


# Indole: The After Next Scaffold of Antiplasmodial Agents?

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**Abstract:** Malaria remains a global public health problem due to the uphill fight against the causative *Plasmodium* parasites that are relentless in developing resistance. Indole-based antiplasmodial compounds are endowed with multiple modes of action, of which inhibition of hemozoin formation is the major mechanism of action reported for compounds such as cryptolepine, flinderoles, and isosungucine. Indole-based compounds exert their potent activity against chloroquine-resistant *Plasmodium* strains by inhibiting hemozoin formation in a mode of action different from that of chloroquine or through a novel mechanism of action. For example, dysregulating the sodium and osmotic homeostasis of *Plasmodium* through inhibition of PfATP4 is the novel mechanism of cipargamin. The potential of developing multi-targeted compounds through molecular hybridization ensures the existence of indole-based compounds in the antimalarial pipeline.

**Keywords:** indole, antimalarial agents, hemozoin inhibition, PfATP4, multi-target approach

## Introduction

Malaria is one of the most prevalent and deadliest diseases in the world. World history tells us malaria has claimed the lives of millions of people in the past, and current reports reveal that malaria remains among the top three persistent foes to people living in Africa. According to world malaria report 2019, 213 million or 93% of malaria cases were in Africa, followed by 3.4% cases from South-East Asia region. Two hundred twenty-eight million malaria cases were reported in 2018 compared to 251 million cases in 2010, clear proof that the reduction in prevalence of malaria has stalled after several years of decline. The plateauing in the number of malaria cases for the past two years and the estimated 405,000 deaths, globally, in 2018 is affirmation of the treatment gap in malaria.<sup>1-3</sup>

The constitutive element for the rejuvenation of malaria has been the development and spread of drug-resistant *Plasmodium* strains. Development of effective treatments against drug-resistant *Plasmodium* strains is the greatest challenge in the chemotherapy of malaria. Poor understanding of mechanisms of emergence of resistance, few chemotypes in the antimalarial pipeline and the associated cross resistance between similar chemotype hampered new antimalarial drug development.<sup>4</sup> Recently, resources and spotlights aimed at malaria including initiatives such as Medicines for Malaria Venture and Multilateral Initiative on Malaria have increased. However, the history of inconsistent support of researches and prevention projects in the endemic areas are one of the major determinants for the current resurgence of malaria.<sup>5-7</sup>

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## Scaffolds in Currently Available Antimalarial Agents

Quinine, from the bark of *Cinchona calisaya* tree, was the first widely used antimalarial drug. The quinoline nucleus in quinine (**1**, Figure 1) is the classical yet the loftiest scaffold that have relieved the human race from the aftermath of *Plasmodium* infections for more than 400 years. The benefactions from quinoline-based antimalarials was prolonged by synthetic derivatives such as chloroquine (**2**), which was widely used as the first-line antimalarial agent. The clinical indications of **2**, however, is falling at an accelerated rate owing to a rapid surge in resistance. Furthermore, the immediate quinoline-based prospects including amodiaquine are suffering from cross-resistance with their predecessors and development of full resistance is on the horizon.<sup>8,9</sup>

The endoperoxide scaffold in (+)-artemisinin (**3**) and its analogues represents the elite among mid-term prospects. Artemisinin-based combination therapies are currently the mainstay treatments for uncomplicated malaria, albeit they suffer from incomplete oral absorption associated with poor dissolution in the intestinal fluids, short half-lives and emergence of resistant strains of *Plasmodium*.<sup>10</sup> Undoubtedly, new scaffolds capable of filling the highly unmet medical needs in the treatment of malaria are urgently needed. Efficacy, low toxicity and affordability were the most principal virtue for the early bird success of **2**. In addition, new drugs should be safe, effective against resistant strains, provide cure within a reasonable period of time, and have appropriate formulations for oral use.<sup>9,11</sup> The criteria for new antimalarial agents are likely to be met if attentions are diverted from the established and exceedingly evaluated scaffolds to chart less exploited galaxies of scaffolds in the antimalarial chemical space.<sup>12,13</sup> In this review, the indole

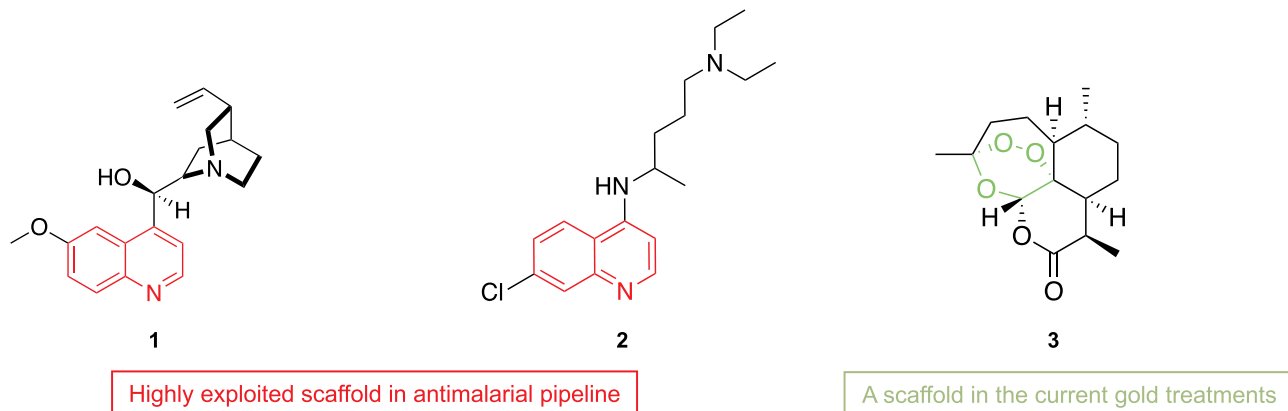
scaffold as a lead for the development of novel antiplasmodial agents meeting the above-mentioned ideal characteristics are discussed. Though numerous indole-based compounds endowed with antimalarial activity are reported and reviewed,<sup>14–16</sup> this mini-review focuses only on recently reported compounds with drug-like properties and promising activity against resistant strains of *Plasmodium*.

## Propitious Indole-Based Antiplasmodial Agents

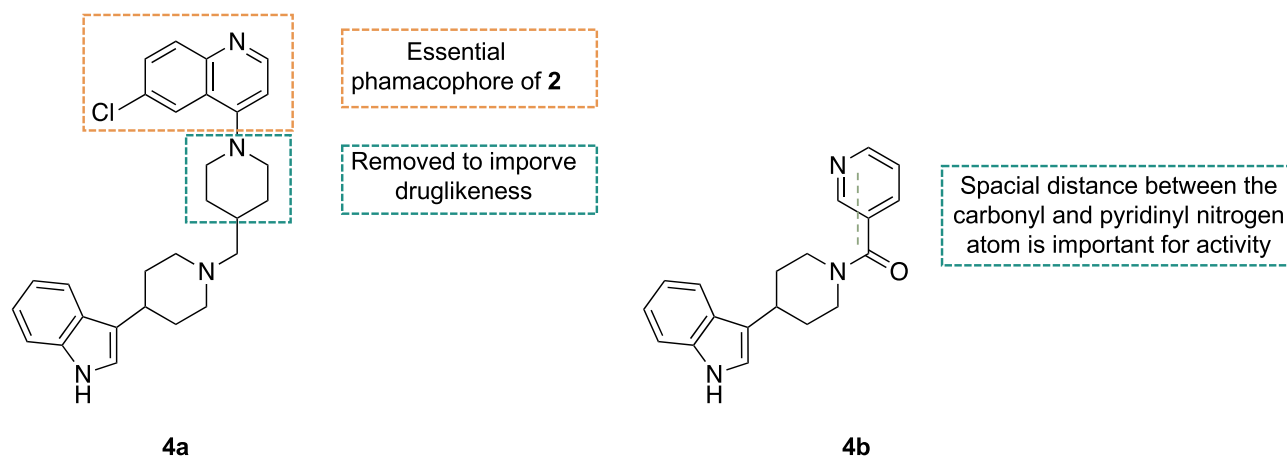
Diverse indole-based antiplasmodial agents of natural sources or synthetic origin are reported. Piperidine indole, spiroindolone, bisindole, or aminoindole are examples of scaffolds in the novel chemical classes of antiplasmodial agents exhibiting multiple modes of action, of which, targeting the formation of hemozoin in *P. falciparum*, PfATP4 and melatonin receptor are the most commonly recognized modes of actions. Indole derivatives in respective chemical classes having promising antiplasmodial activity, acceptable drug likeness and preclinical profiles are discussed below.

### Piperidine Indoles

Screening of the recently publicized Tres Cantos Antimalarial Set (TCAMS) of GlaxoSmithKline (GSK) led to the discovery of a piperidine indole derivative TCMD-134281 (**4a**, Figure 2), which has an EC<sub>50</sub> of 0.034 μM against chloroquine (CQ) sensitive *P. falciparum* 3D7 strain. However, poor drug-like properties and cross-resistance with CQ were demises of **4a**, which were addressed by removing one of the piperidin-4-yl and the 4-aminoquinolonyl fragments. The modifications reduced the overall lipophilicity and molecular weight, thereby improving the druglikeness of **4a**. Specifically,



**Figure 1** The chemical structures of quinoline in quinine (**1**) and chloroquine (**2**), and the endoperoxide scaffold in (+)-artemisinin (**3**).



**Figure 2** Non-essential structural features in TCMD-134,281 (**4a**) which were moved in the design of TCMD-134,281 (**4b**).

removal of the 4-aminoquinolonyl fragment, the essential pharmacophore of CQ, is considered responsible for retaining activity against CQ resistant *P. falciparum* Dd2 strain. Among the simplified analogues, compound **4b** has lead-like properties, with cLog P of 2.42 and molecular weight of 305, and overcome the cross-resistance against Dd2 strain. The relative spatial distance between the carbonyl group and the pyridinyl nitrogen atom is required for activity. Compound **4b** represents a new chemotype for further optimizations towards novel and affordable antiplasmodial drugs.<sup>17</sup>

## Bisindoles

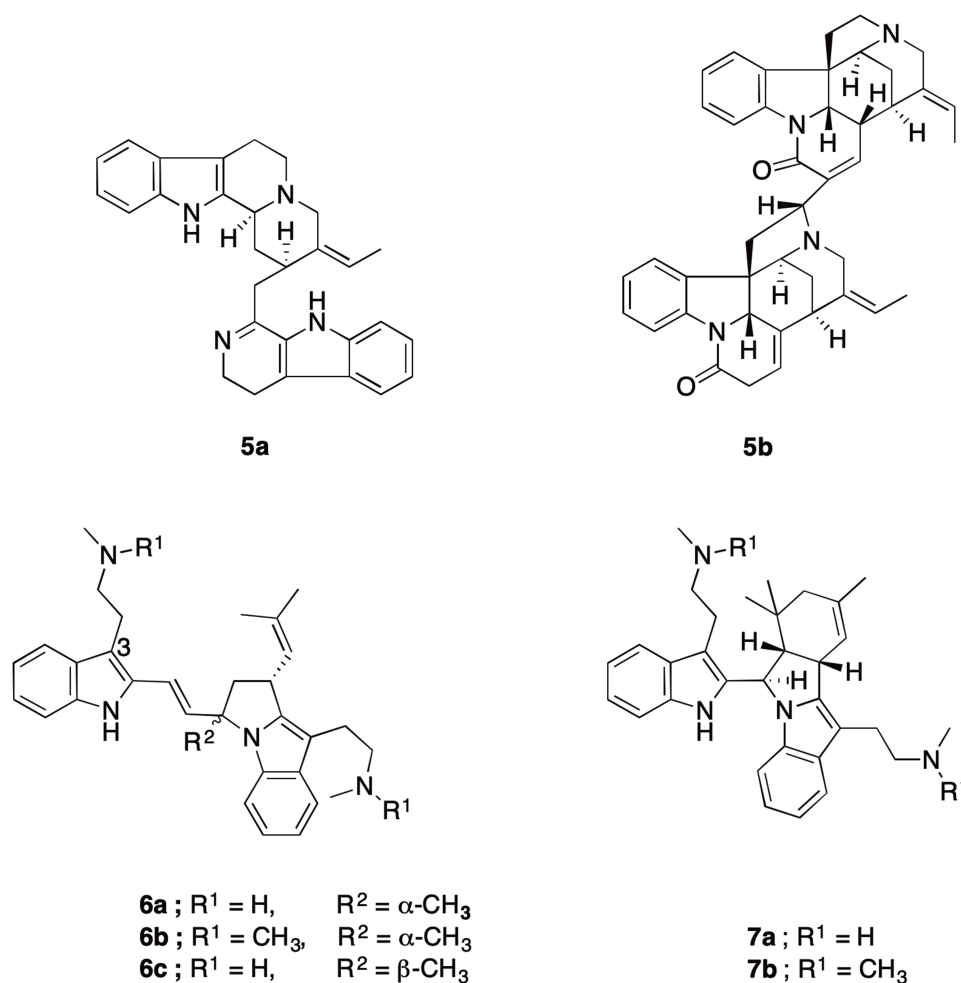
Various compounds, having bisindole moiety embedded somewhere in their structure, have shown potent antiplasmodial properties. Dihydrusamabarensine (**5a**, Figure 3) isolated from *Strychnos usambarensis* exhibited more potency against CQ-resistant strains ( $IC_{50} = 0.032 \mu\text{M}$ ) than CQ-sensitive strains ( $IC_{50} = 0.857 \mu\text{M}$ ). Additional bisindole alkaloids with a moderate activity such as usambarensine, strychnopentamine and isostrychnopentamine were isolated from other *Strychnos* species. Pygmies from Cameroon have a tradition of treating malaria fever with the use of *S. icaja*. An oxygenated bisindole alkaloid with  $IC_{50}$  value of  $0.168 \mu\text{M}$  against CQ-resistant strains, isosungucine (**5b**), was considered as the underlying compound in the bioactivity of *S. icaja*.<sup>16</sup> Modest basicity is essential for the antiplasmodial activity of *Strychnos* alkaloids. Monomers of *Strychnos* alkaloids do not have antiplasmodial properties. However, polymerization increases the basic nature of the monomers and confers antiplasmodial activity.<sup>18</sup>

A novel bisindole alkaloid, flinderole A (**6a**), was discovered from the Australian plant *Flindersia acuminata*.

Chemically related and almost equipotent analogues, flinderole B–C (**6b–6c**), were isolated from the extract of *F. amboinensis*. Flinderoles **6a–c** (Figure 3) have  $IC_{50}$  values of  $0.08–1.42 \mu\text{M}$  against Dd2 *P. falciparum* strain.<sup>19</sup> Methylation of the C-3 ethylamine side chain enhanced the antiplasmodial property of flinderoles. Although in vitro  $\beta$ -hematin (hemozoin) formation assay indicated their direct binding to heme, flinderoles and related bisindole analogues such as isoborreverine (**7a**) and dimethylisoborreverine (**7b**) inhibit hemozoin formation in a different way to CQ. *P. falciparum* trophozoites treated with **7b** showed abnormal morphology of the digestive food vacuole and a reduced formation of hemozoin. Interrupting hemozoin degradation through a novel mechanism might explain their activity against CQ resistant strains<sup>18,20,21</sup> and highlights the versatile antiplasmodial properties of indole-based compounds.

## Spiroindolones

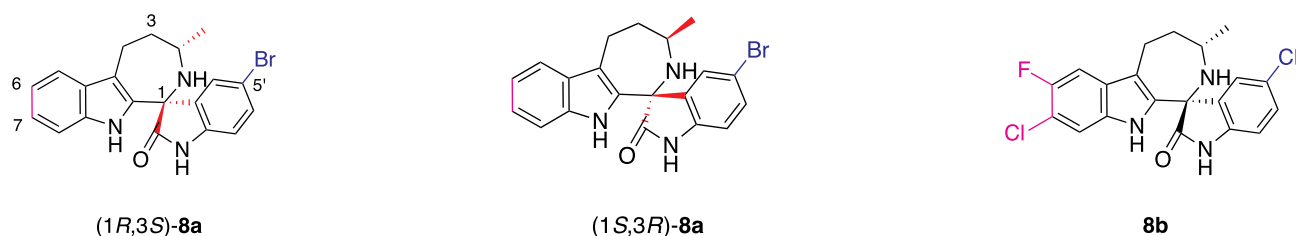
Spiroindolones or spirotetrahydro- $\beta$ -carbolines are a new class of antimalarial agents identified using a whole cell high-throughput screening. Spiroindolones have a mode of action distinct from clinically available antimalarial agents via inhibition of PfATP4, a plasmodial plasma membrane  $\text{Na}^+$ -ATPase that regulates sodium and osmotic homeostasis by exporting  $\text{Na}^+$  ion, while simultaneously importing  $\text{H}^+$  ion. Yeung et al<sup>22</sup> identified a racemic spiroazepineindole (**8a**, Figure 4) with a moderate potency against CQ resistant K1 strain and administration of a single dose of  $100 \text{ mg kg}^{-1}$  to *P. berghei* infected mice reduced parasitemia by 96%. The spirocenter in **8a** was found essential for activity and its removal led to almost complete loss of



**Figure 3** Chemical structures of dihydrousamabarensine (**5a**), isosungucine (**5b**), flinderoles A–C (**6a–6c**), isoborreverine (**7a**) and dimethylisoborreverine (**7b**).

- Chiral centers at C1 and C3 are essential for activity
- Only  $\text{CH}_3$  or  $\text{CF}_3$  were tolerable at 3-position

- C6 and C7 positions are susceptible to oxidation
- Chloro and fluoro substituents can be used as metabolic blocker



5'-Chloro in lieu of 5'-bromo promoted balance between potency, favourable pharmacokinetics and ease of synthesis

**Figure 4** Chemical structures of **(1R,3S)-8a** and **(1S,3R)-8a** enantiomers of a spiroazepineindole derivative and cipargamin (**8b**).

activity. In vitro testing against the wild-type NF54 *P. falciparum* strain revealed the eutomer, where **(1R,3S)-8a** was 250-fold more potent than **(1S,3R)-8a**. Replacing

the 5'-bromo with a 5'-chloro moiety promoted optimal balance between potency, favourable pharmacokinetics and synthesis accessibility. Simplification by removing

the chiral center at 3-position led to an inactive analogue. However, bulkier substituent in lieu of the 3-methyl decreased activity and only a methyl or trifluoromethyl group was tolerable. Structural manipulations of **8a** following the above and more structure-activity relationships led to the synthesis of NITD609 (renamed KAE 609 and now rebranded as cipargamin). The C6 and C7 positions stood as susceptible metabolic positions more prone to oxidation and hence, faster clearance. Accordingly, 6-fluoro and 7-chloro substituents were added in cipargamin (**8b**). Cipargamin is currently under Phase II clinical trial and meets the general criteria set for new antimalarial drugs; kill parasite blood stage, active against drug resistant parasites, safe and has pharmacokinetic properties that allow once-daily oral dosing.<sup>22–24</sup> Cipargamin has a sub-nanomolar potency against blood stage malaria parasite, which is more effective than the current gold standard treatment. Cipargamin is effective against both sexual and asexual stages of *P. falciparum*. Moreover, as it was revealed in <sup>35</sup>S-radiolabeled methionine and cysteine ([<sup>35</sup>S]-Met/Cys) incorporation assay, **8b** blocks protein synthesis in *P. falciparum*.<sup>23</sup>

## Conjugated Indole Analogues

Molecular hybridization gave a new impetus to the science of medicinal chemistry and drug design. The methodology involves the rational design of drugs in which two or more different pharmacophoric units are covalently linked into a single entity to form potentially dual-acting compounds.<sup>25</sup>

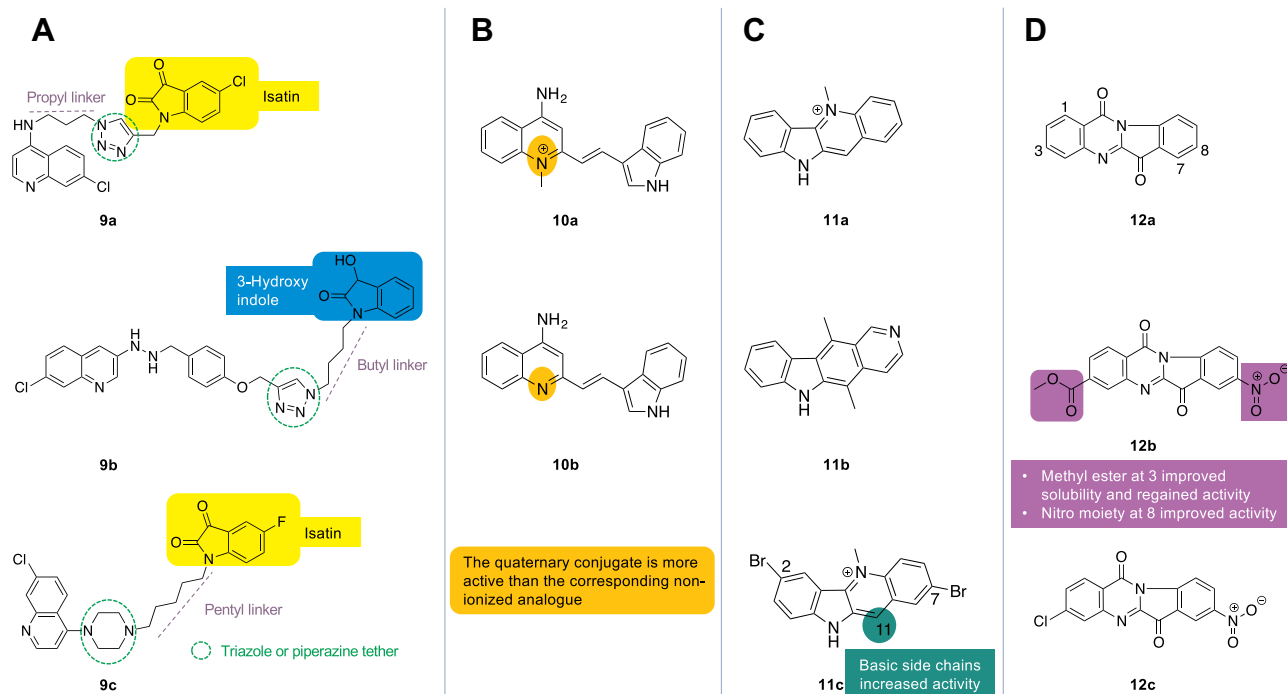
Isatin, 1*H*-indole-2,3-dione, is the most prevalent indole derivatives in natural products and pharmaceuticals. Series of 1*H*-1,2,3-triazole-tethered isatin-7-chloroquinoline and piperazine-tethered isatin-7-chloroquinoline using a molecular hybridization approach was reported by Raj et al<sup>26–28</sup> and the length of the alkyl spacer and a substitution at the C-5 position of the isatin were determinants of antiplasmodial activity. In 1*H*-1,2,3-triazole-tethered 7-chloroquinoline-isatin hybrids, the most potent derivative **9a** (Figure 5) with IC<sub>50</sub> of 1.2 μM against the CQ-resistant W2 *P. falciparum* strain has a propyl spacer and a chloro substituent at C-5 of the isatin moiety. In a series of 1*H*-1,2,3-triazole-tethered 3-hydroxyl-indole-7-chloroquinone, on the other hand, the most active compound **9b** with IC<sub>50</sub> value of 0.069 μM against W2 strains has a reduced imine and a butyl linker. The most potent compound **9c** in piperazine-tethered series of compounds has a pentyl linker and a fluoro substituted isatin moiety.<sup>26–28</sup>

Indole-quinoline conjugation with an indol-3-yl linked to the 2-position of a 4-aminoquinoline moiety represents another

example of molecular hybridization for novel antimalarial agents with promising activity against *P. falciparum*. The indole-quinoline conjugate with a quaternary nitrogen on the quinolone, compound **10a** (Figure 5), showed an improved activity against K1 strain (IC<sub>50</sub> = 0.12 μM) but the corresponding nonquaternized analogue **10b** was found to be relatively less active against the K1 strain. How compound **10a**, with a quaternary quinolinium, crosses the cell membrane is interesting. However, the uptake of quinolinium conjugates was thought to be slow since potencies were lowered after a brief exposure to parasites. Like delocalized cationic compounds endowed with a cell-based activity and oral bioavailability, delocalization of net positively charged quinolinium conjugates may retain the membrane permeability.<sup>29</sup> Furthermore, alkylation of the 4-amino moiety improved activity against 3D7 strain. A unique mechanism of antiplasmodial activity by compromising mitochondrial function is suggested for indole-quinoline conjugates.<sup>30</sup>

Structure complexity is a major limitation of indole conjugates mentioned above. Derivatives of cryptolepine and tryptanthrin represent a class of monomeric heterocycle conjugates which are attractive for chemical optimization. Cryptolepine (**11a**) is an indoloquinoline isolated from the root of *Cryptolepis sanguinolenta*, a plant frequently used as an antimalarial and febrifuge in Central and Western Africa. Mechanistic studies demonstrated that **11a** is capable of inhibiting heme crystal growth and the structurally related analogue, ellipticine (**11b**), directly interacts with heme to inhibit hemozoin formation. Ellipticine was isolated from the bark of the Amazonian tree *Aspidosperma vargasii*, which is used as a traditional antimalarial treatment. Ellipticine and cryptolepine are effective against K1 strain. The circumstances that **11a–11b** do not exhibit cross-resistance with CQ suggest additional mechanisms, and intercalation with DNA and inhibition of topoisomerase II have surfaced. More active semi-synthetic analogues of **11a** (IC<sub>50</sub> = 0.114 μM) were reported. Of this, the most interesting 2,7-dibromocryptolepine (**11c**) is nine times more active against K1 strain than **11a** and suppressed 90% of parasitemia in mice infected with *P. berghei*.<sup>16,31</sup> Furthermore, more potent analogues of **11a** bearing diamino-alkane side chain at C-11 were synthesized depending on the observation that a basic side chain of CQ was important for accumulation in the acidic vacuole of the *Plasmodium* species.<sup>32</sup>

The crystal structure of cryptolepine–DNA complex (Figure 6) identified intercalation into the CG-rich sequence of DNA with non-alternating CC sites. 2,7-Dibromocryptolepine does not appear to intercalate into DNA, albeit cytotoxic activity similar to the parent



**Figure 5** Chemical structures of conjugated indole analogues; **(A)** The optimal length of the linker connecting the triazolyl or piperazinyl tether to isatin or 3-hydroxyindole varies from ethyl to pentyl alkyl in isatin-chloroquine conjugates (**9a–9c**); **(B)** Conjugates of indol-3-yl with quinoline (**10a**) or quinolinium (**10b**); **(C)** The chemical structures of cryptolepine (**11a**), ellipticine (**11b**) and 2,7-dibromocryptolepine (**11c**); **(D)** The chemical structure of tryptanthrin (**12a**), and its more soluble 3-methyl ester (**12b**) and 3-chloro (**12c**) derivatives.

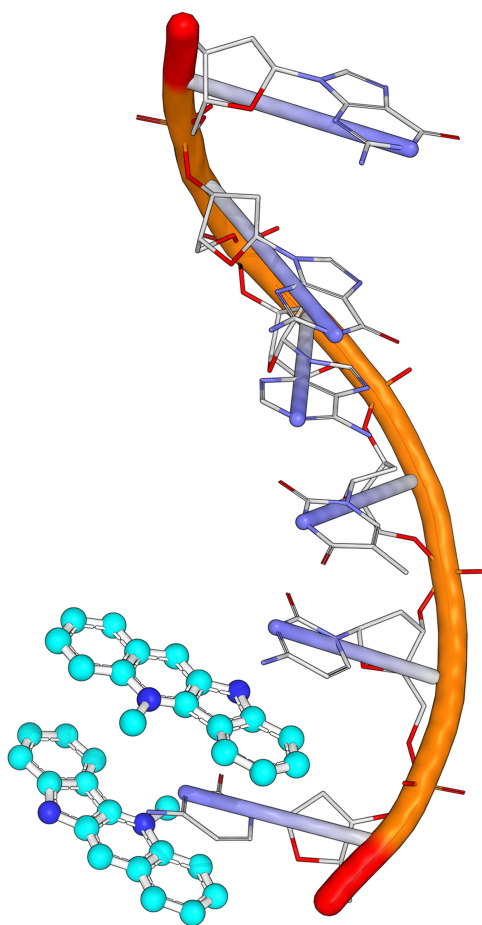
**11a** was reported. An additional mode of action is suggested for **11c** since the increased activity compared to **11a** was not due to more potent inhibition of hemozoin formation nor its increased accumulation inside the acidic food vacuole.<sup>33,34</sup>

Tryptanthrin (**12a**) is an indoloquinazoline conjugate isolated from different plant sources and has broad biological properties, including anti-tubercular and anti-trypanosomal activities. Unlike **2** that showed 13 fold difference in activity between CQ-sensitive NF54 and CQ-resistant Dd2 strains of *P. falciparum*, **12a** (Figure 5) showed similar activity against NF54 and Dd2 strains with IC<sub>50</sub> values of 0.288 μM and 0.114 μM, respectively, though poor solubility has impeded its development. Addition of carboxylic group at position 3 improved solubility but with a costly price of reduced antiplasmodial activity. Inability to cross the biological membrane due to ionization at physiological pH was the reason for the reduced activity. Accordingly, analogues with a carboxylic acid methyl ester such as **12b** have regained antiplasmodial activity. Furthermore, substitution by a halogen or nitro moiety at position 8 was critical for improving antiplasmodial activity.<sup>16</sup> Onambele et al<sup>35</sup> reasoned a further development of a better soluble derivative **12c** with IC<sub>50</sub> values of 0.033 μM and 0.031 μM against

NF54 and Dd2 strains, respectively, which was also considered as a transmission-blocking candidate as it prevented the maturation of early- to late-stage gametocytes and inhibited exflagellation of microgametocytes.

## Aminoindoles

Aminoindole is a novel scaffold with a potent activity against *P. falciparum*.<sup>36</sup> Hit screening of almost 70,000 compounds, against Dd2 and 3D7 *P. falciparum* strains from the Broad Institute and ICCB-L compound collection of Harvard Medical School led to the identification of Genz-644442 (**13a**, Figure 7) as a promising hit compound, with IC<sub>50</sub> values of 0.2 and 0.285 μM against Dd2 and 3D7, respectively. Compound **13a** cured mice infected with *P. berghei* with an efficacy of greater than 99%. In addition to its potent antiplasmodial properties, In vitro ADME assays revealed that **13a** has high membrane permeability, high solubility, better stability in liver microsomes and hepatocytes, and low affinities for all CYP450 isozymes. Accordingly, a major hit-to-lead filtering campaign generated 321 analogues of **13a**. The enantiomers of **13a** which were resolved using supercritical fluid chromatography displayed similar in vitro potency against *P. berghei*. One of the enantiomer (absolute configuration



**Figure 6** Intercalation into the CG-rich sequence of DNA by cryptolepine (PyMOL was used to regenerate the picture from the crystal structure with PDB accession number 1K9G). Cyan color: cryptolepine (19).

not disclosed) was far less active against *P. berghei* in vivo and lower plasma clearance in mice was reported as an underlying reason. Structural manipulations of **13a** depending on the structure-activity relationships developed from the 321 analogues yielded a more potent Genz-668,764 (**13b**) with  $IC_{50}$  of 0.028  $\mu\text{M}$  (against 3D7) and 0.065  $\mu\text{M}$  (against Dd2). Similar to enantiomers of **13a**, enantiomers of **13b** differed in their in vivo efficacy against *P. berghei*. The mechanism of action of aminoimidazole is not established at present, though the trophozoite stage appeared to be the target based on the microscopic examinations of synchronized in vitro *P. falciparum* cultures.<sup>15,37</sup>

### Indole-3-Glyoxyl Tyrosine Derivatives

Promoted by the potent antiplasmodial activity of indole-based compounds from GSK, Vasconcelos et al<sup>38</sup> synthesized the conjugate of indole with a series of tyrosine

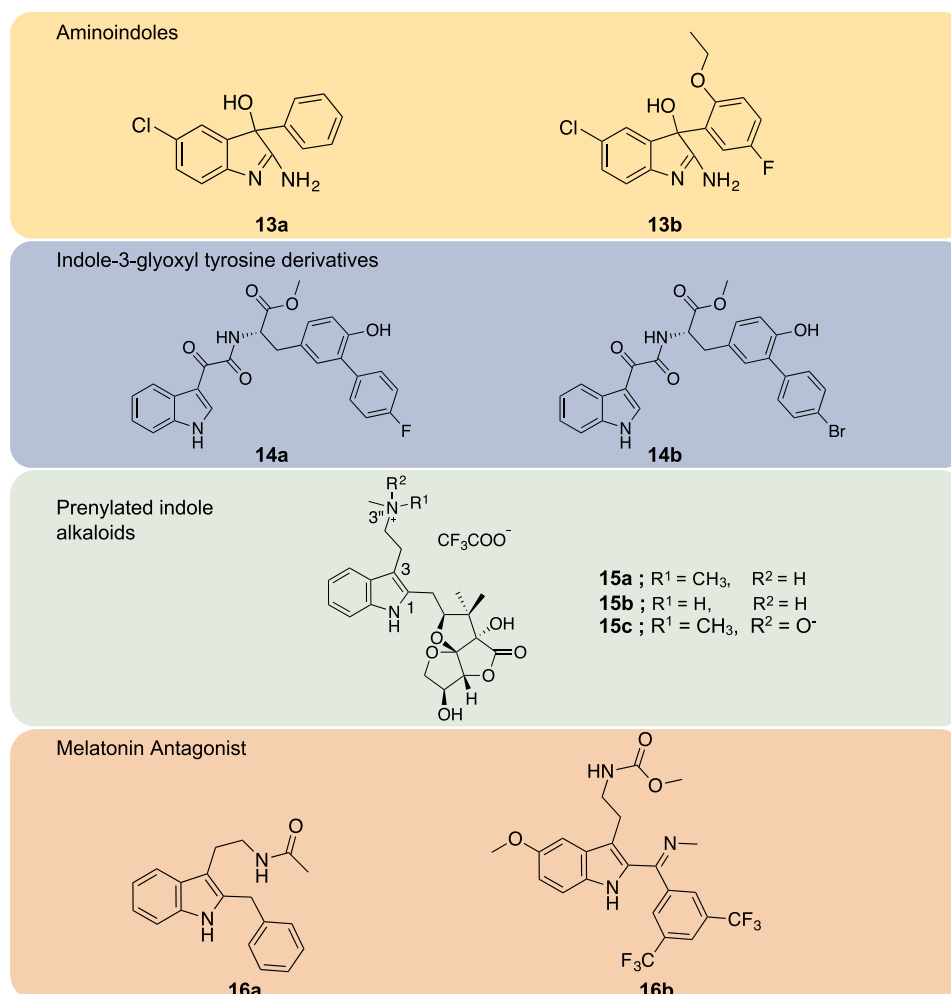
residues. Compounds **14a** ( $IC_{50}$  = 1.3  $\mu\text{M}$ ) and **14b** ( $IC_{50}$  = 3.7  $\mu\text{M}$ ) displayed potential antiplasmodial property against 3D7 strains. Conformational analysis indicated that potent indole-3-glyoxyl tyrosine derivatives including **14a** and **14b** (Figure 7) have linear conformation, unlike the U-shaped conformation by less active analogues. Vasconcelos et al<sup>38</sup> reported that intramolecular hydrogen bonding between indole and tyrosine residues or hydrophobic interaction of  $\pi$ - $\pi$  type are considered responsible for the U-conformation.

### Prenylated Indole Alkaloids

Plants from the genus *Flindersia*, including the aforementioned *F. acuminata* and *F. amboinensis*, have been a source of antimalarial alkaloids of the indole and quinoline types. Pimentelamine A–C (**15a–15c**, Figure 7) represent a new class of indole alkaloids isolated from *F. pimenteliana* with a biosynthetic origin via cyclization of 2-prenyl-*N,N*-dimethyltryptamine with semidehydroascorbic acid radical. Pimentelamine C (**15c**) exhibited a moderate activity against 3D7 and Dd2 strains with  $IC_{50}$  values of 3.6 and 2.7  $\mu\text{M}$ , respectively. The polar *N*-oxide moiety at C3'' in **15c** may enhance bioactivity and solubility, unlike the inactive analogues **15a–15b**, and is responsible for the antiplasmodial activity.<sup>39</sup>

### Indole as a Lead for Multi-Targeted Drugs

The “one target, one drug” approach, where a drug with a high degree of selectivity to a single biological target is designed in order to avoid unwanted side effects, has dominated drug discovery in the past. Genomics has challenged this drug discovery paradigm by unveiling the complexity and interdependence of biological pathways through target-target networks. Consequently, blocking a single process may have a diminishing effect since the system compensates by enhancing alternative pathways.<sup>31</sup> In this post-genomic era, the multi-target drug concept is gaining momentum. The complexity of the current incurable pathologies such as cancer and psychosis are witnesses that single-target drugs are inadequate to effect cures. The anti-neoplastic turmeric and the atypical anti-psychotic clozapine are commonly employed examples for multi-target approaches in drug design. Twenty-one percent of new molecular entities approved by FDA from 2015–17 are multi-target drugs, clear proof of the success of this approach.<sup>40,41</sup>



**Figure 7** Chemical structures of aminoindole derivatives (**13a–13b**), indole-3-glyoxyl tyrosine derivatives (**14a–14b**), prenylated indole alkaloids (**15a–15c**), and melatonin antagonist (**16a–16b**).

Multiple modalities of action of indole-based compounds, as alluded to above, is considered responsible for their efficacy against multiple CQ-resistant strains. The resistant *Plasmodium* spp. are still susceptible to a drug having a different or additional mechanism of action. Antiplasmodial compounds with multiple modes of action are crucial to remain ahead of the relentless *Plasmodium* spp. in developing resistance to antimalarial treatments. It is important to note that the indole nucleus alone does not guarantee antiplasmodial activity. Indeed, alkaloids from species of *Pandaca*, *Bonafousia* or *Rauwolfia*, for example, contain indole structures, and none are active against *Plasmodium* parasites.<sup>42</sup> Accordingly, designing the indole scaffold to simultaneously consolidate the essential structure-activity relationships for inhibiting hemozoin formation, PfATP4 or melatonin receptors may lead to analogues having multiple modes of action. For example, molecular

hybridization in which the pharmacophore units of hemozoin inhibitors and PfATP4 inhibitors are covalently linked to an indole scaffold may lead to dual-acting antiplasmodial agents.

## Inhibition of Hemozoin Formation

Inhibiting plasmodial heme detoxification pathway to hemozoin is considered the most promising and ideal target. Hemoglobin decomposition within the parasite digestive food vacuole releases a large quantity of free heme (ferriprotoporphyrin IX). The large quantity of the free heme is thought to be toxic to the *Plasmodium* through membranes disruptions, lipid peroxidation, and protein and DNA oxidation. Accordingly, *Plasmodium* spp. use a heme detoxification system called biocrystallization that polymerizes heme into an insoluble hemozoin. Hemozoin is chemically and structurally identical to  $\beta$ -



hematin ( $\text{Fe}^{\text{III}}$ -protoporphyrin-IX)<sub>2</sub>. Hemozoin is believed to consist of strands of  $\text{Fe}^{\text{III}}$ -protoporphyrin units, linked into a polymer through coordination complexes between the carbonyl group of a propionate side chain of one monomer and the  $\text{Fe}^{3+}$  atom in the porphyrin ring of another monomer. In addition to the toxic effects of free heme, the ability of the parasite to maintain cationic gradients is impaired by the accumulation of a significant concentration of the free hematin and hematin–antimalarial drug complex, leading to the death of the parasite.<sup>34,43</sup>

The underlying mechanism for the action of quinoline antimalarial agents against hemozoin formation is not clearly known, though a surface-drug interaction is proposed as the origin of action. The same uncertainty surrounds the mechanism of inhibition of hemozoin formation by indole based antiplasmodial agents, albeit hemozoin inhibition, as mentioned above, was elucidated as a major mechanism for the antiplasmodial properties of indole alkaloids including cryptolepine. Some indole derivatives are capable of directly binding with ferriprotoporphyrin IX, thus disrupting the polymerization of heme to hemozoin. Moreover, indole alkaloids with weak basic properties were active antiplasmodial agents, whereas structurally related alkaloids with different acid–base profiles were inactive, suggesting a similar mode of action to that of quinolines.<sup>44,45</sup> Therefore, the above and other pertinent structure-activity relationships of quinolone based antimalarial agents may improve the inhibition of hemozoin formation by indole scaffold, i.e. scaffold hopping.

## PfATP4 Inhibition

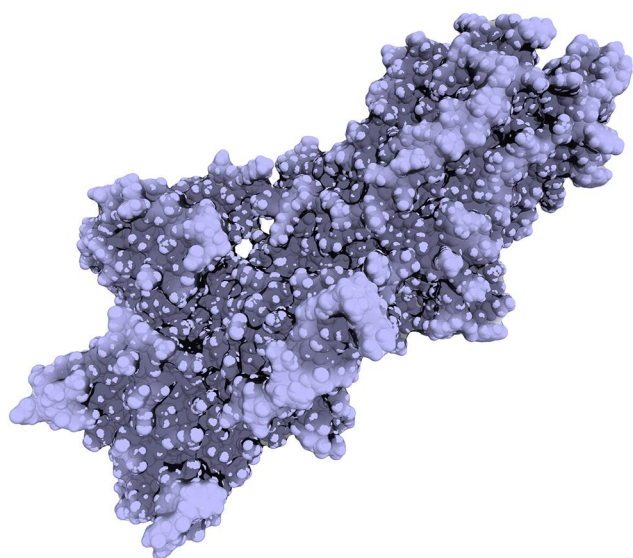
Ensuing entry, *Plasmodium* spp. establish a new permeability pathway in the plasma membrane of the host erythrocytes for the uptake of important nutrients, while simultaneously allowing the influx of  $\text{Na}^+$  ion down the concentration gradient. Despite the increased  $\text{Na}^+$  ion concentration in the erythrocyte cytosol, the intraerythrocytic *Plasmodium* maintains a low cytosolic  $\text{Na}^+$  ion concentration by extruding  $\text{Na}^+$  ion against the inward gradient via PfATP4, a P-type ATPase of *P. falciparum*. PfATP4 has a sequence similarity to the  $\text{Na}^+$  pump of the lower plants' ENA (exitus natrus) P-type  $\text{Na}^+$  ATPase. The ENA ATPases are closely related to Sarcoplasmic/Endoplasmic Reticulum  $\text{Ca}^{2+}$  ATPases (SERCA) and Plasma Membrane  $\text{Ca}^{2+}$  ATPase (PMCA). PfATP4 belongs to the same class (PII class) with SERCA and share a common structural feature of large cytoplasmic domains connected to several transmembrane helices. This

superfamily of proteins is restricted to lower eukaryotes, making it an attractive drug target.<sup>46–48</sup>

PfATP4 is a target for the spiroindolone class of antiplasmodial agents. Spiroindolones inhibit parasite growth via disruption of  $\text{Na}^+$  homeostasis, that was attenuated in *Plasmodium* spp. bearing resistance conferring mutations in PfATP4. In addition to spiroindolones, PfATP4 is identified as a major mechanism for the antimalarial activity of novel chemotypes that have progressed to preclinical evaluations, aminopyrazoles and dihydroisoquinolones.<sup>49</sup> A homology model of PfATP4 (Figure 8), using SERCA (PDB 3TLM) as a template<sup>50,51</sup> and the amino acids sequence of PfATP4 imported from Uniprot,<sup>52</sup> is reported, and can be used for the design, evaluation of structure-activity relationships and optimizations of PfATP4 inhibitors. Another homology model using a rabbit  $\text{Ca}^{2+}$  ATPase (PDB 2DQS) as a template is reported for the binding interactions of indole-based chiral bicyclic lactam derivatives.<sup>53</sup> Structural features essential in the spiroindolones or isoquinoline series of PfATP4 inhibitors and in the quinoline based inhibitors of hemozoin formation can be hybridized for optimization of the indole scaffold as a dual-acting antimalarial agent.

## Melatonin Receptors Modulation

As an additional novel mode of action, targeting melatonin receptor further revealed the potential of developing indole-based multi-targeting drug candidates. Melatonin is established as essential for synchronization of the malaria parasitic cell cycle. Its role in regulating genes involved in ubiquitin/proteasome system is vital to regulate cell cycle and transcriptional activity in *Plasmodium* and increase its mature schizont percentage.<sup>54</sup> Malaria promotes hepatocyte apoptosis by inducing mitochondrial pathology and mitochondrial oxidative stress, an effect prevented by high dose of melatonin.<sup>55</sup> Hotta et al<sup>40</sup> showed that the indole-based melatonin antagonist, luzindole (**16a**), was able to inhibit trophozoites by disrupting the rhythmicity of parasite cell cycle. Smear results from mice treated with **16a** (Figure 7) exhibited altered percentage of *Plasmodium* forms with lower number of trophozoites. Those limited number of trophozoites left following luzindole treatment were unable to produce ring forms in their subsequent developmental stage. The antimalarial effect of luzindole was also found to be statistically significant ( $p < 0.05$ ) in ring stage of the parasite. Further, luzindole significantly abolished the increase in cAMP concentration in both ring form and late trophozoite stage by antagonizing melatonin.<sup>56</sup>



**Figure 8** Homology model of PfATP4 regenerated using the crystal structure of SERCA (PDB 3TLM) as a template.

Besides luzindole, Luthra et al<sup>57</sup> reported the promising antimalarial property of C2-arylmino tryptamine derivatives as novel class of melatonin receptor antagonists, of which **16b** showed the most potent blockade of trophozoite stage growth via disrupting synchronization of the parasite with a considerable affinity to melatonin receptor. Moreover, compound **16b** and luzindole exhibited a 47% and 48% inhibition of parasitemia, respectively, at 5  $\mu$ M dose. As a novel approach to tackle malaria, harmonized administration of high dose of melatonin and its antagonist is considered.<sup>58,59</sup>

Further ensuring their future in antimalarial pipeline, indole alkaloids showed synergistic/additive interactions with conventional antimalarial agents. Mice treated with **16a** (15mg kg<sup>-1</sup>) and suboptimal dose of **2** (1.5mg kg<sup>-1</sup>), for instance, had a drastic reduction in the number of intraerythrocytic parasites.<sup>60</sup> In the in vitro interaction assay, **11a** exhibited additive effect with **2** and lumefantrine while synergism was noted with amodiaquine.<sup>61</sup> This encouraging interaction is augmented by acceptable safety profile of the combinations. Cryptolepine in combination with 4 mg kg<sup>-1</sup> dose of artemisinin showed no significant biochemical and histopathology indices variations compared to the control group.<sup>62</sup>

## Preclinical Profiles

Parallel to pharmacological screening, toxicity profiles of indole alkaloids have been heavily investigated. Extracts rich in indole alkaloids revealed the absence of

genotoxicity, cardiotoxicity as well as adverse effect in the respiratory system.<sup>63</sup> Substantiating this finding, indole analogues such as indole-3-glyoxyl tyrosine derivatives, **11b**, **15c** and piperidine indoles appeared to have an acceptable safety profile.<sup>31,38,63</sup> However, some indole candidates such as **16a** and **11a** were found to impair cardioprotection and cause cytotoxicity, respectively.<sup>64</sup>

Cryptolepine, an extensively studied indole alkaloid, was reported to cause in vivo toxicity in mice.<sup>65</sup> A recent study,<sup>66</sup> moreover, showed that **11a** induces malformations in zebrafish embryo. Despite these reported toxicities, Forkuo et al<sup>62</sup> reported that liver, spleen, stomach, and kidney histopathology of cryptolepine treated *P. berghei* infected mice were not different from control mice. Acute toxicity study of indole alkaloids isolated from *Alstonia scholaris*, with antiplasmodial activity attributed to bisindole alkaloids, was also done on rats, mice and dogs. In dogs, a treatment related short-lasting emesis and reddening of perioral mucosa was noted following a single dose. Due to a possible toxicity to neuromuscular and nervous system, acute dose administration of these indoles caused shortness of breath, wheezing and convulsion in mice but not in rats.<sup>67</sup> A one-week treatment of rats with the aminoindole **13a**, on the other hand, resulted in a 70% reduction in reticulocytes, which recovered a few days following cessation.<sup>37</sup>

Cryptolepine was further demonstrated to exert a cytotoxic and genotoxic effect, in vitro. It caused a dose-dependent reduction in viability of the V79 cell line, a Chinese hamster lung fibroblast,<sup>68</sup> and non-melanoma skin cell lines, SCC-13 and A431.<sup>69</sup> Some bisindole alkaloids were also found to be cytotoxic to MRC-5 cells.<sup>70,71</sup> Cytotoxic effect against 3T6 cell line was exerted by piperazine-tethered 7-chloroquinoline-isatin conjugates. Likewise, a derivative of tryptanthrin, **12c**, showed a strong inhibition of MCF-7 cells. But, due to its higher selectivity index, similar to the parent molecule, it was considered for further animal studies. Moreover, tryptanthrin and its derivatives did not show any disruption of red blood cell membrane integrity, which is a severe side effect of the conventional antimalarial agents.<sup>35,72</sup>

It has been demonstrated that cytotoxicity of indole alkaloids is mainly due to their interference with topoisomerase II-DNA complex stability as well as a tendency to intercalate into DNA (Figure 8). It was also noted that indoles can directly inhibit topoisomerase in the absence of DNA. In addition, **11b** forms cytochromes P450 and peroxidases mediated DNA adducts.<sup>64,73–75</sup>

The observed toxicity in this class of compounds partly hindered further preclinical development of indoles.<sup>64</sup> Most of the reported side effects are associated with long term exposure to indoles. As a result, chronic toxic effect of indole derivatives could be precluded as far as malarial treatment regimen extends only for a couple of days.<sup>61</sup> Moreover, as the antiplasmodial mechanism of most indole alkaloids, exemplified by **11a**, is independent of DNA, it is plausible to prepare analogues with less DNA intercalation propensity. Accordingly, several analogues of **11a** showed less cytotoxicity and in vivo adverse effects while retaining the antimalarial potency. Of these, **11c** displayed an increased potency with apparently no cytotoxicity in mice, evidenced by less affinity to DNA.<sup>75,76</sup> Hence, this is proof that such reported toxicity of promising indole alkaloids is amenable to wane through chemical modifications. Modifications such as having an open ring in place of a six-membered oxygen heterocycle in the tertiary bisindole alkaloid longicaudatine, make the derivative 40 times more selective to the parasite than the parent molecule.<sup>19</sup>

Besides possessing attractive drug-like attributes and comparable efficacy to artemisinin derivatives,<sup>23</sup> cipargamin revealed a promising safety profile of indoles, clinically. Safety and tolerability study in healthy male adult patients showed that **8b** is generally well tolerated with transient gastrointestinal and genitourinary adverse events of mild to moderate intensity. Cipargamin has a higher selectivity index with a very minimal risk of cardiotoxicity and genotoxicity. Unlike older antimalarials, it did not significantly bind to human proteins such as hERG, suggesting a low potential for arrhythmia.<sup>22</sup> Equipped with a remarkable antimalarial potency and low toxicity profile, **8b** is on the verge of joining the existing antimalarial armamentarium.

## Conclusion and Future Perspectives

Multiple modalities of antimalarial action, including inhibition of hemozoin formation, PfATP4 and melatonin are associated with natural and synthetic compounds having an indole scaffold. This property makes indole a compelling scaffold for the currently trending paradigm in drug discovery, the multi-targeted approach. Synthesis of novel indole analogues bearing pharmacophore units of both hemozoin inhibitors and PfATP4 inhibitors, through molecular hybridization, may be insurmountable to the *Plasmodium* spp., which are otherwise relentless in developing resistance to drugs with a single mode of action.

## Abbreviations

CQ, chloroquine; IC<sub>50</sub>, half maximal inhibitory concentration; PfATP4, *Plasmodium falciparum* P-type ATPase; PMCA, plasma membrane Ca<sup>2+</sup> ATPase; SERCA, sarcoplasmic/endoplasmic reticulum Ca<sup>2+</sup> ATPases; TCAMS, Tres Cantos Antimalarial Set.

## Disclosure

The authors declare no conflict of interest.

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