ACS Medicinal Chemistry Letters

Letter

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Design, Synthesis, and Antiplasmodial Activity of Hybrid Compounds Based on (2*R*,3*S*)-*N*-Benzoyl-3-phenylisoserine

Peter M. Njogu,[†,∥] Jiri Gut,[‡] Philip J. Rosenthal,[‡] and Kelly Chibale*[,†,§]

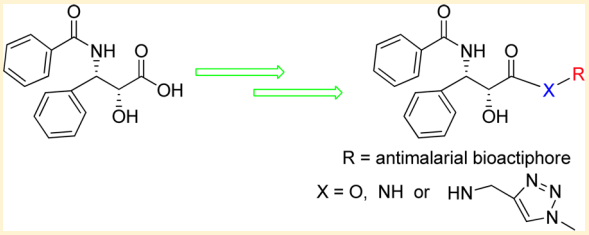
[†]Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa

[‡]Department of Medicine, San Francisco General Hospital, University of California, San Francisco, California 94143, United States

[§]Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch 7701, South Africa

Supporting Information

ABSTRACT: A series of hybrid compounds based on (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine, artemisinin, and quinoline moieties was synthesized and tested for in vitro antiplasmodial activity against erythrocytic stages of K1 and W2 strains of *Plasmodium falciparum*. Two hybrid compounds incorporating (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine and artemisinin scaffolds were 3- to 4-fold more active than dihydroartemisinin, with nanomolar IC_{50} values against *Plasmodium falciparum* K1 strain.



KEYWORDS: *(2R,3S)-N-Benzoyl-3-phenylisoserine, artemisinin, quinoline, hybrids, antiplasmodial activity*

Malaria is a pre-eminent tropical parasitic disease that is the most deadly protozoan infection of humans.[1,2] It is caused by apicomplexan parasites of the genus *Plasmodium* that are transmitted through bites of infected female anopheline mosquitoes.[3] Humans are the definitive host to four plasmodial species: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*.[4] *P. falciparum* accounts for almost all malaria fatalities in sub-Saharan Africa.[5] In spite of concerted efforts at preventive and curative control measures, malaria remains a major health issue, especially in the developing world, as attested to by high annual morbidity and mortality. For example, the World Health Organization's 2011 world malaria report estimated the annual burden of malaria as 216 million clinical cases and 655,000 deaths.[6] A major contributor to the burden from malaria is antimalarial drug treatment failure due to resistance.[7]

Two major contributory factors to treatment failure in drug therapy are dose-dependent toxicities of most chemotherapeutic agents that limit the dose that can be administered and acquired resistance to previously effective drugs. One strategy to improve chemotherapeutic efficacy is the combination of two or more drugs in treatment regimens.[8,9] Combination therapy can entail administration of a cocktail of drugs in the form of two or more individual pills. However, the benefits of this approach are often compromised by poor patient adherence to full treatment regimens.[10] A second approach that is rapidly gaining currency is the coformulation of two or more individual drugs in a single pill as fixed-dose combinations (FDCs) aimed at simplifying treatment regimens and improving in patient compliance.

In view of the emphasis on FDCs, medicinal chemists are increasingly considering the concept of hybrid molecules.[10−12] In this approach, two or more drugs are covalently linked into a single chemical entity so as to exert dual drug action. Hybrid molecules offer advantages over FDCs, including dosage compliance, minimized toxicity, and cheaper preclinical evaluation while pursuing the ultimate objective of delaying or circumventing the development of drug resistance.[12]

(2*R*,3*S*)-*N*-Benzoyl-3-phenylisoserine is a structural component of the antimicrotubular drug paclitaxel (Taxol). Paclitaxel **1** (Figure 1) is a complex taxane diterpenoid initially isolated

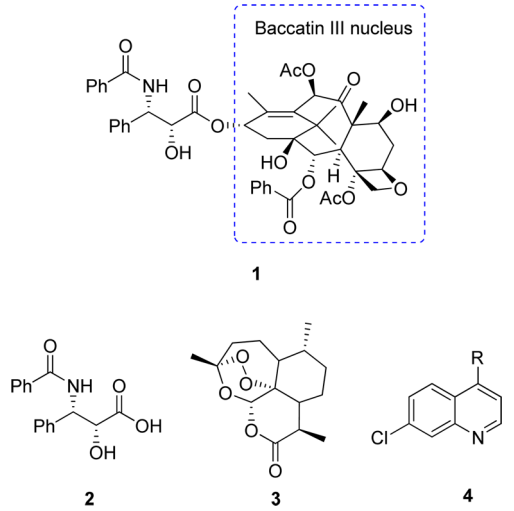


Figure 1. Chemical structures of paclitaxel **1**, (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine **2**, artemisinin **3**, and 7-chloro-4-substituted quinoline **4**.

Received: May 1, 2013
Accepted: May 22, 2013
Published: May 22, 2013

from the bark of the Pacific yew *Taxus brevifolia* Nutt. in 1967 through a screening program for antitumor natural products coordinated by the National Cancer Institute of the United States of America.[13] Its pharmacological effects arise through inhibition of microtubular function during cell division.[14,15] In addition to its strong antitumor activity, previous studies have demonstrated its potential antimalarial efficacy.[16] It has also been proven that both the (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine moiety and the baccatin III nucleus are essential for the antimicrotubular activity of paclitaxel, whereas individually they are devoid of any appreciable activity.[17]

Paclitaxel may therefore be regarded a hybrid molecule designed by nature in which the (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine and the baccatin III nucleus act synergistically to produce antimicrotubular activity. Hence, (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine **2** renders itself as a potential template for design and synthesis of novel antimalarial chemical entities. However, the bioactive artemisinins and quinoline scaffolds (**3** and **4**, respectively, Figure 1) are suitable hybridization partners due to their well established antimalarial efficacy. Thus, (2*R*,3*S*)-*N*-benzoyl-3-phenyl-isoserine was hybridized with appropriately derivatized artemisinin and quinoline scaffolds and the hybrid compounds evaluated for in vitro antiplasmodial activity.

The hybrid compounds were designed in such a manner as to bear the (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine moiety coupled to appropriately derivatized artemisinin or quinoline scaffold via ester, amide, or triazole linkages as captured in Figure 2. For proof-of-concept studies, initial diversity was restricted to the nature of the linker (alkyl chain, triazole, amide, and ester) and the presence or absence of acetylation of the isoserine hydroxyl group.

The artemisinin-bearing target hybrid molecules **7a** and **7b** were accessed via the synthetic protocol illustrated in Scheme 1. In brief, their synthesis commenced with the borohydride-mediated reduction of the carbonyl group in artemisinin **3** to dihydroartemisinin **5**. Acetylation of (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine **2** using acetic anhydride in pyridine yielded the acetylated derivative (2*R*,3*S*)-*N*-benzoyl-*O*-acetyl-3-phenylisoserine **6**. The coupling of (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine **2** and (2*R*,3*S*)-*N*-benzoyl-*O*-acetyl-3-phenylisoserine **6** to dihydroartemisinin **5** was carried out in dichloromethane in the presence of the coupling reagent 1,3-diisopropylcarbodiimide (DIC), auxiliary nucleophile 1-hydroxybenzotriazole (HOBt) and acylation catalyst 4-dimethylaminopyridine (DMAP) to furnish the corresponding target hybrids **7a** and **7b** in 42% and 47% synthetic yields, respectively. As it has previously been established, the acylation reaction furnished α-esters exclusively.[18]

Quinoline-bearing target hybrids **10a−f** were obtained following the synthetic procedure shown in Scheme 2. In all cases, the crucial starting reagent was the 4,7-dichloroquinoline. Diversity was imparted by the insertion of ethyl and propyl linkers at the C-4 position of the quinoline ring. Synthetic efforts toward the ester hybrids made use of aminohydroxyalkyl linkers, while the amide hybrids required use of diaminoalkyl linkers. Successful execution of the coupling reactions was confirmed spectroscopically. Of particular note, the ^{1}H NMR spectra of the target compounds **10a−f** displayed two (or three) pairs of diastereotopic peaks assignable to the aliphatic protons in the alkyl linker. This diastereotopicity arose from the stereogenic influence of the chiral phenylisoserine moiety.

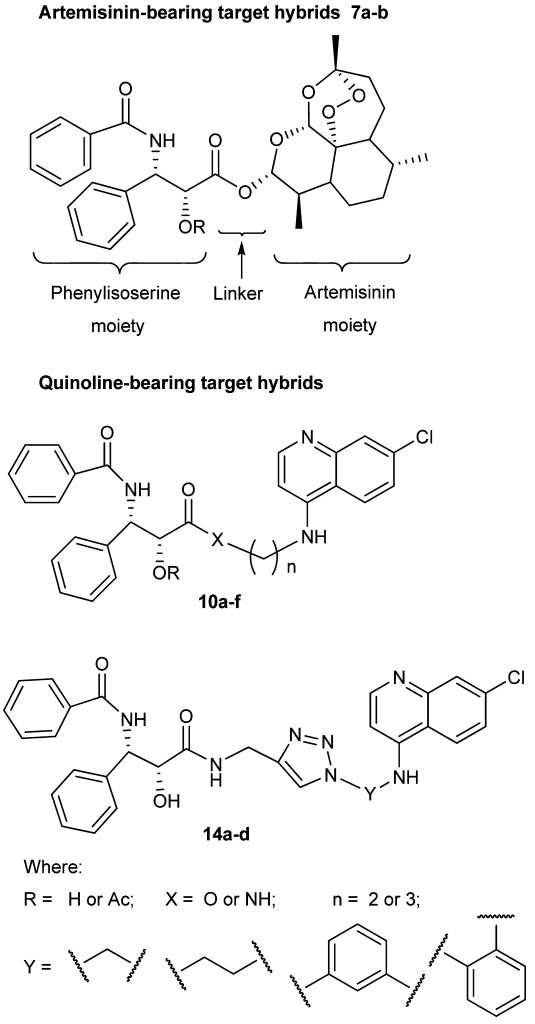


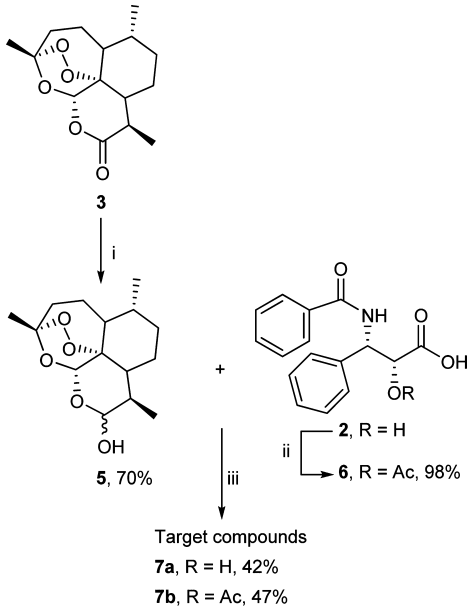
Figure 2. Design of target hybrid compounds based on (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine.

Target hybrid compounds **14a−d** were accessed via a synthetic scheme depicted in Scheme 3 that made use of the Huisgen 1,3-dipolar cycloaddition of azides and terminal alkynes as the hybridization strategy. The first step involved insertion of aminohydroxyalkyl (and aryl) moieties at the C-4 position of the quinoline ring to obtain aminoalcohols **8a−d**. Functional group interconversions gave chloro derivatives **11a−d**, and then the quinolinyl alkyl azides **12a−d**. All intermediates were obtained in moderate to good yields. The acetylene moiety was inserted into (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine **2** via carbodiimide-mediated coupling to propargylamine to give a terminal acetylene-functionalized (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine aminopropyne **13** in 80% yield. The Cu^I-catalyzed cycloaddition of azides **12a−d** and the aminopropyne **13** furnished the triazole-linked target hybrids **14a−d** in moderate to good 50−86% synthetic yields.

All synthesized compounds were evaluated in vitro for efficacy against erythrocytic stages of two *P. falciparum* strains: the chloroquine-resistant IndoChina W2 strain and the multidrug-resistant Thailand K1 strain. The compounds were also subjected to in vitro cytotoxicity screening against the rat skeletal myoblast L-6 cell line. Artemisinin, dihydroartemisinin, chloroquine, and podophyllotoxin were used as positive controls. For each compound, a selectivity index (SI) was calculated by comparing cytotoxicity against the L6 cell-line to

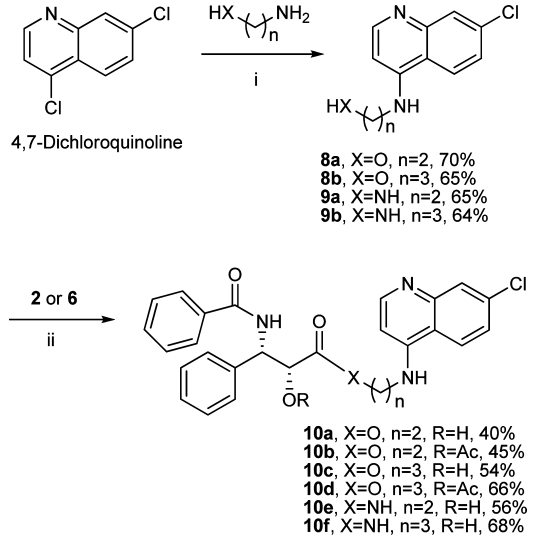
dx.doi.org/10.1021/ml400164t | *ACS Med. Chem. Lett.* 2013, 4, 637−641

Scheme 1. Synthetic Protocol Towards Target Hybrids 7a and 7b[a]



[a]Reagents and conditions: (i) MeOH, $NaBH_4$, 0−5 °C, 2 h; (ii) pyridine, Ac_2O, N_2, 0 °C, 1 h; 20−25 °C, 24 h; (iii) DCM, DIC, HOBt, DMAP, 0 °C, 0.5 h; 20−25 °C, 12 h.

Scheme 2. Synthesis of Target Hybrids 10a−f[a]

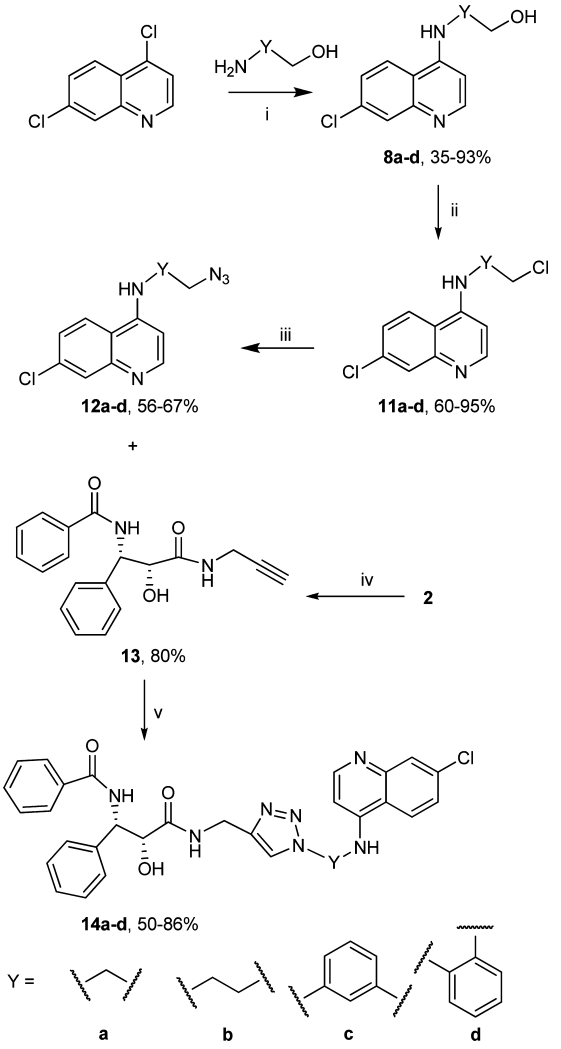


[a]Reagents and conditions: (i) 80 °C, 1 h; 130 °C, 7 h; (ii) DCM/DMF, DIC (or EDC·HCl), HOBt, DMAP, 0 °C, 0.5 h; 22 °C, 12 h.

antiplasmodial activity against the W2 strain of *P. falciparum*. The results are presented in Table 1.

The in vitro antiplasmodial activity of hybrids **7a** and **7b** was 3−4 times greater than that of dihydroartemisinin against the K1 *P. falciparum* strain. This implies potential synergistic interaction between the artemisinins and the isoserine moieties for antiplasmodial activity. Further, the selectivity indices of the hybrid compounds (**7a**, SI = 206; **7b**, SI = 166) were comparable to that of dihydroartemisinin (SI = 243). This implies that the selectivity profile of dihydroartemisinin toward antiplasmodial cells as opposed to mammalian cells is preserved in the hybrid molecules. These observations give credence to

Scheme 3. Synthesis of Target Hybrids 14a−d[a]



[a]Reagents and conditions: (i) neat/EtOH, reflux, 80 °C, 1 h; 130 °C, 3−7 h; (ii) $SOCl_2$, cat. DMF, 0−25 °C, 3−5 h; (iii) DMF, NaN_3, 100 °C, 5−10 h; (iv) DCM/DMF, propargyl amine, EDC·HCl, HOBt, DMAP, 0 °C, 0.5 h; 20 °C, 4 h; (v) DCM/water (2:1), $CuSO_4$ (0.2 equiv), sodium ascorbate (0.6 equiv), 25 °C, 12 h.

potential application of molecular hybridization in antimalarial drug discovery.

However, the apparent antiplasmodial synergy exhibited by the hybrid molecules **7a** and **7b** against the K1 strain was not seen with the W2 strain. This could be due to a number of factors, including differences in the sensitivity of the two strains to the assay compounds and interlaboratory variations in the assay conditions employed. First, since the two strains are genetically different, their susceptibility to the assay compounds might vary. Second, there were differences in the assay conditions such as levels of parasitaemia utilized and duration of drug exposure. Further, variation in the composition of the assay media may contribute to variable protein binding of the experimental compounds and hence different levels of free drug available to exert biological effect(s).

For the quinoline-based series, hybrid compounds **10c** and **10d** were the most active (IC_{50} = 0.13 and 0.16 μM, respectively) against the W2 strain. However, their antiplasmodial activities were considerably lower than that of the control drug chloroquine (IC_{50} = 0.05 μM). The activities of the other

dx.doi.org/10.1021/ml400164t | ACS Med. Chem. Lett. 2013, 4, 637−641

Table 1. In Vitro Antiplasmodial Activity of the Synthesized Compounds[a]

entry	*P. falciparum* IC_{50} (μM) W2[b]	*P. falciparum* IC_{50} (μM) K1[c]	cytotoxicity IC_{50} (μM) L6[d]	SI[e]
2	>10	>10	>100	
6	>10	>10	>100	
7a	0.005	0.0007	1.03	206.0
7b	0.005	0.0005	0.83	166.0
artemisinin	0.015	0.0032		
dihydroartemisinin	0.003	0.0018	0.73	243.3
8a	0.55	nd	nd	
8b	0.41	nd	nd	
8c	0.3	0.30	21.12	70.4
8d	0.04	nd	nd	
10a	0.39	nd	nd	
10b	0.22	0.25	25.19	114.5
10c	0.13	nd	nd	
10d	0.16	0.22	24.19	151.2
10e	0.56	0.36	15.13	27.0
10f	0.48	1.00	28.33	59.0
12a	0.29	nd	nd	
12b	0.17	0.33	9.48	55.8
12c	0.55	1.11	76.6	139.3
12d	0.22	nd	nd	
14a	2.71	1.11	85.61	31.6
14b	1.69	0.39	88.18	52.2
14c	0.28	2.44	67.87	242.4
14d	1.05	3.24	30.06	28.6
chloroquine	0.05	0.34	nd	
podophyllotoxin			0.02	

[a] IC_{50} values represent a mean of triplicate assays repeated at least once. [b] Chloroquine-resistant *P. falciparum* IndoChina W2 strain. [c] Multidrug-resistant *P. falciparum* Thailand K1 strain. [d] Rat-skeletal myoblasts. [e] Selectivity index [IC_{50}(L6 cell-line)/IC_{50}(W2)]; nd = not determined; structures of all intermediates and target compounds can be found in the Supporting Information.

hybrid compounds were comparable or only marginally improved over those of their respective intermediate compounds. Thus, it is discernible from the available data that although the inherent pharmacological activity of the 4-amino-7-chloroquinoline moiety was preserved, hybridization with the (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine moiety did not appreciably improve the in vitro antiplasmodial activities of the quinoline-based hybrid molecules.

In summary, the (2*R*,3*S*)-*N*-benzoyl-3-phenylisoserine scaffold has been used as a template to synthesize hybrid molecules based on artemisinin and quinoline scaffolds. Notably, the artemisinin-based hybrids **7a** and **7b** had equipotent in vitro activity as dihydroartemisinin against Indochina chloroquine-resistant W2 strain and approximately 3- to 4-fold greater potency against the multidrug resistant Thailand K1 strain of *P. falciparum*. They were also selective for plasmodial over mammalian cells. The available data imply that the concept of molecular hybridization might yield novel bioactive molecules with enhanced antiplasmodial activity. It is expected that, in vivo, the ester-linked hybrids will be metabolically cleaved faster than the amide-linked hybrids to release the constituting units, which may act separately and/or individually.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic experimental procedures, characterization of final compounds, and details regarding biological assay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(K.C.) Email: kelly.chibale@uct.ac.za. Tel: +27 21 650 2553. Fax: +27 21 650 5195.

Present Address

[‖]Department of Pharmaceutical Chemistry, University of Nairobi, 19676, Nairobi 00202, Kenya.

Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Marcel Kaiser (Swiss Tropical and Public Health Institute) for performing antiplasmodial assays against *P. falciparum* Thailand K1 strain and cytotoxicity against the L6 cell line. We gratefully acknowledge financial support from the following sources: the South African National Research Foundation (NRF) and Carnegie Foundation of New York (to P.M.N.); the South African Research Chairs Initiative (SARChI) of the Department of Science and Technology (DST), the South African Medical Research Council (MRC), and the University of Cape Town (to K.C.).

■ ABBREVIATIONS

Ac_2O, acetic anhydride; DCM, dichloromethane; DIC, 1,3-diisopropylcarbodiimide; DMAP, 4-dimethylaminopyridine; EDC·HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; FDCs, fixed-dose combinations; HOBt, 1-hydroxybenzotriazole; IC, inhibitory concentration; SI, selectivity index

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