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Novel benzopsoralen analogues: Synthesis, biological activity and molecular docking studies

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ABSTRACT

New benzopsoralen analogues were synthesized and their inhibitory effect on the growth of tumour-tumour cell lines (MDA MB231 and TCC-SUP) was evaluated. The *in vitro* antitumour activity of the new benzopsoralen analogues was discussed in terms of structure–activity relationship. Molecular docking studies with human-CYP2A6 enzymes were also carried out with the synthesized compounds to evaluate the potential of these molecules to interact with the haem group of the enzymes. The results demonstrated that the compounds that are able to interact with the iron ion of the haem cofactor and at the same time with active site Asn297 are those that have better anti-proliferative activity.

1. Introduction

Psoralens are naturally occurring furocoumarins present in many plant families such as *Apicaceae* and *Umbelliferae* [1]. Furocoumarins extracted from plants are commonly used as food additives, in phytomedicine and as cosmetics [2]. Additionally, Psoralen and 8-methoxypsoralen (8-MOP, named Xanthotoxin) are used for the treatment of Psoriasis in combination with UVA irradiation (PUVA therapy) [3]. It is important to notice that these compounds have also been reported to have an anti-cancer effect independently of its photoactivation [4,5]. It has been reported that Bergapten (5-methoxypsoralen) enhances p53 gene expression and induces apoptosis in human breast cancer cells [6].

Psoralens have been developed as pharmaceuticals for a wide range of disorders (vitiligo, psoriasis, skin cancers) that require cell division inhibitors [7] and are known to interrupt drug metabolism due to their ability to competitive and/or mechanistically inhibit a variety of human cytochrome P450 enzymes, including CYP1A2, CYP2A6 and CYP3A4/5 [8,9]. Xanthotoxin, Bergapten and Psoralen have been reported to suicide-inactivate the human CYP2A6 (enzyme involved in the coumarin metabolism) [10,11] and they contribute to the programmed cancer cell death and increased sensitivity to chemotherapy [10,11].

Some psoralens, e.g. Bergapten, have been demonstrated to exert an anti-cancer effect against different cancers, independently of its photoactivation [6]. For instance, Lee et al. [12] reported the chemopreventive role of Bergapten in a human hepatocellular carcinoma. The authors propose that there are at least three modes of suppressive effects shown by Bergapten, namely killing the cells directly; inducing apoptosis by arresting cells at the G2/M phase in the cell cycle; and inducing apoptosis through an independent pathway with cell cycle arrest at a given exposure time. It is also pertinent to mention that structurally related Bergapten exerts its anticarcinogenic properties by a cytotoxic effect, inducing apoptosis and inhibiting cell proliferation. In the current work, no mechanistic studies have been included, therefore only considerations regarding cytotoxicity and a potential effect on cell proliferation can be advanced.

The human CYP2A6 protein is involved in the metabolism of coumarins [13] and is known to be overexpressed in several cancer cells [14,15], including breast and bladder cancers. Therefore, it is an adequate model for molecular docking studies of the compounds herein synthesized. Previously, we reported that these compounds interact very closely with the iron ion of the haem group of the enzyme [4,5]. Ye and Zhang [16] demonstrated that the depletion of iron (haem deficiency) caused the apoptosis of HeLa cells, which

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involved the release of cytochrome c and the activation of caspase 3 (involved in the programmed cell death). It appears that haem deficiency inhibits cell growth by selectively interfering with the progression of the S phase of the cell cycle. Indeed, the haem altered metabolism has been associated with numerous diseases, including cancer.

Earlier in our group Psoralen derivatives were synthesized and their anti-proliferative effects on different human tumour cell lines were demonstrated [4,5]. The molecular docking results of these compounds with the CYP2A6 enzyme showed that, in general, the compounds carrying few bulky groups attached to the coumarin moiety adopted a conformation that allows the carbonyl group of the coumarin moiety to interact very closely with the iron ion of the haem cluster. Interestingly, these compounds were found to have a better ability to inhibit the proliferation of the three cell lines studied (MDA MB231, HeLa, TCC-SUP).

In the current work, two angular benzopsoralen analogues derived from 4-hydroxydibenzothiophene **1** were synthesized (compounds **2** and **3**, Scheme 1) and their biological activities were evaluated. Other two linear benzopsoralen analogues derived from 2-hydroxydibenzofuran and 2-hydroxycarbazole (compounds **4** and **5**, Scheme 2) were also synthesized and evaluated. One compound devoid of a coumarin moiety, (*E*)-ethyl 3-(3-hydroxy-9*H*-carbazol-9-yl)acrylate (**10**) (Scheme 3), was also obtained and tested.

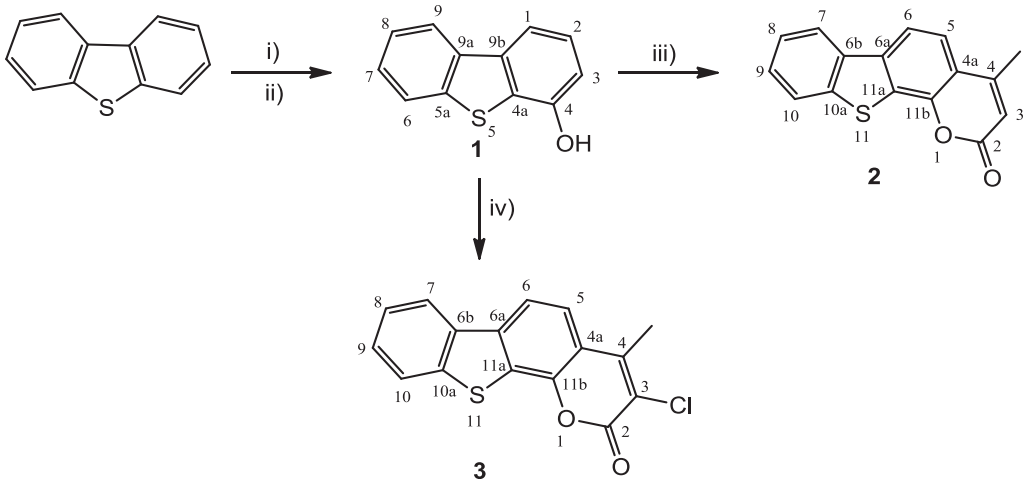
2. Results and discussion

2.1. Chemistry

Benzopsoralen derivatives with sulphur nucleus were obtained by Pechmann reaction using 4-hydroxydibenzothiophene **1** as the precursor. Compound **1** was prepared by the reaction of dibenzothiophene (commercial) with TMEDA, $B(OBu)_3$ and *n*-BuLi in the presence of 30% H_2O_2, in dry diethyl ether [17]. Although a good resolution of the multiplicity of signals was not achieved, in the ^1H NMR spectrum it was possible to identify the signal corresponding to the hydroxyl group at δ 9.95 ppm and also the other seven aromatic proton signals (δ 7.02–8.28 ppm). By Pechmann reaction of 4-hydroxydibenzothiophene **1** with ethyl acetoacetate and ethyl 2-chloroacetoacetate in the presence of concentrated H_2SO_4 compounds **2** and **3** were obtained in 21 and 53% yields, respectively (Scheme 1). The NMR data for compound **2** are consistent with the proposed structure, namely the presence of two doublets at 8.05 and 7.68 ppm, J = 8.4 Hz belonging to protons H-5 and H-6, respectively. The methyl group appears as a doublet at 2.56 ppm due to the long range coupling with H-3 and therefore the signal of H-3 is as an apparent doublet 6.37 ppm. For compound **3** ^1H NMR spectrum showed the signals of protons H-5 and H-6 as doublets at 8.08 (J = 8.4 Hz) and 7.69 ppm (J = 8.7 Hz), respectively, a singlet at 2.70 for the methyl group and absence of H-3 signal, that confirms the formation of the product.

The benzopsoralen analogues **4** and **5** were also prepared under Pechmann conditions (Scheme 2). The compound **4** was obtained, in 14% yield, from 2-hydroxydibenzofuran and ethyl 2-chloroacetoacetate. Its formation was confirmed by analysis of the ^1H NMR spectrum that showed the presence of singlets at 7.89 (H-11), 7.79 (H-5) and 2.70 (CH_3) ppm. The reaction of 2-hydroxycarbazole with ethyl 2-chloroacetoacetate afforded compound **5** in 16% yield. By analysis of the ^1H NMR spectrum the presence of two singlets at 8.59 and 7.38 ppm for the protons H-5 and H-11, respectively, and a singlet at 2.67 (CH_3) was observed.

Compound **6** was prepared by reaction of cyclohexanone with 4-methoxyphenyl hydrazine according to the method described by Rogers and Corson [18], in a yield of 71% (Scheme 3). ^1H NMR spectrum showed a singlet at δ 7.56 corresponding to –NH and a doublet at 6.93 ppm J = 2.4 Hz corresponding to H-4 and the expected signals for the aliphatic protons. Dehydrogenation reaction of compound **6** in *p*-cymene/water and in the presence of 10% Pd/C, under reflux, afforded the carbazole **7** in 53% yield (Scheme 3). The introduction of the –CHO group at 4-position of carbazole **7** was attempted under the conditions of Vilsmeier-Haack formylation ($POCl_3$ in dry DMF) and a solid was obtained in 76% yield. However, after analysis of the ^1H NMR spectrum the absence of the signal corresponding to the –NH and the presence of a singlet at 9.80 ppm, led to the conclusion that *N*-formylation had occurred with formation of compound **8** (Scheme 3). Demethylation of **8** with a 1 M solution of BBr_3 in CH_2Cl_2 under nitrogen atmosphere gave compound **9**, in 48% yield after purification by column chromatography (Scheme 3). In the proton NMR spectrum the disappearance of the OCH_3 signal was observed together with the presence of a singlet at δ 9.60 ppm (OH group). The alkene **10** was prepared by Wittig reaction between **9** and $Ph_3PCHCOOEt$ (in *N*,*N*-diethylaniline, 15 h, reflux) (Scheme 3). The proton NMR spectrum of the product showed two doublets at 8.54 (H-3) and 6.32 (H-2)



Scheme 1. Reagents and conditions: i) TMEDA, *n*-BuLi, rt → reflux, 1 h, dry ether; ii) $B(OBu)_3$, 30% H_2O_2, reflux, 90 min; iii) ethyl acetoacetate, conc. H_2SO_4, 2 h, rt; iv) ethyl 2-chloroacetoacetate, conc. H_2SO_4, 8 h, rt.