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Combretastatin linked 1,3,4-oxadiazole conjugates as a Potent Tubulin Polymerization inhibitors

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ABSTRACT

A new class of combretastatin linked 1,3,4-oxadiazoles were designed, synthesized and screened for their cytotoxic activity against five human cancer cell lines such as HeLa, DU-145, A549, MDA-MB-231 and B16. These compounds showed significant cytotoxicity with IC_{50} values in the range 0.118–54.32 μM. Conjugate **5m** displayed potent antiproliferative activity against DU-145 cell line. Flow cytometric analysis revealed that these compounds arrested the cell cycle in G2/M phase. Moreover, the tubulin polymerization assay and immunofluorescence analysis indicate that **5m** exhibits potent inhibitory effect on the tubulin assembly. Further, DNA fragmentation and Hoecst staining assays confirm that **5m** induces apoptosis. Molecular docking studies and competitive binding assay indicated that **5m** effectively bind at the colchicine binding site of the tubulin.

1. Introduction

Microtubules are essential for various cellular processes including motility, cell signalling and division, shape maintenance and intracellular transport [1,2]. They are formed by the polymerization of tubulin heterodimers and dissociate back during normal functioning of the cell. This process is highly aberrant in case of cancer thereby rendering it to be an attractive target for the development of newer anticancer agents [3,4]. Drugs such as paclitaxel and vinblastine that are used for the treatment of several types of cancers act by interfering with microtubule dynamics. Their clinical success indicates the immense potential of this therapeutic target and attracted interest of numerous research groups across the globe to find out microtubule targeting agents [5,6].

Combretastatin A-4 (CA-4,**1**, Fig. 1) is a *cis*-stilbene natural product isolated by Pettit and coworkers from the bark of *Combretum caffrum* [7]. It showed cytotoxicity against a wide range of human cancer cell lines including multidrug-resistant cancer cells [8]. It is a microtubule-destabilizing agent and interacts strongly with the β-tubulin by binding into the colchicine binding site [9]. Structure–activity relationship (SAR) studies revealed that 3,4,5-trimethoxyphenyl ring (ring-A) and *cis* configuration of the stilbene connecting the ring A to ring B are essential for antiproliferative activity [10–12]. Whereas the 3′-hydroxy group of ring B is considered to be nonessential for the interaction of CA-4 with the tubulin and therefore it has been replaced by other suitable moieties however, CA-4 suffers from bioavailability and stability issues [13]. The *cis* double bond readily isomerizes to the stable *trans* form that leads to loss of both cytotoxicity as well as antitubulin activity [14]. Therefore, several attempts made to overcome the stability issues led to the development of phenstatin and *iso* CA-4 derivatives (**1b-e**) [15,16]. Similarly, strategy of replacing the olefinic bridge with several carbocycles and heterocycles has also been adapted to overcome the isomerization. Some of the heterocycles include pyrazole, isoxazole, oxadiazole, imidazole, aminothiazole, benzofuran etc. This replacement was found to be fruitful as both cytotoxicity and antitubulin activity have not only been retained but also improved in most of the cases [17–19].

On the other hand, oxadiazoles are known to possess various biological activities such as antibacterial, *anti*-inflammatory and anticancer etc. [20–24]. 1,3,4-Oxadiazole moiety shows lower lipophilicity and high metabolic stability, hERG inhibition and aqueous solubility compared to 1,2,4-oxadiazole [25]. One of the compounds containing an oxadiazole ring, zibotentan (**2**) is a pyridine based sulfonamide that exhibits remarkable antiproliferative activity [26]. Considering their biological potential we have

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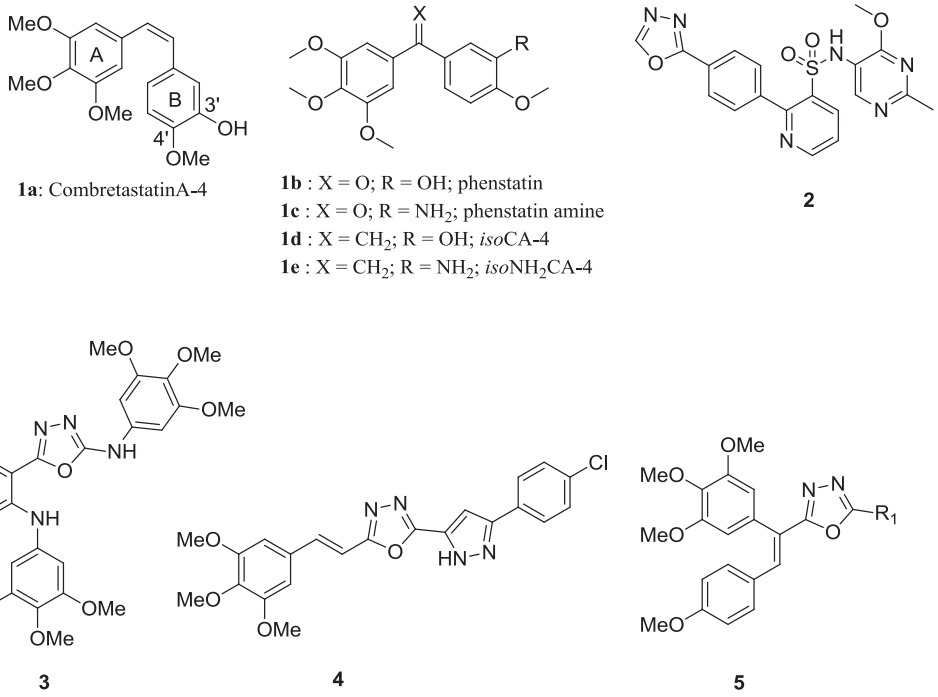

Fig. 1. Chemical structures of Combretastatin A-4 (**1a**), phenstatin (**1b**), phenstatin amine (**1c**), *iso*combretastatin A-4 (**1d**), *iso*combretastatin A-4 amine (**1e**), Zibotentan (**2**), anilinonicotinyl–oxadiazole conjugate (**3**), pyrazole–oxadiazole conjugate (**4**), combretastatin linked oxadiazole conjugates (**5**).

previously developed and reported newer anticancer agents by conjugating it to other pharmacophores such as 2-anilinonicotine and pyrazole [27,28]. These conjugates effectively inhibited tubulin polymerization which indicates the immense potential of oxadiazole structure to be useful in the development of microtubule destabilizing agents. Therefore, in order to develop newer anticancer agents based on CA-4 scaffold an attempt has been made to rigidify its unstable ethenyl bridge by introducing substituted oxadiazole moiety adjacent to the ring A. We herein report the synthesis and cytotoxic activities of these oxadiazole linked combretastatin congeners along with a brief study to determine their mode of action.

2. Results and discussion

2.1. Chemistry

The desired target molecules (**5a-p**) were obtained from the starting material trimethoxyphenylacetic acid (**6**) prepared by Perkins condensation of 3,4,5-trimethoxyphenylacetic acid (**6**) and 4-methoxybenzaldehyde (**7**) in the presence of acetic anhydride and triethylamine under refluxing conditions to afford (*E*)-3-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylic acid (**8**) [29]. The hydrazide intermediate (**11**) was prepared from substituted aromatic and hetero aromatic carboxylic acids by esterification followed by their reaction with hydrazine hydrate and H_2SO_4 in EtOH [30]. The formation of oxadiazole scaffold from carboxylic acids and hydrazides has been well investigated by various researchers [31–35]. Therefore, the desired combretastatin-oxadiazoles conjugates (**5a-p**) were obtained by following one of the known methods i.e., condensation of aroylhydrazides (**11a-p**) and acid (**12**) in $POCl_3$ (Scheme 1).

2.2. Antiproliferative activity

The synthesized conjugates **5a-p** were evaluated for their antiproliferative activity against five human cancer cell lines namely cervix (HeLa), prostate (DU-145), lung adenocarcinoma (A549), breast adenocarcinoma (MDA-MB-231) and mouse macrophages (B-16) cell line using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [36]. The results expressed as IC_{50} values are summarized in Table 1 and CA-4 has been employed as a positive control for comparison. Results indicate that some of the compounds exhibit significant cytotoxicity in almost all the tested cell lines with IC_{50} values ranging between 0.118 and 54.3 μM. Particularly, compounds **5l** and **5m** showed significant cytotoxicity against all the tested cell lines displaying IC_{50} below 3 μM. In fact, **5m** containing 3,4,5-rimethoxy substituent was found to be the most effective compound from the series against the prostate cancer cell line (IC_{50} = 0.118 μM). Similarly, **5k** exhibited significant cytotoxicity particularly against HeLa, DU-145 and A549 cells.

Based on the cytotoxicity data, the structure–activity relationship (SAR) for these combretastatin linked 1,3,4-oxadiazole conjugates has been elucidated (Fig. 2). Keeping the stilbene pharmacophore constant the effect of modifications on the phenyl ring at the second position of 1,3,4-oxadiazole moiety has been examined. The phenyl ring containing various substituents with electron donating and electron withdrawing nature have been employed in this study. In addition, the effect of hetero aromatic rings such as thiophenyl, 2-furfuryl and 3-benzofuran have also been investigated. Compounds containing electron donating groups exhibited very good activity. Compound **5m** having trimethoxy substituent on C-ring displayed excellent anticancer efficacy towards DU-145 cell line with IC_{50} value 0.118 μM. The cytotoxicity was found to decrease with the removal of methoxy group which is evident from the activities of **5l** and **5k** containing 3,4-dimethoxy and 4-methoxy substituents respectively (IC_{50} = 0.274 and 0.538 μM respectively against DU-145). Further decrease in the activity was observed when methoxy group is replaced by a N,N-dimethylamino and methyl group as in **5i** and **5j** (IC_{50} = 2.138 and 5.108 μM respectively against DU-145). Compounds with weak electron withdrawing groups such as halogen substituents exhibited moderate efficacy. It was observed that cytotoxicity decreases with the increase in size of the halogen atom and thus cytotoxicity for fluoro, chloro and bromo is in the