Bioorganic & Medicinal Chemistry 26 (2018) 3610–3618

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Access to new highly potent antileukemia, antiviral and antimalarial agents via hybridization of natural products (homo)egonol, thymoquinone and artemisinin

Aysun Çapcı Karagöz[a], Christoph Reiter[a], Ean-Jeong Seo[b], Lisa Gruber[b], Friedrich Hahn[c], Maria Leidenberger[d], Volker Klein[a], Frank Hampel[a], Oliver Friedrich[d], Manfred Marschall[c], Barbara Kappes[d], Thomas Efferth[b], Svetlana B. Tsogoeva[a,*]

[a] Organic Chemistry Chair I and Interdisciplinary Center for Molecular Materials (ICMM), Friedrich-Alexander University of Erlangen-Nürnberg, Nikolaus-Fiebiger-Straße 10, 91058 Erlangen, Germany
[b] Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, University of Mainz, Staudinger Weg 5, 55128 Mainz, Germany
[c] Institute for Clinical and Molecular Virology, Friedrich-Alexander University of Erlangen-Nürnberg, Schlossgarten 4, 91054 Erlangen, Germany
[d] Institute of Medical Biotechnology, Friedrich-Alexander University of Erlangen-Nürnberg, Paul-Gordon-Straße 3, 91052 Erlangen, Germany

ARTICLE INFO

Keywords:
Natural product hybrid
Artemisinin
Thymoquinone
Egonol
Antileukemia activity
Antimalarial activity
Antiviral activity

ABSTRACT

Hybridization of natural products has high potential to further improve their activities and may produce synergistic effects between linked pharmacophores. Here we report synthesis of nine new hybrids of natural products egonol, homoegonol, thymoquinone and artemisinin and evaluation of their activities against *P. falciparum* 3D7 parasites, human cytomegalovirus, sensitive and multidrug-resistant human leukemia cells. Most of the new hybrids exceed their parent compounds in antimalarial, antiviral and antileukemia activities and in some cases show higher *in vitro* efficacy than clinically used reference drugs chloroquine, ganciclovir and doxorubicin. Combined, our findings stress the high potency of these hybrids and encourages further use of the hybridization concept in applied pharmacological research.

1. Introduction

Nature is a great source of medicinal drugs or their precursors.[1] Many approved drugs are based on natural products. Most of the time, natural products able to cross the biological barriers and penetrate cells, which enables them to be ignition points for the leads in drug discovery with desirable pharmacokinetic properties.[2] Combination therapy is an important treatment option, which may not be restricted to one distinct targeting mechanism, but utilizes several different mechanisms of action, to treat a single disease. For example, the artemisinin-based combination therapy has been accepted as a first-line treatment for uncomplicated malaria by the World Health Organization.[3] However, the combination therapy of artesunate-mefloquine has started to fail due to the emerging drug resistance, which increases the risk of morbidity and mortality from malaria.[4] Besides combination therapy, the new concept is molecular hybridization of natural products and/or bioactive compounds towards novel leads able to overcome drug resistance.[5–8] Hybridization is a powerful concept which attracts attention in terms of improved pharmacological properties of parent compounds. In general, the concept of compound hybridization based on natural products gives the opportunity to make use of an additional unit serving as linker moiety. The linker might similarly be any pharmacophore or any unit to improve the properties of the individual compound fragments, for example: their stability, solubility, lipophilicity or bioavailability.[5–9] The reasons for improvements in drug efficacy can be multifaceted when comparing individual examples and may include a beneficial mechanistic complexity, a simultaneous uptake of the hybrid's two active subunits, synergy dosage effects or resistance evasion. Recently, we and other groups have designed, synthesized and analyzed new hybrid molecules in order to generate improved candidate compounds with broad biological activities, specifically strong *in vitro* against malaria parasites, cancer cell proliferation and cytomegalovirus infection.[10–22] In the present work, we designed and synthesized nine new hybrid molecules (Fig. 2) based on four different natural products (Fig. 1) and evaluated their activities against *P. falciparum* strain 3D7, leukemia cells (wild-type CCRF-CEM and P-glycoprotein overexpressing CEM/ADR5000 human leukemia cells) and human cytomegalovirus (HCMV) (Tables 1–3). Our natural product library has very effective examples such as egonol, homoegonol, thymoquinone and artemisinin derivatives (Fig. 1).

Abbreviations: DHA, dihydroartemisinin; DMAP, 4-(dimethylamino)-pyridine; DCC, *N,N′*-dicyclohexylcarbodiimide; DIAD, diisopropyl azodicarboxylate; EC_{50}, half maximal effective concentration; EtOAc, ethylacetate; eq, equivalent; HCMV, human cytomegalovirus; TMSOTf, trimethylsilyl trifluoromethanesulfonate; TLC, thin layer chromatography; TQ, thymoquinone; THF, tetrahydrofuran; PPh_3, triphenylphosphine; *P. falciparum*, *Plasmodium falciparum*; UV, ultraviolet

* Corresponding author.
E-mail address: svetlana.tsogoeva@fau.de (S.B. Tsogoeva).

https://doi.org/10.1016/j.bmc.2018.05.041
Received 10 April 2018; Received in revised form 21 May 2018; Accepted 23 May 2018
Available online 24 May 2018
0968-0896/

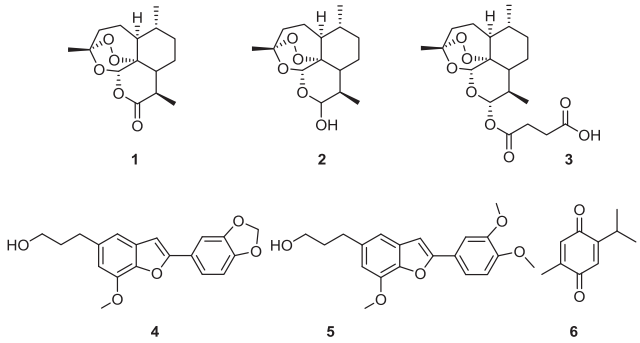

Fig. 1. Bioactive natural products and semisynthetic derivatives: artemisinin (**1**), dihydroartemisinin (**2**), artesunic acid (**3**), egonol (**4**), homoegonol (**5**) and thymoquinone (**6**).

Egonol and homoegonol are natural products that exist in *Styrax* species. These natural products were isolated from *Styrax officinalis* L.[23,24] which is known from ancient times as herbal medicine. These pharmacologically active compounds are formed in the 2-aryl-benzofuran chemical structure. Egonol and homoegonol have been studied by several researchers up to now. In previous studies, it has been reported that egonol and homoegonol show significant bioactivities such as anti-inflammatory, antioxidant, antimicrobial and anticancer activity.[25–28] Moreover, homoegonol is known to suppress the production of nitric oxide and proinflammatory mediators, and it is suggested as a therapeutic drug for allergic asthma.[29] Homoegonol has also been investigated for its intravenous and oral pharmacokinetics in rats, however, it displayed a very low bioavailability and high plasma protein binding.[30] Because of their notable pharmacological activities, the total synthesis of egonol and homoegonol have been achieved.[31–35]

Thymoquinone is a bioactive compound isolated from *Nigella sativa*.[36,37] According to numerous pharmacological studies, thymoquinone has been found to be a very promising anticancer natural product.[38,39] Artemisinin is an enantiomerically pure sesquiterpene lactone found in *Artemisia annua* L. Its isolation earned the Nobel Prize in 2015 to Youyou Tu.[40,41] Artemisinin and its derivatives are very important plant-originating compounds and they are of great interest for their remarkable wide range of biological properties.[42,43] Recently, target identification experiments have been performed and putative inhibitory mechanisms of artemisinin-related compounds were studied.[44–46] Moreover, its unique 1,2,4-trioxane ring has been investigated many times to enlighten its mechanism of action, which is not fully understood so far.[47–50] Herein, we present our results obtained from three different fields of analysis (antimalarial, antiviral and anticancer) with nine new hybrids prepared for the first time from above mentioned natural products. The results are described and discussed, to highlight hit compounds of this study and to provide further support for the broad usefulness of the natural product hybridization concept.

2. Results and discussion

2.1. Chemistry

The preparation of the precursors **17–24** (Fig. 3) of designed hybrids (Fig. 2) was performed according to synthetic methods we and other groups previously reported.[12,19,51–53] The synthesis of hybrid molecules **7–16** is outlined in Scheme 1. These new hybrid compounds were prepared from functionalized derivatives of artemisinin, egonol, homoegonol, thymoquinone (compounds **17–19**, **22–24**) and the anticancer drug chlorambucil, utilizing their functional groups such as carboxylic acid, primary alcohol, amine or aldehyde moieties (Fig. 3). In 2014, we published the first artesunic acid-egonol hybrid **10** which we have synthesized via esterification reaction.[12] As this hybrid was found to be more potent than its precursors artesunic acid and egonol; further hybrids **7** and **9** were designed and synthesized for the current study. We performed two different etherification reactions, which were previously reported[51,54] to obtain artemisinin-egonol and artemisinin-homoegonol hybrids **7** and **8**. First, artemisinin-egonol hybrid **7** was synthesized via TMSOTf (trimethylsilyl trifluoromethanesulfonate) catalyzed coupling reaction of *O*-acetyldihydroartemisinin (**17**) with egonol (**4**) at 0 °C (Scheme 1). This reaction provides good yield of **7** (78%) under mild conditions, however, it needs preliminary preparation of *O*-acetyldihydroartemisinin (**17**). An alternative etherification reaction, which does not need functionalization of dihydroartemisinin (DHA), was applied for the synthesis of hybrid **8**. Thus, we performed a direct coupling of DHA (**2**) and homoegonol (**5**), catalyzed by $H_3[P(W_3O_{10})_4]\cdot xH_2O$ (phosphotungstic acid hydrate) at room temperature to obtain artemisinin-homoegonol hybrid **8** with 81% yield (Scheme 1). Another advantage of this etherification method is that no aqueous work-up is required. In both protocols, hybrids have been obtained with high yields in short reaction time (1 h and 2 h, respectively). The X-ray diffraction analysis was performed for the natural product egonol (**4**) and hybrid **8**. We obtained the crystals by vapour diffusion crystallization in CH_2Cl_2 (solvent) and hexane (antisolvent)[55] afterwards their structures were unambiguously determined by X-ray crystallography (Fig. 4).[56] To our knowledge, we report the crystal structure of natural product egonol (**4**) and artemisinin-homoegonol hybrid **8** for the first time. To accomplish synthesis of hybrid **9**, C-10 nonacetal artemisinin-derived carboxylic acid **18** was used as precursor. This derivative lacks oxygen at the C-10 position, which pertains to the so called C-10 nonacetal artemisinins. The C-10 nonacetal derivatives of artemisinin were reported as more effective against malaria parasites than C-10 acetals, which was explained by their higher stability.[57] The hybrid **9**, comprising C-10 nonacetal artemisinin-derived acid and egonol was synthesized via Steglich esterification reaction in 45% yield (Scheme 1). Next, we incorporated the ferrocene unit/linker into hybrid molecules using derivatives of ferrocene. This idea came up with the drug candidate ferroquine which has reached Phase II clinical trials after its discovery in 1994.[58] The two component (reaction of **20** and **24**) or three component (reaction of **19**, **20** and **24**) transformations in the presence of DMAP resulted in desired thymoquinone-ferrocene-thymoquinone hybrid **11** and artemisinin-ferrocene-thymoquinone hybrid **12** in 23 and 26% yields, respectively (Scheme 1). We applied Mitsunobu esterification reaction in dry THF using DIAD and PPh_3 to generate thymoquinone-ferrocene hybrid **13** in 49% yield. Semisynthetic aldehyde and amine derivatives **22** and **23**, obtained from natural products egonol and thymoquinone, gave us the possibility to couple them via an imine bond with a yield of 37%. We also designed the artesunic acid-derived dimer molecule **15**, which consists of two artesunic acid moieties linked via 1,4-butynediol (**25**) using Steglich esterification method. Use of the internal alkyne was inspired by the drugs efavirenz (used to treat and prevent HIV infection/AIDS) and terbinafine (an antifungal agent). Moreover, several artemisinin dimers have been reported up to now with potent biological activities.[57,59,60] Chlorambucil, as originally derived from nitrogen mustard, is a powerful chemotherapeutic medication used to treat leukemia.[61] Besides the specific cytotoxic effects towards tumor cells, chlorambucil additionally showed an unwarranted broader toxic side effect against non-tumor cells and tissues. To possibly overcome such intrinsic limitations, like nonspecific toxicity, drug resistance and low bioavailability as well as to improve its activity, we prepared hybrid **16** via Steglich esterification reaction (Scheme 1). Subsequently, we have examined all new hybrids' biological activities in comparison to their parent compounds and reference drugs, against leukemia cells (Table 1), malaria parasites (Table 2) and viruses (Table 3).

2.2. Biology

2.2.1. In vitro anticancer activity

The hybrids **7–14** and their precursors, have been tested against wild-type CCRF-CEM and P-glycoprotein overexpressing CEM/