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Original article

Conformationnally restricted naphthalene derivatives type *iso*combretastatin A-4 and *iso*erianin analogues: Synthesis, cytotoxicity and antitubulin activity

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ABSTRACT

A novel series of dihydronaphtalene, tetrahydronaphtalene and naphtalene derivatives as restricted analogues of *iso*CA-4 were designed, synthesized and evaluated for their anticancer properties. High cell growth inhibition against four tumour cell lines was observed at a nanomolar level with dihydronaphtalenes **1d**, **e** and **1h**, tetrahydronaphtalene **2c** and naphtalene **3c**. Structure–activity relationships are also considered. These compounds exhibited a significant inhibitory activity toward tubulin polymerization ($IC_{50} = 2–3$ μM), comparable to that of *iso*CA-4. The effect of the lead compounds **1e** and **2c** on the cancer cells tested was associated with cell cycle arrest in the G_2/M phase. Docking studies reveal that these compounds showed a binding mode similar to those observed with their non-constraint *iso*CA-4 and *iso*erianin congeners.

1. Introduction

Microtubules found in cytoskeleton of almost all eukaryotic cell types are hollow tubes formed by self-assembly of α and β-tubulin heterodimers. They are directly involved in a variety of cellular functions, such as cell movement, transport of organelles inside the cell, maintenance of cell shape as well as mitosis and cell replication. Consequently, perturbation of tubulin assembly/disassembly is a popular target for new chemotherapeutic agents [1,2]. The vinca alkaloids, typified by vinblastine and vincristine which inhibit microtubules assembly [3] as well as the taxanes, such as paclitaxel and docetaxel which promoted microtubules polymerization and inhibits microtubules depolymerization [4,5], are the mostly used antimicrotubules agents introduced in clinical oncology [6]. However, despite their potent antitumour activities, these drugs have undesirable side effects [7,8] and are subject to multidrug resistance [9,10]. These last decades, there has been a strong enthusiasm for discovering tubulin polymerization inhibitors of small size, easy synthesis and low side effects. Combretastatin A-4 (CA-4, Fig. 1), a *cis*-stilbene extracted from the South African willow *Combretum caffrum* [11,12] is arguably the most studied substance that displays a nanomolar level of cytotoxicity against a variety of human cancer cells, including multidrug resistant cell lines [13,14]. CA-4 binds at or near colchicine binding site of β-tubulin and strongly interferes with the assembly of tubulin, leading to cell death [15]. It also exerts highly selective effects in proliferating endothelial cells and, as a consequence, demonstrates strong suppressive activity on tumour blood flow leading to tumour necrosis [16]. Two derivatives are currently in clinical trials: CA-4 disodium phosphate CA-4P [17,18], a water soluble prodrug of CA-4 and the amino-combretastatin prodrug AVE-8062 (**3**) [19,20]. To date, CA-4P [21] either as a single agent or in combination therapy is undergoing several advanced clinical trials worldwide for the treatment of age-related macular degeneration or anaplastic thyroid cancer.

Despite its remarkable anticancer activity, the main disadvantage of CA-4 is the ready isomerization of the *Z*-double bond to its inactive *trans*-form during storage, administration [22] and metabolism [23]. In an ongoing project aimed at developing novel tubulin assembly inhibitors [24–29], we recently discovered *iso*-combretastatin A-4 (*iso*CA-4), a structural isomer of the natural product, which holds biological activities comparable to that of CA-4 [30]. This substance having a 1,1-diarylethylene scaffold is easy to synthesize [31–33] at a multi grams scale without the need to

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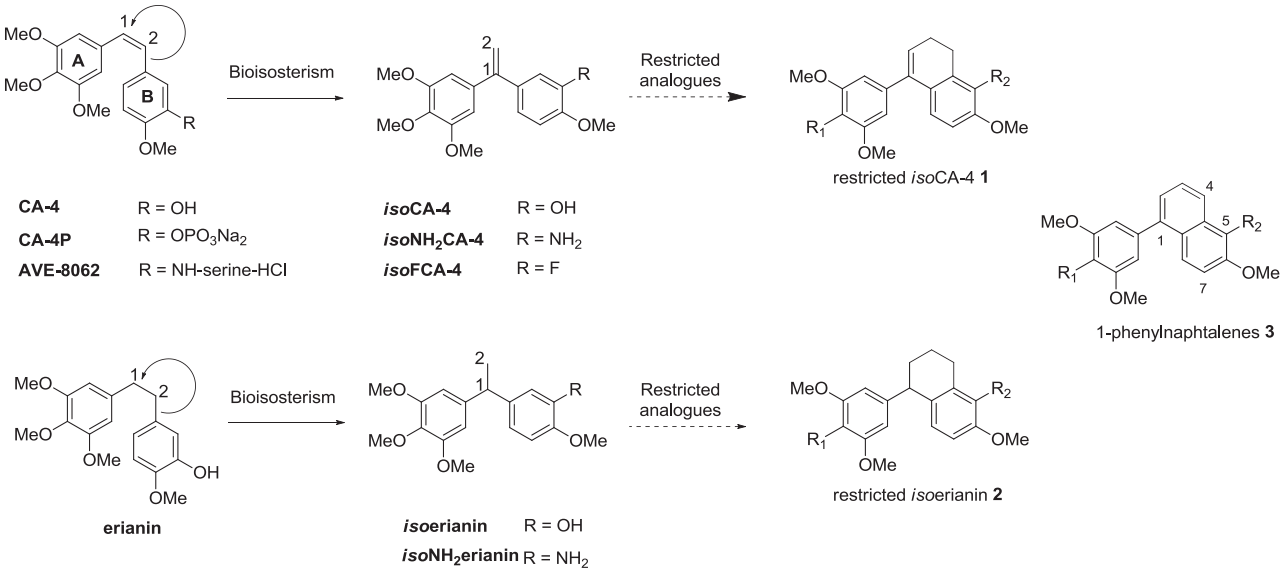

Fig. 1. Representative tubulin binding agents and general structure of the synthesized analogues **1**–**3**.

control the olefin geometry and then definitively solving the *Z*-double bond isomerization problem [34].

By structural modifications on the B-ring, we have also identified other promising antiproliferative agents such as *iso*NH_2CA-4 and *iso*FCA-4 (Fig. 1) [35,36]. We also demonstrated that the bioisosteric replacement of the (*Z*)-1,2-ethylene by the 1,1-ethylene could be apply successfully to natural combretastatins CA-2, CA-3 and CA-5 [30]. On the basis of these bioisosteric considerations, we also showed that isoerianin derivatives having a 1,1-diarylethane scaffold were as active as the natural product erianin [37]. A set of molecular docking calculations was performed with *iso*CA-4 as well as *iso*erianin which showed a binding pose similar to those observed with CA-4 and the co-crystallized DAMA-colchicine in the colchicine binding site [38]. In addition, the dihedral angles between the planes of the two A/B-aromatic nucleus in *iso*CA-4 and *iso*erianin (68° and 77°, respectively) [30,37] were found to be close to that of CA-4 (53°) [39]. From all of these considerations, we are planning to rationalize the synthesis of three new series of rigid analogues of *iso*CA-4 and *iso*erianin namely, 4-aryldihydronaphtalenes **1** (e.g.; dihedral angle = 69° for **1e**), 4-aryltetrahydronaphtalenes **2** (e.g.; dihedral angle = 79° for **2c**) and 1-arylnaphtalenes **3** (e.g.; dihedral angle = 70° for **3d**) with reduced mobility of the B-ring. We hypothesized that constrained analogues **1**–**3** with dihedral angles close to those of *iso*CA-4 and *iso*erianin would be as active as their non restricted congeners. In this paper we would like to describe the synthesis and evaluation of compounds **1**–**3** in terms of inhibition of tubulin assembly along with cytotoxicity studies against various cancer cell lines.

2. Results and discussion

2.1. Chemistry

Scheme 1 outlines the convergent synthetic routes followed for the synthesis of the novel restricted-analogues **1**–**3**. The projected incorporation of a variety of substituents at the C3 position of the B-ring trusts in the tractability of a C̃-Br bond, which can further be engaged into diverse coupling reactions. Thus, the preparation of the pivotal brominated precursors **1a** and **1b** was achieved from 5-bromo-6-methoxytetralone **4b** [40] which was heated in EtOH at 50 °C with $TsNHNH_2$ in the presence of PTSA. The resulting *N*-tosylhydrazone **5b** was next coupled with aryl iodides under palladium catalysis [30,41,42] to afford the key intermediates **1a**, **b**. By securing the required skeleton for the dihydronaphtalene analogues, the stage was ready for the installation of various functionalities in place of the bromine atom. The C(sp^2)-NH_2 bond of **1d** and **1i** was formed from **1a** and **1b**, respectively, using sodium azide as the amino source [43,44] in the presence of a catalytic amount of CuI. Treating **1a**, **b** with KOH in the presence of Pd_2dba_3, *t*BuXPhos in a mixture dioxane/H_2O: 1/1 at 90 °C [45] delivered in good yields the C3′-hydroxy substituted analogues **1e** (63%) and **1h** (62%). For the introduction of alkyne substituents on the B-aromatic ring [36], we examined the Sonogashira–Linstrumelle reaction [46] of **1a** with propargylic and homopropargylic alcohols. The coupling reaction of these alcohols with **1a** proceeded in the presence of $PdCl_2(PPh_3)_2$ and CuI catalysts under microwave irradiation (MWI) at 120 °C to give the corresponding alkynes **1f**, **g** in good yields. Similarly, **1a** underwent Heck coupling with methyl acrylate using [1,3-bis(2,6-diisopropylphenyl) imidazol-2-ylidene](3-chloropyridyl)palladium(II) dichloride (PEPPSI) as the catalyst in NMP at 140 °C to afford the corresponding 3′methyl (*E*)-cinnamate **1j** in an unoptimized 35% yield.

Having achieved the preparation of dihydronaphtalenes **1**, we next focused our attention on their catalytic reduction to give restricted *iso*erianin analogues **2**. Thus, 4-aryltetrahydronaphtalenes **2a**–**e** were obtained in acceptable yields using H_2 in the presence of Pd/C in MeOH. Finally, aromatization of compound **1a** into naphtalene derivative **3a** was attempted using a variety of oxidizing species, including Pd/C, SeO_2, *o*-chloranil, *p*-chloranil, and SO_3-pyridine complex. However, the oxidation reactions were unsuccessful and gave **3a** in very poor yields (<10%). After several trials, we found that the oxidation of **1a** with DDQ in CH_2Cl_2 afforded the desired naphtalene **3a** but in a moderate 33% yield. Introduction of various substituents on **3a** was next achieved in a similar manner as described above for **1d**–**j** from **1a**, **b**. Using a similar route for the synthesis of **1d**, dihydronaphtalene **1l** bearing a NH_2 substituent at the C7 position was prepared from 7-bromo-6-methoxytetralone **4c** [47] for structure–activity relation study (Scheme 2).

2.2. Biological results

In vitro antiproliferative activity of the synthesized naphthalene derivatives **1**–**3** was first determined against the human colon carcinoma cell line (HCT116) using CA-4 [48] and *iso*CA-4 [30] and