Bioorganic & Medicinal Chemistry Letters 28 (2018) 289–292

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis, *in vitro* antimalarial activities and cytotoxicities of amino-artemisinin-ferrocene derivatives

Christo de Lange [a], Dina Coertzen [b], Frans J. Smit [a], Johannes F. Wentzel [a], Ho Ning Wong [a], Lyn-Marie Birkholtz [b], Richard K. Haynes [a,*], David D. N'Da [a,*]

[a] Centre of Excellence for Pharmaceutical Sciences, North-West University, Potchefstroom 2520, South Africa
[b] Department of Biochemistry, Institute for Sustainable Malaria Control, University of Pretoria, Private Bag X20, Hatfield 028, South Africa

ARTICLE INFO

Article history:
Received 7 October 2017
Revised 20 December 2017
Accepted 23 December 2017
Available online 26 December 2017

Keywords:
Amino-artemisinin
Ferrocene
Redox
Hybrid
Malaria

ABSTRACT

Novel derivatives bearing a ferrocene attached via a piperazine linker to C-10 of the artemisinin nucleus were prepared from dihydroartemisinin and screened against chloroquine (CQ) sensitive NF54 and CQ resistant K1 and W2 strains of *Plasmodium falciparum* (*Pf*) parasites. The overall aim is to imprint oxidant (from the artemisinin) and redox (from the ferrocene) activities. In a preliminary assessment, these compounds were shown to possess activities in the low nM range with the most active being compound **6** with IC_{50} values of 2.79 nM against *Pf* K1 and 3.2 nM against *Pf* W2. Overall the resistance indices indicate that the compounds have a low potential for cross resistance. Cytotoxicities were determined with Hek293 human embryonic kidney cells and activities against proliferating cells were assessed against A375 human malignant melanoma cells. The selectivity indices of the amino-artemisinin ferrocene derivatives indicate there is overall an appreciably higher selectivity towards the malaria parasite than mammalian cells.

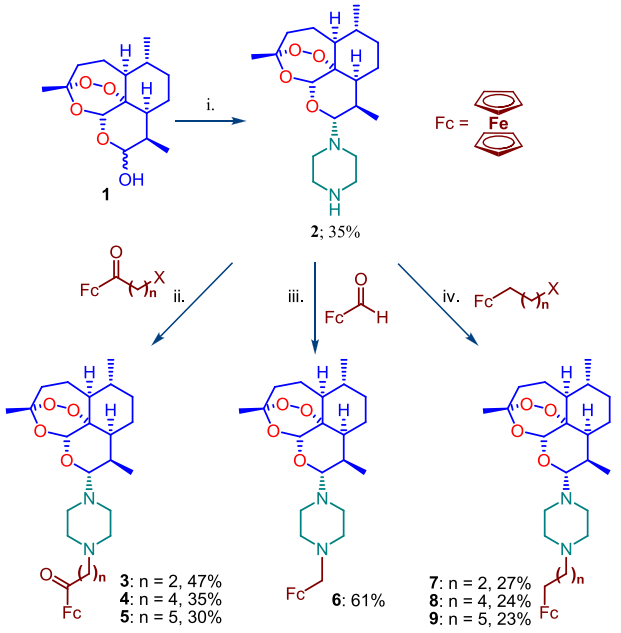
According to the World Health Organization (WHO), there were about 212 million cases of malaria amounting to approximately 429,000 deaths in 2015, of which the majority of deaths were recorded in Africa.[1] The most important parasite, *Plasmodium falciparum* (*Pf*) has acquired resistance to most drugs, including most recently the clinically-used artemisinins.[2–6] As an example, in some areas of Western Cambodia the ACT comprising dihydroartemisinin (DHA)-piperaquine failed to cure half of all patients treated.[2,3] In general, DHA **1** is either used as such, or is the active metabolite of the other clinically used artemisinins artemether and artesunate due to the metabolism of the C-10 methyl ether or facile hydrolysis of the succinate ester respectively.[4] It appears that DHA is implicated in artemisinin resistance.[5,6] Thus, it is necessary to avoid the formation of this metabolite, and so it is best to consider new derivatives not bearing an oxygen atom attached to C-10. We have shown elsewhere that artemisone, a derivative bearing an amino group at C-10 is not metabolized to DHA.[7] Further, in terms of their *in vitro* activities, C-10 substituted amino-artemisinins in general appear to be optimal substrates in direct comparison with C-10 O- and C-substituted counterparts.[8] We have shown that artemisinins act as *oxidant* drugs through the ability of the peroxide group to rapidly oxidize intracellular components such as reduced flavins of flavin disulfide reductases, and thereby perturb redox homeostasis in the malaria parasite.[9,10] The peroxide is reduced irreversibly through accepting electrons from the reduced flavin. Thus, a possible further means to address resistance is to seek additional modes of action by modifying the structure of the artemisinin through attachment of groups that may act as pharmacophores in their own right. The ferrocene pharmacophore acts as a redox centre that undergoes redox cycling. The ferrocene-Fe^{2+} may be oxidized by free or labile Fe^{3+} to form ferrocenium (ferrocene-Fe^{3+}).[11–13] In the last case, the labile Fe^{2+} thereby generated is oxidized by oxygen to form superoxide; subsequent reaction with Fe^{2+} via the Fenton pathway generates hydroxyl radicals. Thus, the increased production of reactive oxygen species (ROS) leads to perturbation of parasite redox homeostasis. Importantly, ferrocenium is reduced to ferrocene by metalloproteins (ferrocytochrome c), NADH and thiols such as glutathione (GSH).[14–17] Given that thiols are capable reductants, ferrocenium very likely is reduced also by reduced flavins (that like thiols rapidly reduce labile Fe^{3+}), although evidently this has yet to be demonstrated.[18–20] Thus, by attaching the redox active moiety to the oxidant artemisinin, the overall ability of the ensemble of oxidant and redox centres to enhance oxidative stress is greatly increased; that is, once the

* Corresponding authors.
E-mail addresses: richard.haynes@nwu.ac.za (R.K. Haynes), david.nda@nwu.ac.za (D.D. N'Da).

https://doi.org/10.1016/j.bmcl.2017.12.057
0960-894X/

peroxide pharmacophore is reduced, the ferrocene is able to continue the cycle of oxidation and reduction thereby maintaining oxidative stress in the parasite. It is noted that artemisinin-ferrocene derivatives have been prepared previously and their antimalarial activities have been assessed essentially in terms of their binding to heme.[21,22] The most noteworthy aspect of these hybrids is the evidently mutual compatibility of the peroxide with the ferrocene ferrous iron, even though free ferrous iron, with its admittedly lower oxidation potential than that of ferrous iron in ferrocene or ferrous iron in heme, are popularly ascribed to 'activating' the peroxide to induce formation of 'toxic' free radicals from the artemisinin peroxide.[23] This toxic radical concept has been thoroughly dissected on the basis of the well-established chemistry of carbon-centred free radicals and is difficult to reconcile with the behaviour of artemisinins in presence of free or hemeligated ferrous iron.[24] Be that as it may, as an exploratory venture into probing efficacies of artemisinin-ferrocene hybrids in terms of the oxidant-redox activity concept, we sought here to exploit the unique activities of C-10 amino-artemisinins through attachment of the redox-active ferrocene pharmacophore to the amino group.

DHA **1** in toluene in the presence of catalytic dimethyl sulfoxide was quantitatively converted by oxalyl chloride into the 10β-chloride and the latter treated *in situ* with piperazine to provide 10α-(1′-piperazino)-10-deoxo-10-dihydroartemisinin **2** (Scheme 1).[25] That this and the final derivatives possessed the 10α-stereochemistry was confirmed by the coupling constant of *J* 10.2 Hz between H-10 and H-9 in **2**, which is consistent with an *anti*-periplanar (*trans*-diaxial) arrangement of these protons in a chair pyranose ring.[26] This intermediate proved to be sufficiently stable for attachment of the ferrocene moiety. Friedel-Crafts acylation of ferrocene with the corresponding acyl halides gave 1-ferrocenyl-3-chloropropan-1-one (27%), 1-ferrocenyl-5-bromopentan-1-one (79%) and 1-ferrocenyl-6-bromohexan-1-one (83%) according to literature methods.[27] These intermediates were condensed with the piperazine derivative **2** in the presence of DBU



Scheme 1. Synthesis routes for amino-artemisinin-ferrocene derivatives and yields i. **1** (1 eq.), toluene, dimethyl sulfoxide (0.1 eq.), N_2, oxalyl chloride (1.1 eq.), room temperature, 2 h; piperazine (5 eq.), dichloromethane, N_2, room temperature, 16 h, 35%. ii. **2** (1 eq.), acetonitrile, ferrocenoyl halide (X = Cl, Br, see text) (1 eq.), DBU (0.225 eq.), room temperature, 24 h. iii. Ferrocenecarboxaldehyde (1 eq.), **2** (3.1 eq.), THF, N_2, sodium triacetoxyborohydride (2.5 eq), room temperature, overnight. iv. **2** (1 eq.), acetonitrile, ferrocene alkyl halide (1 eq.), DBU (2 eq.), 0.1 mL DMF, microwave.

to give the derivatives **3**–**5**. 1-Ferrocenyl-2-chloroethan-1-one and 1-ferrocenyl-4-chlorobutan-1-one were also prepared, but although the final piperazine derivatives were obtained after condensation with **2**, these could not be purified, thus precluding their further examination. For preparation of the ferrocene methyl derivative **6**, the piperazine intermediate **2** was submitted to reductive alkylation with ferrocene-carboxaldehyde and sodium triacetoxyborohydride to cleanly deliver this product (61%). In order to prepare the other alkylation products **7**–**9**, the ferrocenoyl halides were reduced with borane *tert*-butylamine and aluminium chloride to give the ferrocenyl alkyl halides (84–98%).[28] However, alkylation of the piperazine intermediate **2** with these alkyl halides was not straightforward, and microwave radiation had to be used to drive the reactions so as to provide the final derivatives **7**–**9** in approximately 20% yields.

In vitro antiplasmodial activity was determined against the chloroquine sensitive (CQS) NF54 strain and chloroquine resistant (CQR) K1 and W2 strains of *P. falciparum* (*Pf*) using the SYBR Green I based fluorescence assay to measure parasite proliferation.[29] The resistance index (RI) for each drug resistant strain (ratio of the IC_{50} values of the resistant to sensitive strains IC_{50} K1/ IC_{50} NF54 and IC_{50} W2/ IC_{50} NF54) was calculated as an indication of potential cross-resistance formation. The *in vitro* cytotoxicity assay was performed on human embryonic kidney cells Hek293 and anti-tumour screening was carried out on the human malignant melanoma cell line A375 as described previously.[30] The selectivity indices (SI) indicate the selectivity of the compounds towards *Pf* or cancer cells compared to mammalian cells *in vitro*. The amino-artemisinin-ferrocene derivatives showed good activity on asexual parasites with IC_{50} values in the low nM range (Table 1). The ferrocene derivatives in general tend to be less active than the comparator drugs dihydroartemisinin and artesunate (Table 1). The RI values of all of the amino-artemisinin-ferrocene derivatives were smaller than 1, which indicates a low potential for cross resistance and similar to the indices for the artemisinin reference compounds (Table 1). With the exception of compound **5**, most compounds showed good selectivity for *Pf* parasites, with SI indices >9000. Compound **5**, the least active compound towards *Pf*, was more cytotoxic towards mammalian cells than cancer cells. It is noted that compounds **4**, **8** and **9** were relatively poorly soluble in the culture medium, and meaningful data could not be obtained for compound **4**. The solubility issues aside, the three most active amino-artemisinin-ferrocene derivatives were **3**, **6** and **7**. Not unexpectedly, electron withdrawing substituents attached to the ferrocene decrease the ease of oxidation of the Fe^{2+} centre and electron donating groups have the reverse effect.[31–34] However, even though the atom adjacent to the ferrocene ring comprises different functional groups, namely electron withdrawing carbonyl for compound **3**, and electron-donating amino-methylene for compound **6** and methylene for compound **7**, activities do not vary significantly, indicating that such effects are insignificant in these screens. Conversion of DHA **1**, that has IC_{50} values of 4 ± 1 μM on the Hek293 cell line and 1 ± 0.1 μM on the A375 cell line, via the amino-artemisinin **2** into the derivatives **3**, **6** and **7** results in greatly enhanced selectivities with respect to the malaria parasites (Table 1).[35,36] In terms of overall accessibility and activities, compound **6** is identified as a hit compound.

Thus, in summary, we have demonstrated that the amino-artemisinin ferrocene derivatives retain good antimalarial activities and display good selectivities *in vitro* towards *Pf*. Clearly, in order to evaluate any role played by the ferrocene pharmacophore, it is necessary to conduct *in vivo* assays to establish if there are indeed any differences between the parent amino-artemisinin and the amino-artemisinin-ferrocene conjugates wherein redox cycling of the ferrocene moiety should continue once the peroxide of the artemisinin is reduced *in vivo*. Overall, the work provides a useful