24% for untreated control cells).

With the above results in hand, we investigated the influence of various substituents on the benzene ring of the benzocyclobutene unit. Substrates substituted by a methoxy group on the *para* position of the nitrogen atom and with unsubstituted nitrogen, were chosen for this study.

With reference to **4a** ($IC_{50} = 9.7$ μM) it appeared that a 6-hydroxy group induced a total loss of activity (**4d** $IC_{50} > 50$ μM). On the contrary, a 4-hydroxy substituent



Scheme 1. (i) DHP, PTSA, CH_2Cl_2, 30 °C; (ii) 3-Cl,4-$MeOC_6H_3NH_2$, C_6H_6 reflux; (iii) $NaNH_2$:*t*-BuONa (5:2), THF, 0 °C–rt[5] then H_2O, HCl 10% Me_2CO; (iv) twice $PhCH_2SH$:$AlCl_3$ (20:1.5), 0 °C;[9] (v) $POCl_3$, pyridine, 0 °C–rt, then H_2–Pd:C (5%); (vi) $NaNH_2$:*t*-BuONa (9:4), THF 0–10 °C, then 2-ZC_6H_4Br (Z = H, $CH(OMe)_2$,[6,8] then HCl 10% MeOH; (vii) H_2 (50 Pa), $Pd(OH)_2$/C (20%), AcOEt–AcOH, rt; (viii) $H_2N(CH_2)_2$ NMe_2, CH_2Cl_2, 0 °C, then $NaBH_4$, MeOH; (ix) $CuSO_4/SiO_2$, MeC_6H_5, reflux; (x) DDQ, C_6H_6, reflux.

Table 1. Antiproliferative activity

Compounds	*F* (°C)	IC_{50}[a] L1210 (μM)	% of L1210 cells in the G_2+M phase[b] (μM)	Compounds	*F* (°C)	IC_{50} L1210 (μM)	% of L1210 cells in the G_2+M phase[b] (μM)
VP 16		0.23	83% (0.5 μM)				
Adriamycin		0.025	80% (0.1 μM)				
1	220	312.2	ne[c]	**4d**	159	>50	ne
4e	88	0.6	No specific	**4b**	98	24.9	ne
4a	88	9.7	66% (100 μM)	**7g**	105	36.5	ne
7f	118	45.8	ne	**7c**	104	10.1	40% (25 μM)
7a	144	>50	ne	**7d**	108	5.3	40% (50 μM)
7b	93	40.4	ne	**7h**	143	16.7	ne
5	162	>50	ne	**10a**	186	17	ne
8	109	>50	ne	**10b**	97	50	ne
4c	189	16.6	68% (50 μM)	**10c**	119	34.1	ne

[a]A solution of product 10^{-2} M in DMSO was diluted to 100 μM (1% DMSO) in the culture medium. Then cells were exposed to graded concentrations of the compound for 48 h. Care was taken that no precipitation took place.
[b]24% of untreated control L1210 cells were in the G_2M phase of the cell cycle.
[c]ne, not evaluated.

only slightly decreased the cytotoxicity (**4c** $IC_{50} = 16.6$ μM) and seemed to improve the cellular cycle interaction (68% of the L1210 cells in the $G_2 + M$ phase at 50 μM compared to 66% at 100 μM for **4a**). Replacement of a 4-hydroxy by a 4-methoxy (**4b** $IC_{50} = 24.9$ μM) or a 4-hydroxymethyl group (**7g** $IC_{50} = 36.5$ μM) resulted in a decrease of the cytotoxicity. Surprisingly, while a 4-formyl group had no effect on the IC_{50} when $R^1 = OMe$ and $R^2 = H$ (**7c** $IC_{50} = 10.1$ μM), it substantially improved the activity when $R^1 = OMe$ and $R^2 = Me$ (**7d** $IC_{50} = 5.3$ μM compared to >50 μM for the unformylated counterpart **7a**).

In the hope of reinforcing the interactions with DNA, the formyl group was used to introduce a 4-*N*,*N*-dimethylaminoethylaminomethyl unit. Unfortunately, the resulting compound **7h** was less active than **7d** with an IC_{50} of only 16.7 μM.

Finally, from the data reported in Table 1, it appears that the position of the benzocyclobutene unit plays a substantial part since **10a** and **10c** were found to be less active than **4a** and **7d**, respectively.

In conclusion, we have presently described the synthesis and the in vitro antiproliferative activity of new benzo[3,4]cyclobuta[1,2-*a*] and [1,2-*c*]carbazol derivatives. Some of these compounds exhibit significant cytotoxicity with concomitant accumulation of the L1210 cells in the $G_2 + M$ phase of the cell cycle, opening the door to promising pharmacomodulations.

Acknowledgements

Warm thanks are expressed to ADIR for financial support.

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