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Synthesis and anti-proliferative activity of a small library of 7-substituted 5*H*-pyrrole [1,2-*a*][3,1]benzoxazin-5-one derivatives

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ABSTRACT

In this study, we investigate the anti-proliferative activity of a small library of 7-substituted 5*H*-pyrrolo [1,2-*a*][3,1]benzoxazin-5-one derivatives, against a panel of human cancer cell lines. We reported the synthesis of these compounds in a previous work. 7-Bromo-5*H*-benzo[*d*]pyrrolo[2,1-*b*][1,3]oxazin-5-one showed a promising anti-proliferative effect. As starting material for Suzuki-Miyaura cross coupling reaction, it was selected for the design and the synthesis of six further derivatives, with the aim to better define structure-activity relationships. The anti-proliferative MTT assay revealed a dose-dependent reduction of cell viability, especially for 7-([1,1′-biphenyl]-4-yl)-5*H*-benzo[*d*]pyrrolo[2,1-*b*][1,3]oxazin-5-one. Cell cycle and western blotting analysis suggested apoptosis as possible mechanism for its anti-proliferative activity. These preliminary results encourage our interest for further optimizations.

Heterocycles containing benzoxazinone skeleton have been always attracting scaffolds in the field of medicinal chemistry (Fig. 1).

Since their discovery as secondary metabolites of the grasses over 50 years ago, benzoxazinoids have been extensively studied. Different aspects of their chemistry and a wide range of their diverse biological activities have been published. Originally, they were found playing an important role in the chemical defense of plants, acting as natural pesticides and exhibiting allelopathic properties.[1,2] They also could function as genotoxins for human cells.[3] Inhibiting the bacterial type IIa topoisomerase, a series of 6-substituted benzoxazinone derivatives showed a rapid bactericidal activity.[4] Furthermore, benzoxazinoids showed thrombin and cyclooxygenase inhibitory properties, as well as antimicrobial and anti-inflammatory activities.[5,6]

Moreover, it has been demonstrated that benzoxazinoids could be useful for the treatment of cardiovascular diseases and cancer.[7,8]

The benzoxazinone skeleton contains three different potential areas for functionalization, the carbon atom in position 3 (C3), the nitrogen in position 4 (N4) and the aromatic ring. One of the modification that involved both the C3 and N4 atoms was their introduction in a fused pyrrole ring. A number of methods have been reported for the synthesis of 4*H*-pyrrolo[2,1-*c*][1,4]benzoxazin-4-one frame.[9,10]

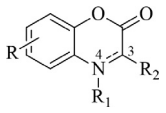

Fig. 1. General structure of heterocycle containing benzoxazinone skeleton.

Two derivatives of this class of compounds were identified as selective antagonists of G protein-coupled estrogen receptor (GPER), inhibiting the proliferation of SkBr3 cells and the migration of cancer-associated fibroblasts (CAFs) induced by 17β-estradiol and G-1.[11] Thus, heterocycles containing benzopyrroloxazinone frame can be considered as privileged scaffolds for the development of potential new drugs, including the treatment of cancer. The isomer 5*H*-pyrrolo[1,2-*a*][3,1]benzoxazin-5-one is not very studied yet. Recently, we reported the synthesis of a small library of 5*H*-pyrrolo[1,2-*a*][3,1]benzoxazine-5-one derivatives.[12]

In this study, our interest was to investigate about the anti-proliferative activity of those compounds against different human breast cancer cell lines with the aim to further develop an understanding of the SAR study of this class of compounds. From a preliminary screening, 5*H*-naphtho[2,3-*d*]pyrrolo[2,1-*b*][1,3]oxazin-5-one (**2**), and 7-bromo-5*H*-benzo[*d*]pyrrolo[2,1-*b*][1,3]oxazin-5-one (**3**), bearing a bromine atom on C7, showed a good anti-prolifera-

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tive activity against all used cell lines. On the contrary, the unsubstituted 5*H*-pyrrolo[1,2-*a*][3,1]benzoxazin-5-one (**1**) and 5*H*-pyrrido[2,3-*d*]pyrrolo[2,1-*b*][1,3]oxazin-5-one (**4**) didn't show the same effect. Since compound **4** moderately inhibited the growth of the used cancer cells, the presence of benzene ring in the derivatives may be necessary for their anti-proliferative activity. Furthermore, the presence of substituents on the aromatic ring of compound **1** may improve the cytotoxicity of this class of compounds (Fig. 2).

Continuously to our interest in the synthesis of 5*H*-pyrrolo[1,2-*a*][3,1]benzoxazin-5-one derivatives, having biological and pharmacological activities, we designed and synthesized a new series of 7-substituted analogs and their *in vitro* anti-proliferative activity was evaluated, with the aim to better define the SAR, determining which moieties are essential for the anti-proliferative effect (Fig. 3).

Due to its promising anti-proliferative activity, compound **3** was chosen as starting material, suitable substrate for coupling reaction. In particular, we performed Suzuki-Miyaura cross coupling, adjusting the reaction conditions for the synthesis of our derivatives.[13] To validate the importance of the substitutions on the aromatic ring, variously substituted phenyl, phenol and pyridines moieties were selected to decorate the new compounds. Then, a preliminary cytotoxicity was evaluated and the possible mechanism of action of this class of compounds was investigated.

Firstly, 5-bromo-2-(1*H*-pyrrol-1-yl)benzoic acid (**II**) was prepared by the reaction of **I** with 4-chloropyridine hydrochloride and 2,5-dimethoxytetrahydrofuran. The subsequent cyclization was performed using activated MnO_2, leading to compound **3** (Scheme 1).[12]

Compounds **3a**–**3f** were obtained in good yields and with a fast purification procedure, through crystallization, using a similar approach (Scheme 2). We adjusted the Suzuki-Miyaura cross coupling reaction, to optimize the synthesis of our derivatives. Since all the reactions were performed in water, TBAB was added to improve the solubility of the reactants and arylboronic acids were used as alcoholic solution. The quick reaction lasted about 4 h and led to appreciable quantities of all the derivatives, except in the case of compound **3f**, due to the poor solubility of the starting (2-methoxypyridin-3-yl)boronic acid in the alcoholic solution and in others organic media.

To establish the biological profile of all compounds, cell viability and proliferation were evaluated in MCF-7, MDA-MB 231, and SkBr3 breast cancer cell lines. To evaluate the possible toxicity of

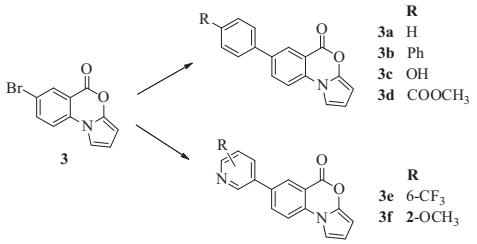
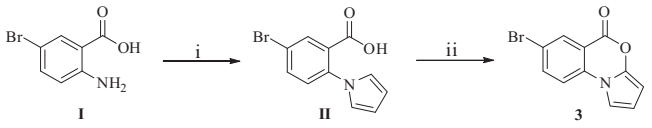
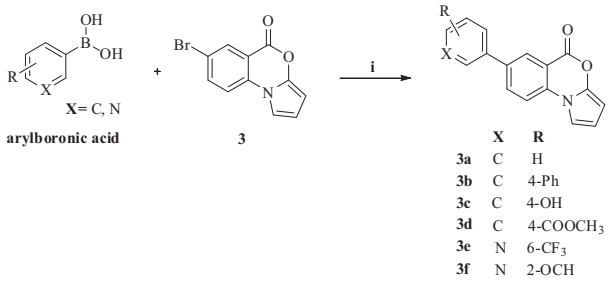


Fig. 3. Compounds of interest to further investigate the SAR of 7-substituted 5*H*-pyrrolo[1,2-*a*][3,1]benzoxazine-5-one analogs.



Scheme 1. Reagents and conditions: (i) 4-Chloropyridine hydrochloride (1 equiv.), 2,5-dimethoxytetrahydrofuran (1.2 equiv.), 1,4-dioxane, reflux, 24 h; (ii) MnO_2 (5 equiv.), dry toluene, reflux, 24 h, **3** 30%.



Scheme 2. Reagents and conditions: (i) alcoholic solution of arylboronic acid (1.5 equiv.), **3** (1 equiv.), PPh_3 (0.3 equiv.), $Pd(OAc)_2$ (0.1 equiv.), TBAB (0.1 equiv.), 1 M Na_2CO_3 (0.2 mL), water, 130 °C, 4 h, **3a** 60%, **3b** 80%, **3c** 86%, **3d** 51%, **3e** 30%, **3f** 5%.

the derivatives, a mammary epithelial cell line (MCF-10A) was used.

As shown in Fig. 4, most of the new synthesized compounds demonstrated a moderately improved anti-proliferative effect compared to the parent compound **3**. The presence of the unsubstituted phenyl ring in **3a** and the ethanone moiety in *para* in **3d**, did

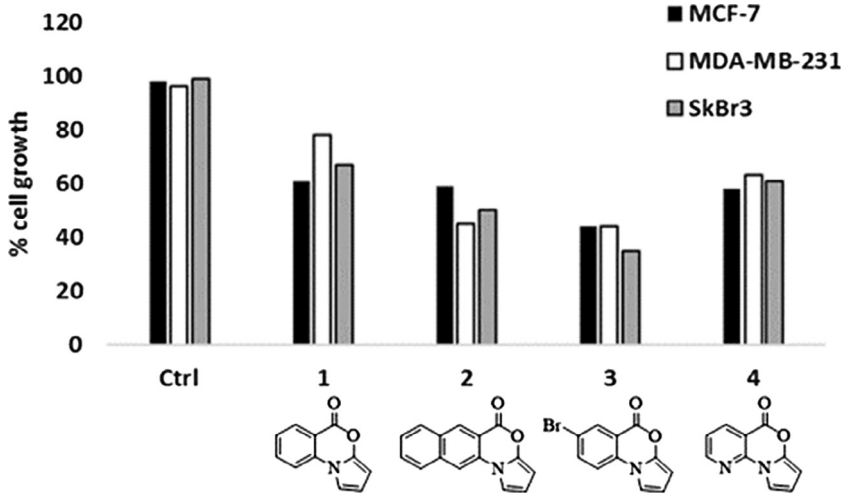


Fig. 2. Some of 5*H*-pyrrolo[1,2-*a*][3,1]benzoxazin-5-one analogs previously prepared and their respective cytotoxicity at 50 μM.