**Enantioselective Synthesis and Profiling of Potent, Non-linear Analogues of Antimalarial Tetraoxanes E209 and N205**

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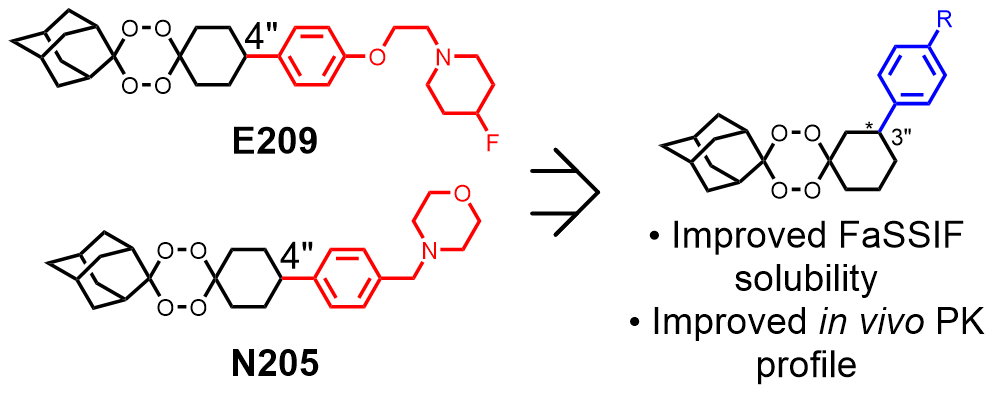
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**Abstract**

Synthetic endoperoxide antimalarials, such as 1,2,4-trioxolanes and 1,2,4,5-tetraoxanes are promising successors for current front-line antimalarials, semi-synthetic artemisinin derivatives. However, limited solubility of second-generation analogues in biological relevant media represents a barrier in clinical development. We present methodology for the synthesis of non-linear analogues of second generation tetraoxane antimalarials E209 and N205 to investigate reduced molecular symmetry on *in vitro* antimalarial activity and physicochemical properties. Whilst maintaining good antimalarial activity and metabolic stability, head-to-head comparison of linear and non-linear counterparts showed up to ten-fold improvement in FaSSIF solubility for three of the four analogues studied. Pharmacokinetic studies in the rat comparing a selected non-linear analogue **14a** and its parent N205 showed improvement on oral absorption and exposure *in vivo* with more than double the AUC and a significant increase in oral bioavailability (76% versus 41%). These findings provide support for further *in vivo* efficacy studies in preclinical animal species.

**KEYWORDS**: Endoperoxide, antimalarial, *Plasmodium falciparum*, asymmetry, melting point, solubility

Artemisinin combination therapies (ACTs) are currently the front-line treatment for uncomplicated malaria. All World Health Organisation (WHO) approved ACTs contain a fast acting semi-synthetic short half-life artemisinin (ART, Figure 1) derivative alongside a partner drug with a longer half-life to maintain a prolonged parasiticidal effect.1 Whilst effective, ART derivatives suffer from poor pharmacokinetic (PK) profiles, with the short half-life being the main limiting factor preventing deployment in dosing regimens of less than 3 days.2 Due to this, it is unlikely that semi-synthetic artemisinin derivatives can achieve the requisite target of a single-dose cure of malaria even in combination, the preferred target candidate profile 1 (TCP1) as defined by the Medicines for Malaria Venture (MMV).3 Additionally, cases of ACT treatment failure have been reported across the Greater Mekong subregion spreading from the Thai-Cambodian border into Vietnam.4,5 The K13 C580Y gene mutation is used as a primary marker of ART resistance in *Plasmodium falciparum* malaria with the proposed mechanism of resistance pointing towards altered endocytosis of hemoglobin, which is required for parasite growth and artemisinin activation.6 Due to this, there is a pressure for new fast-killing antimalarials to take the place of failing ART-derivatives in ACT programmes.



Figure 1: Structures of artemisinin and fully-synthetic endoperoxide antimalarials

Presently, OZ439 (Figure 1, known as Artefenomel) is the most developed fully-synthetic second generation endoperoxide antimalarial currently undergoing phase IIb clinical trials in combination with the longer acting partner drug Ferroquine.7,8 OZ439 is a 1,2,4-trioxolane containing antimalarial possessing potent fast-killing activity against plasmodium parasites and crucially has been shown to achieve a single dose 100% cure in the *P. berghei* mouse model of malaria infection.9 The structure activity relationship of OZ439 centres around the 1,2,4-trioxolane endoperoxide unit which mimics the 1,2,4-trioxane moiety of ART allowing bioactivation by Fe(II), while the spiro-adamantane and adjacent 4”-phenyl moieties restrict the accessibility of the O-O linkage enabling greater stability to serum Fe(II).10 While OZ439 has demonstrated outstanding efficacy, there are known issues with variable pharmacokinetics (PK) *in vivo* owing to solubility issues and formation of colloids.11 OZ439 has been formulated as a mesylate salt due to its high aqueous solubility, however recent studies have suggested that in the digestion process OZ439-mesylate can undergo anion transfer with chloride ions in the stomach causing precipitation of colloidal OZ439-hydrochloride.11 Furthermore, in the less acidic environment of the small intestine the free-base form of OZ439 has also been observed to precipitate reducing the amount of solubilised OZ439 available for absorption. 11–13



Figure 2: Structure of OZ277 (arterolane) and regioisomeric analogues from amide (**1**)14 and carbamate (**2**) 15 series

Recently, a series of antimalarials based on the early trioxolane arterolane (**OZ277**, Figure 2), approved for use in combination with piperaquine as Synriam in 2012,16 with regioisomeric substitutions have been synthesised and evaluated. These differ from the parent compound by possessing a 3” substitution pattern on the cyclohexyl ring affording control over the conformation-dependent activity of the trioxolane pharmacophore.14,15 Amide (**1**) and carbamate (**2**) series of compounds have been described with compounds possessing potent antimalarial activity *in vitro* and up to 5 fold more potent PD100 values in the *P. berghei* mouse model of malaria.14,15 Though the *in vivo* activity of these 3” analogues appear promising, the sidechains explored in both series are similar to OZ277 and likewise display short half-lives with the carbamate compound **2** being extensively cleared by mouse liver microsomes contributing to its shorter half-life of just 9 minutes. 14,15

Previously, we have reported second generation 1,2,4,5-tetraoxane containing antimalarials E209 and N205 (Figure 1) with both compounds possessing potent fast killing of *P. falciparum* as well as a 66% cure in the *P. berghei* mouse model after a single dose of 30 mg/kg (2/3 mice cured).17,18 Tetraoxane antimalarials work by a similar mechanism of the bioactivation of the 1,2,4-trioxolane peroxides by interaction with Fe(II).19 Like OZ439 these compounds are amphiphilic and poorly water soluble, particularly in fasted state simulated intestinal fluid (FaSSIF), suggesting room for improved intestinal absorption.17 It is well established that the melting point is related to molecular symmetry in that high molecular symmetry is often accompanied by a high melting point. By the same logic, reducing molecular symmetry is likely to reduce melting point; this is rationalised by a larger entropy change being observed in the fusion of high symmetry systems.20 Furthermore, melting point has been shown to be useful in the prediction of solubility curves for a range of small molecules with a lower melting point being correlated with improved solubility.21 For medicinal chemistry, a decrease in molecular symmetry may translate into improved drug-like properties including solubility and absorption leading to a greater systemic exposure to a drug.22 We present a methodology for the synthesis of chiral tetraoxane containing antimalarials and have assessed structure activity relationships and structure property relationships of the synthesised analogues in comparison to the established endoperoxides OZ439, E209 and N205.



Scheme 1: Retrosynthetic analysis of 2nd generation tetraoxane 3”-substituted analogues

As mentioned above, non-linear analogues of the trioxolane antimalarial OZ277 have been reported opting to introduce non-linearity at the 3” position on the cyclohexyl ring.14 Similarly, for our tetraoxane scaffold the 3”-position offers synthetic access to non-linear analogues. Retrosynthetic analysis of second generation tetraoxane 3”-substituted analogues (Scheme 1) identified two chiral conjugate addition products as key intermediates analogous to those in previously reported syntheses. Movement to a non-linear scaffold introduces a chiral centre into the previously achiral scaffold, hence it was determined that the analogues should be synthesised and evaluated as both individual enantiomers and racemic mixtures.

Several conditions exist for enantioselective aryl conjugate addition, for our purposes we chose to use Rh(I) catalysis for the conjugate addition of aromatic boronic acids. This was chosen due to the high reported yields and enantiomeric excesses in previous literature as well as the synthetic flexibility for the preparation of substituted cyclohexanones. Furthermore, the use of chiral ligands allows control over the geometry of the generated chiral centre as well as allowing racemic syntheses through use of a racemic ligand.23,24



|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compound | R | Ligand | Yield/ % | %ee | Product |
| **3a** | OBn | *R*-BINAP | 57 | > 99 | *R* |
| **3b** | OBn | *S*-BINAP | 59 | > 99 | *S* |
| **3c** | OBn | rac-BINAP | 46-59 | - | Racemate |
| **4a** | CO2Me | *R*-BINAP | 91 | > 99 | *R* |
| **4b** | CO2Me | *S*-BINAP | 91 | > 99 | *S* |
| **4c** | CO2Me | rac-BINAP | 88-93 | - | Racemate |

Table 1: Summary of yields and enantiomeric excesses obtained in the synthesis of conjugate addition intermediates. a) Rh(I)(acac)(C2H4)2, KOH, Dioxane:H­2O, 100°C 5h

Conjugate addition was carried out using Rh(acac)(C2H4)2 as the Rh(I) source and BINAP as the chiral ligand with yields summarised in Table 1. Benzyloxyphenylboronic acid was used as a route to the 4-acetoxyphenyl intermediate in the synthesis of E209 analogues. All conjugate addition products were obtained in modest to high yields that are sufficient to continue synthesis with conversion evidenced by disappearance of alkene protons on 1H NMR. Enantiomeric excesses of the chiral products were all determined to be > 99% by chiral normal phase HPLC. The absolute stereochemistry of these products has not been determined however DFT calculations using phenylboronic acid as a model substrate (Table S1) showed a clear energetic preference of 4.48 kcal/mol for the favoured transition state which would yield the assigned stereochemistry. The origin of stereoselectivity comes from steric interaction of the cyclohexenone with the bulky phenyl substituents on the phosphine. This produces a preference for the cyclohexenone to interact with the rhodium (I) centre in a position which minimises steric interactions enabling formation of a transition state with a bond to the aryl group forming on a specific face.



Scheme 2: b) H2, 10 % Pd/C, EtOAc, rt 3h. c) Ac2O, NEt3 DCM, rt, o/n. d) 50 % H2O2, HCO2H, MeCN, 0°C to rt 30 min. e) 2-adamantanone, Re2O7, DCM, 0°C to rt o/n. f) LiOH, THF:H2O, rt 2h. g) Alkyl chloride, K2CO3, MeCN, reflux o/n

As carbonyl containing substrates have been seen to afford higher yields in tetraoxane formation, the benzyl protected intermediate required conversion to an acetyl protected phenol for the synthesis of E209 analogues. Synthesis was carried out identically for both the pure enantiomers and racemate. This was achieved by catalytic hydrogenation of **3a-c** (Scheme 2) over 10 % palladium on carbon to afford the free phenol **5a-c**, which following workup was subsequently acetylated by treatment with acetic anhydride and triethylamine. Workup afforded **6a-c** in quantitative yield over two steps. The tetraoxanes **7a-c** were synthesised from **6a-c** by dihydroperoxide formation promoted by formic acid followed by Re2O7 catalysed condensation with the 2-adamantanone.25 The yield of this step was relatively low but consistent with yields obtained in other literature.26 Formation was evidenced by appearance of characteristic peaks on 13C NMR corresponding to the two tetraoxane carbons at 100-110 ppm. Quantitative acetyl deprotection by treatment with LiOH in THF and water afforded free phenols **8a-c**. Treatment with the required alkyl halide and potassium carbonate in acetonitrile afforded the E209 analogues **9a-c** and **10a-c**. Enantiomeric excess, summarised in Table 2, was found to be at least 98 % for all chiral analogues suggesting that no racemisation had occurred during synthesis. All compounds were obtained as amorphous solids.



Scheme 3: h) 50 % H2O2, HCO2H, MeCN, 0°C to rt 30 min. i) 2-adamantanone, Re­2O7, DCM, 0°C to rt o/n j) LiAlH4, THF, 0°C 30 min. k) Methane sulfonyl chloride, NEt3, DCM, 0°C 1 hour l) Amine or amine hydrochloride, NEt3, DCM, rt o/n.

In the synthesis of N205 analogues, the obtained methoxycarbonyl conjugate addition products **4a-c** (Scheme 3) were used directly to synthesise the required tetraoxanes **11a-c** through formic acid dihydroperoxide formation and subsequent condensation with 2-adamantanone. Similar to the analogous reaction for E209 analogues, the yields were relatively low but afforded enough products to continue synthesis. Subsequent reduction of **11a-c** by lithium aluminium hydride afforded benzyl alcohols **12a-c** in quantitative yield, which were subsequently converted to benzyl mesylates **13a-c** by treatment with methane sulfonyl chloride and triethylamine in DCM. Coupling of **13a-c** with the desired cyclic amine was achieved by treatment with triethylamine in DCM to afford the N205 analogues **14a-c** and **15a-c**. The synthesised analogues were analysed by chiral HPLC to determine enantiomeric excess. Enantiomeric excess, summarised in Table 2, was found to be at least 98 % for all enantiomerically pure analogues suggesting that no racemisation had occurred during synthesis. All compounds were obtained as amorphous solids.



|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Compound | X | C-3 Stereochemistry | %ee | m.p.  (°C) | IC50 D10  (nM)  a | IC50 W2  (nM) | Reference |
| **9a** | CHF | *R* | > 99 | 68-71 | 17.46 | 19.20 | - |
| **9b** | CHF | *S* | > 99 | 65-69 | 20.77 | 22.46 | - |
| **9c** | CHF | Racemate | - | 58-61 | 12.92 | 12.80 | - |
| **E209 b** | CHF | - | - | 182-183 | 8.96 | 8.49 | 17 |
| **10a** | O | *R* | 99 | 61-65 | 17.38 | 15.98 | - |
| **10b** | O | *S* | 98 | 56-62 | 15.96 | 15.82 | - |
| **10c** | O | Racemate | - | 56-58 | 16.39 | 17.68 | - |
| **16** | O | - | - | 106-108 | 12.37 | 10.12 | 26 |
| **14a** | O | *R* | 98 | 93-95 | 12.49 | 11.25 | - |
| **14b** | O | *S* | 99 | 93-95 | 15.22 | 15.20 | - |
| **14c** | O | Racemate | - | 73-75 | 15.43 | 11.22 |  |
| **N205b** | O | - | - | 180 | 9.38 | 8.65 | 18 |
| **15a** | CHF | *R* | >99 | 89-92 | 10.58 | 10.28 | - |
| **15b** | CHF | *S* | 98 | 83-86 | 16.46 | 14.88 | - |
| **15c** | CHF | Racemate | - | 74-76 | 15.68 | 14.44 | - |
| **N214** | CHF | - | - | 122-124 | 16.15 | 11.74 | 18 |
| **OZ439** | - | - | - | 105-109 | 5.10 (3D7) | - | 17 |
| **CQ** | - | - | - | - | 31.92 | 462.02 | - |
| **DHA** | - | - | - | - | 2.59 | 1.02 | - |

Table 2: Summary of enantiomeric excesses, melting points and antimalarial activity of synthesised analogues and literature compounds. a Unless stated IC50 values were determined against the D10 chloroquine sensitive strain of P. falciparum (Method is in Supporting Information file). The IC50 values are the mean of three different experiments, each performed in duplicate. b Tested as mesylate salt

In comparison to linear counterparts, the melting points of the chiral analogues (Table 2) were found to be significantly lower. This reduction in melting point is presumably due to interference with crystal packing making fusion a more entropically favourable process.20 As expected, the racemates possessed lower melting points than enantiomerically pure compounds.

To assess antimalarial activity, all analogues were screened against the chloroquine (CQ) sensitive strain D10 *P. falciparum* and theCQ resistant strain W2 *P. falciparum*;the IC­50 values for which are summarised in Table 2. Firstly, all analogues maintain low nanomolar potency against W2 *P. falciparum* suggesting the introduction of non-linearity does not interfere with the mechanism of action of the tetraoxane antimalarials. Notably in the CQ sensitive D10 strain all non-linear analogues were found to outperform CQ by approximately two-fold. Despite this, a reduction in activity was seen for all non-linear analogues in comparison to their linear counterparts. No significant difference was seen in antimalarial activities between individual enantiomers which we expected as the proposed mechanism for bioactivation involves mainly the peroxide containing moiety, with the tetraoxane substituents attenuating peroxide stability and physicochemical properties.19



|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Cmpd | X | Stereo-chemistry | Aqueous Solubility pH 7.4 (µM) | Rat Hepatocyte CLint (µl/min/ 106) | Human Microsome CLint (µl/min/mg) | cLogPa |
| **9a** | CHF | *R* | 0.1 | 13.5 | 19.8 | 5.73 |
| **9b** | CHF | *S* | 0.1 | 16.7 | 26.3 | 5.73 |
| **10a** | O | *R* | 0.1 | 21.6 | 22.2 | 4.79 |
| **10b** | O | *S* | 0.1 | 49.2 | 22.1 | 4.79 |
| **14a** | O | *R* | 0.1 | 26.2 | 27.1 | 4.48 |
| **14b** | O | *S* | 0.1 | 50.2 | 19.7 | 4.48 |
| **15a** | CHF | *R* | 0.1 | 19.2 | 35.9 | 5.34 |
| **15b** | CHF | *S* | 0.1 | 30.7 | 26.7 | 5.34 |
| **E209** | - | - | 0.106 | 12 | 23 | 6.06 |
| **N205** | - | - | < 0.1 | - | 41 | 4.5 |
| **OZ439** | - | - | 0.085/0.18128 | 14 | 68 | 5.44 |

Table 3: Summary of DMPK data obtained for individual enantiomers of chiral analogues. The DMPK property data described above were measured once through a high-throughput platform provided by AstraZeneca UK. The methods of the five assays, including LogD7.4, aqueous solubility, plasma protein binding, and microsome and hepatocyte clearance measurements, have been reported previously 27 aCalculated using ChemDraw professional 19.1.1.21

The enantiomerically pure enantiomers were screened for DMPK parameters as summarised in Table 3. Intrinsic clearance by human liver microsomes showed high metabolic stability for all analogues except for **15a**which possessed moderate metabolic stability. In comparison, clearance by rat hepatocytes was found to be more variable with **9a, 9b, 10a, 14a** and **15a** displaying high metabolic stability while **10b, 14b** and **15b** displayed moderate metabolic stability. While the substituent on the cyclic amine was not seen to produce significant differences in metabolic stability, for most analogues there were some noticeable differences between pairs of enantiomers, especially in the rat hepatocyte clearance. Non-linear analogues of E209 scaffold were found to display similar levels of metabolic stability with only **9b** appearing significantly less stable in rat hepatocytes compared to the parent molecule. Non-linear analogues of the N205 scaffold were all found to be more stable to metabolism by human liver microsomes compared to the parent molecule. In comparison to OZ439 all analogues were found to be more stable in human liver microsomes. It should however be noted that, in contrast to its long half-life of >40 hours, OZ439 possesses paradoxically low stability in human liver microsomes *in vitro* and as such improvements in stability aren’t necessarily indicative of improved *in vivo* efficacy.28



|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compound | | **9b** | **10b** | **E209** | **OZ439** |
| X | | CHF | O | - | - |
| Stereochemistry | | *S* | *S* | - | - |
| m.p. (°C) | | 65-69 | 56-62 | 182-183 | 105-109 |
| pKaa | | 8.4 | 7.09 | 8.43 | 7.09 |
| Concentration (µg/ml) | 0.1M HCl | 1271 | 284 | 1789 | - |
| FaSSIF Media | 413 | 69 | 215 | 120 |
| pH at equilibrium | 0.1M HCl | 1.14 | 1.11 | 1.13 | - |
| FaSSIF Media | 6.43 | 6.42 | 6.42 | 6.6 |

Table 4: Summary of solubility measurements for non-linear tetraoxane containing antimalarials based on the E209 scaffold and reference compounds. a Calculated using ChemDraw professional 19.1.1.21 N.B. basicity of pKa values is likely overpredicted using in silico methods

Although initially all analogues were seen to possess poor aqueous solubility in PBS buffer at pH 7.4 with no improvement over second generation tetraoxane antimalarials, when selected compounds were screened for solubility in 0.1M HCl and FaSSIF media the effects of non-linearity on solubility in model physiological fluids were clearly demonstrated. Table 4 summarises the obtained solubility data for compounds **9b** and **10b** as well as the reference compounds **E209** and **OZ439**. As mentioned, while E209 displayed good solubility at lower pH values, solubility in FaSSIF media was limited indicating potential poor absorption in the fasted state. **9b** similarly displayed good solubility in 0.1M HCl but also displayed improved solubility in FaSSIF media showing a 2-fold increase compared to **E209** as well as a 3.5-fold increase compared to **OZ439**. Disappointingly, **10b** displayed poorer solubility in both 0.1M HCl and FaSSIF media. While we expected the solubility of both analogues to increase with the introduction of asymmetry, we hypothesise that the varied effect could be rationalised by the flexibility of the ethylene linker which through its flexibility could make any structural changes to the core of the molecule less significant to its crystal packing.



|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Compound | | **14a** | **14b** | **N205a** | **15a** | **15b** | **N214 a** |
| X | | O | O | O | CHF | CHF | CHF |
| Stereochemistry | | *R* | *S* | *-* | *R* | *S* | - |
| m.p. (°C) | | 93-95 | 73-75 | 180 | 89-92 | 83-86 | 122-124 |
| pKab | | 7.42 | 7.42 | 7.44 | 8.74 | 8.74 | 8.76 |
| Concentration (µg/ml) | 0.1M HCl | 188 | 165 | 21.2 | 95 | 60 | 101 |
| FaSSIF Media | 324 | 248 | 24 | 195 | 189 | 14.8 |
| pH at equilibrium | 0.1M HCl | 1.12 | 1.13 | 1 | 1.12 | 1.14 | 1 |
| FaSSIF Media | 6.43 | 6.42 | 6.1 | 6.43 | 6.44 | 6.1 |

Table 5: Summary of solubility measurements for non-linear tetraoxane containing antimalarials based on the N205 scaffold and reference compounds. a Solubility determined for the mesylate salt. b Calculated using ChemDraw professional 19.1.1.21 N.B. basicity of pKa values is likely over predicted using in silico methods

Table 5 summarises the solubility data obtained for the benzyl amine analogues **14a**, **14b**, **15a** and **15b**. For each of the non-linear analogues FaSSIF solubility was found to be significantly improved in comparison to the parent compounds; 10 – 13.5-fold for **14a** and **b** and 13-fold for **15a** and **b**. This represents a greater increase in FaSSIF solubility than the analogues on the **E209** scaffold which may be due to the comparably lower flexibility of the benzyl amine scaffold. It should be noted that while solubility for an enantiomeric pair should be the same, variations in FaSSIF measurements between batches has been observed and appropriate care should be taken when interpreting results.29

In order to further investigate the effect asymmetry on absorption, a pair of compounds, **14a** and **N205** that showed a significant difference (>13 folds) in terms of FaSSIF solubility were selected to be assessed for their pharmacokinetic (PK) profiles through both IV and oral administration routes in the rat. As showed in Table 6, in the 10 mg/kg single oral dosing experiment, a number of PK parameters associated with drug absorption were noticeably improved when comparing between the non-linear analogues **14a** and its parent **N205**. For example, the mean Tmax of **14a** was shortened to 2 hours and the mean Cmax was increased to 410 ng/ml in the study dosing with **14a** in comparison to 4.33 hours and 136 ng/ml for **N205** respectively. The overall drug exposure, indicated by the mean AUCinf., was also increased from 608 hr\*ng/ml (**N205**) to 1438 hr\*ng/ml (**14a**) and the oral bioavailability was improved from an acceptable level of 41.3% (**N205**) to 76.3% (**14a**) at 10 mg/kg oral dosage. All these improved oral PK parameters strongly suggested that the enhanced FaSSIF solubility of the non-linear analogues has translated to improved oral absorption and exposure *in vivo* providing solid proof of principle for this approach.

|  |  |  |
| --- | --- | --- |
| Compound | **14a** | **N205** |
| Dosage and administrative route a | 10 mg/kg, oral | 10 mg/kg, oral |
| Tmax (hr) b | 2.0 ± 0.0 | 4.33 ± 1.15 |
| Cmax (ng/ml) b | 410 ± 14.8 | 136 ± 43.4 |
| AUCinf. (hr\*ng/ml) b | 1438 ± 363 | 608 ± 65.2 |
| Bioavailability (%) b | 76.4 ± 19.3 | 41.3 ± 4.43 |

*Table 6: Summary of selected oral PK data from PK studies in the rat. a Single oral dose using 10%DMSO +90%(5%Tween80 in 20 mM phosphate buffer (pH 3.0)) as formulation with male SD rats. b Mean values ± SD, n = 3.*

In conclusion, we have developed a methodology for the synthesis of chiral non-linear analogues of second generation tetraoxane containing antimalarials. The synthesised analogues all possessed low nanomolar activity against two strains of *P. falciparum* with no significant difference in in vitro potency between enantiomeric pairs. Introduction of non-linearity successfully decreased melting point and displayed significant improvements in FaSSIF solubility. This effect was more profound for the N205 benzyl amine analogues examined in this study. Head-to-head oral PK studies in the rat between the selected non-linear analogue, **14a** and its parent, **N205** demonstrated the enhanced FaSSIF solubility of the non-linear analogues had positive impacts to the oral absorption and exposure *in vivo*. Further *in vivo* screening of the synthesised analogues is required to determine whether introduction of non-linearity to tetraoxane antimalarials would be beneficial to *in vivo* efficacy.

ASSOCIATED CONTENT

Supporting Information

Supporting information is provided on computational chemistry, in *vitro* biological testing, Fassif solubility measurments, synthesis and HPLC traces for key intermediates and final products, and *in vivo* pharmacokinetics study.

The Supporting Information is available free of charge on the ACS Publications website.

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Author Contributions

The manuscript was written through contributions of all authors. P.M.O., W.D.H. and CW designed research; CW, NB, SP, KO, and TH performed the experimental research; C.W., W.D.H., G.L.N., N.B., S.P., K.O., T.H. and P.M.O. analysed data.

Notes  
The authors declare no competing financial interests.

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**ABBREVIATIONS**

ACT, artemisinin combination therapy; ART, artemisinin; AUCinf, AUC from 0 to infinity; Clint, intrinsic clearance; Cmax, maximum concentration observed; CQ, chloroquine; MLM, mouse liver microsomes; MMV, Medicines for Malaria Venture; PD100, dose required for 100 % cure of parasitaemia; TCP1, target candidate profile 1; Tmax­, time of maximum concentration; WHO, world health organisation

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