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Brief report

Spirocyclic chromanes exhibit antiplasmodial activities and inhibit all intraerythrocytic life cycle stages

Bracken F. Roberts [a], Iredia D. Iyamu [b], Sukjun Lee [c], Eunyoung Lee [c], Lawrence Ayong [c], Dennis E. Kyle [d], Yu Yuan [e], Roman Manetsch [b], Debopam Chakrabarti [a, *]

[a] Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL, USA
[b] Department of Chemistry and Chemical Biology and Department of Pharmaceutical Sciences, Northeastern University, Boston, MA, USA
[c] Institut Pasteur Korea, Seongnam-si, Gyeonggi-do, Republic of Korea
[d] Department of Global Health, College of Public Health, University of South Florida, Tampa, FL, USA
[e] Department of Chemistry, University of Central Florida, Orlando, FL, USA

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ABSTRACT

We screened a collection of synthetic compounds consisting of natural-product-like substructural motifs to identify a spirocyclic chromane as a novel antiplasmodial pharmacophore using an unbiased cell-based assay. The most active spirocyclic compound UCF 201 exhibits a 50% effective concentration (EC_{50}) of 350 nM against the chloroquine-resistant Dd2 strain and a selectivity over 50 using human liver HepG2 cells. Our analyses of physicochemical properties of UCF 201 showed that it is in compliance with Lipinski's parameters and has an acceptable physicochemical profile. We have performed a limited structure-activity-relationship study with commercially available chromanes preserving the spirocyclic motif. Our evaluation of stage specificities of UCF 201 indicated that the compound is early-acting in blocking parasite development at ring, trophozoite and schizont stages of development as well as merozoite invasion. SPC is an attractive lead candidate scaffold because of its ability to act on all stages of parasite's aexual life cycle unlike current antimalarials.

1. Introduction

There are more than 200 million global clinical cases of malaria resulting in over 600,000 deaths each year (Murray et al., 2012; World Health Organization et al., 2014). In addition to contributing significantly towards overall childhood mortality in the poorest nations, the disease is estimated to cause considerable reduction in economic growth in countries that bear a heavy malaria burden (Gallup and Sachs, 2001). The situation is dire because widespread prevalence of drug resistant parasites is making most of the available drugs for treatment to rapidly lose their effectiveness (Greenwood, 1995; Rieckmann, 2006). Most of the drugs that are currently being used for malaria treatment were developed more than 30 years ago, and many are derivatives of older drugs. Furthermore, only a handful of antimalarials primarily belonging to three main chemical classes (4- and 8-aminoquinolines, antifolates. and artemisinin-based agents) are being used for therapy (Grimberg and Mehlotra, 2011). While artemisinin-based combination treatments (ACTs) have played an effective role in controlling the disease in many malaria endemic areas, the appearance of resistant parasites to artemisinin derivatives in wide area of Southeast Asia encompassing south Vietnam to central Myanmar underscores the fragility of malaria treatment measures (Cui, 2011; Miotto et al., 2013; Ashley et al., 2014). Therefore, there is a pressing need for novel therapeutic options to treat resistant malaria. Historically, natural products were the most important source for the majority of therapeutics, including antimalarials (Li and Vederas, 2009). Quinine and artemisinin and their synthetic derivatives are prime examples in the malaria field (Woodward and Doering, 1944; Klayman, 1985). Current trends in lead discovery are biased towards high-throughput screening (HTS) of synthetic compound collections instead of the testing of natural products (NPs). The complexity of natural product structures are often accompanied by synthetic challenges and supply problems, whereas compound collections of commercial sources or “in-house” origin are easily accessible. Nevertheless, because NPs are known to occupy biologically important chemical space, they continue to be valuable for

* Corresponding author.
E-mail address: dchak@ucf.edu (D. Chakrabarti).

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the discovery of new biologically active compounds. The synthesis of NP-inspired compound libraries is a promising alternative covering chemical space similar to NPs while being synthetic tractable (Cordier et al., 2008; Rishton, 2008; Vasilevich et al., 2012). Critical evaluation of known drugs and NPs has been used to identify NP-based substructural motifs, termed as "BioCores" (Kombarov et al., 2010), which have been incorporated into the scaffolds of a BioDesign synthetic library to increase drug-likeness of compounds. In an effort to identify novel antimalarial scaffolds we have screened a subset of the anti-infective BioDesign collection of the vendor Asinex that incorporates "BioCore" features and structures (e.g., enriched oxygen and saturated rings), which were filtered against peroxide bridges that are present in many current antimalarials. Herein, we report that spirocyclic chromane compounds demonstrate potent antiplasmodial activities with excellent selectivity. Furthermore, we demonstrate that this chemotype exhibits a cellular mechanism of action distinct from current antimalarials.

2. Materials and methods

2.1. Plasmodium falciparum culture and EC_{50} determination

Plasmodium falciparum Dd2 and 3D7 strains were maintained at 37 °C in 5% CO_2 and 95% air using a modified Trager and Jensen (Trager and Jensen, 1976) method in RPMI media with L-glutamine (Invitrogen) and supplemented with 25 mM HEPES, 26 mM $NaHCO_3$, 2% dextrose, 15 mg/L hypoxanthine (Sigma–Aldrich), 25 mg/L gentamicin (Life Technologies), and 0.5% Albumax II (Life Technologies). Different dilutions of the compound in RPMI 1640 (Life Technologies) from a stock of 10 mM in dimethyl sulfoxide (DMSO) were added to the culture at a 1% parasitemia and 2% hematocrit in 96-well black plates (Santa Cruz Biotechnology). Maximum DMSO concentration in the culture never exceeded 0.125%. Chloroquine at 1 μM was used as a positive control to determine the baseline value. Following 72 h incubation at 37 °C, the ability of the compounds to inhibit growth of the parasite was determined by a SYBR green I-based DNA quantification assay (Bennett et al., 2004; Smilkstein et al., 2004; Johnson et al., 2007). EC_{50} was calculated (n = 3) from a dose response curve that was generated from a concentration range of 0–20 μM using GraphPad Prism v5.0.

2.2. Cytotoxicity determination

Compounds were evaluated for cytotoxicity using HepG2 human hepatoma cells. A 384-well clear bottom plate (Santa Cruz Biotechnology) was seeded with 2500 cells/well and incubated for 24 h. Serial dilutions (0–40 μM concentration range) of the compound were added to the plate followed by incubation for an additional 48 h. Viability of cells were assessed by MTS [(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) cell proliferation assay (CellTiter 96® Aqueous non-radioactive cell proliferation assay, Promega) (Cory et al., 1991).

2.3. Physicochemical parameters

The aqueous solubility at pH 7.4 was determined by UV–visible absorption based method (Avdeef, 2001). The permeability was assessed by the *in vitro* double-sink parallel artificial membrane permeability assay (Kansy et al., 1998) that is a model for the passive transport from the gastrointestinal tract into the blood stream. The microsomal stability (Janiszewski et al., 2001) was determined in the presence of mouse liver microsomes with or without NADPH.

2.4. Determination of cellular mechanism of action

P. falciparum Dd2 cultures were tightly synchronized using a combination of magnetic separation of schizonts (Ribaut et al., 2008) followed by sorbitol treatment at the early ring stage (Lambros and Vanderberg, 1979). Inhibitory effects of UCF 201 on parasite development, merozoite egress and invasion were analyzed by treating the synchronized culture (1.5% hematocrit and 5% parasitemia) at four different time-points post-merozoite invasion (6, 18, 30, and 42 h post invasion or hpi) at 5×EC_{50} final concentration for a duration of 36 h (or 24 h for treatments at the 42 hpi) under regular culture conditions. Giemsa-stained thin smears were prepared at 12 h intervals for microscopic evaluations of the intraerythrocytic maturation of parasite.

2.5. Synthesis of spirocyclic chromane UCF 201

In order to meet the amount of compound needed for *in vivo* efficacy studies, hit compound UCF 201 has been prepared "in-house" (Fig. 1). Compound **1** was synthesized via an acetylation of

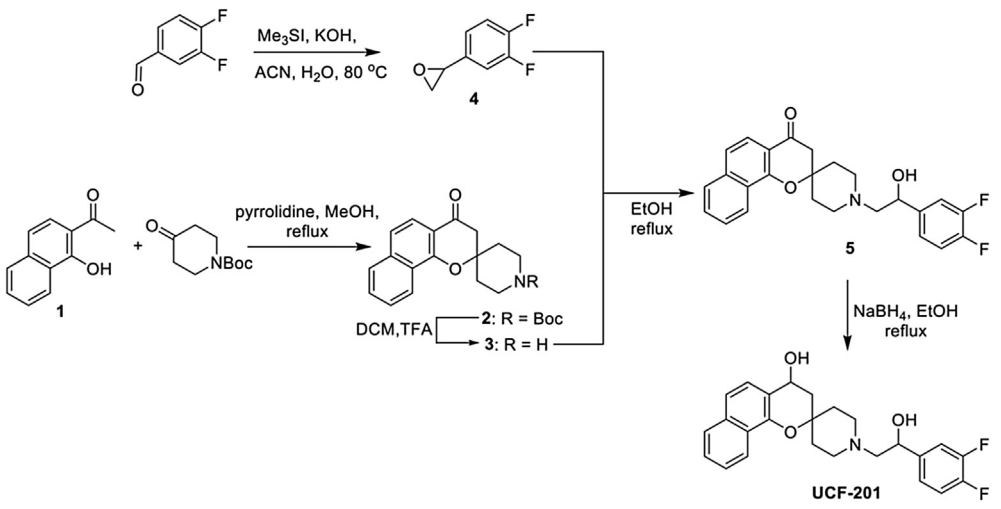

Fig. 1. Synthetic Scheme of UCF 201. Starting from commercially available α-naphthol, UCF 201 was synthesized in 6 steps in good yields.