European Journal of Medicinal Chemistry 122 (2016) 232–246

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Synthesis of a series of novel dihydroartemisinin monomers and dimers containing chalcone as a linker and their anticancer activity

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ARTICLE INFO

Article history:
Received 23 December 2015
Received in revised form
17 June 2016
Accepted 19 June 2016
Available online 22 June 2016

Keywords:
Artemisinin
Monomer and dimer
Apoptosis
Leukemia
Synthesis

ABSTRACT

A new series of monomer and dimer derivatives of dihydroartemisinin (DHA) containing substituted chalcones as a linker were synthesized and investigated for their cytotoxicity in human cancer cell lines HL-60 (leukemia), Mia PaCa-2 (pancreatic cancer), PC-3 (prostate cancer), LS180 (colon cancer) and HEPG2 (hepatocellular carcinoma). Some of these derivatives have greater antiproliferative and cytotoxic effects in tested cell lines than parent compound DHA. The structures of the all compounds were confirmed by IR, ^{1}H NMR and mass spectral data. Among the new derivatives, compounds **8**, **14**, **15**, **20** and **24** were found to be more active than parent DHA against tested human cancer cell lines. DHA derivatives were found to be most active in human leukemia cell lines with compounds **8**, **14**, **15**, **20** and **24** showed IC_{50} values less than 1 μM for 48 h whereas DHA has IC_{50} value of 2 μM at same time period. The most potent compounds **8** with $IC_{50} = 0.3$ μM (at par with doxorubicin ($IC_{50} = 0.3$ μM)) and **15** with $IC_{50} = 0.4$ μM, of the series, six and three times active than DHA (with $IC_{50} = 2$ μM) respectively were selected for further mechanistic work in human leukemia HL-60 cells.

1. Introduction

Artemisinin (**1**), isolated from *Artemisia annua* L. [1] contains a 1, 2, 4-trioxane moiety (Fig. 1). Artemisinin and its derivatives dihydroartemisinin (DHA, **2**), artemether (**3**), arteether (**4**), and artesunate (**5**) have been developed as a new type of antimalarial drugs [2a]. Recently we have synthesized twenty five ether and ester derivatives having significant antimalarial activity [2b]. In addition to their antiparasitic properties, it is of considerable interest to note that artemisinin derivatives are cytotoxic towards cancer cell lines *in vitro* [3]. Interestingly, the recent discovery of artemisinins as anticancer agents against various cancer cell lines have evoked many interests on this class of compounds [4-21a]. Artemisinin (**1**) and its derivatives are significantly cytotoxic towards murine lymphocytic leukemia (P-388), human lung carcinoma (A-549), human colon adenocarcinoma (HT-29) and other tumour cell lines [21b]. However, one of its derivatives appeared to be more cytotoxic than artemisinin (**1**) towards Ehrlich ascites tumour (EAT) cells [21c,21d].

Artemisinin dimers are obtained by joining two artemisinin molecules without destroying their endoperoxide bridge. Endoperoxide bridge in artemisinin is the key factor for its outstanding medicinal value. Due to sensitive nature of endoperoxide ring of artemsisinin majority of artemisinin analogues were synthesized via chemical modification of artemisinin at its C-10/C-13 position [22,23]. Beekman et al. synthesized C-10 acetal dimers where two artemisinin units are connected through an ether-linkage possessing good anticancer activity [24]. Posner et al. synthesized a series of C-10 acetal artemisinin dimers linked through a polyethylene glycol or carbon chain link or disulfide linker, with varying length and flexibility [25,26]. Lee et al. reported that introduction of a sulfur atom to an artemisinin moiety affords new derivatives which selectively control tumor related angiogenesis [27,28].

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http://dx.doi.org/10.1016/j.ejmech.2016.06.035
0223-5234/

Although different classes of cytotoxic artemisinin derivatives have been prepared, identification of the key factors contributing to their cytotoxicities and rational design of new classes of cytotoxic artemisinin analogues remain difficult. In this connection, it is of importance to conduct systematic structure-activity relationship (SAR) studies to assist the design and synthesis of new cytotoxic artemisinin derivatives in a rational manner.

Chalcone is an open chain flavonoid α, β-unsaturated carbonyl group and is one of the important compound groups derived from nature identified in *Angelica Keiskei* and exhibit interesting pharmacological activities [29,30]. Chemically, they are open-chained molecules bearing two aromatic rings linked by a three-carbon enone [31]. Both natural and synthetic chalcones exhibit various activities like antimicrobial, antimalarial, anti-inflammatory, anticancer, antioxidant and many more [29]. These activities are largely being attributed due to the unsaturated ketone moiety. Chalcone and its derivatives have been reported to be cytotoxic for cancer cells including leukemia cells [32].

Some of the new dimeric trioxane also have selective and potent anticancer activity [33]. Increasingly widespread evidence indicates that human cancer cells, richer than normal cells in iron-transport transferrin receptors [34,35], selectively activate trioxanes to produce various cytotoxic intermediates; this process is similar to that in the triggering of trioxanes by heme iron in malaria-infected human erythrocytes [8]. The anticancer properties of trioxanes have been reviewed [36–40]. Here we disclose that some of the new dimeric trioxane and monomers powerfully inhibit the growth (submicromolar IC_{50} values) of various cancer cells *in vitro*.

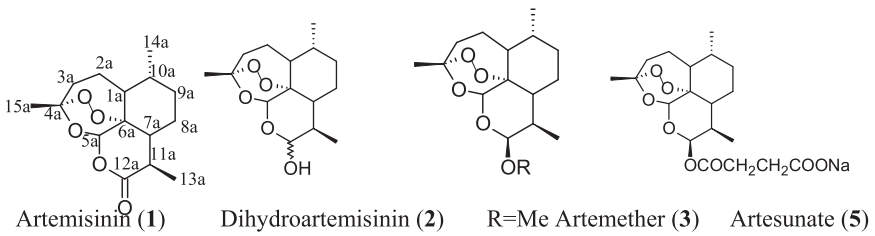
As more analogs were evaluated for antitumor activity, the unsymmetrical DHA acetal dimer was reported as being highly cytotoxic and more potent than cis-platin [1], while the symmetrical DHA acetal dimer also exhibited pronounced cytotoxic effects [41a]. In continuation of our work on artemsinin and chalcone in the area of antimalarial and anticancer drug discovery [41b-41e], these findings stimulated an interest in preparing additional DHA acetal dimers with various linkers. Synthesis and evaluations of many diversified dimers have been reported. However chalcone dimers are new and reported for the first time in this paper. We designed a new group of DHA monomers and dimers containing chalcone with a different substituent, linked by ether and chalcone as a linker in dimers and examined their *vitro* cytotoxic activities on human leukemia HL-60 cells, pancreatic cancer Mia PaCa-2 cells, prostate cancer PC-3 cells, colon cancer LS180 cells, hepatocellular carcinoma HEP G2. Since chalcones are known to have a wide variety of biological activities including anti-proliferative activity against leukemia cells, this study seems is an approach to develop drug like candidates.

In general, the cytotoxicities of artemisinin derivatives depends on type of linker [42,43] which decides efficient diffusion of compounds through membranes [44]. Yet, the effects of solubility, the stereochemistry (configuration and conformation) [42,45–51] and nature of functional groups of artemisinin derivatives could not be distinguished [47–51]. Therefore, structure modification might improve their anti-cancer activities. Several groups have performed modifications at the C-12 position of artemisinin and reported that the addition of an alkyl carbon chain (C_8H_{17} to $C_{16}H_{33}$) or a cyanoarylmethyl group significantly improved its antitumor activities [51]. In addition, it has been found that the endoperoxide in artemisinin is required for the cytotoxic activity. These data provide a rationale for the modification of artemisinin's structure in order to improve its antitumor activity.

2. Results and discussion

We have designed and synthesized a new group of DHA monomers and dimers containing chalcone moiety with a different substituent, linked by C-12 ether linkage/s. The synthetic pathways are shown in Scheme 1. Firstly, various chalcone based (basic structure: 1, 3-diphenyl-2-propene-1-one, Fig. 2.) analogues were synthesized by Claisen-Schmidt condensation based on a method reported previously [52,53]. In this process, an acetophenone was reacted with the corresponding aldehyde under acid/base catalyst. In the second step, the DHA prototype, 2-(12β-dihydroartemisinoxy)-ethyl bromide (**2P**) was synthesized according to a reported procedure [40], by the reaction of bromoethanol and DHA in presence of $BF_3.OEt_2$ in dichloromethane. The intermediate compound **2P** was crystallised with methanol and filtered as white crystals. Finally, in the third step the targeted artemisinin monomers **6**–**20** (using 1 eq. of **2P** and 1 eq. of respective chalcone) (Scheme 1.) and dimers, **21**–**25** (using 2 eq. of **2P** and 1 eq. of respective chalcone) (Scheme 2.) were successfully obtained by the reaction of the DHA prototype, **2P** and appropriate chalcones in the presence of K_2CO_3 and KI in *N*,*N*-dimethylformamide (DMF) at 60 °C, respectively. The products obtained were purified by silica gel column chromatography.

The stereochemistry (αH-12 or βH-12) of these compounds was confirmed by the application of ^{1}H NMR technique which analyzes the chemical shift of H-12 and the coupling constant between H-11 and H-12. The DHA aliphatic ethers are 12β-isomers as indicated by a chemical shift (4.60–4.90 ppm) and a small coupling constant (J = ~3.3 Hz) [28]. The geometrical configuration of the chalcone (α, β-unsaturated ketone) side chain was determined to be *trans* by observing the coupling constant (J = ~15.6 Hz) between H-α (H-2) and H-β (H-3). Among the synthesized compounds, **8**, **14**, **15**, **20** and **24** were found to be more active than parent DHA against tested human cancer cell lines. DHA derivatives were found to be most active in human leukemia cell lines with compounds **8**, **14**, **15**, **20** and **24** showed IC_{50} values less than 1 μM for 48 h whereas DHA has IC_{50} value of 2 μM at same time period. It is evident from Table 2 that a progressive increase in electron density on ring A/B of chalcone resulted in progressive enhancement in anticancer potency, with the para hydroxyl substituted hybrids **8** and **15**



Artemisinin (**1**) Dihydroartemisinin (**2**) R=Me Artemether (**3**) Artesunate (**5**)

R=Et Arteether (**4**)

Fig. 1. Artemisinin and its derivatives.