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Anti-Cancer Activities of a New Family of Ethacrynic Acid Derivatives †

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Abstract: The present invention relates to a new class of small anti-cancer molecules derived from ethacrynic acid (symbolized by AE). The invention relates to the in vitro and in vivo anti-cancer activities and to the methods for producing the new AE family. New AE analogues were synthesized and then the in vitro cytotoxic activities thereof were evaluated on the P815 tumour cell line using the MTT test. The AE derivative, which exhibited the best in vitro cytotoxicity, was then tested in vivo using the DBA2/P815 (H2d) mouse model. At 30 mg/kg, the effective dose, the animals showed general tolerance with a percentage survival of around 80%, and no significant weight loss was observed.

Keywords: Ethacrynic acid; anti-cancer; cytotoxicity

1. Introduction

Remarkable advances have been made in the field of chemotherapy, with the introduction of new molecules such as thalidomide, lenalidomide and bortezomib. Despite this, cancer remains an incurable disease. The efficacy of chemotherapy remains to be improved, by reducing the toxicity and side effects of treatments. In addition, the intrinsic or acquired resistance of a large number of tumors to chemotherapy is also a major obstacle to the efficacy of anticancer treatments. Several mechanisms of cellular resistance to different active substances have been identified (Moscow and Cowan 1988). Taking this into consideration, the search for new effective chemotherapy agents capable of treating different types of cancer is still essential. According to the World Health Organization, cancer is one of the leading causes of death worldwide. Lung, liver, stomach, colon and breast cancers are the most common in the world. Given this diversity of cancer types, the development of new anticancer agents is very important in the field of oncology. In addition, the development of specific molecules to combat this disease while circumventing the obstacle of cellular resistance is necessary.

Microsomal glutathione S-transferase 1 (mGSTl) and glutathione S-transferase pi(GSTpi) are often overexpressed in tumors, thus conferring resistance to a number of chemotherapeutic agents, such as cisplatin and doxorubicin (DOX) [1]. These enzymes catalyze the conjugation of glutathione and act as detoxification enzymes. EA or 2,3-dichloro-4-(2-methylenebutryl)-phenoxyacetic acid, which is a well-known diuretic, is used in the treatment of hypertension and swelling caused by diseases such as congestive heart failure, liver failure, and kidney failure. It is also known as a good inhibitor of pi-class glutathione S-transferase. EA has an acidic function and an a,b-unsaturated carbonyl unit

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that reacts with nucleophiles, such as the thiol of glutathione S-transferase Pl-1 (GSTP1-1, GSTpi). Moreover, it has been recently confirmed that EA inhibits Wnt/beta catenin signaling which plays an important role in the regulation of cell proliferation, differentiation and apoptosis [2,4].

2. Discussion

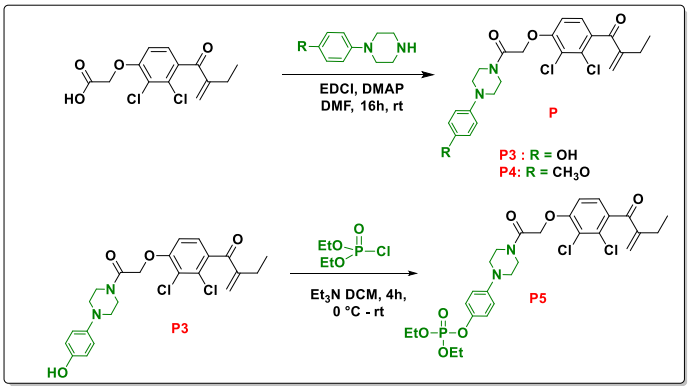
In order to improve the ability of AE to inhibit cancer cell growth in vivo while maintaining its good glutathione S-transferase inhibition activity, we propose in this invention a new synthesis of potent and original anticancer agents. Thus, based on our very encouraging results concerning the antitumor activities of various AE analogues in vitro on a panel of cell lines [5], we propose in this invention, the synthesis and in vivo evaluation of the best analogues by performing structural modifications on the basic skeleton of the AE molecule. These chemical transformations result in the formation of amide bonds between the carboxylic acid function of AE and primary and secondary amines. The acrylate part, for its part, has remained intact.

2.1. Synthesis of Target Molecules

The AE derivatives are prepared from commercially available AE (Scheme 1). The treatment of AE under the conditions of a peptide reaction with different amines in a DCM/DMF mixture in the presence of l-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and 4-dimethylaminopyridine (DMAP) at room temperature provides the desired products in moderate yields.

The presence of the phenol function on the modified AE was used for nucleophilic substitution with chlorophosphates. Thus, the treatment with different chlorophosphates in dichloromethane (DCM) in the presence of trimethylamine as an organic base allows to obtain the desired compounds in moderate yields.

General method for the preparation of molecules **P3** and **P4**. To a mixture of EDCI (1.2 equiv), DMAP (in catalytic quantity) and 1 equivalent of AE in DMF anhydride (5 mL), 1 equivalent of the amines (4-hydroxyphenyl piperazine or 4-methoxyphenyl piperazine) is added at 0 °C. The reaction mixture is stirred overnight at room temperature, then ethyl acetate (100 mL) is added and the organic phase is washed with water (2 × 50 mL) and brine (3 × 50 mL), dried over anhydrous $MgSO_4$ and concentrated using a rotary evaporator. The residue obtained is purified by flash chromatography.



Scheme 1. Synthesis of AE derivatives **P3**, **P4** and **P5**.

2.2. Cytotoxicity Test

Before performing the cytotoxicity test, viable cells are counted by trypan blue exclusion. The aim is to obtain a suspension of 4 x 104 cells/mL to be incubated in 100 pL of complete culture medium per flat-bottomed well of 96-well microculture plates. This microculture thus obtained is incubated for 24 h before carrying out the cytotoxicity tests. The latter is then carried out by applying decreasing doses of the molecules (**P3**, **P4** and

P5) obtained by half-by-half dilutions, in 100 pL of DMEM medium. Each test is carried out in duplicate and repeated three times with the positive and negative controls. The three molecules **P3**, **P4** and **P5** are first solubilized in DMSO, the final concentration of which, during the test, will not exceed 0.5% (this concentration having no effect on cell growth). These micro-cultures are incubated at 37 °C in a humid atmosphere containing 5% CO_2 for 48 h.

The determination of cytotoxic activity is carried out by evaluating the concentration of the molecules tested inhibiting 50% of cell growth (IC_{50}) compared to a control cultured under the same conditions in the absence of the compound studied. This simple and rapid test allows for a rapid selection of molecules exhibiting an activity likely to limit or stop the growth of cancer cells. The revelation of cytotoxic action is carried out using the MTT test: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide [6,7]. This test is performed as described and modified by Mosmann, 1983. After 48 h of incubation under the culture conditions cited below, 20 µL of MTT solution (5 mg/mL of PBS) are added. After 4 h of incubation under the same culture conditions, the purple crystals formed following the reduction of MTT by the mitochondrial dehydrogenases of living cells are solubilized by adding 100 µL of HCl/Isopropanol solution (24:1). The optical density (OD) is then read at two wavelengths 540 nm and 630 nm using the MultisKan EX microplate spectrophotometric reader. Thus, the effect of **P3**, **P4** and **P5** on cell viability.

3. Results

The in vitro cytotoxic activity was measured by the MTT test against the P815 tumor line (Figure 1). This cytotoxicity begins at low concentrations and increases in a dose-dependent manner for all the molecules tested. The latter present a very significant cytotoxic activity with an IC_{50} between 0.15 and 9.2 pM (Table 1), and it is the P4 molecule that showed a very strong cytotoxic effect compared to the other molecules P3 and P4 with an IC_{50} = 0.15 pM. Thus, the P4 compound was evaluated for its antitumor effect.

Antitumor Activity: Preclinical Studies

In vivo tests represent an important step in the study of the antitumor activity of our molecule. The objective is to move on to a test in conditions that are as close as possible to the reality of the disease. For this purpose, DBA-2 (H2d) mice bearing P815 solid tumors were used to test the in vivo antitumor effect of the P4 molecule, which showed very significant cytotoxic activity compared to the other molecules tested PI and P3. The tests were carried out by oral administration (gavage) of the molecules dissolved in vegetable oil (table oil) to 6–8 week old mice every 48 h for a period of 14 days. The results obtained are presented in Figure 2. Reading this figure, the treatment of mice with the P4 molecule induced a significant decrease in tumor volume. Administration by gavage of the P4 molecule at doses of 10, 20 and 30 mg / kg induced a significant reduction in tumor volume after 28 days of treatment compared to control mice (Figure 2). In addition, no significant difference was observed between the doses used. However, it was noted that mice treated with the 30 mg/kg dose showed tolerance to this dose with a survival rate of approximately 80% (Figure 4), and no significant impact on body weight loss (Figure 3) compared to mice treated with the 10 and 20 mg/kg doses ($p < 0.05$). Also, no significant difference was noted in the decrease in tumor volume after treatment with the three doses (10, 20 and 30 mg/kg) ($p < 0.05$). Thus, we could consider from an efficacy point of view that the 30 mg/kg dose of compound P4 is more effective compared to other doses tested.

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References

1. Johansson, K.; Ito, M.; Schophuizen, C.M.S.; Thengumtharavil, S.M.; Heuser, V.D.; Zhang, J.; Shimoji, M.; Vahter, M.; Ang, W.H.; Dyson, P.J.; et al. Characterization of new potential anticancer drugs designed to overcome glutathione transferase mediated resistance, *Mol. Pharm.* **2011**, *8*, 1698–1708.
2. Zhao, G.; Liu, C.; Wang, R.; Song, D.; Wang, X.; Lou, H.; Jing, Y. The synthesis of a,b-unsaturated carbonyl derivatives with the ability to inhibit both glutathione S-transferase P1-1 activity and the proliferation of leukemia cells, *Bioorg. Med. Chem.* **2007**, *15*, 2701–2707.
3. Jin, G.; Lu, D.; Yao, S.; Wu, C.N.; Liu, J.X.; Carson, D.A.; Cottam, H.B. Amide derivatives of ethacrynic acid: Synthesis and evaluation as antagonists of Wnt/bcatenin signaling and CLL cell survival. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 606–609.
4. Janovská, P.; Bryja, V. Wnt signalling pathways in chronic lymphocytic leukaemia and B-cell lymphomas. *Br. J. Pharmacol.* **2017**, *174*, 4701–4715.
5. Mignani, S.; Nabil El Brahmi, N.; El Kazzouli, S. A novel class of ethacrynic acid derivatives as promising drug-like potent generation of anticancer agents with established mechanism of action. *Eur. J. Med. Chem.* **2016**, *122*, 656–673.
6. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *16*, 55–63.
7. Tilaoui, M.; Mouse, H.A.; Jaafari, A.; Zyad, A. Differential effect of artemisinin against cancer cell lines. *Nat. Prod. Bioprospect*. **2014**, *4*, 189–196.