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Studies on indolizines. Evaluation of their biological properties as microtubule-interacting agents and as melanoma targeting compounds

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ABSTRACT

With the aim of investigating new analogues of phenstatin with an indolizin-3-yl unit, in particular as the B-ring, three new series of compounds (**6**–**8**, **9**–**34** and **54**) were synthesized and tested for interactions with tubulin polymerization and evaluated for cytotoxicity on an NCI-60 human cancer cell lines panel. The replacement of the 3′-hydroxy-4′-methoxyphenyl B-ring of phenstatin with substituted indolizine unit results in the conservation of both antitubulin and cytotoxic effect. Indolizines **9** and **17** were the most effective in the present study and showed the highest antiproliferative effect on melanoma cell lines MDA-MB-435 (GI_{50} = 30 nM) and could serve as new lead compounds for the development of anti-cancer therapeutics.

1. Introduction

Tubulin is a heterodimer of closely related and tightly linked globular α- and β-tubulin proteins. α- and β-Tubulin heterodimers are polymerized in hollow tubes called microtubules, forming the mitotic spindle. Their importance in cell division makes microtubules an important target for anti-cancer drugs [1]. Tubulin binding agents interfere with the dynamic instability of microtubules, and thereby arrest mitotic cells in the G2/M-phases of the cell division cycle, leading to induction of apoptosis [2]. Colchicine and its analogues predominantly bind to a high affinity site, called the colchicine binding site, located at the interface of homodimers α/β [3].

A known tubulin polymerization inhibitor that binds to the colchicine site of the protein is phenstatin which resulted from a SAR-study on combretastatin A-4 (CA-4) and exhibited potent inhibition of tubulin polymerization and of cancer cell growth [4].

The literature abounds of pharmacomodulations performed on known inhibitors of tubulin polymerization. The majority of structural modifications of the phenstatin have concerned either the carbonyl bridge or the B-ring; the A-ring (3,4,5-trimethoxyphenyl unit) was generally kept intact and it has long been considered essential to the biological activity [5]. However, some modulations on the A-ring are permitted without reducing the biological potency [6].

We were puzzled by the recent discovery of phenstatin analogs with 5,6-fused bicyclic heteroaromatic scaffolds as B-ring, and we have pointed out that only four indolizin-3-yl-(3,4,5-trimethoxyphenyl)methanones were described [7–11] (compounds **1**–**4**, Fig. 1). Indolizine **1** showed potent anti-mitotic and anti-proliferative activity, and authors concluded that this compound could serve as important tool for the development of anti-cancer therapeutics [7]. Concerning compound **2**, only synthesis details and physico-chemical properties are available in the

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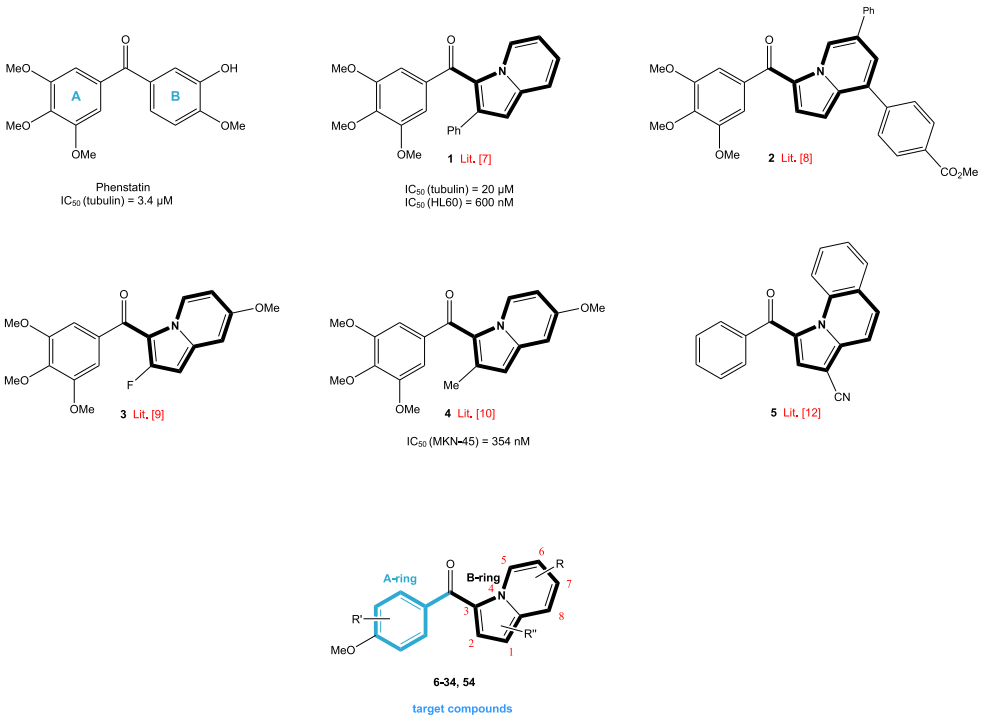

Fig. 1. Structure of reference phenstatin, described indolizin-3-yl-(3,4,5-trimethoxyphenyl)methanones **1**–**4**, ketone **5** and target derivatives **6**–**34** and **54**.

literature [8]. The biological evaluation of this compound could provide useful information for the development of new chemical entities in this family of microtubule-interacting agents. Indolizine **3** is described as a tubulin binding anti-cancer agent [9]. However, its potential on tubulin and on cancer cell growth is not reported. Finally, the indolizin-3-yl-(3,4,5-trimethoxyphenyl)methanone **4** showed a growth inhibitory activity *in vitro* on gastric carcinoma MKN-45 cells [10,11]. On the other hand, some closely related ketones such as compound **5** (Fig. 1) acts as apoptosis inducer [12]; that enhances the biological potential of products with this type of structure.

Our recent identification of phenstatin analogues bearing an indolizin-3-yl scaffold as B-ring [6c], and the absence of a complete SAR study in these series encouraged us to explore some structure–activity relationships in this new family of tubulin polymerization inhibitors. Therefore, we present here our synthetic strategies for the replacement of the classical 3′-hydroxy-4′-methoxyphenyl B-ring with substituted indolizines and the biological evaluation of target compounds (compounds **6**–**34** and **54**, Fig. 1).

2. Results and discussion

2.1. Chemistry

The objective of the present study was to highlight the substituents on the indolizine ring that may play an important role in the biological activity. In order to obtain diverse substituents on the indolizine B-ring, a [3 + 2] cycloaddition synthesis was privileged for the target compounds.

The bromoacetyl-4-methoxybenzene **35A** was commercially available, and the other starting bromoacetyl derivatives **35B**–**D** were synthesized by reacting the corresponding di- or trimethoxyacetophenone with bromine in acetic acid. Accordingly, bromoacetyl-3,4-dimethoxybenzene **35B** was obtained in 69% yield from 3,4-dimethoxyacetophenone, and bromoacetyl-3,4,5-trimethoxybenzene **35C** was formed in 43% yield from 3,4,5-trimethoxyacetophenone. In this last reaction, bromoacetyl-2-bromo-3,4,5-trimethoxybenzene **35D** was also isolated in 10% yield [13]. Now, substituted bromoacetyl substituted benzenes **35A**–**D** were reacted with pyridines **36a**–**i** to give pyridinium salts **37**–**50** (Scheme 1 and Table 1). The construction of the indolizine unit in products **6**–**34** was then achieved by [3 + 2] cycloaddition reaction of the corresponding ylide **51 a**–**n**, generated *in situ* by base treatment of pyridinium salts **37**–**50**, with ethyl propiolate, acrylonitrile, dimethyl or diethyl acetylenedicarboxylate, followed by spontaneous aromatization of intermediates (Scheme 1). Noteworthy, the first synthesized product, compound **32**, was obtained with a low 10% yield by using DBU as the base, thus all the other reactions were performed in higher yields, by using triethylamine; however, the mesomeric effect (+M) of dimethylamino group decreases the reactivity of the corresponding pyridinium salt in the cycloaddition reaction and leads therefore to indolizines in modest yields.

The reaction conditions that we used are much easier than those described in literature for analogue syntheses of indolizines; particularly, the aromatizations did not require tetrapyridinecobalt (II) dichromate or MnO_2, but only oxygen from the atmosphere.

Generally, these cycloaddition reactions provided a unique final product. However, one exception needs to be noted when treating the β-picolinium salt **42** with ethyl propiolate, two different isomers **11** and **12** were obtained in 21% and 26%, respectively (Scheme 2).

In order to complete the preliminary study and to establish structure–activity relationships, we become interested in the synthesis of compounds with a free carboxylic group on the indolizine unit. Compound **54** was thus synthesized by saponification of ethyl ester **9** (Scheme 3). Finally, a structural modification, leading to conformationnally blocked products **55** and **56**, was realized by