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Research paper

4,5-Diaryl-3*H*-1,2-dithiole-3-thiones and related compounds as combretastatin A-4/oltipraz hybrids: Synthesis, molecular modelling and evaluation as antiproliferative agents and inhibitors of tubulin

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ABSTRACT

A new series of 4,5-diaryl-3*H*-1,2-dithiole-3-thiones and related compounds were designed and synthesised as combretastatin A-4/oltipraz hybrids. We evaluated the antiproliferative activities, inhibition of tubulin polymerization, and cell-cycle effects of these compounds. Several compounds in this series, such as **4d** and **5c**, displayed significant activity against SGC-7901, KB and HT-1080 cell lines, as determined using MTT assays. The most active compound, **4d**, markedly inhibited tubulin polymerization, with an IC_{50} value of 4.44 μM being observed. In mechanistic studies, **4d** caused cell arrest in G2/M phase, induced apoptotic cell death, and disrupted microtubule formation. Molecular docking studies revealed that **4d** interacts and binds efficiently with the tubulin protein.

1. Introduction

Microtubules formed by the polymerization of α- and β-tubulin heterodimers are essential for a variety of fundamental cell functions, including mitosis, cell replication, maintenance of cell shape, cellular transport and motility [1,2]. Therefore, tubulin represents a proven target for anticancer drug development. Combretastatin A-4 (CA-4, **1a**), a natural *Z*-stilbene isolated from the South Africa bush willow *Combretum caffrum*, strongly inhibits the polymerization of tubulin by binding to the colchicine site, and it exhibits excellent antiproliferative activity against a wide variety of cell lines, including multidrug-resistant lines, by arresting the cell cycle in G2/M phase [3,4]. Recently, CA-4 (**1a**) has drawn significant attention due to its potent and selective effect on established tumour vasculature. The sodium phosphate of CA-4 (CA-4P, **1b**), a water-soluble prodrug, has yielded promising results in human clinical trials as a vascular disrupting agent that selectively hinders blood flow by occluding tumour vasculature, resulting in hypoxia and, ultimately, tumour necrosis [5–10]. Because of this agent's interesting bioactivity and simple structure, a wide number of CA-4 (**1a**) analogues have been synthesised and evaluated [11–16]. Several structure-activity relationship (SAR) studies demonstrated that a 3,4,5-trimethoxy substituted A-ring and a 4-methoxy substituted B-ring linked by a two-atom bridge with *cis* configuration are important for the potent antitubulin and antiproliferative activities of CA-4 (**1a**) and related compounds [17].

Over the years, molecular hybridization strategy has been employed to discover promising chemical architectures displaying significant anticancer profiles. Chlorambucil, nitrogen mustard/

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phenylbutyrate hybrids, have served as the front-line therapy for CLL/SLL for several decades given as low-dose continuous treatment or higher dose intermittent therapy [18]. Estramustine phosphate (EMP), nitrogen mustard/estradiol-17β phosphate hybrids which were introduced in the early 1970s, have been predominantly evaluated as a second-line option for patients with CRPC [19]. One viable strategy for developing novel CA-4 (**1a**) analogues focuses on hybrid compounds that combine the structural features of CA-4 (**1a**) and other antiproliferative agents Several types of hybrid compounds have been designed and synthesised, including combretastatin A-4/lamellarin T hybrids, combretastatin A-4/nitrogen mustard hybrids, combretastatin A-4/*N*-phenyl-*N*′-(2-chloroethyl)urea hybrids, combretastatin A-4/flavone hybrids and combretastatin A-4/lamellarin D hybrids, and these compounds displayed significant antiproferative activity [20–24].

Oltipraz (OPZ, **2**), which was originally developed as an antischistosomal agent, is a synthetic derivative of the naturally occurring dithiolthiones found in cruciferous vegetables, and it is currently being evaluated in clinical studies as a chemopreventive agent for hepatocarcinogenesis [25]. In addition to cancer chemopreventive activity, OPZ (**2**) possesses potent antiangiogenic activity in vitro, *ex vitro* and *in vivo*. In athymic mice xenografted with angiosarcoma cells, OPZ treatment (**2**) decreased tumour mass by 81% [26–28].

In our efforts to search for new antiproliferative agents, we designed a set of 4,5-diaryl-3*H*-1,2-dithiole-3-thiones (**3a**-**3g**) and related compounds, 4,5-diaryl-3*H*-1,2-dithiole-3-ones (**4a**-**4g**) and 4,5-diaryl-3*H*-1,2-dithiole-3-one oximes (**5a**-**5f, 6a**-**6d**), as combretastatin A-4/oltipraz hybrids. 1,2-Dithiole-3-thione, -one or -one oxime ring,the core of OPZ (**2**), was utilised to mimic the *cis* double bond in CA-4 (**1a**). A 3,4,5-trimethoxy phenyl unit, the A-ring of CA-4 (**1a**), was attached at the C_4- or C_5-position of the central 1,2-dithiole-3-thione, -one or -one oxime ring; the other aryl ring, the B-ring of CA-4 (**1a**), was linked at the C_5- or C_4-position (see Fig. 1).

In this study, we report the synthesis and in vitro antiproferative activities of CA-4/OPZ hybrids as well as the inhibitory effects of the most active compounds on tubulin polymerization and cell survival. Furthermore, we performed molecular modeling studies for one of the most potent CA-4/OPZ hybrids, **4d**, which binds into the colchicine binding site of α, β-tubulin.

2. Results and discussion

2.1. Chemistry

The 4,5-diphenyl-1,2-dithiolane-3-thione skeleton can be synthesised via the reaction of β-ketoester and P_2S_5 [29]. It also can be prepared from α-methylstyrene and S powder [30]. The first method was used to construct the target 4,5-diphenyl-1,2-dithiolane-3-thiones. According to the procedure described in Scheme 1, commercially available methyl phenyl acetates (**7a**-**7c**) were treated with substituted benzoic acids (**8a**-**8d**) in the presence of CDI/NaH in anhydrous DMF to give β-ketoesters (**9a**-**9f**) in 70–75% yield [31,32]. Treatment of respective β-ketoesters (**9a**-**9e**) with 4-fold excess of P_2S_5 in refluxing toluene at 110 °C produced the 4,5-diaryl-3*H*-1,2-dithiole-3-thiones (**3a**, **3b**′, **3c**, **3e** and **3f**) in 60–70% yield. Then, these compounds were converted to corresponding 4,5-diaryl-3*H*-1,2-dithiole-3-ones (**4a**, **4b**′, **4c**, **4e** and **4f**) using potassium permanganate in THF at room temperature [33]. The resulting compounds could be obtained in high yields of up to 90–93% when the reaction time was extended to 24 h. The 4,5-diaryl-3*H*-1,2-dithiole-3-one oximes (**5a**, **5b**, **5c**′, **5e**, **6a**, **6b**′ and **6c**) were prepared in approximately 90%–95% yield from the corresponding 4,5-diaryl-3*H*-1,2-dithiole-3-thiones by treatment with hydroxylamine hydrochloride or *O*-methylhydroxylamine in refluxing ethanol [34]. Removal of *p*-methoxybenzyl PMB in **3b**′ and **4b**′ in a refluxing 3:1 mixture of EtOH/1 N HCl (3:1) afforded the target compounds, **3b** and **4b** [35], in approximately 85–90% yield.

The selective reduction of nitro groups in the presence of disulphide bond is a major concern, and therefore, we carefully screened and selected the required reductants. Apart from zinc dust/glacial acetic acid, several other reducing agents were introduced, including $SnCl_2$, $NaBH_4$ and H_2/Pd–C. However, this reaction led to a complex mixture. Finally, sodium hydrosulfite demonstrated high efficiency for the reduction of aromatic nitro groups and high selectivity in the presence of the other reducible moiety [36]. With this background, the substituted aromatic nitro compounds (**3c**, **3f**, **4c**, **4f**, **5c**′, **5e**, **6b**′, **6c**) were reduced to the corresponding aromatic amine compounds (**3d**, **3g**, **4d**, **4g**, **5c**, **5f**, **6b**, **6d**) by treatment with sodium hydrosulfite in a refluxing 3:1 mixture of acetone/water in approximately 85–90% yield.

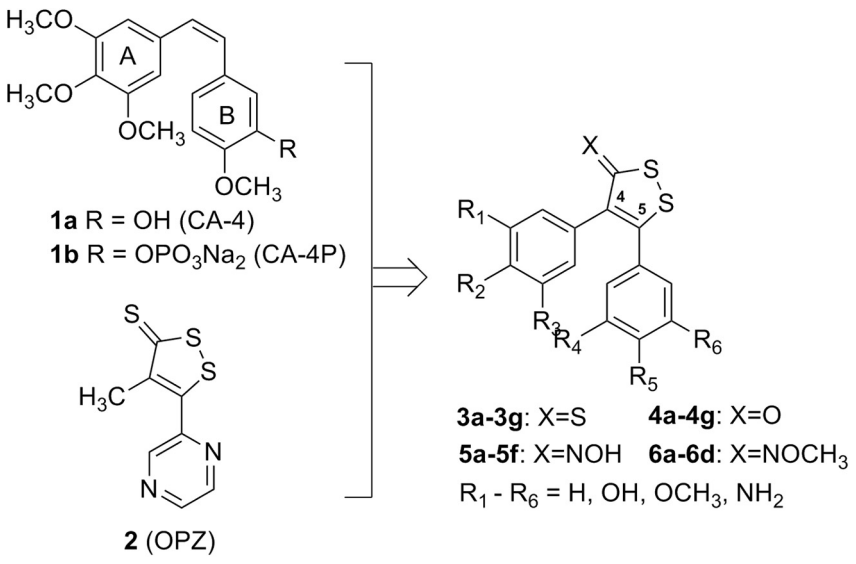

Fig. 1. Structures of combretastatin A-4 (CA-4, **1a**), the sodium phosphate of CA-4 (CA-4P, **1b**), Oltipraz (OPZ, **2**) and novel CA-4/OPZ hybrids.