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Original article

Synthesis and study of cytotoxic activity of 1,2,4-trioxane- and egonol-derived hybrid molecules against *Plasmodium falciparum* and multidrug-resistant human leukemia cells

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ABSTRACT

Malaria and cancer cause the death of millions of people every year. To combat these two diseases, it is important that new pharmaceutically active compounds have the ability to overcome multidrug resistance in cancer and *Plasmodium falciparum* strains. In search of effective anti-cancer and anti-malaria hybrids that possess improved properties compared to their parent compounds, a series of novel 1,2,4-trioxane-based hybrids incorporating egonol and/or ferrocene fragments were synthesized and tested *in vitro* against *P. falciparum* strains, CCRF–CEM cells and the multidrug-resistant P-glycoprotein-over-expressing CEM/ADR5000 cells. The most active compounds against *P. falciparum* strains were artesunic acid homodimers **12** and **13** (IC_{50} of 0.32 and 0.30 nM, respectively), whereas novel hybrids **7** (1,2,4-trioxane–ferrocene–egonol), **9** (1,2,4-trioxane–ferrocene) and **11** (artesunic acid–egonol) showed a remarkable cytotoxicity toward CCRF–CEM cells (IC_{50} of 0.07, 0.25 and 0.18 μM, respectively). A cooperative and synergistic effect of the three moieties 1,2,4-trioxane, ferrocene and egonol in hybrid molecule **7** is significant and is obviously stronger than in hybrids **9** (1,2,4-trioxane–ferrocene) and **11** (artesunic acid–egonol), which comprises of only two of the three considered parent compounds. Interestingly, hybrid **9** containing a 1,2,4-trioxane and a ferrocene fragment has shown to be the most effective among the studied hybrids against the tested multidrug-resistant leukemia CEM/ADR5000 cells (IC_{50} of 0.57 μM) and possesses a degree of cross-resistance of 2.34.

1. Introduction

Malaria infections caused by *Plasmodium falciparum* annually lead to more than one million deaths, mainly in Africa, Asia and South America [1]. After the appearance of *P. falciparum* strains, which are resistant to well-established anti-malarial drugs (e.g. chloroquine (CQ)), new drug candidates capable to combat drug resistance are urgently needed [2].

In addition to malaria, another big challenge in human medicine is the fight against cancer, especially the overcoming of multidrug resistance. This disease also causes the death of millions of people every year [3].

A promising redox-directed, multidrug resistance overcoming anti-malarial [4] and anti-cancer [5] compound is artemisinin (**1**) (Fig. 1) – a natural product and well known representative of the 1,2,4-trioxane family from the plant *Artemisia annua* L. Artemisinin (**1**) contains an endoperoxide bridge, that can be activated and fragmented by intracellular Fe(II) leading to the formation of peroxyl free radicals and reactive oxygen species (ROS), which induce oxidative stress, DNA damage, alkylation of target proteins and apoptosis [6,7]. In case of malaria a disruption of the cellular redox cycling [8], an inhibition of a calcium-ATPase (SERCA, pFATP6) as

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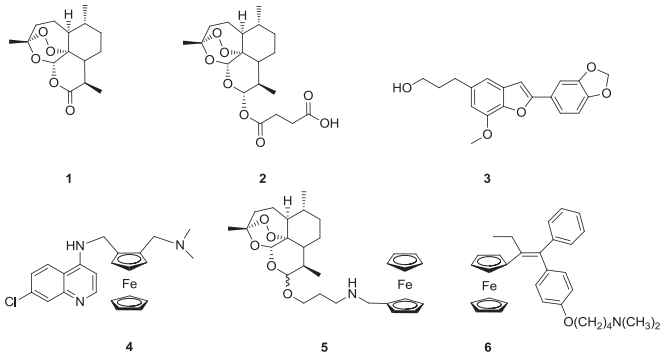

Fig. 1. Compounds with anti-malaria and/or anti-cancer activity.

well as a depolarization of the mitochondrial potential is discussed furthermore [9]. In artemisinin's mode of action against cancer also the inhibition of metastasis [10], cancer-related signaling pathways [11] and inhibition of angiogenesis occurs [5g,12].

A major remarkable advantage of artemisinin is its lack of side effects on normal cells [13]. The applicability of artemisinin (**1**) as drug is, nevertheless, limited by its low solubility and poor oral bioavailability [14]. A solution for these problems may be derivatives of artemisinin (**1**), such as artesunic acid (**2**) (Fig. 1). This compound has also been shown to induce apoptosis [5a,15] and inhibit angiogenesis [5g].

To develop even more effective drug candidates than artemisinin (**1**) and artesunic acid (**2**) against cancer and malaria chemical hybridization as a known and promising approach may be applied: Two or more natural product fragments are linked with each other and thereby new structures are obtained that possess improved properties compared to their parent compounds [16].

Several homodimer and hybrid molecules based on artemisinin (**1**) are described in literature [17], but homodimers containing artesunic acid (**2**) have been scarcely reported as yet [18], although they possess great therapeutic potential.

As a continuation of our interest in the exploration of the potential of 1,2,4-trioxanes (including artesunic acid) for the preparation of potent anti-cancer and anti-malaria homodimers and hybrid molecules [18], we envisioned the synthesis of novel target trioxane-based hybrids incorporating egonol (**3**) and/or ferrocene fragments (compounds **7**–**11**, Fig. 2).

Egonol (**3**) is a natural benzofuran that is widely distributed in *Styrax* species and possesses interesting biological properties such as insecticidal, fungicidal, anti-microbial, anti-proliferative, and cytotoxic activities toward cancer cell lines [19], as well as cyclooxygenase inhibitory [20] and anti-oxidant properties [21].

Since ferrocene derivatives have proven to be active anti-malaria (e.g. ferroquine (**4**) and hybrid **5**) [22] and anti-cancer (e.g. ferrocifen (**6**), Fig. 1) [23] agents, we selected ferrocene as a promising hybrid subunit and/or linker. In the presence of ferrocene-linker the oxidative cleavage of the endoperoxide bond of the trioxane fragment might be triggered more facially and consequently a higher amount of ROS with involvement of Fenton chemistry [6e] might be formed, leading to the intrinsic cell death pathway.

Here we wish to report the synthesis of the designed trioxane-derived hybrids **7**–**11** (Fig. 2) from easily accessible starting materials in a few chemical steps, as well as the results of their biological tests against drug-sensitive CCRF–CEM leukemia cells, their multidrug-resistant sub-line CEM/ADR5000 and against the *P. falciparum* 3D7 strain. The evaluation of the anti-malaria activity of our previously reported homodimers **12** and **13** (highly active against leukemia) [18b] will be also discussed in the present work.

2. Results and discussion

2.1. Chemistry

Artemisinin (**1**) and its derivatives are active anti-malarial compounds, especially since they can also successfully combat complicated and severe forms of malaria and overcome resistance of Plasmodia to other anti-malarial drugs [24]. Artemisinin-type drugs also inhibit cancer growth *in vitro* and *in vivo* [5g,5j,5k,25]. First clinical reports indicate that they are active in cancer patients [26].

One strategy to kill otherwise unresponsive *Plasmodia* or cancer cells is to use compounds with novel modes of action. The generation of hybrid molecules has been described during the past decade as an attractive approach in pharmaceutical and medicinal chemistry [16]. In the present investigation we synthesized and analyzed 1,2,4-trioxane and egonol-derived novel hybrid molecules (Fig. 2) for their activity toward malaria parasites (*P. falciparum* 3D7) and leukemia cancer cells (CCRF–CEM and multidrug-resistant CEM/ADR5000 cells).

All compounds of the present work (Fig. 2) were accessible starting from commercially available artesunic acid (**2**), from plants (egonol (**3**)) or easily obtained precursors (1,2,4-trioxane-derived alcohol **16**). The literature known alcohol **16** [17g,27] was chosen as a building block for some of our novel hybrid molecules (e.g. **7** and **9**) as it has been shown that C-10 non-acetal substances are 15- to 22-times more stable and reveal a greater bioavailability than recently established drugs like artemether or arteether [28]. Additionally, this precursor was already applied for the synthesis of very effective anti-cancer and anti-malaria agents [27e].

Based on 1,2,4-trioxane-derived alcohol **16** and natural product egonol (**3**), hybrids **7**–**10** were prepared. The approach of linking 1,2,4-trioxanes with ferrocene is rare, but does have precedent (e.g. anti-malaria hybrid **5**, Fig. 1) [22b], yet to our knowledge there are no reports on hybridization of egonol (**3**) with 1,2,4-trioxane and/or ferrocene compounds.

The egonol-derived hybrid **11** was synthesized for the first time starting from artesunic acid (**2**). The synthesis and studies of cytotoxic activity of artesunic acid homodimers **12** and **13** against human leukemia cells were recently reported by us [18b]. In the present work we studied the anti-malaria activity of these homodimers **12** and **13** for the first time.

Generally, the applied syntheses of this research work can be divided into three groups (Scheme 1): At first, attempts were made to synthesize the novel hybrids **7** and **8** using a Mitsunobu reaction. These approaches did not yield any of the desired products. Therefore, an alternative synthetic route had to be found. To get a very reactive precursor, ferrocene dicarboxylic acid dichloride **15** was synthesized in 85% yield using oxalyl chloride and pyridine in dry DCM. As a follow-up, dichloride **15** was reacted with the corresponding alcohols (1.0 eq of each alcohol for hybrid **7** and 2.0 eq of egonol (**3**) for hybrid **8**) in DCM using DMAP (4.0 eq) as a base. Applying these reaction conditions, 1,2,4-trioxane–ferrocene–egonol hybrid **7** was obtained in 30% yield and egonol–ferrocene–egonol hybrid **8** in 97% yield.

As the second reaction type a Mitsunobu reaction, to obtain 1,2,4-trioxane–ferrocene hybrid **9** and egonol–ferrocene hybrid **10** between the corresponding alcohol (1,2,4-trioxane-derived alcohol **16** or egonol (**3**), 1.0 eq) and ferrocene monocarboxylic acid **17** (1.1 eq with alcohol **16** and 1.0 eq with egonol (**3**)) in the presence of DIAD (1.0 eq) and PPh_3 (1.0 eq) in THF proved to be successful. The yields were moderate to good (68% for **10** and 77% for **9**) for the two compounds.

A Steglich esterification as the last of the three reaction types led to artesunic acid–egonol hybrid **11**. Artesunic acid (**2**) (1.0 eq) was