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Synthesis and biological activity of carbocyclic lexitropsins with a bioreductive fragment

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Abstract

Carbocyclic oligopeptides containing of two, three or four aromatic rings with *N*,*N*-dimethylpropyl-1,3-diamine group as C-terminus fragment of compounds and 5-[bis(2-chloroethyl)amino]-2,4-dinitrobenzamide as N-terminal were synthesized. These lexitropsins present antitumour activity on the neoplastic cells hepatoblastoma HEP G2. These experiments were evaluated in hypoxic and oxygen conditions. Significant differences of activity in oxygen and hypoxic conditions were shown only in compound *N*-(3-dimethylaminopropyl)-*N*′-({3-[5-bis(2-chlorethyl)amino]-2,4-dinitrobenzamide})-phenyl]urea dihydrochloride **1** ($IC_{50} = 8545$ nM in oxygen vs. $IC_{50} = 710$ nM in hypoxia). The rest of compounds (**2**–**6**) do not indicate differences of activity in oxygen and hypoxia.

Keywords: Lexitropsins; Hypoxic-selective cytotoxins; Nitrogen aromatic mustards

1. Introduction

Hypoxic cells are for a variety of reasons resistant to both radio- and chemotherapy and may be an important limiting factor in clinical cancer treatment. One of the strategies of overcoming hypoxia problem is development of hypoxic-selective cytotoxins (HSCs) [1,2]. The most common design of HSCs is the prodrug approach, where a nontoxic precursor molecule is converted to a toxic form by an activation process.

In recent years, a interest connected with the development of nitrogen aromatic mustards has grown. The 'classical' nitrogen mustards, in particular melphalan and cyclophosphamide, are important as components of drugs. However, they have a number of disadvantages common to all alkylating agents. Their high chemical reactivity and reversible binding affinity to DNA lead to rapid decay of drugs [1]. Therefore, it seems necessary to search the way of improvement of the therapeutic effects of mustards by the masking of their cytotoxicity and the formation of prodrugs [3–6]. The mustard moiety can be deactivated by attachment of nitro group to an aromatic ring. The nontoxic nitromustard precursor molecule diffuses efficiently in unchanged form to the hypoxic cells. After the reduction of the nitro group by using endogenic enzymes (i.e. nitroreductase) to the amine or hydroxylamine groups, nitrogen mustards are activated to a form which is capable to form cross-links with DNA [1].

One of the most hypoxia-selective compounds is 5-[*N*,*N*-bis(2-chloroethyl)amino]-2,4-dinitrobenzamide (SN 23562). This compound shows about 60-fold higher cytotoxic effect to the hypoxic UV4 cells than to the same cells under aerobic conditions in vitro [4].

In this paper we describe the synthesis and the biological evaluations of the series of carbocyclic lexitropsins with benzamide dinitroarenemustard as N-terminal group (Fig. 1). Lexitropsins are synthetic analogs of natural olipeptides antibiotics-netropsin and distamycin, which clinical application is limited due to their toxicity [7,8]. Netropsin and distamycin and their analogs are excellent carriers of moiety with known activities [6].

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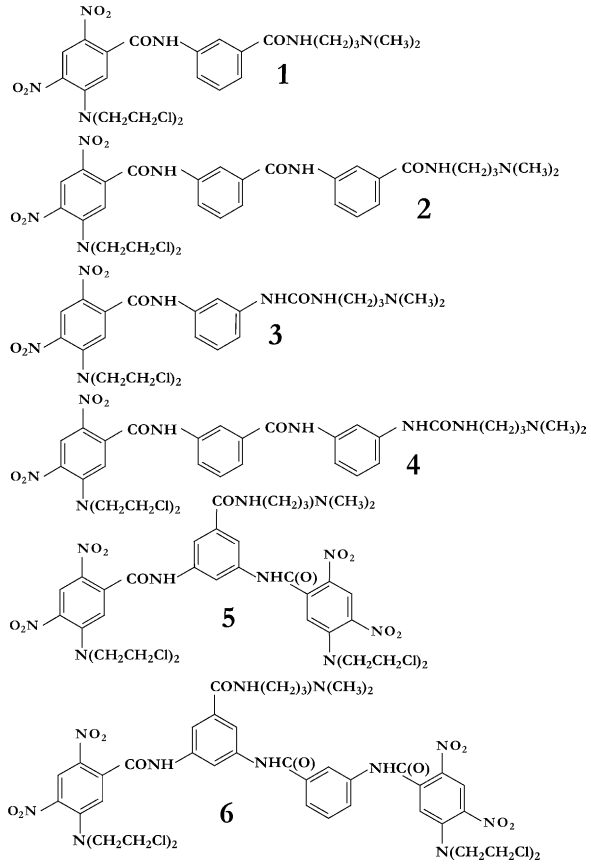
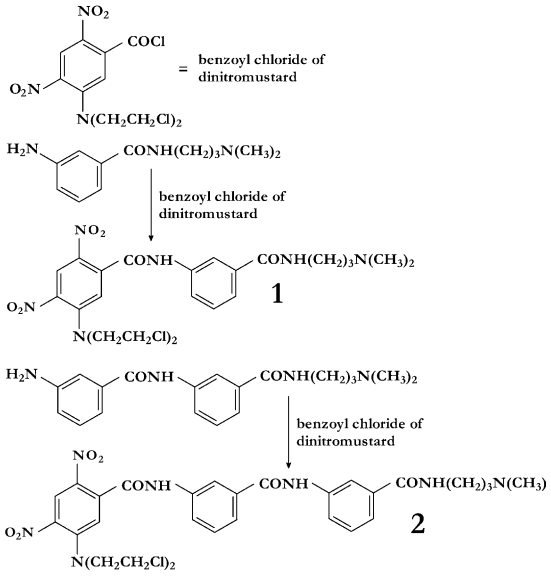


Fig. 1. Compounds **1**–**6**.

2. Experimental

2.1. Chemistry

Compounds **1**, **3**, **5** and **6** were prepared according to the general procedure described in this paper (Scheme 1). Compounds **2** and **4** were prepared using a method previously described [9–11]. The starting material 5-bis(2-chloroethyl)amino-2,4-dinitrobenzoyl chloride was prepared according to the literature [5]. Yields and physicochemical properties of synthesized compounds are reported in Table 1(Schemes 2 and 3).



Scheme 1. Synthesis of compounds **1**–**2**.

The structures of synthesized compounds were confirmed by analyses of their ^{1}H and ^{13}C NMR spectra. The spectra were recorded on Bruker AC 200F spectrometer, using TMS (tetramethylsilane) as an internal standard. Chemical shifts are expressed in δ value (ppm).

Thin-layer chromatograms were prepared on precoated plates (Merck, silica gel 60F-254). Solvent system 1% concentrated NH_{3aq} in MeOH was used to TLC.

The UV light was used from detection of all compounds.

The identification of aromatic primary amine was confirmed with solution containing 1 g dimethylaminobenzaldehyde (DMAB), 10 ml 95% EtOH, 30 ml concentrated HCl and 180 ml *n*-butanol. Compounds containing N-terminal aromatic mustard group were detected by spraying with 5% solution NBP (γ-(4-nitrobenzyl)-pyridine) in C_3H_6O, heating in 100 °C for 20 min and then spraying with 20% solution of Et_3N in C_3H_6O [12]. Aromatic nitrogen mustard groups were also identified with Dragendorff's reagent (solution A: 0.85 g basic bismuth nitrate in 10 ml glacial acetic acid and 40 ml water; solution B: 8 g potassium iodide in 20 ml water). The chromatograms were sprayed with a mixture of the solutions A and B (1:1), 1 ml; glacial acetic acid, 2 ml and water, 10 ml [13].

Solvents used in the experiments were dried and distilled.

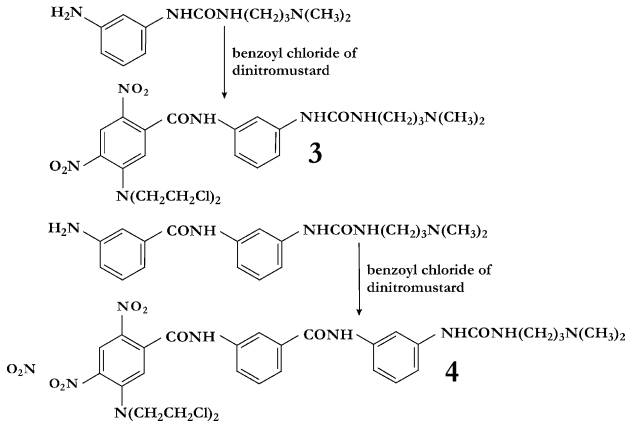
Silica gel 60 (Merck, 230–400 mesh) was used for column chromatography.

2.2. General procedure for the preparation of compounds *1–6*

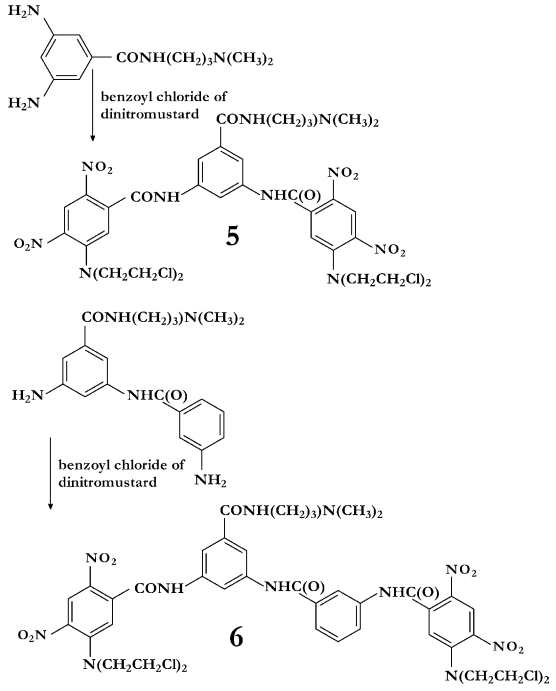
To a suspension of amine (1 mol), 4-(*N*,*N*-dimethylamino)pyridine (DMAP) (1/80 mol) and methylene chloride was added Et_3N (1 mol). Dinitromustard benzoyl chloride (1 mol) was added dropwise to the solution. The reaction mixture was stirred in room temperature for 1 h. The reaction progress was controlled on TLC plates and spraying DMAB and Dragendorff's reagent. After finishing the reaction precipitate of hydrochloride of Et_3N was filtered off and the resulting solution was concentrated under reduced pressure. The residue was dissolved in mixture MeOH–water (1:1) and a solution of 1 M HCl was added dropwise until the pH reached 6. Water and MeOH were removed under reduced pressure. The crude product was purified by column chromatography in a mixture C_6H_{14}–C_3H_6O (1:2) with a MeOH gradi-

Table 1
Yields and physicochemical properties of compounds **1**–**6**

Comp.	Molecular formula	M.p. (°C)	Rf	Yield (%)	^{1}H NMR (ppm) DMSO-d_6	^{13}C NMR (ppm) DMSO-d_6
1	$C_{23}H_{28}Cl_2N_6O_6 \cdot 2HCl$	187–188	0.42	47	1.9–1.99 (m, 2H, CCH_2C), 2.72 (s, 6H, $N(CH_3)_2$), 3.06–3.17 (m, 4H,CH_2N,$CONHCH_2$), 3.71–3.84 (2 × t, 8H, $ArNCH_2CH_2Cl)_2$), 7.15–7.72 (m, 6H, Ar–H), 8.59 (s, 1H, CONH), 9.17 (s, 1H, N·HCl), 10.6 (s, 1H, N·HCl), 10.69 (s, 1H, CONH)	24.86 (CCH_2C); 31.01 ($CONHCH_2$); 38.24 ($N(CH_3)_2$); 41.93 (CH_2N); 52.66, 54.46 ($Ar–N(CH_2CH_2Cl)_2$); 108.9, 112.5, 113.3, 120.95, 124.37, 128.74, 135.61, 137.16, 137.85, 138.92, 140.95, 147.36 (12 × C–Ar); 155.48 (CONH); 162.66 (CONH)
2	$C_{30}H_{33}Cl_2N_7O_7 \cdot 2HCl$	197–198	0.34	62	1.9–1.98 (m, 2H, CCH_2C), 2.7–2.73 (d, 6H, $N(CH_3)_2$), 3.03–3.07 (m, 2H, CH_2N), 3.32–3.35 (m, 2H, $CONHCH_2$), 3.73–3.86 (2 × t, 8H, $Ar–(NCH_2CH_2Cl)_2$), 7.2–8.62 (m, 10H, Ar–H), 8.76 (t, 1H, $CONH–CH_2$), 10.56 (s, 1H, CONH), 10.83 (s, 1H, N·HCl), 11.03 (s, 1H, CONH)	24.11 (CCH_2C); 30.68 ($CONHCH_2$); 36.43 ($N(CH_3)_2$); 41.86 (CH_2N); 52.62, 54.39 ($Ar–(NCH_2CH_2Cl)_2$); 119.4, 119.95, 121.09, 122.24, 122.77, 122.98, 123.19, 124.54, 128.44, 128.82, 134.91, 135.45, 135.59, 136.85, 137.93, 138.92, 139.18, 147.45 (18 × C–Ar); 163.08 (CONH); 165.46 (CONH); 166.35 (CONH)
3	$C_{23}H_{29}Cl_2N_7O_6 \cdot 2HCl$	158–159	0.39	65	1.9–1.98 (m, 2H, CCH_2C); 2.71–2.73 (s, 6H, $N(CH_3)_2$); 3.03–3.13 (m, 2H, CH_2N); 3.43–3.5 (m, 2H, $CONHCH_2$); 3.6–3.88 (2 × t, 8H, $Ar–(NCH_2CH_2Cl)_2$); 7.41–8.62 (m, 6H, Ar–H); 8.77–8.82 (s, 1H, $CONHCH_2$); 10.75, 11.033 (s, 1H, NHCONH)	24.16 (CCH_2C); 36.48 ($CONHCH_2$); 41.92 ($N(CH_3)_2$); 48.58 (CH_2N); 52.66, 54.46 ($Ar–(NCH_2CH_2Cl)_2$);119.21, 121.09, 122.44, 124.58, 128.72, 135.12, 135.12, 135.62, 136.93, 137.95, 138.83, 147.5 (12 × C–Ar), 163.07 (CONH), 166.24 (CONH)
4	$C_{30}H_{34}Cl_2N_8O_7 \cdot 2HCl$	210–211	0.23	52	1.76–1.84 (m, 2H, CCH_2C), 2.67 (s, 6H, $N(CH_3)_2$), 2.94–3.02 (m, 2H, CH_2N), 3.1 (s, 2H, 2 × HCl), 3.14–3.17 (m, 2H, $CONHCH_2$), 3.73–3.86 (2 × t, 8H, $Ar–(NCH_2CH_2Cl)_2$), 6.61–8.19 (m, 6H, Ar–H), 8.95, 10.29, 10.95 (3 × s, 3H, CONH, NHCONH)	25.24 (CCH_2C); 38.24 ($CONHCH_2$); 41.78 ($N(CH_3)_2$); 42.31 (CH_2N); 52.62, 54.39 ($Ar–(NCH_2CH_2Cl)_2$); 106.84, 109.96, 113.49, 119.26, 120.93, 122.9, 124.57, 128.56, 128.76, 135.47, 135.9, 136.9, 137.91, 138.8, 139.32, 140.74, 141.92, 147.5 (18 × C–Ar·); 155.46 (CONH); 163.05 (CONH); 165.28 (CONH)
5	$C_{34}H_{38}Cl_4N_{10}O_{11} \cdot 3HCl$	195–194	0.13	45	1.67–1.74 (m, 2H, CCH_2C), 2.27 (s, 6H, $N(CH_3)_2$), 2.92 (s, 2H, CH_2N), 2.92 (s, 2H, $CONHCH_2$), 3.72–3.85 (m, 8H, $Ar–(NCH_2CH_2Cl)_2$), 7.35–8.63 (m, 10H, Ar–H), 8.64 (t, 1H, $CONH–CH_2$), 10.85 (s, 1H, CONH), 10.91 (s, 1H, CONH)	26.13 (CCH_2C); 32.06 ($CONHCH_2$); 42.32 ($N(CH_3)_2$); 52.59 (CH_2N); 55.79, 56.63 ($Ar–(NCH_2CH_2Cl)_2$); 114.16, 120.85, 124.53, 132.52, 134.22, 135.48, 136.92, 137.92, 138.96, 139.09, 147.49, 148.06 (18 × C–Ar); 163.04 (CONH); 163.55 (CONH), 166.08 (CONH)
6	$C_{41}H_{43}Cl_4N_{11}O_{12} \cdot 3HCl$	223–224	0.11	23	1.92 (m, 2H, CCH_2C), 2.07–2.11(d, 6H, $N(CH_3)_2$), 2.72–2.94 (m, 2H, CH_2N), 2.94 (s, 3H, 3 × HCl), 3.06–3.16 (m, 2H, $CONHCH_2$), 3.73–3.85 (2 × t, 8H, $Ar–(NCH_2CH_2Cl)_2$), 7.53–8.21 (m, 10H, Ar–H), 8.54 (t, 1H, $CONH–CH_2$), 8.63 (s, 1H, CONH), 8.87 (m, 2H, CONH, CONH)	24.11 (CCH_2C); 36.20 ($CONHCH_2$); 41.91 ($N(CH_3)_2$); 52.56, 54.45 ($Ar–(NCH_2CH_2Cl)_2$); 55.76 (CH_2N); 113.52, 114.18, 119.25, 120.93, 122.93, 122.42, 124.51, 128.75, 129.16, 135.45, 136.82, 136.91, 137.02, 137.87, 138.12, 138.81, 140.86, 147.44 (24 × C–Ar); 155.65 (CONH); 163.02 (CONH); 164.46 (CONH); 165.47 (CONH)



Scheme 2. Synthesis of compounds **3**–**4**.



Scheme 3. Synthesis of compounds **5**–**6**.

ent. Compound **1** was present in fraction with 34 ml of MeOH and 100 ml mixture of C_6H_{14}–C_3H_6O, compound **2** with 37 ml MeOH and 100 ml mixture as above, compound **3**—35 ml MeOH, compound **4**—36 ml MeOH, compound **5**—47 ml MeOH and compound **6**—49 ml MeOH. The isolated dinitromustard of carbocyclic oligopeptide was recrystallized from MeOH.

3. Pharmacology

The obtained compounds **1**–**6** were tested for their antitumour activity on the neoplastic cells hepatoblastoma HEP G2. Experiments were evaluated in hypoxic and oxygen conditions.

Table 2
Anticancer activity of tested compounds

Comp.	IC_{50} (nM)	
	Oxygen condition	Hypoxia condition
1	8545±538	710±61
2	54±3	46±4
3	836±73	> 10000
4	29±2	28±3
5	133±8	57±5
6	37±2	16±1

Hepatocellular carcinoma HepG2 were purchased from ATCC (American Type Culture Collection) (Manassas VA) Hep G2 line were routinely maintained in DMEM (Dulbecco's Modified Eagle's Medium) containing 10% FBS (Fetal Bovine Serum), 50 μg/ml penicilin, 50 μg/ml streptomycin, 2 mM glutamine at 37 °C in 95% air, 5% CO_2 and 95% humidity. Cells were grown in Falcon flasks and subconfluent cells were detached with 0.05% trypsin, 0.02% EDTA (Ethylenediaminetetraacetic acid disodium salt) in calcium-free phosphate buffered saline and seed in 1 ml of growth medium in 32 well dishes. Cells for the experiments reached about 80% of confluency at day 3. HepG2 cells were treated with compounds **1**, **2**, **3**, **4**, **5** and **6** in a dose dependent manner for 24 h. in normoxic (20% O_2, 5% CO_2, 75% N_2) and hypoxic (1% O_2, 5% CO_2, 94% N_2) condition. All experiments were performed in triplicate. The viability of cells was determined by the thrypan blue exclusion test. There was an accounted percentage of nonviable cells for every concentration of the drug. The IC_{50} data are presented in Table 2.

4. Results and discussion

Examination of the conventional alkylating agents and their mechanisms has provided some useful information for the design of new chemotherapeutics. The nitrogen mustards produce lesions at N7 position of guanine [1,2]. Because of this limited sequence specificity and the low affinity of these agents for DNA, combining an alkylating functionality and DNA sequences-recognition binding ligand is reasonable [14].

The designed compounds have linear chains like natural antibiotics distamycin and netropsin or branched carbocyclic oligopeptide chains. The replacement of heterocyclic rings by carbocyclic rings yields lexitropsins which in comparison with distamycin and netropsin have the reduced affinity to A–T pairs and have the increased affinity to G–C pairs [15,16].

The branching of the chain of oligogopeptides can cause the consolidation of the binding of the ligand to DNA, through creation of additional van der Waals'

interactions [17]. If one of the benzene rings of the oligopeptide forms 1,3-diaminobenzene system (*m*-fenylenodiamine), the compound is of 'BIGBEN' type [18]. This type is similar to lexitropsins and also shows ability to the binding to DNA.

We also introduced unsymmetrical substitute fragment of urea in synthetized linear lexitropsins, to check the influence of this element on the activity of the synthesized compounds. Derivatives of bis-naphthalene–bis-netropsin are new compounds with antiviral activity, where symmetric fragment of urea is a linker. This compound inhibits replication of the HIV virus and is proposed as a drug preventing from viral infections during heterosexual contacts [19].

We obtained six of carbocyclic lexitropsins with N-terminal bioreductive fragment which was characterized by considerable antitumor activity. We expected that values IC_{50} of compounds in hypoxic conditions would be lower in comparison to IC_{50} appointed in oxygen conditions. It was noticed that lower values IC_{50} in hypoxia conditions step out only for compounds **1** ($IC_{50} = 8545$ nM in oxygen vs. $IC_{50} = 710$ nM in hypoxia). This compound **1** inhibits 50% of colony formation in concentration about 12-times lower in hypoxia conditions than in oxygen. In compound **5** we observed the weaker effect of bioactivation ($IC_{50} = 133$ nM in oxygen vs. $IC_{50} = 57$ nM in hypoxia). But activation effect was not confirmed in compounds **2**, **4** and **6**. In the case of compound **3** we observed that the values for oxygen and hypoxic conditions were reversed, what was unexpected. This phenomenon is inexplicable for this experiment.

Compound **4** (and also **3**) contains in its structure an unsymmetric fragment of urea (–NHCONH– instead of –CONH–). No differences connected with this change in activity in oxygen and hypoxic conditions were noticed.

The question, if carbocyclic lexitropsins with bioreductive fragment can be potential anticancer agents needs further investigations.

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