European Journal of Medicinal Chemistry 122 (2016) 656–673

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

A novel class of ethacrynic acid derivatives as promising drug-like potent generation of anticancer agents with established mechanism of action

Serge Mignani [a, **], Nabil El Brahmi [b, c, f], Saïd El Kazzouli [b, ***], Laure Eloy [d], Delphine Courilleau [e], Joachim Caron [b, c, f], Mosto M. Bousmina [b], Anne-Marie Caminade [c, f], Thierry Cresteil [d, e, ****], Jean-Pierre Majoral [c, f, *]

[a] Laboratoire de Chimie et de Biochimie pharmacologiques et toxicologique, CNRS UMR 860, Université Paris Descartes, PRES Sorbonne Paris Cité, 45, rue des Saints Pères, 75006, Paris, France
[b] Euromed Research Institute, Engineering Division, Euro-Mediterranean University of Fes, Fès-Shore, Route de Sidi Hrazem, 30070, Fès, Morocco
[c] Laboratoire de Chimie de Coordination CNRS, 205 route de Narbonne, BP 44099, 31077, Toulouse Cedex 4, France
[d] Institut de Chimie des Substances Naturelles, CNRS UPR2301, Avenue de la terrasse, 91190, Gif sur Yvette, France
[e] IPSIT Faculté de Pharmacie, Université Paris Sud, 92290, Chatenay-Malabry, France
[f] Université de Toulouse, UPS, INPT, F-31077, Toulouse Cedex4, France

ARTICLE INFO

Article history:
Received 22 March 2016
Received in revised form
9 May 2016
Accepted 29 May 2016
Available online 11 June 2016

Keywords:
Anticancer agents
Ethacrynic acid
Ethacrynic acid derivatives
Glutathione S-Transferase π

ABSTRACT

The well-known diuretic Ethacrynic acid (**EA**, Edecrin), showing low anti-proliferative activities, was chemically modified at different positions. The new **EA** derivatives have been tested *in vitro* in anti-proliferative assays on both tumor KB (epidermal carcinoma) and leukemia HL60 (promyelocytic) cells suitable targets for anticancer activity. Reduction of the α-β double bond of **EA** completely abolished anti-cancer activities, whereas introduction of either 2-(4-substituted phenyl)ethanamine (series A) or 4-(4-substituted phenyl)piperazine (series B) moieties generated compounds showing moderate to strong anti-proliferative activities against human cancer cell lines. Several substitutions on the phenyl of these two moieties are tolerated. The mechanism of action of the **EA** derivatives prepared in this study is more complex than the inhibition of glutathione S-transferase π ascribed as unique effect to **EA** and might help to overcome tumor resistances.

1. Introduction

Cancer is the second leading cause of death in North America and Europe [1]. Although an armada of anticancer agents has received FDA approval over the past two decades [2], the inefficiency of their discovery and development is no longer sustainable and the pipeline of new cancer agents is slim. Today, the outcome of patients with advanced metastasis, for instance regarding lung, colorectal, prostate and breast cancers, remains very poor [3]. The development of anticancer agents for several decades was based on the identification of active compounds, with cytostatic or cytotoxic activity on tumor cell lines, but with many side effects. Consequently, new anticancer agents within new chemical families are urgently needed to allow an extension of this cancer-fighting armada. In addition to the discovery and development of new anticancer agents, an improved understanding of each cancer patient's needs is also essential to enhance the ability of standard treatments to kill cancer cells without significantly affecting normal cells [4].

One important consideration in the development of cancer treatment regimens is resistance against anticancer drugs, which remains a serious obstacle [5]. Indeed, microsomal glutathione transferase 1 (mGST1) and glutathione transferase π (GST π) are often overexpressed in tumors and confer resistance against a

* Corresponding author. Laboratoire de Chimie de Coordination CNRS, 205 route de Narbonne, BP 44099, 31077, Toulouse Cedex 4, France.
** Corresponding author.
*** Corresponding author.
**** Corresponding author. IPSIT Faculté de Pharmacie, Université Paris Sud, 92290, Chatenay-Malabry, France.
E-mail addresses: serge.mignani@parisdescartes.fr (S. Mignani), s.elkazzouli@ueuromed.org (S. El Kazzouli), thierry.cresteil@u-psud.fr (T. Cresteil), jean-pierre.majoral@lcc-toulouse.fr, majoral@lcc-toulouse.fr (J.-P. Majoral).

http://dx.doi.org/10.1016/j.ejmech.2016.05.063
0223-5234/

number of cytostatic drugs, such as cisplatin and doxorubicin (DOX) [6]. To address this point, the diuretic drug ethacrynic acid (**EA**, Edecrin) **1**, an inhibitor of π class glutathione S-transferase, has been tested against multiple myeloma, and as adjuvant in clinical trials [7]. On the basis of these considerations, Dyson et al. [8] and Osella et al. [9] synthesized and characterized the bifunctional **EA**-Pt(IV) complex **2** (ethacraplatin) and the **EA**–Pt(II) complex **2′** (cis-diaminobis(ethacrynato)platinum(II) (Fig. 1), both of them able to release a cytotoxic platinum (II) agent (inducing apoptosis of cancer cell) and two **EA** moieties (inhibiting glutathione S-transferase (GST) and overcoming drug resistance) via hydrolysis or reduction, respectively. Ethacraplatin **2** resulted to be an excellent inhibitor of GST in several tumor cell lines such as A549 lung adenocarcinoma epithelial cell line, MCF7 and T47D breast tumor cell lines and HT29 colon cancer cell line albeit demonstrating a moderate anti-proliferative activity. Unfortunately, the bifunctional conjugates **2** and **2′** did not offer any advantage over cisplatin for the treatment of malignant pleural mesothelioma (MPM) cells, since the increase of intracellular glutathione (GSH) counteracts the modest inhibition of GST [10]. Recently, Yang et al. constructed biodegradable nanoparticles to codeliver **EA** and dichloro(1,2-diaminocyclohexane)platinum(II) (DACHPt) which is a precursor of oxaliplatin as a promising approach to overcome the drug resistance in cancer chemotherapy [11]. In vitro studies showed that these hybrid nanoparticles could release both **EA** and DACHPt enhancing of up to ~5 fold of the anticancer efficacy *versus* DACHPt alone. Interestingly, in vivo studies showed better anticancer activities than the simple combination of **EA** and DACHPt.

The development of new and potent anticancer agents based on **EA** will undoubtedly offer new opportunities to tackle cancer and some reports in this direction appeared recently reviewed by us and others [12,13]. In order to improve the poor anti-proliferative activity of **EA** [14], we designed original **EA** derivatives based on modifications of the **EA** core structure and tested the resulting derivatives for their capacity to inhibit cell growth *in vitro*. For this initial screening, we tested the chemicals on three cell lines: two actively dividing cell lines derived from human cancer, human KB carcinoma and human leukemia HL60, and the non-dividing quiescent endothelial progenitor cells (EPC) from Cyprinus carpio. Cell number and cellular NADH content evaluated after 72 h chemical exposure provided relevant information concerning viability. The two different tumor cell lines have been selected as representative 'models' of the two common types of cancer: solid tumor cancers (*e.g.* breast, lung, colon, etc.) and blood-based cancers (*e.g.* leukemia, lymphoma, myeloma etc.). According to a recent analysis, the percentage of new patients annually diagnosed for solid tumor cancers and blood-based cancers is 34% and 9%, respectively, whereas the percentage of cancer related deaths is 43% and 9%, respectively [15].

Herein, we report the design, preparation and anti-proliferative activity of original **EA** derivatives active upon cancer cell lines and exemplifying a promising class of potent anticancer agents. The preparation of **EA** derivatives and their synthetic pathways are described in Schemes 1–6 and their mode of action was proposed. The mechanism of action of the **EA** derivatives prepared in this study is more complex that the potent inhibition of π class glutathione S-transferase attributed to **EA** and could potentially overcome tumor resistances.

2. Chemistry

The initial strategic design of these **EA** derivatives can be divided into two parts as shown in Fig. 2: part 1: modification of the 3-methylenepentan-2-one unit; and part 2: modification of the carboxylic acid unit. In this study, we decided not to modify the core of the **EA**.

As a first strategic attempt to identify a strongly toxic **EA** derivative and to investigate SAR, we targeted the α,β unsaturated carbonyl moiety of **EA** (part 1) as shown in Scheme 1.

The synthesis of compounds **3** and **4** was initiated from the commercially available **EA**. The treatment of **EA** with hydrogen (3 bars) in the presence of Pd/C in isopropanol for 20 min provided compound **3** in quantitative yield. Compound **4** was prepared in three steps. Thus, the treatment of **EA** with methanol in the presence of *p*-toluenesulfonic acid (PTSA) at room temperature led to the corresponding methyl ester. This intermediate was subjected to the Luche reduction with $NaBH_4$ in presence of $CeCl_3$ in a mixture of H_2O/methanol. Then, the reaction mixture was treated with sodium hydroxide in methanol to furnish the free acid **4**.

Then, to further explore SAR in the **EA** series, we directed our efforts to the preparation of two original **EA** derivative series based on modifications in the part 2 (Fig. 3): series A (2-(4-substituted phenyl)ethanamine) and series B (4-(4-substituted phenyl)piperazine) and for this purpose **EA** derivatives **5**–**22** were prepared.

The synthesis of **EA** derivatives **5**–**9**, **19** and **22** is shown in Scheme 2, whereas the synthesis of **10**–**17** is shown in Scheme 3. The preparation of original derivatives **5**–**9**, **19** and **22** was achieved in one step synthesis from **EA** and various amines using

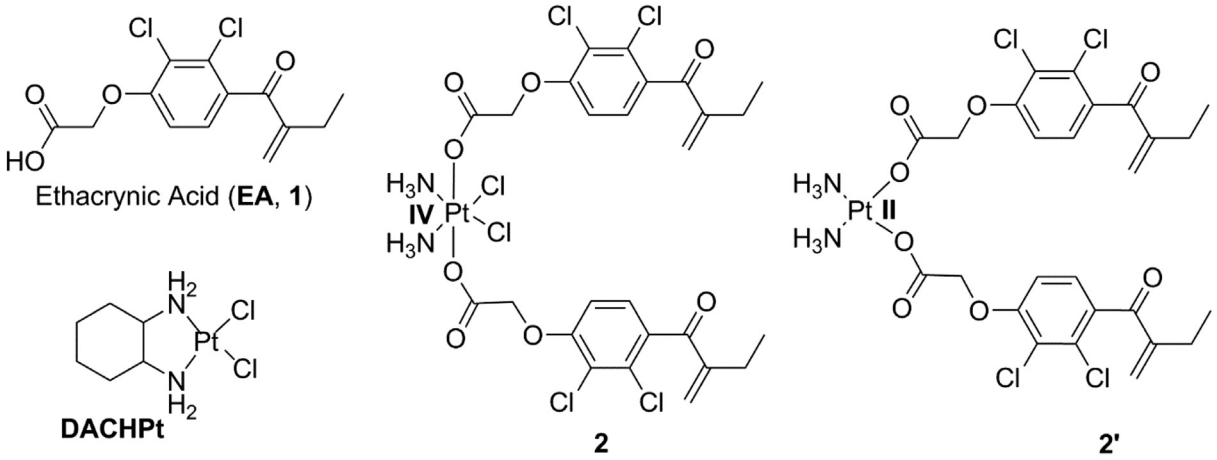

Fig. 1. Chemical structure of ethacrynic acid (**EA**, **1**), DACHPt, Pt(IV) and Pt(II) ethacrynic derivatives (**2** and **2′**).