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Multidimensional optimization of promising antitumor xanthone derivatives

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ABSTRACT

A promising antitumor xanthone derivative was optimized following a multidimensional approach that involved the synthesis of 17 analogues, the study of their lipophilicity and solubility, and the evaluation of their growth inhibitory activity on four human tumor cell lines. A new synthetic route for the hit xanthone derivative was also developed and applied for the synthesis of its analogues. Among the used cell lines, the HL-60 showed to be in general more sensitive to the compounds tested, with the most potent compound having a GI_{50} of 5.1 μM, lower than the hit compound. Lipophilicity was evaluated by the partition coefficient (K_p) of a solute between buffer and two membrane models, namely liposomes and micelles. The compounds showed a $\log K_p$ between 3 and 5 and the two membrane models showed a good correlation ($r^2 = 0.916$) between each other. Studies concerning relationship between solubility and structure were developed for the hit compound and 5 of its analogues.

1. Introduction

Xanthones (or xanthen-9-ones) constitute a class of O-heterocycles with a dibenzo-γ-pyrone scaffold commonly found as secondary metabolites in higher plants, fungi and lichens.[1] They can also be obtained by synthesis and several strategies to achieve this goal have been described in the literature.[2] The members of the xanthone classes bear different types of substituents that are able to interact with several biological targets exerting different pharmacological activities.[3] Indeed, the xanthone core is a rigid heteroaromatic tricyclic platform which may be considered as a 'privileged structure' since it can provide potent and selective ligands through modification of functional groups, allowing them to interact with different pharmacological targets.[4] The more frequent chemical groups found in natural and synthetic xanthones are hydroxyl, methoxyl, methyl, chloro, prenyl and carboxyl.[1–3] For synthetic xanthones, other substituents have also been introduced including aminoalkyl, aminoalcohol, azido and furanyl groups.[2,3]

Prenylated xanthones are the most abundant family of naturally occurring xanthones.[5,6] This family comprises compounds that include one or more isoprenic moieties which can be found as an open chain or cyclized to give a fused furan or pyran ring. More importantly, this family of compounds has been described to possess a great variety of biological activities, in particular they have been considered as promising antitumor agents.[5–8] Representative members of prenylated xanthones with potent antitumor activity are α-mangostin (**1**) and γ-mangostin (**2**) (Fig. 1) which have been isolated from the pericarp of the mangosteen fruit.[9] These compounds have previously been shown to exert a potent growth inhibitory activity towards several tumor cell lines,[10] being capable of inducing apoptosis in leukemia cell lines through a mechanism

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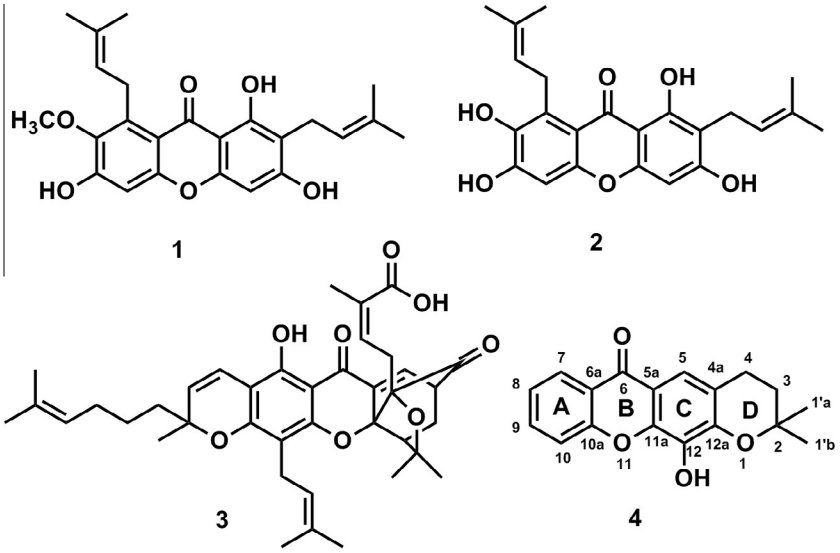

Figure 1. Prenylated xanthones with promising antitumor activity.

which involves activation of caspases-3 and -9.[11,12] Another well-known family of prenylated xanthones with promising antitumor activity are xanthones bearing a 4-oxo-tricyclo[4.3.1.0^{3,7}]dec-8-en-2-one, from which gambogic acid (**3**) is the most representative member of the so-called 'caged xanthones' (Fig. 1).[13]

In recent years, our research group has been synthesizing a small library of prenylated xanthones[14–18] and evaluating their biological activity in different cell lines. This approach was shown to be fruitful with many of the synthesized compounds presenting encouraging antitumor activity. Among them, compound **4** (12-hydroxy-2,2-dimethyl-3,4-dihydropyran[3,2*b*]xanthene-6(*2H*)-one—Fig. 1) was considered one of the most promising, exhibiting antiproliferative and pro-apoptotic activities in leukemia cell lines.[17] Interestingly, it also showed an enhancement of the anti-estrogenic effect of 4-hydroxytamoxifen in an estrogen-dependent (MCF-7) tumor cell line.[18] Therefore, this molecule has the potential to be optimized and may be used as the starting point in the search for more potent antitumor agents. To increase the likelihood of success, attention must also be paid to their pharmacokinetic behavior.[19] Consequently, it was decided to optimize this compound following a multidimensional approach looking, in parallel, at the activity and physicochemical properties, with the latter being used as a tool to predict the pharmacokinetic behavior.[20]

Compound **4** is composed of four fused rings that create a linear tetracyclic system. This structure has an unsubstituted ring A (Fig. 1) in which different groups can be introduced to increase the interactions with a putative target, and consequently, its potency. Furthermore, the introduction of substituents may also be used to improve the pharmacokinetics. The described methodology for the synthesis of compound **4** through a heterogeneous catalysis methodology has only been employed with relative success for the formation of a fused 2,2-dimethyl-3,4-dihydropyran ring in a few simple substituted xanthones,[16–18] flavonoids,[21] benzophenones[22] and phenols.[23] Moreover, low yields were generally obtained and in some cases no regioselectivity was observed. Furthermore, the scope and limitation of this reaction has not been thoroughly evaluated. In fact, for the synthesis of more complex molecules, this methodology is expected to show serious limitations. Therefore, in this paper the synthesis of compound **4** is reported by a new and more effective route and its application for the synthesis of 17 structural analogues. The targeted modifications envisaged the introduction of different functional groups on ring A and/or ring D orientation (Fig. 2). The newly synthesized analogues were evaluated for their in vitro growth inhibitory activity on four human tumor cell lines, namely MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), A375-C5 (melanoma) and HL-60 (acute myeloid leukemia).

In addition to the study of cell growth inhibitory activity, a preliminary estimation of the pharmacokinetic behavior of compound **4** analogues was made by evaluating their lipophilicity and solubility. Lipophilicity is one of the first physicochemical properties to be evaluated in the early phases of a drug discovery program[24] and has been correlated with several other physicochemical and pharmacokinetic properties, for example, solubility,[25] permeability,[26] plasma protein binding,[27] metabolism,[28] CNS penetration,[29] volume of distribution[25] and clearance.[28] Consequently, it may extensively influence the success of a drug discovery program. In fact, compounds with high lipophilicity have shown an increased risk of attrition during the clinical trials.[30] Lipophilicity is commonly evaluated by the partition coefficient of a solute in a biphasic octanol–water system which has some limitations since it fails to create the anisotropic media that is found on biomembranes and encode some important interactions that take place between the solute and the membranes.[31] Therefore, other models have been developed such as liposomes and micelles[32,33] which have proved to be advantageous when compared to octanol–water.[31] Consequently, it was decided to evaluate the lipophilicity on these two models. Solubility is emerging as one of the major issues in drug discovery and development of new chemical entities.[34] In fact, compounds with low solubility have a higher risk of attrition in the drug discovery pipeline as well as a higher cost during the drug development stage.[34] Therefore, the thermodynamic solubility in water at pH 7.4 (HEPES buffer) of compound **4** and five of its analogues (**36a–e**) was evaluated.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of building blocks

Pyranoxanthones are generally obtained by two classic strategies: Chemical modification of simple oxygenated xanthones or total synthesis using a benzopyran derivative as building block.[2,5,8] The former is more commonly used and was already applied for the synthesis of compound **4**.[18] However, the reaction used has some limitations and should not be applied for the synthesis of compound **4** analogues bearing substituents on ring A. As a result,