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Research paper

Synthesis and biological evaluation of isomeric methoxy substitutions on anti-cancer indolyl-pyridinyl-propenones: Effects on potency and mode of activity

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ABSTRACT

Certain indolyl-pyridinyl-propenone analogues kill glioblastoma cells that have become resistant to conventional therapeutic drugs. Some of these analogues induce a novel form of non-apoptotic cell death called methuosis, while others primarily cause microtubule disruption. Ready access to 5-indole substitution has allowed characterization of this position to be important for both types of mechanisms when a simple methoxy group is present. We now report the syntheses and biological effects of isomeric methoxy substitutions on the indole ring. Additionally, analogues containing a trimethoxyphenyl group in place of the pyridinyl moiety were evaluated for anticancer activity. The results demonstrate that the location of the methoxy group can alter both the potency and the mechanism of cell death. Remarkably, changing the methoxy from the 5-position to the 6-position switched the biological activity from induction of methuosis to disruption of microtubules. The latter may represent a prototype for a new class of mitotic inhibitors with potential therapeutic utility.

1. Introduction

Glioblastoma multiforme (GM) remains a lethal cancer due to rapid progression and limited treatment options, namely surgical removal of the tumor followed by combined radiotherapy and chemotherapy with temozolomide [1,2]. Recurrence of disease is usually untreatable as a result of acquired drug-resistance and invasive dissemination of the tumor. Temozolomide relies on triggering programmed cell death via activation of apoptosis [3]. However, GM cells harbor specific mutations in genes that are required to promote an efficient apoptotic response [4,5]. Stimulation of nonconventional cell death pathways offers a possible solution for treating drug-resistant cancers that are able to circumvent apoptosis [6,7]. Methuosis is a recently identified caspase-independent form of cell death that displays characteristics distinct from other types of non-apoptotic cell death, such as necroptosis or autophagy [8,9]. In cultured glioblastoma cells, methuosis begins with defective macropinocytotic trafficking, causing the formation of large fluid-filled vacuoles. Accumulation of vacuoles ultimately displaces the cytoplasm and the cell membrane loses integrity and ruptures. While dysfunctional vesicular trafficking and accumulation of vacuoles appear to contribute to cell death, there is evidence that additional metabolic or cellular insults are required for execution of the methuosis cell death program [8,10,11].

The methuosis phenotype was initially observed by the ectopic expression of activated Ras and Rac GTPases in GM cells [12,13].

abbreviations: EtOAc, ethyl acetate; GM, glioblastoma multiforme; h, hour; IPP, indolyl-pyridinyl-propenone; min, minute; NaH, sodium hydride; SAR, structure-activity relationship; SRB, Sulforhodamine B; PBS, phosphate-buffered saline; rt, room temperature; TBAB, tetrabutylammonium bromide; TFA, trifluoroacetic acid.

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More recent studies have focused on the pursuit of small molecules with the potential to induce this form of cell death in a therapeutic context. An initial search for compounds reported to induce cellular vacuolization led us to an indolyl-pyridinyl-propenone (IPP, also referred to as indole-derived chalcone) as a potential lead [13]. Associated structure-activity relationship (SAR) studies revealed that the optimized scaffold for induction of methuosis consists of a 2,5-disubstituted indole and a pyridine in the *para*-configuration, bridged by an α,β-unsaturated ketone [11,14,15]. Our previously reported IPP compounds, and their modes of biological activity, are summarized in Fig. 1.

To date, compounds **1a**-**1e** are the most potent inducers of methuosis, possessing activity between 2 and 3 μM when assayed against the human glioblastoma cell line, U251. Among these compounds **1a** has been studied most thoroughly. Comparisons of structurally similar IPP's have revealed intriguing and unexpected results suggesting that the morphological appearance of vacuoles in the treated cells is not always associated with cell death. For instance, analogues with larger aliphatic substitutions (**2e**-**2g**) on the 2-indolyl position caused vacuolization but had surprisingly less cytotoxicity than the vacuole-inducing compounds with Me (**1a**) or Et (**1b**) at this position (Fig. 1) [11]. Similarly, certain 5-substituted analogues (**2a-2c**), as well as the 2-des-methyl derivative **2d**, also induced vacuole formation but were not cytotoxic [15]. While 5-methoxy (**1a**) and 5-propoxyindole (**1e**) analogues triggered cell death by methuosis, their structurally similar counterparts, namely 5-ethoxy (**2a**) and 5-isopropoxyindole (**2b**) caused cytoplasmic vacuolization without cell death. Studies are currently underway with this series of analogues to explore the mechanistic basis for their differential cytotoxicity.

Another novel insight into the biological effects of the IPP compounds was gathered from derivatives containing electron-withdrawing functionalities at the 2-indolyl position [15]. Derivatives containing trifluoromethyl (**3a**) or alkyl carboxylate (**3b**-**3d**) substitutions caused minimal to no vacuolization but remained highly cytotoxic. Morphologically, cells treated with the latter series of compounds did not resemble cells undergoing methuosis. Instead, the cells displayed features consistent with disruption of tubulin polymerization and microtubule architecture. Cell cycle analysis demonstrated an accumulation of cells in the G2/M phase, with eventual death by mitotic catastrophe. In this respect, **3a**-**3d** were quite distinct from the methuosis-inducing compounds, which did not disrupt microtubules or cause mitotic arrest at the same concentrations. The redirection of cytotoxicity from methuosis to microtubule disruption for derivatives **3a**-**3d** was associated with a significant increase in growth inhibitory potency.

While our previous synthetic work focused on substitution at either the 2- or 5-indole positions, a lack of information exists for substitutions at the 4-, 6-, or 7- positions (Scheme 1). We noted that the importance of a 5-methoxy group for either methuosis or microtubule disruption is further dependent upon the electron withdrawing properties of the 2-substituent. In the present study we have synthesized and evaluated methoxy isomers of **1a** to sequentially survey the 4-, 6- and 7-positions while initially holding the 2-position constant. Upon finding significant anti-mitotic activity for the 6-position isomer **9b**, we immediately prepared its 2-trifluoromethyl version (**15**) by analogy to **3a** where this type of functionality also led to microtubule disruption [15]. Finally, drawing from several reports describing *N*-methyl-indole-based trimethoxyphenyl chalcones as compounds affecting tubulin polymerization, we examined replacing the *para*-pyridine in our structural template with a trimethoxyphenyl group and, likewise, separately examined the effect of adding a methyl group to the indole nitrogen. The results reveal that the position of the methoxy group on the indole ring and the *para*-pyridine are critical determinants of the biological activities of the IPP compounds.

2. Results and discussion

2.1. Chemistry

Scheme 1 illustrates the synthesis of isomers of **1a** at the 4-, 6- or 7- indole position (**9a**-**9c**). A disubstituted 5,6-dimethoxy derivative (**9d**) was also synthesized. From commercially available **4a**-**4d**, the indole nitrogen was protected with benzenesulfonyl chloride (**5a**-**5d**). The benzenesulfonyl group ensured regioselective methylation at the 2-indolyl position (**6a**-**6d**), which was accomplished under conditions of *tert*-butyllithium and iodomethane [16]. Removal of benzenesulfonyl in a mixed solvent system of EtOH/aqueous NaOH provided **7a**-**7d**. Formylation reactions utilizing Vilsmeier conditions (**8a**-**8d**) followed by Claissen-Schmidt condensation reactions produced target compounds **9a**-**9d**. This approach generally provided compounds in reasonable yields; however, intermediate **7b** was not stable under these conditions

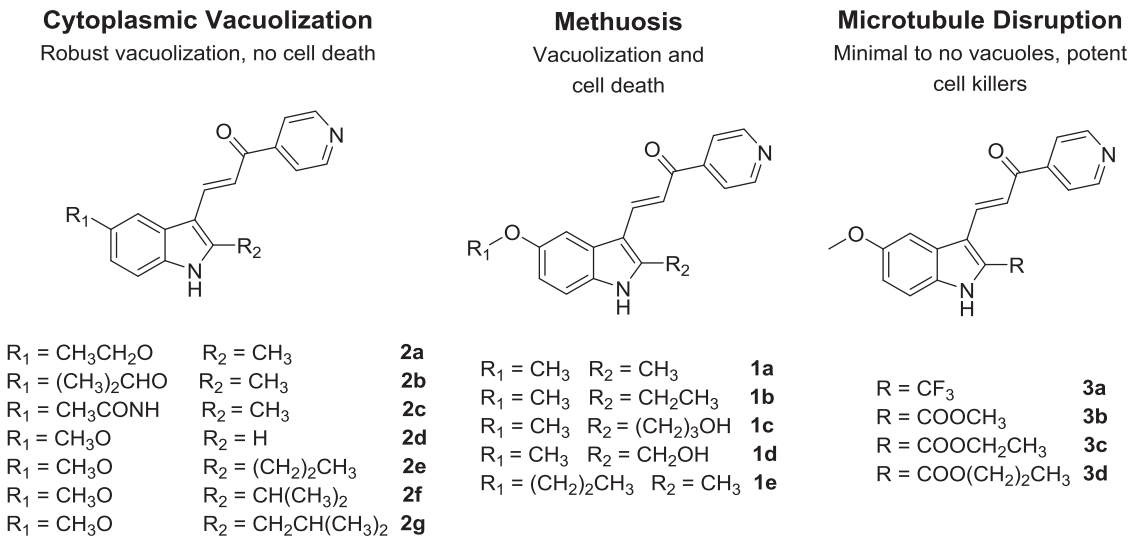

Fig. 1. Previously reported analogues illustrating the various biological activities of substituted IPP's.