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IL FARMACO 59 (2004) 211–214
IL FARMACO
www.elsevier.com/locate/farmac

Synthesis and cytotoxic effect of carbocyclic potential minor groove binders

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Received 26 February 2003; accepted 8 November 2003

Abstract

New carbocyclic potential minor groove binders were synthesised, using 3-nitrobenzoyl chloride and aliphatic α,ω-diamines with three, four and five methylene fragments. The half structures, compounds **IV–VI** can be compared to bis-amidines, compounds **X–XII** to bis-netropsin. All of the compounds were investigated antiproliferative and cytotoxic effects in the standard cell line of mammalian tumour MCF-7.

Keywords: Minor groove binders; Bis-netropsin; Carbocyclic analogues of netropsin

1. Introduction

Cell-permeable small molecules with the ability to target predetermined DNA sequences would be valuable tools in molecular biology and potentially in human medicine. A number of natural and synthetic compounds are known to bind to DNA double helix in a non-intercalative manner [1]. This is possible because most DNA-binding molecules possesses cationic functional groups, complementary in size to one of the grooves, have an aromatic ring system, or a combination factors of these [1]. Netropsin, bis-netropsin or bis-amidines (e.g. pentamidine), DNA minor groove binders, could provide a significant improvement in cancer management increasing gene specificity. There are known a lot of synthetic compounds that binds specifically to many different DNA sequences—they all have the name lexitropsins [2]. Due to the high selectivity of DNA interaction, lots of lexitropsin were used in the recent past as a DNA sequence-selective vector of alkylating functions [3,4].

Studies on benzene-containing and C-terminus-modified analogues of netropsin and distamycin, afforded compounds with antiproliferative and cytotoxic effects in standard cell lines of mammalian tumour MCF-7 [5]. Data from the ethidium displacement assay showed that these compounds were able to bind in the minor groove-binding mode in AT sequences of DNA [6]. They can use as carriers of alkylating elements—carbocyclic analogues with chlorambucil moiety were synthesised and investigated [7]. Comparison values of K_{app} tri- and dibenzene analogues suggested that the optimum number of benzene units for binding to the DNA could be two. In addition, in order to rationalise the experimental findings, computer molecular modelling studies were performed with an appropriate B-DNA on the basis of molecular mechanics and molecular dynamics calculations.

As a part of ongoing rational drug design programme aiming at development of carbocyclic minor groove binders, six novel compounds **IV–V** and **X–XII** were synthesised and evaluated (Scheme 1).

2. Experimental

2.1. Chemistry

Compounds **IV–VI** and **X–XII** were prepared according to the general procedure described in this paper (Scheme 1). Yields and physico-chemical properties of synthesised compounds are reported in Table 1.

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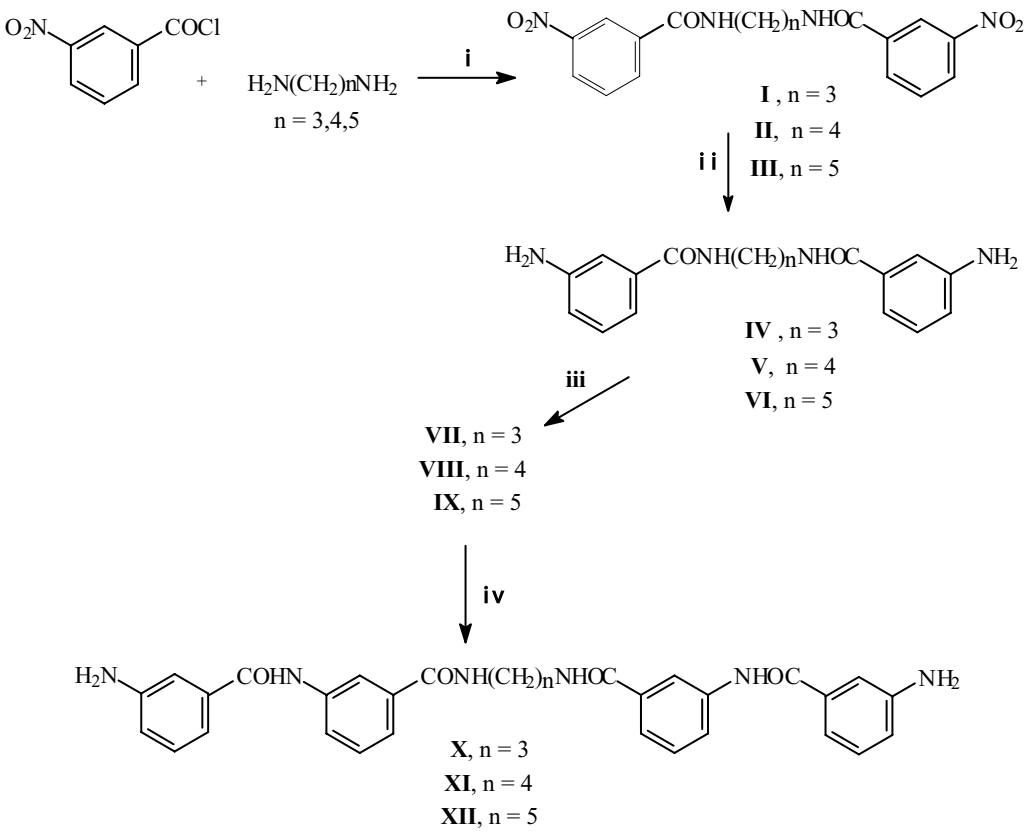
doi:10.1016/j.farmac.2003.11.005



Scheme 1. Synthesis of carbocyclic analogues of minor groove binders. Reagents and conditions: (i) CH_2Cl_2, 2 h, room temperature; (ii) H_2/Pd, MeOH, 1.5 h, room temperature; (iii) 3-nitrobenzoyl chloride, Py/CH2Cl_2, DMAP, 5 h, room temperature; (iv) H_2/Pd, MeOH, 2.5 h, room temperature.

The structures of synthesised compounds were confirmed by analyses of their ^{1}H NMR and ^{13}C NMR spectra. The spectra were recorded on a Bruker AC 200F spectrometer, using TMS as an internal standard. Chemical shifts are expressed in δ value (ppm). The results of elemental analyses for C and H were within ±0.4% of the theoretical values. Melting points were determined on Buchi 535 melting points apparatus and were uncorrected.

The reaction progress was controlled on thin-layer chromatography (TLC) plates and spraying dimethylaminobenzaldehyde (DMAB) solution. TLC was performed on Silica Gel 60 F254 (Merck) and visualised with UV. The identification of aromatic primary amine was confirmed with solution containing 1 g DMAB, 10 ml 95% EtOH, 30 ml concentrated hydrochloric acid (HCl) and 180 ml *n*-butanol; 5% NH_{3aq} in methanol was used as a solvent to TLC.

Solvents used in the experiments were dried and distilled.

Silica gel 60 (Merck, 230–400 mesh) was used for column chromatography.

*2.1.1. General procedure for preparation of compound **XII***

The starting materials for synthesis, 3-nitrobenzoyl chloride (2.2 mmol), was dissolved in methylene chloride and added dropwise to the solution 1,5-diamine (1 mmol) in dry pyridine with 4-dimethylamino-pyridine (DMAP). The reaction mixture was stirred in room temperature for 2 h. After finishing the reaction, the precipitated crude product was filtered off and crystallised from acetone to give pure compound **III** with good yield (85.02%).

The nitro groups were reduced by catalytic hydrogenation of compound **III** (0.85 mmol), which was dissolved in methanol. The reaction mixture was stirred in room temperature and at atmospheric pressure for 1.5 h. Then a black precipitate of catalyst was filtered off, and HCL (10 ml in 100 ml of MeOH) was added to filtrate until the pH reached 5. The resulting solution was concentrated under reduced pressure. The crude product was purified by column chromatography in chloroform with a MeOH gradient. The concentration of fractions containing product of reduction yielded compound **VI** (80.60%).

To solution of diamine **VI** (0.68 mmol) in pyridine with DMAP was added dropwise 3-nitrobenzoyl chloride (1.50 mmol) dissolved in methylene chloride. The mixture was stirred at room temperature for 5 h and gave nitro compounds **IX**. Recrystallisation of this product gave pure solid of **IX** (yield 56%). Catalytic reduction of the nitro groups to the amine **XII** preceded satisfactorily under the reaction conditions described earlier (yield 73.87%).

2.2. Pharmacology

2.2.1. MCF-7 cells

Stock cultures of breast MCF-7 cancer cells (purchased from the American Type Culture Collection, Rockville, MD)

Table 1
Physico-chemical properties of compounds (in the form of dihydrochlorides)

Compound	Formula (molecular weight)	Yield (%)	Melting point (°C)	R_f	^{1}H NMR, δ (ppm) (d_6-DMSO)	^{13}C NMR, δ (ppm) (d_6-DMSO)
IV	$C_{17}H_{20}N_4O_2$ (312.37)	78.70	84–85	0.44	1.88 (m, 2H, C–CH_2–C); 3.37 (m, 2 × 2H, $CONHCH_2$); 4.58 (bs, 2 × 2H, NH_2); 7.72–8.68 (2 × 4H, Ar-H); 9.10 (t, 2 × 1H, CONH)	25.01 (C–CH_2–C); 36.01 (2C, $CONHCH_2$); 121.96 (C_2); 125.69 (C_6); 130.04 (C_4); 133.60 (C_5); 135.88 (C_1); 147.74 (C_3); 163.97 (CONH)
V	$C_{18}H_{22}N_4O_2$ (326.40)	82.84	163–164	0.43	1.54 (m, 2H, C–CH_2–C); 3.22 (m, 2 × 2H, $CONHCH_2$); 5.47 (bs, 2 × 2H, NH_2); 6.71–7.10 (2 × 4H, Ar-H); 8.29 (t, 2 × 1H, CONH)	26.79 (C–CH_2–C); 38.91 (2C, $CONHCH_2$); 113.41 (C_2); 115.00 (C_6); 116.83 (C_4); 128.62 (C_5); 135.71 (C_1); 147.49 (C_3); 166.84 (CONH)
VI	$C_{19}H_{24}N_4O_2$ (340.43)	80.60	246–247	0.34	1.40 (m, 2H, C–CH_2–C); 1.54 (m, 2 × 2H, C–CH_2–C); 3.15 (s, 2 × 2H, NH_2); 3.26 (m, 2 × 2H, $CONHCH_2$); 7.20–8.35 (2 × 4H, Ar-H); 8.59 (t, 2 × 1H, CONH)	23.99 (C–CH_2–C); 28.81 (2C–CH_2–C); 39.22 (2C, $CONHCH_2$); 119.61 (C_2); 122.66 (C_6); 122.92 (C_4); 129.35 (C_5); 136.03 (C_1); 137.21 (C_3); 165.60 (CONH)
X	$C_{31}H_{30}N_6O_4$ (550.62)	72.12	155–156	0.27	1.80 (m, 2H, C–CH_2–C); 3.36 (m, 2 × 2H, $CONHCH_2$); 5.36 (bs, 2 × 2H, NH_2); 6.75–8.23 (2 × 8H, Ar-H); 8.52 (t, 2 × 1H, CONH); 10.23 (s, 2 × 1H, CONH)	29.32 (C–CH_2–C); 39.74 (2C, $CONHCH_2$); 116.73 (C_2); 119.70 (C_6); 119.80 (C_4); 122.96 ($C_{4'}$); 128.87 ($C_{5'}$); 129.16 (C_5); 130.25 ($C_{3'}$); 131.44 (C_1); 139.47 ($C_{1'}$); 147.71 ($C_{6'}$); 148.86 (C_3); 166.47 (CONH); 167.90 (CONH)
XI	$C_{32}H_{32}N_6O_4$ (562.63)	69.59	195–196	0.25	1.61 (m, 2 × 2H, C–CH_2–C); 3.28 (m, 2 × 2H, $CONHCH_2$); 6.03 (bs, 2 × 2H, NH_2); 6.85–8.50 (2 × 8H, Ar-H); 8.93 (t, 2 × 1H, CONH); 10.23 (s, 2 × 1H, CONH)	26.71 (C–CH_2–C); 38.98 (2C, $CONHCH_2$); 113.78 (C_2); 115.68 (C_6); 117.62 (C_4) 119.71 ($C_{4'}$); 121.88 ($C_{5'}$); 122.78 (C_5); 128.81 ($C_{3'}$); 135.35 (C_1); 135.65 ($C_{1'}$); 139.32 ($C_{6'}$); 147.48 (C_3); 166.21 (CONH); 167.69 (CONH)
XII	$C_{33}H_{32}N_6O_4$ (576.66)	73.87	209–211	0.19	1.38 (m, 2H, C–CH_2–C); 1.55 (m, 2 × 2H, C–CH_2–C); 3.28 (m, 2 × 2H, $CONHCH_2$); 5.51 (s, 2 × 2H, NH_2); 6.76–7.92 (2 × 4H, Ar-H); 8.22 (t, 2 × 1H, CONH); 10.24 (s, 2 × 1H, CONH)	23.99 (C–CH_2–C); 28.86 (2C–CH_2–C); 39.34 (2C, $CONHCH_2$); 113.20 (C_2); 114.98 (C_6); 117.09 (C_4); 119.72 ($C_{4'}$); 121.91 ($C_{5'}$); 122.81 (C_5); 128.34 ($C_{3'}$); 135.39 (C_1); 135.61 ($C_{1'}$); 139.35 ($C_{6'}$); 148.52 (C_3); 166.24 (CONH); 167.82 (CONH)

were maintained in continuously exponential growth by weekly passage in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% FBS (Sigma), 50 μg/ml streptomycin, 100 U/ml penicillin at 37 °C in a humid atmosphere containing 5% CO_2. Cells were cultivated in Costar flasks and subconfluent detached with 0.05% trypsin and 0.02% EDTA in a calcium-free phosphate-buffered saline. The study was carried out using cells from passages 3–7, growing as monolayer in six-well plates (Nunc) (5×10^5 cells per well and preincubated 24 h without phenol red.

2.2.2. Determination of apoptotic index and cell viability

The compounds were dissolved in sterile water and used at concentrations of 25, 50, 100 and 150 μM.

Microscopic observations of cell monolayers were performed with a Nikon optiphot microscope. Wright–Giemsa staining was performed using the Fisher Leuko Stat Kit. Adherent MCF-7 cells grown in six-well plates were stained after induction of apoptosis with a dye mixture (10 μM acridine orange and 10 μM ethidium bromide, prepared in phosphate-buffered saline). At the end of each experimental time point, all of the media was removed and cells were harvested by incubation with 0.05% trypsin and 0.02% EDTA for 1 min and washed with the medium. Then, 250 μl of cell suspension was mixed with 10 μl of the dye mix and 200 cells per sample were examined by fluorescence microscopy, according to the following criteria:

- viable cells with normal nuclei (a fine reticular pattern stained green in the nucleus and red-orange granules in the cytoplasm);
- non-viable cells with normal nuclei (bright orange chromatin with organised structure).

Antitumour activity investigated compounds expressed as percentage of non-viable MCF-7 mammal tumour cells was shown in Table 2.

2.2.3. Statistical analysis

The results were submitted to statistical analysis using the method of the smallest squares, accepting coefficient of determination in the range $0.9800 < R^2 < 1$. The IC_{50} data are presented in Table 2.

3. Results and discussion

Described compounds were tested for their cytotoxic and antiproliferative activity in the standard cell line of mammalian tumour MCF-7. The compound concentration that inhib-

Table 2
Antitumour activity of tested compounds

Compound	Concentration (μM)				IC_{50} (μM)	Statistical data (model $y = ax + b$)		
	25	50	100	150		*a*	*b*	R^2
IV	3%[a]	5%	20%	35%	209.80	0.2664	–5.8983	0.9815
V	5%	10%	20%	30%	250.00	0.2000	0.0000	1.0000
VI	5%	10%	25%	30%	235.89	0.2102	0.4237	0.9581
X	3%	8%	20%	30%	238.64	0.2224	–3.0678	0.9953
XI	5%	10%	15%	20%	406.62	0.1153	0.7565	0.9796
XII	7%	10%	20%	30%	258.30	0.1878	1.4915	0.9950

[a] Percent of non-viable MCF-7 mammalian tumour cells.

its 50% of colony formation is in the range 209.80–406.62 μM.

The binding of all compounds, as well analogues of bis-amidines **IV–VI**, as products **IX–XII**, is weaker than that of DAPI (IC_{50} = 176 μM) and Hoechst 33258 (IC_{50} = 55 μM) [8], likewise carbocyclic mono-lexitropsin (24.43 μM) [5]. As can be seen analogues **X–XII** produced related reduction in cell viability in breast cancer MCF-7 cells to compounds **IV–VI**. This suggests that only one part of molecules of analogues of bis-netropsin **X–XII** binds to minor groove, but second one stays behind minor groove. Conformation of introduced linkers is such inflexible that bis(two-benzene) compounds **X–XII** do not have shapes that fits to minor groove. It can be explained also by the fact that the compounds are weakly basic anilines, at variance with strong basic netropsin and pentamidine.

The IC_{50} values for synthetic analogues of minor groove binders, **IV–VI**, suggest that these binders can serve as potential carriers of other strong acting elements, e.g. alkylating groups. Free amino group of these compounds can be connected with such elements. Compounds **X–XII** do not give a lot of hope to be useful.

Method of synthesis is simple and convenient. Further investigations on the mechanisms of cytotoxicity and inhibition of DNA topoisomerases for compounds **IV–VI** will be investigated, compounds **X–XII** are too week to be studied more.

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