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Novel (*S*)-1,3,4,12a-tetrahydropyrazino[2,1-*c*][1,4]benzodiazepine-6,12(2*H*,11*H*)-dione derivatives: Selective inhibition of MV-4-11 biphenotypic B myelomonocytic leukemia cells' growth is accompanied by reactive oxygen species overproduction and apoptosis

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ABSTRACT

A series of optically pure (*R*)- and (*S*)-1,3,4,12a-tetrahydropyrazino[2,1-*c*][1,4]benzodiazepine-6,12(2*H*,11*H*)-dione derivatives was designed and synthesized as novel anthramycin analogues in a three-step, one-pot procedure, and tested for their antiproliferative activity on nine following cell lines: MV-4-11, UMUC-3, MDA-MB-231, MCF7, LoVo, HT-29, A-549, A2780 and BALB/3T3. The key structural features responsible for exhibition of cytotoxic effect were determined: the (*S*)-configuration of chiral center and the presence of hydrophobic 4-biphenyl substituent in the side chain. Introduction of bromine atom into the 8 position (**8g**) or substitution of dilactam ring with benzyl group (**8m**) further improved the activity and selectivity of investigated compounds. Among others, compound **8g** exhibited selective cytotoxic effect against MV-4-11 (IC_{50} = 8.7 μM) and HT-29 (IC_{50} = 17.8 μM) cell lines, while **8m** showed noticeable anticancer activity against MV-4-11 (IC_{50} = 10.8 μM) and LoVo (IC_{50} = 11.0 μM) cell lines. The cell cycle arrest in G_1/S checkpoint and apoptosis associated with overproduction of reactive oxygen species was also observed for **8e** and **8m**.

Anthramycin (**1**) and structurally related tricyclic pyrrolobenzodiazepine (PBD) antibiotics produced by various actinomycetes, possess unique features, which permit them to bind covalently through N10-C11 bond to *C*2-NH_2 group of guanine within the minor groove of DNA (Fig. 1).[1,2] This class of compounds exhibits antibacterial properties and selective cytotoxicity toward tumor cells, and in consequence, it received a great attention of medicinal chemists as a possible source of anticancer and anti-MRSA agents.[3–5] One of the dimeric anthramycin derivatives, SJG-136, capable of formation of DNA cross-links, successfully completed Phase I clinical trials and progressed to Phase II.[6,7] Even early reports revealed two main structural features of PBDs responsible for binding to the DNA's minor groove: presence of a reactive, alkylating group (imine, carbinoloamine or carbinoloamine methyl ester) and an *S* configuration on the chiral center at the C11a-position, causing the longitudinal twist and isohelicity with the minor groove of DNA. Some later reports revealed that even compounds deprived of the alkylating group at the N10-C11 bond could still bind and interact with DNA strands, by non-covalent interactions, when the appropriate geometry of binding molecule was preserved. This phenomenon was mainly investigated on PBDs possessing amide[8,9] or amidine group.[10] Derivatives **2a**[8] and **4**[10] (Fig. 1) elevated the melting temperature of DNA by 3.3 ± 0.8 K and 0.7 ± 0.1 K, respectively. Recent report[9] presented a *C*2-aryl pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione type **3** library, which exhibited a strong, non-covalent binding to DNA, and the newly introduced *C*2-aryl substituents to the PBD dilactam skeleton significantly enhanced helix stabilization, in comparison to the unsubstituted PBD dilactam. The possible application of PBD's dilactams as antitumor compounds was initially reported by

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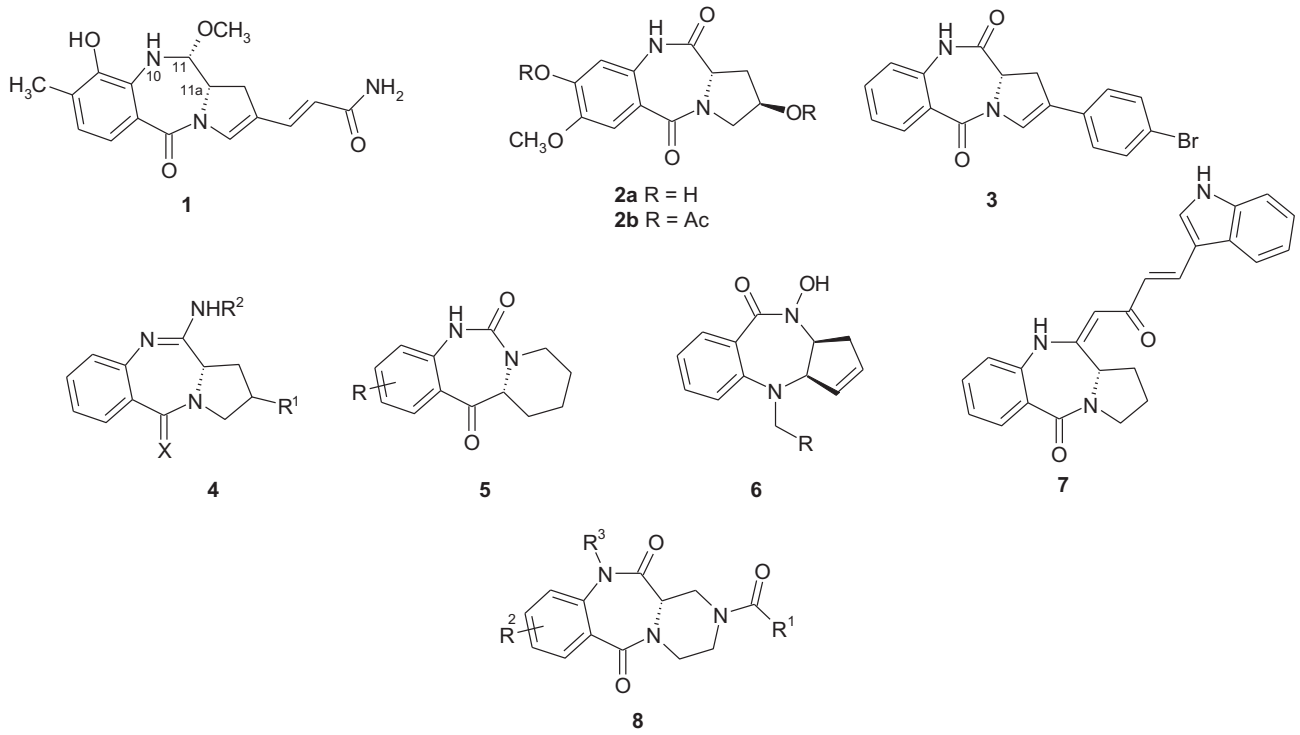

Fig. 1. Anthramycin (**1**) and its analogues **2**–**8**.

Kaneko et al.[11] who observed, that diacetate **2b** had a significant *in vivo* antitumor activity in the P388 lymphocytic leukemia mouse model. During cytotoxic studies, derivative **3** exhibited a high antitumor activity against A498 cell line (renal cancer), with GI_{50} = 0.51 μM, the effect comparable with the reference compound – anthramycin (GI_{50} = 0.195 μM).

Further experiments revealed that some other anthramycin analogues, such as 7,8,9,10-tetrahydrobenzo-[*e*]pyrido[1,2-*a*][1,4]-diazepin-12(6a*H*)-ones type **5**[12] or analogues containing hydroxamic acid moiety **6**,[13] could also bind to the calf thymus CT-DNA or exhibit promising anticancer activity against selected cell lines (PC-3, MCF-7). These results demonstrated that although alkylating group present on the benzodiazepine ring could facilitate binding of Anthramycin analogues to DNA, sometimes it could be replaced by non-covalent interactions. Additionally, it was reported that structurally related Fuligocandin B (**7**), isolated in 2004 from the myxomycete *Fuligo candida*,[14] sensitizes leukemia cells to apoptosis induced by tumor necrosis factor–related apoptosis-inducing ligand (TRAIL).[15]

During our research on the design and discovery of novel anticancer compounds based on diverse heterocyclic structures, such as 6*H*-oxazolo[3,2-*f*]pyrimidine-5,7-dione,[16] 3*H*-pyrrolo[2,3-*d*]pyrimidin-2(7*H*)-one,[17,18] furo[2,3-*d*]pyrimidin-2(3H)-one,[18] 5,6-dihydropyrimido[4,5-*c*]pyridazin-7(8H)-one[18] and purine[19] scaffold, we envisioned a possible synthesis of novel, anticancer anthramycin analogues, possessing a fused piperazine ring instead of a pyrrole ring (**8**, Fig. 1). This idea was partially inspired by our previous reports, concerning solid-phase syntheses of novel, rigid, bi- and tricyclic mimetics of peptide β-turn.[20,21] As the solid-phase synthesis possess several disadvantages, including difficulties in scaling-up the synthesis and tracking reaction progress, we decided to develop a solution-phase synthesis of anthramycin analogues, starting from optically pure (*S*)-2-piperazinecarboxylic acid dihydrochloride (**9a**) (Scheme 1).

After several attempts, we found that **9a** dissolved in the dioxane-water mixture, in the presence of 2 eq. of sodium hydroxide, reacts with acyl chlorides, giving predominantly (*S*)-4-acyl-piperazine-2-carboxylic acids **10a-e**. In a similar manner, (*R*)-4-acyl-piperazine-2-carboxylic acids **10f-i** were synthesized from (*R*)-2-piperazinecarboxylic acid dihydrochloride (**9b**) (Scheme 2). Intermediates **10a-i** were then directly treated with an excess of isatoic anhydrides, in the presence of sodium carbonate as a base, which resulted in the formation of (*R*)-/(*S*)-1-(2-aminobenzoyl)-4-acyl-piperazine-2-carboxylic acids **11a-k** (Schemes 1,2). The final cyclization step, leading to the tricyclic dilactam scaffold **8**, was performed with HATU, in the presence of DIPEA as a base.

In further structure-activity relationships studies, the lactam ring of **8e** was alkylated with methyl iodide, benzyl bromide, and benzyl bromoacetate, in the presence of potassium carbonate, which led to the *N*-alkylated derivatives **8l-8n**. The benzyl group in derivative **8n** was removed in hydrogenation reaction, giving *N*-methylocarboxyl substituted product **8o** (see Scheme 3).

The structure of synthesized compounds was confirmed by detailed NMR spectra analysis (1H, 13C, dept135, COSY, HSQC, HMBC and temperature spectra) and crystallographic methods. In general, the obtained anthramycin analogues exist in two interconvertible conformations in deuterated DMSO solution, which resulted in two sets of signals. This dynamic system and vibrations of the molecule in the solution, together with the presence of axial and equatorial, magnetically non-equivalent hydrogens in three methylene groups of piperazine ring, resulted in the extension of signal bands observed as complex multiplexes in both aliphatic and aromatic part of the collected spectra (see SI). The identity of **8a** (crystallized from acetone:water 1:1 v/v mixture) and **8i** (crystallized from ethyl acetate) was proven by the single-crystal X-ray diffraction analysis. It turned out that investigated compounds crystallize in the hexagonal $P6_5$ (**8a**) and triclinic *P*1 (**8i**) space groups, with one (**8a**) or two (**8i**) molecules in the asymmetric units of the corresponding crystal lattices (Fig. 2). The details of crystallographic data and the refinement parameters are summarized in Table 1 of the Supplement file. The full list of values of