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Antitumoral activity of trisubstituted dihydrobenzo(a)carbazoles. Part III

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Two recently synthesized, trisubstituted dihydrobenzo(a)carbazoles were investigated regarding their anti-HIV and antitumoral activity. The compounds showed some activity against melanoma, renal cancer and breast cancer cell lines.

1. Introduction

Benzodihydro(a)carbazoles have been reported as starting compounds for the synthesis of various drugs and possess important biological, pharmacological and medicinal activities [1–9]. The therapeutic efficacy of tamoxifen in the therapy of breast cancer is well-established [10]. Therefore, it is desirable to search for new drugs with greater efficacy or more prolonged duration of response. Von Angerer [3, 4] has previously synthesized and assayed a number of 11-alkylbenzo(a)carbazoles, their dihydro analogues and a series of hydroxy-substituted 2-phenylindoles which showed high binding affinities for the estrogen receptor and inhibited the growth of dimethylbenzanthracene-induced hormone-dependent mammary tumors.

Previously we have described some compounds with this structure which demonstrated antitumoral activity [1, 2]. The influence of structural variations on *in vitro* and *in vivo* activity was studied. First, estradiol receptor binding affinity of these drugs was measured by a competitive binding assay with 17β-[^{3}H]estradiol. Drugs were then tested for their inhibitory activity against rat mammary carcinomas induced by *N*-nitroso-*N*-methylurea. All compounds of the series had similar receptor affinity. However, *in vivo* antitumoral activity was only observed in compounds having two hydroxyl groups as substituents located in rings *a* and *d* at a distance similar to that of the hydroxyls in estradiol as well as an aminoalkyl chain similar to that of tamoxifen. This work is a continuation of studies on the structure-activity relationships of active substituted dihydrobenzo(a)carbazoles.

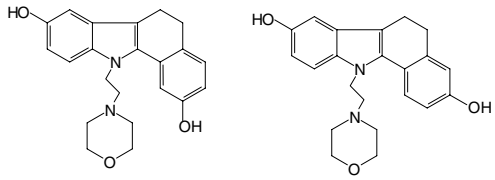
The compounds synthesized have been evaluated for *in vitro* anti-HIV and antitumoral activity at the National Cancer Institute at Bethesda, USA according to the described method [11–12].

Since many cytostatic or cytotoxic polycycles have the ability to intercalate into DNA and inhibit DNA replication, we studied the interaction of active derivatives with DNA as a first approach to determine their pharmacological mechanism.

2. Investigations, results and discussion

The test of anti-HIV activity was performed on T-4 lymphocytes (CEM-SS cell line) uninfected or infected with HIV-1. Cell viability was determined spectrophotometrically, using the tetrazolium assay procedure. These compounds failed to show any activity in the anti-HIV test.

Antitumoral activity of the studied compounds was evaluated using a total of 60 human cell lines derived from nine different cancer types (lung, colon, melanoma, prostate, breast, renal, ovarian, brain and leukemia). Compounds were tested over a broad concentration range (10^{-4} M–10^{-8} M) against every cell line of the panel. Response parameters GI_{50} (concentration exerting 50% inhibition); TGI (concentration exerting total growth inhibition) and LC_{50} (concentration exerting 50% lethal effect)



1 (NCS 686760) 2 (NCS 693112)

were calculated from dose-response curves. Mean panel $\log_{10}$ values (MG-MID) of the three response parameters were obtained by averaging individual values for each cell line. Mean GI_{50}, TGI and LC_{50} values of compounds **1** (NCS686760) and **2** (NCS 693112) are listed in the Table.

Binding to DNA was determined by the alteration in UV spectra produced by adding calf thymus DNA [13], measured over a 40-nm band centered on the maximal absorbance value of each compound after 24 h. Drug-DNA ratio was 1 : 5 (expressed as base pairs). The degree of interaction was expressed by means of the ratio between the final absorbance area (a_{24}) and that of the pure compound at equal concentration (a_0). Areas were calculated automatically by the same measuring device. Values lower than unity indicate progressively more affinity to DNA; thus value 1 indicates a total lack of affinity and value 0 that the entire compound binds to DNA (Table). Ethidium bromide, mitoxantrone and adriamycin served as reference drugs in these experiments.

The data presented in the Table show that, out of the 60 cell lines tested, compound **1** was active on 28, for which individual $-\log_{10} GI_{50}$ values were ≤ 4.6 and **2** was active on 24, for which individual $-\log_{10} GI_{50}$ values were ≤ 4.3. Both compounds exert selective activity against melanoma, renal cancer and breast cancer subpanels.

The most remarkable aspect of this work is the evaluation of cytostatic activity in hormone-sensitive MCF-7 and hormone-independent MDA-MB 231 human breast cancer cells. In this study it became evident that the cytostatic effect on estrogen receptor positive cells is not mediated by the receptor because there is no significant difference in activity between these two cell lines.

UV spectrum differences of DNA in the presence of the dihydroxy-benzodihydro(a)carbazoles gave no hint at any intercalation into DNA or other major changes in DNA structure. Apart from this particular interaction with DNA there are many other possibilities by which cell proliferation can be inhibited, mostly involving enzymes such as DNA polymerases or topoisomerase II. Up to now, the potential biochemical target participating in this specific antitumoral activity of compounds **1** and **2** is unknown. Further studies on the mode of action are in progress.

3. Experimental

3.1. Chemistry

Melting points were determined on an electric variable heater (Kofler) and are uncorrected. Elemental analysis for C, H and N was carried out and

Table: DNA affinity coefficients and *in vitro* screen of compounds 1 and 2 based upon a panel of 60 human cell lines[a]

Compd.	a_{24}/a_0	$-\log_{10}GI_{50}$ Delta[b]	$-\log_{10}TGI$ Delta	$-\log_{10}LC_{50}$ Delta	Panel (Cell line)/$-\log_{10}GI_0$							
					Leukemia	Non-Small Cell Lung cancer	Colon Cancer	CNS Cancer	Melanoma	Ovarian Cancer	Renal Cancer	Breast Cancer
1	0.99	4.64 0.20	4.19 0.32	4.02 0.23	K-562 4.70 MOLT-4 4.71 SR 4.74	HOP-62 4.71 NCI-H226 4.74 NCI-H522 4.76	COLO 205 4.76 HCC-2998 4.77 HCT-116 4.72 HCT-15 4.72	SF-295 4.70 SNB-75 4.71 U251 4.71	LOX IMVI 4.75 M14 4.71 SK-MEL-2 4.75 SK-MEL-28 4.73 SK-MEL-5 4.78 UACC-62 4.78	OVCAR-3 4.68	A498 4.68 RXF-393 4.71 UO-31 4.76	MCF-7 4.73 MDA-MB-231/ATCC 4.75 MDA-MB-435 4.75 BT-549 4.84 T-47D 4.73
2	0.96	4.49 **0.50**	4.06 0.40	4.00 0.01	RPMI-8226 4.54	NCI-H226 4.64 NCI-H23 4.56	KM12 4.68	SF-539 4.56 SNB-19 4.60 SNB-75 4.77	MALME-3M 4.60 SK-Mel-2 4.57 SK-MEL-28 4.53 SK-MEL-5 4.80 UACC-62 4.69	IGROV1 4.96 OVCAR-8 4.69	A498 4.83 ACHN 4.79 CAKI-1 4.61 RXF-393 4.52 UO-31 4.98	MCF-7 4.63 MCF7/ADR-RES 4.68 MDA-MB-231/ATCC 4.30 BT-549 4.50 T-47D 4.51

[a] $\log_{10}$ values of calculated mean molar GI_{50}, TGI, LC_{50} concentration
"Delta" is the number of $\log_{10}$ units by which the most sensitive line(s) differ from the corresponding mean values (MG-MID); [b] Delta is considered low when <1, moderate when >1 and <3, high when >3

found to be within ±0.4% of theoretical values. IR spectra were recorded using KBr pellets on a Brucker IFS 25 spectrometer. ^{1}H NMR spectra were measured on a Bruker MSL-300, using tetramethylsilane as internal standard. TLC analysis were carried out on fluorescent Merck GF_{254} silica gel plates, using chloroform : MeOH (99 : 1) for the first step of the reaction and chloroform : MeOH (9 : 1) for the second, as eluents. Spots were visualized under 254 nm UV illumination. A Shimatzu GCMS-QP100 gas chromatograph was used to determine molecular weights. Compound 1 (NCS 686760) preparation was published previously [1]. Compound 2 (NCS 693112) was prepared following a reported procedure [1] and physico-chemical data are: m.p. 135–137 °C; IR 3320 cm^{-1} (OH), 2820 cm^{-1} (aromatic and aliphatic CH), 1110 cm^{-1} (C–O–C); ^{1}H–NMR [D_6]-DMSO δ (ppm): 2.6 (m, 4 H, $-CH_2-N-CH_2-$); 2.8 (m, 4 H, $-CH_2-CH_2-$); 2.9 (m, 2 H, $CH_2-NC_4H_8O$); 3.7 (m, 4 H, $-CH_2-O-CH_2-$); 4.5 (t, 2 H, $=N-CH_2-$); 6.6–7.5 (m, 6 H, arom); 8.75 (s, 1 H, $-OH$) and 9.5 (s, 1 H, $-OH$) which disappeared with D_2O. MS M^+: 364.

3.2. Binding to DNA

DNA solution: calf thymus DNA (12.5 mg) was slowly stirred magnetically in 5 ml Tris-buffer (10 mM, pH 7.4) for 24 h at 4 °C. From this solution, 0.6 ml were taken and diluted with the same buffer to 25 ml.
The test compound solution was prepared at 10^{-4} M concentration using a minimal ethanol volume and water, then diluted to 2×10^{-5} M concentration, 3 ml of each resulting solution were then mixed with 3 ml of DNA solution described above and their UV spectra were recorded after 24 h using 1 cm cell on a Jasco 7850 spectrophotometer.
Under such experimental conditions, ethidium bromide, a recognized intercalation agent, rendered an a_{24}/a_0 ratio of 0.55, mitoxantrone 0.51 and adriamycin 0.66.

3.3. Cytotoxic evaluation

All compounds were examined against human tumor cell lines by the National Cancer Institute, Bethesda, USA using a reported procedure [11–12].

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