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Short communication

Synthesis of isocryptolepine analogues and their structure–activity relationship studies as antiplasmodial and antiproliferative agents

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ABSTRACT

Novel isocryptolepine analogues have been conveniently synthesized and evaluated for antimalarial and antiproliferative activities. We have found 3-fluoro-8-bromo-isocryptolepine (**1n**) to have the highest activities against chloroquine-resistant K1, chloroquine-sensitive 3D7, and chloroquine- and mefloquine-resistant SKF58 and SRIV35 strains. Several fluorine-substituted analogues (**1b**, **1n**, and **1q**) also showed excellent selectivities while maintaining good to excellent activities against all four *Plasmodium falciparum* strains. Additionally, antiproliferative properties of isocryptolepine derivatives against HepG2, HuCCA-1, MOLT-3 and A549 cancer cell lines are reported for the first time in this study. 2-Chloroisocryptolepine (**1c**) and benzo-fused-2-chloroisocryptolepine (**1i**) showed significant bioactivities whereas several novel fluorinated compounds and 2-chloro-8-bromoisocryptolepine (**1f**) displayed excellent selectivities.

1. Introduction

Malaria and cancer have been two major health issues affecting lives of millions worldwide. Malaria is a major tropical parasitic disease concentrated in both developing and industrialized countries. It has been estimated that in 2012 malaria caused up to 627,000 deaths from an estimated 207 million people infected [1]. Many factors contributing to the wide-spread of malaria include poor control of disease-borne mosquitos, changes in global climate, poor living environments and drug-resistance of several malarial strains, the latter of which has been the main factor which prevents it from being fully eradicated [1,2]. While malaria is a major infectious illness affecting lives of millions, for non-communicable diseases, cancer is among the leading and most dreaded causes of death. According to WHO, 14 million new cases of cancer were diagnosed and around eight million people died from cancer in 2012 [3].

Isocryptolepine (**1a**) is a natural alkaloid isolated from the root of the West and Central African plant *Cryptolepis sanguinolenta* [4] which has been used traditionally to treat a range of illnesses, including diabetes, fungal infection, pain and inflammation, urinary tract and upper respiratory tract infections and malaria [5–11]. In particular, in antimalarial studies, isocryptolepine has shown potential to be developed into a new antimalarial agent. Recently, studies have surfaced which demonstrated the mechanism of cytotoxicity of a related alkaloid, cryptolepine, suggesting a plethora of new indications for this type of structures [12]. Particularly, Wietrzyk and Inokuchi recently reported the cytotoxicities of 6-amino-substituted 11*H*- and 11-methyl-indolo[3,2-c]quinoline derivatives [13]. This has inspired us to investigate both antimalarial and antiproliferative properties of new isocryptolepine analogues.

The core structure of isocryptolepine is an indolo[3,2-*c*]quinoline which can be assembled *via* several published methods [14–19]. These methods included transition metal-catalyzed cross-coupling reactions starting from haloquinoline and haloaniline derivatives [14,15,18]. These syntheses suffered from the lack of

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diversity in commercially available starting materials and the use of transition metal catalysts which may contaminate the products. Murray and co-workers [18] reported that halogen-substituted isocryptolepine analogues, obtained *via* the direct halogenation of the parent isocryptolepine, provided improved antimalarial activities and selectivities. However, the preparation of such analogues by the direct halogenation was severely limited by low regioselecitivities and yields.

Our research group demonstrated that isocryptolepine (**1a**) could be efficiently synthesized in four steps from benzyl azide (**2a**; $R^1 = R^2 = R^3 = H$) and *N*-phenylsulfonyl indole (**3a**; Ar = Ph, $R^4 = R^5 = R^6 = H$) *via* the key TfOH-promoted arylmethyl azide rearrangement reaction [20]. The synthetic sequence provided compound **1a** in 65% overall yield without the need for metal catalysts and harsh reaction conditions (Scheme 1). One could envision that this synthetic route would allow a rapid construction of a library of diverse isocryptolepine analogues simply by starting from both starting materials (**2** and **3**) with various and available structures with pre-defined substitution patterns for further investigation of their biological activities (see Scheme 2).

In this work, we report a library of new isocryptolepine analogues and the investigation of their *in vitro* antiplasmodial activities against four *Plasmodium falciparum* strains, including chloroquine-sensitive, chloroquine-resistant, and mefloquine-resistant strains, and their *in vitro* antiproliferative activities against four primary cancer cell lines as well as the cytotoxicity against a normal human lung cell line for the determination of their selectivity indices. In addition, isocryptolepine analogues with various fluorine substituents were also investigated due to the well-known properties of fluorine substituents in modifying the biological and physicochemical properties of medicinal agents [21].

2. Results and discussion

2.1. Chemistry

In a recently reported preparation of isocryptolepine, our group has utilized a TfOH-promoted rearrangement of arylmethyl azide (**2**) and subsequent interception and annulation of the resulting *N*-aryliminium ion intermediate with a protected indole (**3**) as the key step in obtaining the core cis-fused *N*-tetracyclic structure of the natural product (**4**). DDQ oxidation of compound **4** then provided the fully aromatic tetracycle **5**. The arylsulfonyl group of compound **5** was subsequently removed under the basic conditions (to **6**), followed by the *N*-methylation reaction [22] at the quinoline nitrogen to arrive at the isocryptolepine analogues (**1**). This synthetic sequence has opened an opportunity for us to conveniently and easily synthesize other analogues of isocryptolepine, starting from various arylmethyl azides (**2**) and protected indoles (**3**), both of which are widely available *via* a short synthesis and/or from commercial sources.

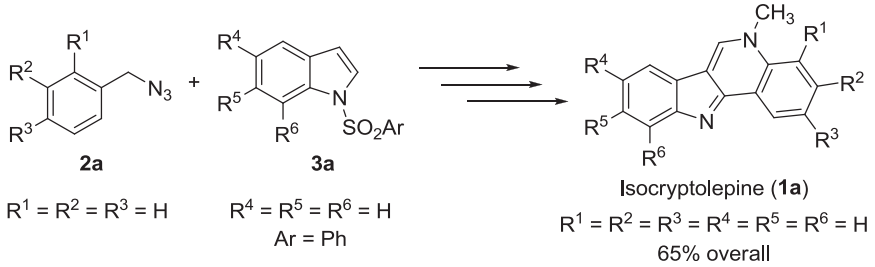
A number of isocryptolepine analogues have been evaluated previously for their antiplasmodial and antiproliferative activities. Murray and co-workers prepared and studied several halogen-substituted analogues for their antiplasmodial activities [18]. The study revealed that inclusion of halogen atoms in the structures could significantly increase the activities of these compounds. Fluorine is particularly of interest since it is well-known in the literature that the presence of fluorine atoms or groups in medicinal compounds can lead to improved biological and physicochemical properties which may be suitable for developing into active pharmaceutical ingredients [21]. Additionally, the studies of the effect of other types of substituents besides halogen atoms are rare in the literature. Based on the scarcity of available structure–activity relationship (SAR) information, we prepared new analogues of isocryptolepine starting from ten arylmethyl azides (**2a**–**2j**) and seven *N*-protected indoles (**3a**–**3g**) as shown in Fig. 1. We were able to successfully prepare a library of nineteen isocryptolepine analogues, including the parent compound (**1a**) and the previously reported **1f**, whose inclusions in this study were for comparison purposes. Compound **1f** was prepared previously by Murray and co-workers [18] and was shown to possess potent antiplasmodial activities against *P. falciparum* 3D7 and W2mef strains with high selectivity index over the normal mouse embryonic fibroblasts (3T3) cell line. The yields of each step of our syntheses are summarized in Table 1.

Of the nineteen isocryptolepine analogues (**1a**–**1s**), seventeen (**1a**–**1q**) were directly prepared from arylmethyl azides **2a**–**2j** and *N*-protected indoles **3a**–**3g**. For the remaining two analogues (**1r**–**1s**) the cis-fused *N*-tetracyclic compounds **4b** and **4m**, obtained from the rearrangement/annulation reactions between indole **3d** and azides **2b** and **2f**, respectively, were first electrophilically chlorinated [23] to give compounds **4r** and **4s** which were subsequently subjected to the remaining reactions in the synthetic sequence (entries 18–19, Table 1) as shown in Scheme 3.

2.2. Biological evaluation

2.2.1. In vitro antiplasmodial assays

In the *in vitro* antiplasmodial assays, the twelve isocryptolepine analogues, as free bases, were subjected to the two standard laboratory *P. falciparum* isolates, chloroquine-sensitive (CQ-S) clone 3D7 and chloroquine-resistant (CQ-R) clone K1. The compounds were also tested against the two recently adapted isolates, SKF58 and SRIV35, which were collected from patients in Srisaket and Kanchanaburi provinces in Thailand in 2013, respectively. Both isolates are chloroquine-resistant (CQ-R) and mefloquine-resistant (MQ-R) phenotypes. In these studies, chloroquine, mefloquine, quinine, and artesunate were employed as positive controls for all strains. The compounds were also tested for their cytotoxicities against the normal human embryonic lung cell, MRC-5, for determination of their selectivity indices.



Scheme 1. Synthesis of isocryptolepine (**1a**) by Tummatorn and co-workers [20].