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Design and synthesis of new antitumor agents with the 1,7-epoxycyclononane framework. Study of their anticancer action mechanism by a model compound

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ABSTRACT

This article describes the design, synthesis and biological evaluation of a new family of antitumor agents having the 1,7-epoxycyclononane framework. We have developed a versatile synthetic methodology that allows the preparation of a chemical library with structural diversity and in good yield. The synthetic methodology has been scaled up to the multigram level and can be developed in an enantioselective fashion. The study *in vitro* of a model compound, in front of the cancer cell lines HL-60 and nMCF-7, showed a growth inhibitory effect better than that of cisplatin. The observation of cancer cells by fluorescence microscopy showed the presence of apoptotic bodies and a degradation of microtubules. The study of cell cycle and mechanism of death of cancer cells by flow cytometry indicates that the cell cycle arrested at the G_0/G_1 phase and that the cells died by apoptosis preferably over necrosis. A high percentage of apoptotic cells at the subG_0/G_1 level was observed. This indicates that our model compound does not behave as an antimitotic agent like nocodazole, used as a reference, which arrests the cell cycle at G_2/M phase. The interaction of anticancer agents with DNA molecules was evaluated by atomic force microscopy, circular dichroism and electrophoresis on agarose gel. The results indicate that the model compound has not DNA as a target molecule. The *in silico* study of the model compound showed a potential good oral bioavailability.

1. Introduction

The 1,7-epoxycyclononane framework is present in natural products with important biological activity.[1] Among them are physalins[2] (Fig. 1), a family of natural *seco*-steroids, with bioactivity in several pharmacological areas. In particular, these compounds have an important cytotoxic activity,[3] which has generated a special interest within the scientific community in both the synthesis and biological testing of

Abbreviations: AFM, atomic force microscopy; ATR, Attenuated total reflectance (IR system); CCC, covalently closed circular DNA; CD, circular dichroism; CT DNA, calf thymus DNA; DEPT, distortionless enhancement by polarization transfer; DMF, Dimethylformamide; DMSO, Dimethylsulphoxide; EDTA, *N,N,N′,N′*-Ethylenediaminotetraacetic acid; ESP-MS, electrospray mass spectrometry; ELISA, enzyme-linked immunosorbent assay; FAB-MS, fast atom bombardment mass spectrometry; FBS, foetal bovine serum; FT-IR, Fourier transformed infrared spectroscopy; HEPES, 4-(2-hydroxyethyl)-1-piperazinemethanesulphonic aci; HLB, Hydrophilic-Lipophilic Balance; LogP, Logarithm of the calculated partition coefficient between *n*-octanol and water (for non-ionic species); LogD, pH dependent partition coefficient (for ionic species at pH = 7.4); LogS, Water solubility logarithm; MR, Molar refractivity; XTT, 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide; MW, Molecular weight; NBA, 3-nitrobenzyl alcohol; OC, open circular DNA; PI, propidium iodide; SAR, structureactivity relationship; SASA, Solvent accessible surface area; SES, Solvent excluded surface; SEV, Solvent excluded volume; VdWSA, Van der Waals surface area; VdWV, Van der Waals volume; TE, culture medium composed of 50 mM NaCl, 10 mM TRIS-HCl and 0.1 mM EDTA; TM-AFM, tapping mode atomic force microscopy; TMS, tetramethylsilane; TRIS-HCl, *tris*-(hydroxymethyl)aminomethane hydrochloride

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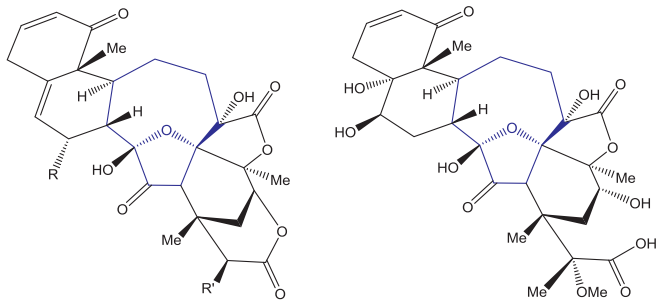

Fig. 1. Physalins with cytotoxic activity.

derivatives and analogues of these natural products.[4]

Following a fragment-based drug design approach and considering cytotoxic physalins as a model family of anticancer compounds, we are studying the structural subunits and the possible pharmacophore responsible for the cytotoxicity. In this context, we envisioned the synthesis of conveniently functionalized molecules having the 1,7-epoxycyclononane framework, with different substituents and organic functions, (introduced according to a rational design to get molecular diversity) as potential hits of a new family of anticancer agents. Thus, continuing with our efforts to develop new organic[5] and metal-based[6] drugs with anticancer activity, we have developed a synthetic methodology to prepare a chemical library of derivatives, in order to carry out in the future SAR studies, based on *in vitro* cytotoxicity assays on selected cancer cell lines. The rationale of choosing the previous core molecules as a model for the design of molecules with anticancer activity was because the SAR studies[2,3] carried out on the aforementioned natural *seco*-steroids showed that the 1,7-epoxycyclononane subunit was fundamental for the anticancer activity of these natural products.

In this work we describe the synthesis of a selected model on anticancer agent having the 1,7-epoxycyclononane framework, but in order to perform future SAR studies it is important to have available a chemical library, as large as possible, with an adequate structural diversity, in order to evaluate the molecular sites, the organic functions and, in general, the stereo-electronic effects that may increase or decrease the bioactivity, and in our case the cytotoxicity. For this purpose, it was relevant to design a very versatile synthetic methodology. In Fig. 2 we illustrate the possibility to get a very wide library of 1,7-epoxycyclononanes by starting from ketones **1′** different from 3-pentanone **1**, where R_1 may be equal or different from R_2. On the other hand, it is possible to work with substituted furans **3′** as dienophiles, having substituents R_3 to R_6 equal or different from each other. Furthermore, the double bond of cycloheptanone **4′** may be derivatized by addition of a wide variety of X-Y reagents. In addition, it is worth to mention that the complete synthetic process may be carried out on an *enantio*-enriched or even enantiopure intermediate **4′**, because the [4 + 3]-cycloaddition reaction may be performed in an enantioselective way by using an adequate chiral auxiliary on the oxyallyl cation or on the furan ring.[7] In addition, chiral organocatalysts may be used instead of chiral auxiliaries.[8] Moreover, if the ketone **5′** would be symmetrical, it may be desymmetrized in the process of formation of the silyl enol ether **6′** by using an enantiopure chiral base,[9] approach that has been carried out previously with success in our lab.[10] This enantioselective version adds value to the here presented synthetic methodology in the case that in the future a chiral recognition of the active principle at the active site of the target molecule should condition the cytotoxicity. That will make necessary the synthesis of the adequate eutomer.[11]

On the other hand, substituents R_1 to R_6 may be modulated, regarding their nature, constitution and size in order to control the regio- and stereoselectivity, in such a way that the number of regio- and stereoisomers formed may be reduced to a minimum, respect to the theoretically possible.[5,6,12–14]

To facilitate the development of this synthetic methodology up to the final 1,7-epoxycyclononane products of interest, we have worked with the simple and commercially available starting materials: 3-pentanone **1** (as precursor of the oxyallyl cation), furan **3** (as dienophile) and acrylic aldehyde **7** as precursor of dibenzyl acetal of propargyl aldehyde **10**. It is worth to mention that this synthetic pathway has been scaled up to multigram scale. Thus, key intermediates **4** and **10** have been prepared at 100 g scale. The scale of work for the other steps of the synthetic pathway, has ranged from a few mg up to 20 g, depending on the strategy of the experiment. We have optimized all steps working at different scale and under different reaction conditions, but we only describe in the experimental section those reaction conditions that afforded the best results.

On the other hand, we have implemented and optimize the protocols and procedures to study *in vitro* these compounds in front of the cancer cell lines HL-60 and MCF-7, in order to evaluate the effects on cells growth inhibition, cell cycle, apoptosis and microtubule distribution in nuclei of cancer cells. In addition, we have performed a series of biochemical, biophysical and *in silico* studies to unveil a possible mechanism of action. At this point of time, we have carried out these studies on our model compound **21a**. The results are described below, which are very promising and encourage us to continue with this research project.

2. Results and discussion

2.1. Synthetic methodology to prepare 1,7-epoxycyclononane derivatives

Our synthetic methodology is illustrated in Fig. 3 and involves as a key step the preparation of a oxabicyclic synthon **4a,b** *via* a [4 + 3] cycloaddition reaction of a furan derivative and the oxyallyl cation generated *in situ* from an α, α′-dihaloketone and a reducing metal.[12,13] This methodology was originally developed by Hoffmann[14] and it has been widely used in our laboratory to obtain a wide variety of molecules in good yields.[5,13,15] The precursor dibromoketone **2** was prepared in 80% yield by bromination of 3-pentanone **1** under acid catalysis.[16] 2,4-Dibromo-3-pentanone was then reacted with furan in the presence of copper powder and sodium iodide. The *in situ* generated oxyallyl cation reacts with furan and affords two possible oxabicyclic isomers: *cis*-diequatorial cycloadduct **4a** and *cis*-diaxial **4b** in 92:8 ratio (respect to the relative position of the methyl groups on C-2 and C-4) in a 98% yield. Both stereoisomers were easily separated by flash column chromatography on silica gel and were physically and spectroscopically characterized. The stereochemistry of the isolated diastereoisomers **4a** and **4b** was stablished by a correlation of ^{1}H- and ^{13}C NMR data.[17] Next step of the synthetic path was the hydrogenation of the double bond between C-6 and C-7. Because in the previous reaction the major product resulted to be the *cis*-diequatorial stereoisomer in a 92:8 ratio, we decided to continue the synthetic pathway with only the major pure isomer **4a**. This approach facilitated the evaluation of the results, since the reaction crude was much simpler than in the case of working with a mixture of stereoisomers **4a**/**4b** as a substrate. The hydrogenation afforded ketone **5a** in 98% yield. Silyl enol ether **6a** was prepared from ketone **5a** by the kinetic silylation method.[18] The conversion of **5a** was complete and the yield of the resulting **6a** was 99%.

In a parallel way, acrolein **7** was reacted first with bromine, in order to dibrominate the double bond, and subsequently with trimethyl orthoformate to protect the aldehyde group as a dimethyl acetal, affording compound **8** in a 86% yield.[19] The next step consisted in a double bromine elimination of **8** by using tetrabutylammonium hydroxide. The resulting dimethyl acetal of propynal, **9**, was isolated in a 50% yield. Dibenzyl acetal intermediate **10** was then synthesized by transacetallization of **9** with benzyl alcohol in the presence *p*-