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Research paper

Synthesis of triazoloquinazolinone based compounds as tubulin polymerization inhibitors and vascular disrupting agents

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ABSTRACT

A series of 1-phenyl-[1,2,4]triazolo[4,3-*a*]quinazolin-5-ones designed as conformationally restricted CA-4 analogues, were tested for their tubulin polymerization and growth inhibitory activities. The 3-hydroxy-4-methoxy derivatives **11d** and **12d** are potent inhibitors of tubulin assembly but only the *N*-methylated amid counterpart **12d** possesses potent anticancer activity in a large panel of cancer cell lines. Upon treatment with compound **12d**, remarkable cell shape changes as cell migration and tube formation were elicited in HUVECs, consistent with vasculature damaging activity.

1. Introduction

Microtubules provide an important framework supporting cellular morphology in interphase, and they are essential in cell division as the key component of the mitotic spindle. Consequently, the microtubule has become an important target for the design of new antimitotic anticancer agents. The antimitotic agents currently in clinical use include vinca alkaloids [1], which inhibit microtubule polymerization, and toxoids [2], which promote microtubule assembly, but the development of drug resistance limits its usefulness [3–5]. Combretastatin A-4 (CA-4, Fig. 1) is another well-known antimitotic agent, derived from the African bush willow *Combretum caffrum* and has been first described over 20 years ago [6]. It is a strong tubulin depolymerizing agent (TDA) and therefore inhibits tumor growth and has antivascular effects [7]. Its prodrug (disodium salt water-soluble phosphate derivative, CA-4P) has now entered clinical trials for both solid and liquid tumors [8]. The encouraging antivascular and antitumor profile of CA-4 has contributed greatly to the current interest in the design and synthesis of several CA-4 analogues [9].

Through SAR studies, it has been established that the cis orientation of both phenyl groups is an essential requirement for efficient tubulin affinity, forcing the two aromatic rings to stay within appropriate angles and distances in the colchicine binding site (Fig. 2A). In fact, the cis double bond of CA-4 or analogues easily undergoes isomerization, leading to trans isomers that display dramatically decreased inhibitions of cancer cell growth and tubulin assembly. In order to stabilize the active configuration, numerous teams have synthesized a wide range of various cis-restricted analogs of CA-4 by replacement of the double bond with other rigid linkers [10] or different cyclic moieties [11], or by introducing other rings [12].

Quinazolinone skeleton, which is considered as a privileged structure [13], has been demonstrated to conduct to antimitotic agents, exerting their antitumor activity through inhibition of the DNA repair enzyme system or dysregulation of cell cycle

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progression of cancer cells [14,15]. Among the various classes of antimitotics, quinazolin-4-ones form an important component of pharmacologically active compounds as they are associated with inhibitory effects on tubulin polymerization and the anticancer activities of 2-styrylquinazolin-4-ones [16], 2-aryl and 2,3-dihydro-2-arylquinazolin-4-ones [14,15,17] and tetrahydropyrido[2,1-*b*] quinazolin-10-ones [18] are well established. These considerations led us to design a new series of conformationally restricted CA-4 analogues by replacing the cis-olefinic bond by a quinazolin-4-one based heterocycle.

In the past recent years, a number of 1-substituted-[1,2,4]triazolo[4,3-*a*]quinazolin-5-ones have revealed antitoxoplasmosis [19], H1-antihistaminic [20], antimicrobial [21] anticonvulsant [22] and PDE4 inhibitory [23] properties. As the two aromatic rings of 1-phenyl-[1,2,4]triazolo[4,3-*a*]quinazolin-5-one derivatives adopt also a conformation in which they are not coplanar (Fig. 2B), the structural similarity between combretastatins and these tricyclic compounds can be expected to lead to an activity on the colchicine site of tubulin in the case of conveniently substituted derivatives. Moreover, such a structure will avoid inactivation resulting from cis to trans isomerization of the double bond of combretastatin derivatives. We therefore decided to investigate the synthesis of 1-phenyl-[1,2,4]triazolo[4,3-*a*]quinazolin-5-one analogues of CA-4 and to study their biological efficiency toward tubulin polymerization, their cytotoxicities toward various cancer cell lines as well as their antivascular effects.

2. Results and discussion

2.1. Chemistry

The synthesis of 1-phenyl-[1,2,4]triazolo[4,3-*a*]quinazolin-5-one derivatives is well described in the literature and followed the general methods depicted in Scheme 1. Methyl anthranilate **1** reacted with thiophosgene to provide the corresponding thioisocyanate **2** which on refluxing in toluene with benzylamine or methylamine 40% aqueous solution yielded the desired 3-substituted-2-sulfanylquinazolin-4-ones **3** and **4** via the thiourea intermediates in good yields. Nucleophilic displacement of sulfanyl group in refluxing ethanol with hydrazine hydrate in large excess yielded the desired 2-hydrazinoquinazolin-4-ones **5** and **6** with respectively, 86% and 89% yield. One of the useful methods for the preparation of fused 1,2,4-triazoles is based on oxidative cyclization of the fused heterocyclic hydrazones with iron(III) chloride as oxidant [24]. The hydrazones **7a-g** and **8a-g** were then prepared by condensation of the corresponding hydrazines with equimolar amount of benzaldehyde derivatives in refluxing methanol and the presence of a catalytic amount of acetic acid. Treatment of the hydrazones with hot ethanolic iron(III) chloride solution resulted in an *in situ* 1,5-electrocyclization to the angularly annelated 1,2,4-triazolo[4,3-*a*]quinazolin-5-ones **9a-g** and **10a-g** in very good yields. Finally, decomposition of ammonium formate with catalytic amount of Pd/C in refluxing ethanol conducted to the hydrogenolysis of the benzylheteroatom bonds but also to the reduction of the nitro group to provide the N- and O-deprotected derivatives **11a-e** and **12d-g**. For compounds **9f** and **9g**, these conditions did not allow hydrogenolysis of the N-benzyl protection, even in the presence of a large amount of ammonium formate and Pd/C; only the benzyloxy and nitro groups were deprotected and reduced providing derivatives **11f** and **11g**.

2.2. In vitro tubulin polymerization and antiproliferative assays

To characterize the possible interaction with the microtubule system of this novel series of triazoloquinazolinone derivatives, compounds **10a-c**, **11a-e** and **12d-g** were evaluated for their *in vitro* inhibition of tubulin polymerization (Table 1). Namely, some recent reports suggest that nonlinear relationship between antiproliferative activity and the effect on tubulin polymerization of described inhibitors, as combretastatin A-4, may occur, where highly cytotoxic compounds are not necessarily potent inhibitors of tubulin polymerization and *vice versa* [25,26].

In this assembly assay, only compounds **11d** and **12d** displayed potent inhibitory activities with IC_{50} values of 4.26 and 0.15 μM respectively whereas the others seemed to be inactive as inhibitors of tubulin polymerization and did not inhibit tubulin assembly at concentrations as high as 10 μM. These findings indicated that the 3-hydroxy-4-methoxyphenyl was crucial moiety for inhibition of tubulin polymerization and was preferential partial structure seen with many combretastatin derivatives. Interestingly, introduction of a 3,4,5-trimethoxyphenyl group in **9c** and **11c**, a typical pharmacophore found in many inhibitors of tubulin polymerization [27] led to a total loss of inhibitory activity. Furthermore, quite other variations of substituents within the aryl moiety resulted in inactive compounds at concentrations as high as 10 μM. The unsubstituted derivative **11d** was less potent than its counterpart **12d** whose substitution with a methyl group at the *N*-4 position of the triazoloquinazolinone seemed to be most tolerant for tubulin polymerization inhibitory effect. Antiproliferative effects of compounds **10a-c**, **11a-e** and **12d-g** were initially evaluated at a high single dose (10 μM) on human colon adenocarcinoma HT29 cell line and the representative results are summarized in Table 1. Percentages of cell proliferation inhibition of tested compounds are consistent with their antitubulin potencies. Indeed, the best antitubulin agent **12d** showed significant antiproliferative activity (75%) whereas its unsubstituted counterpart **11d** displayed weak

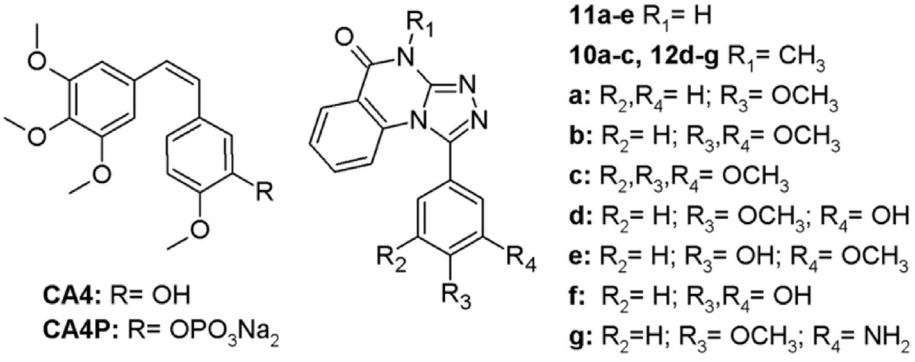

Fig. 1. Structures of reference and synthesized compounds.