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Research paper

Discovery of antitumor anthra[2,3-*b*]furan-3-carboxamides: Optimization of synthesis and evaluation of antitumor properties

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We dedicate this work to the memory of Professor Maria N. Preobrazhenskaya, the eminent scholar in bioorganic and medicinal chemistry.

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ABSTRACT

Anthraquinones and their analogues, in particular heteroarene-fused anthracendiones, are prospective scaffolds for new compounds with improved antitumor characteristics. We herein report the use of a ‘scaffold hopping’ approach for the replacement of the core structure in the previously discovered hit compound naphtho[2,3-*f*]indole-5,10-dione **2** with an alternative anthra[2,3-*b*]furan-5,10-dione scaffold. Among 13 newly synthesized derivatives the majority of 4,11-dihydroxy-2-methyl-5,10-dioxoanthra[2,3-*b*]furan-3-carboxamides demonstrated a high antiproliferative potency against a panel of wild type and drug resistant tumor cell lines, a property superior over the reference drug doxorubicin or lead naphtho[2,3-*f*]indole-5,10-dione **2**. At low micromolar concentrations the selected derivative of (*R*)-3-aminopyrrolidine **3c** and its stereoisomer (*S*)-3-aminopyrrolidine **3d** caused an apoptotic cell death preceded by an arrest in the G2/M phase. Studies of intracellular targets showed that **3c** and **3d** formed stable intercalative complexes with the duplex DNA as determined by spectral analysis and molecular docking. Both **3c** and **3d** attenuated topoisomerase 1 and 2 mediated unwinding of the supercoiled DNA via a mechanism different from conventional DNA-enzyme tertiary complex formation. Furthermore, **3d** decreased the activity of selected human protein kinases *in vitro*, indicating multiple targeting by the new chemotype. Finally, **3d** demonstrated an antitumor activity in a model of murine intraperitoneally transplanted P388 leukemia, achieving the increase of animal life span up to 262% at tolerable doses. Altogether, the ‘scaffold hopping’ demonstrated its productivity for obtaining new perspective antitumor drug candidates.

1. Introduction

Along with the advance of targeted anticancer therapy, the anthracycline antibiotics and their synthetic analogues remain highly effective drugs applicable in many chemotherapeutic schedules [1]. However, the clinical use of these drugs including the ‘gold standard’ doxorubicin **1** (Fig. 1) is frequently limited by organ

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Abbreviations: CDI, 1,1′-carbonyldiimidazole; DCC, *N*,*N*′-dicyclohexylcarbodiimide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; FITC, fluorescein isothiocyanate; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate; PI, propidium iodide; TBTU, O-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate.

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toxicity (mostly heart and bone marrow) and altered drug response. Therefore, studies of new anthracyclines and their analogues with improved chemotherapeutic properties are underway [2]. Next generations of anthracycline based drug candidates have been evaluated in preclinical or clinical studies [3–7].

A variety of chemical modifications have been performed to identify the compounds with defined intracellular targets, decreased general toxicity and circumvention of drug resistance. The most important structural moiety responsible for therapeutic properties of anthracyclines is the anthraquinone core. Therefore, anthraquinones and their analogues are widely used as scaffolds for the design of anticancer drug candidates. Previously we have identified a high potential of hydroxy and amino derivatives of linear hetareneanthracendiones. In particular, a series of pyrrole, furan and thiophene fused derivatives of anthracendione that target duplex or quadruplex DNA, have been designed and evaluated [8–10]. 3-Aminomethylnaphtho[2,3-*f*]indole-5,10-diones with cyclic diamines in the side chain demonstrated a potent inhibition of DNA modifying enzymes topoisomerases 1 and 2 (Top1, Top2) and a high cytotoxicity against wild type and drug resistant tumor cell lines [11,12]. The selected compound **2** (Fig. 1) demonstrated a superior cytotoxicity than the reference drug **1** against mammalian tumor cell lines with determinants of altered drug response (such as Pgp expression or p53 inactivation) as well as promising therapeutic efficacy *in vivo* [12]. However, further development of 3-aminomethylnaphtho[2,3-*f*]indole-5,10-diones is limited by a multistep procedure of synthesis.

In search for more accessible analogues we focused on the core structure replacement ('scaffold hopping') [13]. Our previous studies identified two important moieties of 3-aminomethylnaphtho[2,3-*f*]indole-5,10-diones, namely, a cyclic diamine as a side chain in the position 3 (3-aminopyrrolidine residue in **2**) and hydroxyl groups in the peri-position (4, 11) in the quinone fragment of the core structure. Removal or modifications of these moieties decreased the antiproliferative potency of naphtho[2,3-*f*]indole-5,10-diones. Based on these results, the 'scaffold hopping' approach was employed to obtain new hetarenanthracenediones.

In this study we replaced the core structure of naphtho[2,3-*f*]indole-5,10-dione **2** with an alternative anthra[2,3-*b*]furan-5,10-dione scaffold (Fig. 2) and analyzed the properties of new compounds as antitumor drug candidates. Given its accessibility and potential for diversification, 4,11-dihydroxy-2-methyl-5,10-dioxoanthra[2,3-*b*]furan-3-carboxylic acid can be considered an attractive scaffold. Transformation of this acid into the amides bearing cyclic diamine residues yielded anthra[2,3-*b*]furan-3-carboxamides highly similar to 3-aminomethylnaphtho[2,3-*f*]indole-5,10-diones. We hypothesized that compounds with this scaffold retain the major properties critical for cytotoxicity of anthraquinones, i.e., the ability to bind to the double stranded DNA and inhibit topoisomerases. Additionally, the scaffold hopping led to the modification of the methylene spacer in 3-aminomethylnaphtho[2,3-*f*]indole-5,10-diones into the carboxamide group at the appropriate position on the new scaffolds (Fig. 2), providing an opportunity for drug binding to intracellular targets.

The 'scaffold hopping' drug design strategy helped to identify a new class of potential antitumor agents with the accessible scaffold. The naphtho[2,3-*f*]indole-5,10-dione **2** was synthesized via a linear scheme that included 11 stages whereas the analogues **3a**–**m** based on anthra[2,3-*b*]furan-5,10-dione scaffold can be obtained in 4–5 steps starting from commercially available quinizarine.

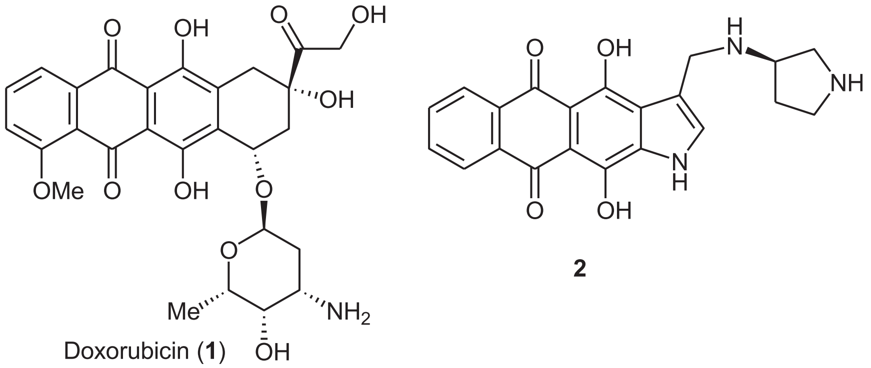
Presented herein is the design, synthesis and antitumor characteristics of a new class of antitumor agents based on anthra[2,3-*b*]furan-3-carboxamides with cyclic diamine moieties attached to carboxamide function. The combination of synthetic methods with 'scaffold hopping' approach led to the discovery of **3d** with improved properties *in vitro* and *in vivo*.

2. Results and discussion

2.1. Chemistry

At the initial step of synthesis of a new series of 4,11-dihydroxy-2-methyl-5,10-dioxoanthra[2,3-*b*]furan-3-carboxamides, the starting anthra[2,3-*b*]furan-3-carboxylic acid was obtained. According to the method of preparation of 4,11-dihydroxy-2-methyl-5,10-dioxoanthra[2,3-*b*]furan-3-carboxylic acid (**6**) [14], the condensation of ethyl acetoacetate with 1,4-hydroxy-2,3-dichloroanthraquinone (**4a**) led to ethyl 4,11-dihydroxy-2-methyl-5,10-dioxoanthra[2,3-*b*]furan-3-carboxylate (**5a**, Scheme 1). The ester **5a** is rather stable to alkaline or acidic hydrolysis, so for its cleavage to anthra[2,3-*b*]furan-3-carboxylic acid **6** a severe heating in concentrated sulphuric acid was used. With this method the efficacy of synthesis of **6** was limited. Mainly, we achieved only 38–42% yield of **5a** compared to 58% in the original procedure [14]. Furthermore, the harsh conditions, big consumption of sulphuric acid for hydrolysis of **5a**, and an insufficient purity of the target acid **6** were disadvantageous.

Therefore, for preparative synthesis of **6** we modified the reported procedure. For heterocyclization we used a more reactive 2,3-dibromoquinizarine **4b** [15] and *tert*-butyl acetoacetate. The addition of the base into the reaction mixture at 120–125 °C increased the yield of **5b** and improved its purity by reducing the formation of tar and angular by-products of heterocyclization. This optimization elevated the yield of *tert*-butyl ester **5b** up to 68% and



Doxorubicin (**1**)

2

Fig. 1. Structures of doxorubicin (**1**) and lead naphtho [2,3-*f*]indole-5,10-dione **2**.