

Cite this: *RSC Adv.*, 2014, 4, 22856

1-Aryl-1,2,3,4-tetrahydroisoquinolines as potential antimalarials: synthesis, *in vitro* antiplasmodial activity and *in silico* pharmacokinetics evaluation†

Joelle Ngo Hanna,^{‡a} Fidele Ntie-Kang,^a Marcel Kaiser,^b Reto Brun^b and Simon M. N. Efang^{*a}

In the present study, twenty-one 1-aryl-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (THIQ) analogues were synthesized by base-catalyzed Pictet–Spengler reaction, and tested *in vitro* against *P. falciparum* using the [³H]hypoxanthine incorporation assay. Two compounds were found to be inactive while seventeen compounds displayed moderate antiplasmodial activity and two compounds were found to be highly active (IC₅₀ < 0.2 µg ml^{−1}). The two highly active compounds, 1-(4-chlorophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline and 6-hydroxyspiro[1,2,3,4-tetrahydroisoquinoline-1:1'-cyclohexane], also displayed low cytotoxicity, against rat skeletal myoblast cells, with CC₅₀ values of 257.6 and 174.2 µM respectively. These results justify further investigation of simple 1-aryl-1,2,3,4-tetrahydroisoquinolines as potential anti-malarial agents.

Received 18th November 2013
Accepted 1st April 2014

DOI: 10.1039/c3ra46791k

www.rsc.org/advances

Introduction

Malaria is a life threatening infectious parasitic disease that inflicts both health and economic losses.¹ The disease is transmitted by mosquitoes of the genus *Anopheles*. The causative agent for malaria is the *Plasmodium* species, and there are four types of human malaria: *P. vivax*, *P. malaria*, *P. ovale* and *P. falciparum*. *P. vivax* and *P. falciparum* are the most common, and *P. falciparum* is responsible for the most deadly type of malaria infection.² Statistics indicate that *P. falciparum* causes more than 300 to 500 million clinical cases of malaria infection, resulting in 1.7 to 2.5 million deaths annually.³ Malaria is a particularly important disease in Sub-Saharan Africa where about 90% of cases and death occur, but is also a serious public health burden in South, East and Central Asia, South and Central America, Caribbean, Oceania, and Middle East regions.⁴ Many attempts have been made to control the disease by using vector control measures and/or chemoprophylaxis, but they have had limited success.⁵ The control of malaria is, among other factors, hampered by the emergence of resistance of the vector (*Anopheles* sp.) to dichlorodiphenyltrichloroethane (DDT) and other insecticides, and the ongoing spread of strains of *P. falciparum* that are resistant to currently used anti-malarial

drugs.⁶ Attempts to address the problem of resistance have resulted in the current shift to artemisinin based combination drug regimens. New anti-malarial drugs, especially those with new structures and novel modes of action against malaria are also needed. This work describes the synthesis of structurally simple tetrahydroisoquinolines (THIQs) and their preliminary evaluation as potential anti-malarial agents. THIQs abound in nature and have attracted significant attention due to their diverse biological activities. The THIQ substructure (Fig. 1) is found in many drugs and alkaloids that exhibit antitumor, cardiovascular, antibacterial, antimicrobial, antitubercular, antifungal, antileishmanial, anti-trypanosomal and antiplasmodial activities.^{7–20} These compounds include the naphthylisoquinolines, benzylisoquinolines and bisbenzylisoquinolines.

The broad spectrum of biological activities of tetrahydroisoquinolines, especially reports on the anti-human immunodeficiency virus (HIV) activity of michellamine B (1) and antiplasmodial activity of dioncophylline C (3), naphthylisoquinolines (Fig. 2) isolated from *Ancistrocladus korupensis*,¹¹ prompted us to investigate 1-aryl-1,2,3,4-tetrahydroisoquinolines, as abbreviated analogues of the naturally occurring compounds, for antiplasmodial activity. Naphthylisoquinoline alkaloids are known to exhibit curative

^aDepartment of Chemistry, Faculty of Science, University of Buea, P.O. Box 63, Buea, South West Region, Cameroon. E-mail: smbuangalefange@gmail.com; Tel: +237 99915610

^bDepartment of Medical Parasitology and Infection Biology Parasite Chemotherapy, Swiss Tropical Institute, Basel, Switzerland

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3ra46791k

‡ Current address: Department of Chemistry, Faculty of Science, University of Douala, P. O. Box 24157, Douala, Littoral Region, Cameroon.

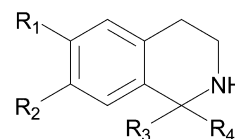


Fig. 1 1,2,3,4-Tetrahydroisoquinoline.

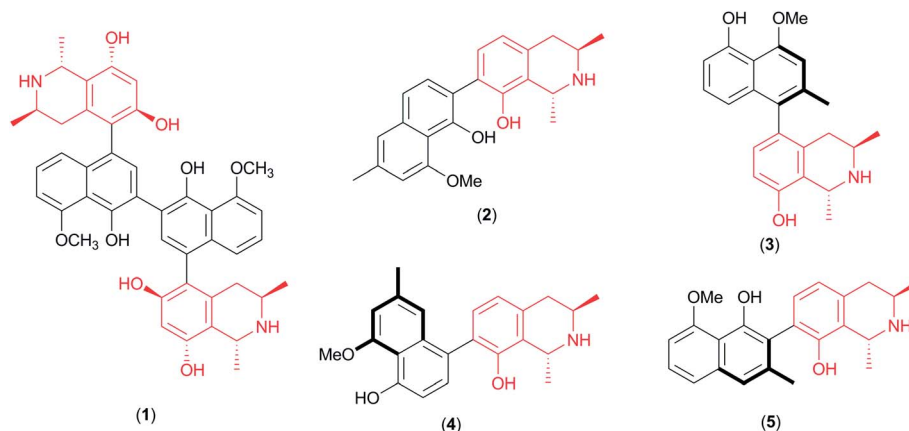


Fig. 2 Michellamine B (1) and dioncophyllines B (2), C(3), D(4) and E(5) with the tetrahydroisoquinoline scaffold shown in red.

potentials against malaria.^{12–14} Dioncophylline E (5) has exhibited good antimalarial activity against both chloroquine-sensitive and -resistant strains of *P. falciparum* *in vitro*,¹³ while dioncophylline C was active against the chloroquine-resistant *P. berghei* Anka CRS parasites¹² with dioncophylline B (2), dioncophylline D (4) and synthetic naphthylisoquinolines also found to display good *in vitro* antiparasmodial activities.¹⁴ A noteworthy example of a structurally simple THIQ is the spirofused compound **IVa**, which was previously described by Kametani *et al.*,²¹ as an abbreviated analogue of erysodine, one member of the *Erythrina* class of alkaloids. Plants of the genus *Erythrina* have been used extensively in ethnomedicine for a number of applications including the treatment of liver disorders,²⁰ as antiparasmodial agents, analgesics, and anti-inflammatory agents,²¹ as well as in the treatment of diseases such as female infertility, stomach pain and gonorrhea.²² Moreover, the alkaloids of *Erythrina* type are characterized by their unique skeleton of a tetracyclic spiroamine.²³ They are classified in two main groups: alkaloids possessing a skeleton of a 6,5,6,6 indoloisoquinoline called erythrinanes and those with a skeleton of 6,5,7-6 indolobenzazepine called schelhammerans or homoerythrinane alkaloids (Fig. 3). *Erythrina* alkaloids are known to display antiparasmodial activity *in vitro*.^{24–26} The rationale behind the design of THIQ analogues as potential anti-malarials is that they seem to be the common denominator of the naturally occurring naphthoisoquinolines (1 to 5) which display anti-malarial activities (marked in red on Fig. 2). It could therefore be easily inferred that the THIQ scaffold could contain the pharmacophore responsible for the anti-malarial activity. Previous studies have revealed that even simple THIQs such as

1-aryl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines and 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines, display anti-HIV¹⁵ and bronchodilator¹⁶ properties. The current study, which forms part of a larger investigation, was undertaken to investigate the antiparasmodial activity of 1-aryl-1,2,3,4-tetrahydroisoquinolines. The THIQ skeleton presents eight positions that can accommodate substituents. The choice of positions 1 and 6 for this study was based on the ease of synthesis of 1-substituted-THIQs (which is offered by the Pictet Spengler) and the presence of alkoxy groups on the aromatic portion of complex THIQs like the naphthoisoquinolines, such as 2–5, which display antiparasmodial activity.

The target 6-hydroxy-1,2,3,4-tetrahydroisoquinoline derivatives (**IIIa** to **IIIu** and **IVa**) were synthesized *via* base-catalyzed Pictet–Spengler cyclization as previously reported.¹⁹ Overall yields over two steps ranged between 4 and 99%. The compounds were characterized by NMR and mass spectrometry. The corresponding hydrochlorides were subsequently tested *in vitro* for antiparasmodial activity against *P. falciparum*. An *in silico* assessment of drug metabolism and pharmacokinetics (DMPK) profiles was also carried out by the use of computed molecular descriptors related to the absorption, distribution, metabolism, excretion and toxicity (ADMET) of compounds.

Results and discussion

Antiparasmodial activity versus cytotoxicity

All compounds were tested *in vitro* against *P. falciparum* as described in the Experimental section. The biological data were plotted to obtain IC₅₀ values which represent the concentration

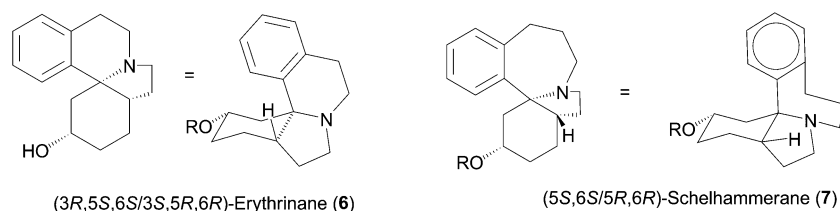


Fig. 3 Homoerythrinane alkaloids.

required to cause 50% inhibition of parasite growth. Activity criteria were as follows: inactive, $IC_{50} > 5 \mu\text{g mL}^{-1}$; moderately active, $0.5 \mu\text{g mL}^{-1} < IC_{50} < 5 \mu\text{g mL}^{-1}$; highly active, $IC_{50} < 0.5 \mu\text{g mL}^{-1}$. The antiparasmodial activity, presented as IC_{50} values, is provided in Table 1. The *P. falciparum* strain K1, known to be resistant to chloroquine and pyrimethamine, was employed for this assay. Chloroquine was used as the reference drug.

Of twenty-one compounds tested, three could be described as inactive following the criteria established. The rest were either moderately active (twelve) or active (seven). Among the active compounds at least three (**IIIb**, **IIIk** and **IVa**) displayed antiparasmodial activity comparable to that of chloroquine, suggesting the need for further evaluation of these compounds *in vivo*. In general, the more active compounds tended to possess electron withdrawing substituents on the pendant phenyl group (**IIIb-e,j,k,q**). However, a number of moderately active compounds contained electron donating substituents on the same phenyl group, thereby obscuring the role of electronic substituent parameters as determinants of antiparasmodial activity in this series. The present demonstration of antiparasmodial activity in the compound would therefore suggest that the key pharmacophoric elements for antiparasmodial activity in this class of alkaloids reside in the skeleton as described by **IVa**.¹⁹

When tested for cytotoxicity, several of the compounds were found to be considerably less toxic against rat skeletal myoblast cells than the reference compound podophyllotoxin. In addition, the tested compounds displayed toxicity at

concentrations that were two orders of magnitude higher than the concentrations at which they displayed antiparasmodial activity. Therefore, structurally simplified 1,2,3,4-tetrahydroisoquinolines may be potentially useful as antiparasmodial agents.

Compounds **IIIb**, **IIIk** and **IVa** were the most active with IC_{50} values 0.70, 0.76 and $0.89 \mu\text{M}$, respectively, and their activity was in the same range as that of chloroquine. In addition, the cytotoxicity of these compounds against myoblast cells could be considered as an index of drug effectiveness. Compounds **IIIb**, **IIIk** and **IVa** had selectivity index values 369.6, 229.1 and >451.5 respectively. These values are up to tenfold lower than the selectivity index value reported for chloroquine but significantly higher than the value of 100 which is required to qualify a compound as a "hit" in the screening of compounds for antiparasmodial activity.

At $2.30 \mu\text{M}$, **IIIa** was moderately active, but the presence of chlorine at C-4 resulted in a significant increase of activity, maybe due to the electron withdrawing property by inductive effect and *ortho* and *para* directive effect. This activity is not maintained in **IIIc**, **IIId**, **IIIe**, **IIIj**, **IIIm**, **IIIn**, **IIIo**, **IIIp** and **IIIq**. When a methoxyl group (electron donating by resonance) is introduced in compound **IIIa** to get **IIIg**, only a slight drop in activity is observed. When additional methoxyl groups are introduced, (**IIIg**, **IIIh**, **IIIi**) the activity drops even further. In general, the effects of both mono- and di-substitution on the pendant phenyl group appeared to be largely dependent on the nature of the substituent(s), but C4-substitution, especially with electron withdrawing groups of moderate size, appeared to be

Table 1 *In vitro* cytotoxicity and antiparasmodial activities of the synthesized 1-aryl-1,2,3,4-tetrahydroisoquinolines, along with selectivity indices^a

Compounds	Substituent (R)	IC_{50} <i>P. falciparum</i> K1 ($\mu\text{g mL}^{-1}$)	IC_{50} <i>P. falciparum</i> K1 (μM)	Cytotoxicity L6 ($\mu\text{g mL}^{-1}$)	Cytotoxicity L6 (μM)	Selectivity index
IIIa	H	0.519	2.304	>90	>400	>200
IIIb	4-Cl	0.181	0.697	66.9	257.6	369.6
IIIc	3-Cl	0.347	1.336	48.9	188.3	140.9
IIId	3,4-Cl ₂	0.395	1.343	29.1	98.9	73.7
IIIe	3-NO ₂	0.347	1.284	>90	>400	>300
IIIg	3-OCH ₃	0.646	2.530	84.9	332.5	131.4
IIIh	2,3-(OCH ₃) ₂	0.892	3.126	>90	>400	>100
IIIi	2,5-(OCH ₃) ₂	1.22	4.276	>90	>400	>100
IIIj	2,6-(OCH ₃) ₂	>5	>20	>90	>400	>200
IVa	<i>Spiro</i>	0.193	0.885	>90	>400	>400
IIIk	4-Br	0.481	1.581	47.44	155.9	98.6
IIIl	4-CF ₃	0.223	0.760	51.1	174.2	229.1
IIIm	4-Phenyl	0.906	3.006	3.29	10.9	3.6
IIIn	2-Br	NA	NA	NA	NA	NA
IIIo	2-F	2.31	9.495	28.3	116.3	12.3
IIIp	3-Br	1.08	3.550	9.06	29.8	8.4
IIIq	3-F	1.59	6.787	33.1	141.3	20.8
IIIr	4-F	0.595	2.446	36.5	150.0	61.3
IIIs	4-Cl-3-NO ₂	2.05	6.727	24.9	81.7	12.1
IIIu	5-Br-2-OCH ₃	0.744	2.226	9.18	27.5	12.3
Chloroquine	2-OH-5-NO ₂	10.3	35.978	19.5	68.1	1.9
Podophyllotoxin	4-CH ₃	0.891	3.955	21.6	95.9	24.2
		0.061	0.192	NA	NA	3000
				0.004		

^a NA: not available.

the most favored substitution pattern (compare **IIIq** vs **IIIp** vs **IIIh**; **IIIb** vs **IIIc** and **IIIj** vs **IIIo**). The favorable antiplasmodial activity of spirocyclohexyl analogue **IVa** may be attributed to the increased rigidity of this analogue.

In silico pharmacokinetics profiles

Molecular modelling was carried out on all synthesised molecules as described in the Experimental section. The computed molecular descriptors are those related to the prediction of DMPK/ADMET properties like the *n*-octanol–water partition coefficient, aqueous solubility, brain–blood partition coefficient, Caco-2 cell permeability, serum protein binding, number of likely metabolic reactions, solvent accessible surface area, blockage of human-ether-a-go-go potassium ion (HERG K⁺) channels and the total volume of the molecule enclosed by the solvent accessible surface area. The bioavailability of a compound depends on the processes of absorption and liver first-pass metabolism.²⁷ Absorption in turn depends on the solubility and permeability of the compound, as well as interactions with transporters and metabolizing enzymes in the gut wall. The computed parameters used to assess oral absorption are the predicted aqueous solubility, log *S*_{wat}, the conformation-independent predicted aqueous solubility, CI log *S*_{wat}, the predicted qualitative human oral absorption, the predicted % human oral absorption and compliance to Lipinski's famous "Rule of Five" (ro5).²⁸ The size of a molecule, as well as its capacity to make hydrogen bonds, its overall lipophilicity and its shape and flexibility are important properties to consider when determining permeability. Molecular flexibility has been seen as a parameter which is dependent on the number of rotatable bonds (NRB), a property which influences bioavailability in rats.²⁹ The blood–brain partition coefficients (log *B*/*B*) were computed and used as a predictor for access to the central nervous system (CNS). Madin–Darby canine kidney (MDCK) monolayers, are widely used to make oral absorption estimates, the reason being that these cells also express transporter proteins, but only express very low levels of metabolizing enzymes.²⁹ They are also used as an additional criterion to predict BBB penetration. Thus, our calculated apparent MDCK cell permeability could be considered to be a good mimic for the BBB (for non-active transport). The efficiency of a drug may be affected by the degree to which it binds to the proteins within blood plasma. Binding of drugs to plasma proteins (like human serum albumin, lipoprotein, glycoprotein, α, β' and γ globulins) greatly reduces the quantity of the drug in general blood circulation and hence the less bound a drug is, the more efficiently it can traverse cell membranes or diffuse. The predicted plasma-protein binding has been estimated by the prediction of binding to human serum albumin; the log *K*_{HSA} parameter (recommended range is −1.5 to 1.5 for 95% of known drugs). Human ether-a-go-go related gene (HERG) encodes a potassium ion (K⁺) channel that is implicated in the fatal arrhythmia known as *torsade de pointes* or the long QT syndrome.³⁰ The HERG K⁺ channel, which is best known for its contribution to the electrical activity of the heart that coordinates the heart's beating,

appears to be the molecular target responsible for the cardiac toxicity of a wide range of therapeutic drugs.³² HERG has also been associated with modulating the functions of some cells of the nervous system and with establishing and maintaining cancer-like features in leukemic cells.³² Thus, HERG K⁺ channel blockers are potentially toxic and the predicted IC₅₀ values often provide reasonable predictions for cardiac toxicity of drugs in the early stages of drug discovery.³³

The results for fifteen (15) relevant descriptors have been shown in Table 2, while 2D scatter plots of selected molecular descriptors are shown in Fig. 4. It was observed that only the points of subfigure C fell outside the recommended range for the dipole moment parameter. Subfigures A and B showed that all the compounds complied with Lipinski's ro5, since the molecular weights (MW) < 500 Da, logarithm of *n*-octanol–water partition coefficient (log *P*) < 5, number of hydrogen bond acceptors (accHB) < 10 and number of hydrogen bond donors (donHB) < 5. It was observed that the other computed molecular descriptors of all the THIQ analogues fell within the recommended range for 95% of known drugs (recommended parameters are shown in the ESI†), except for two compounds (**IIIo** and **IIIq**), as can be seen in subfigures 4D–L, corresponding respectively to the scatter plots for MW against the number of rotatable bonds (#rotor), cohesion index in solids, blood–brain barrier partitioning, HERG K⁺ ion channel blockage, binding affinities to human serum albumin, skin permeability, aqueous solubilities, as well Caco-2 and MDCK permeabilities. As for compound **IIIo**, the dipole moment was computed to be 0.524 Debye, below the recommended range (1.0 to 12.5 Debye), while for compound **IIIq**, the computed value was 0.890 Debye, only slightly below the recommended minimum for 95% of known drugs. The weak dipole moments could be explained by the absence of polar groups in the pendant phenyl ring of the two aforementioned compounds. Dipole moments as high as >6 Debye were observed for compounds **IIIe**, **IIIr** and **IIIt** containing the polarising nitro group in the pendant phenyl ring. It is however important to mention that the most active compounds (**IIIb**, **IIIk** and **IVa**) had interesting predicted pharmacokinetic profiles (with all computed parameters falling within the recommended range for 95% of known drugs, *i.e.*, #stars = 0). We can therefore conclude that these three compounds could be subjected to further investigation as potential anti-malarial drug leads.

Experimental

General experimental procedures

The synthesis of organic compounds was carried out with commercially available chemicals that were used as received (Scheme 1). All target compounds were tested in the form of the hydrochlorides which were obtained by dissolution of the corresponding free base in ethanolic HCl. Nuclear Magnetic Resonance spectroscopy data were recorded using UNITYplus – 500 spectrometer (¹H NMR at 500 MHz). Chemical shifts are denoted in unit parts per million (ppm) relative to the solvent (¹H NMR peak: 3.30 for CD₃OD). The following abbreviations are used to indicate peak multiplicities and characteristics: s

Table 2 Predicted ADME-related properties of the THI-Qs (Fig. 3) computed by QikProp program³¹

Compound	#stars ^b	Ro5 ^c	MW (Da) ^d	log P ^e	HBA ^f	HBD ^g	NRB ^h	log B/B ⁱ	BP ^j _{caco-2} (nm s ⁻¹)	S _{mol} ^k (Å ²)	S _{mol,hfob} ^l (Å ²)	V _{mol} ^m (Å ³)	log S _{wat} ⁿ	log K _{HSA} ^o	log HERG ^p	#metab ^q
IIIa	0	0	225.29	2.37	2	2	1	0.27	496.43	471.63	108.05	791.71	-2.47	0.20	-5.62	4
IIIb	0	0	259.73	2.88	2	2	1	0.43	490.96	490.97	109.67	831.60	-3.11	0.30	-5.47	4
IIIc	0	0	259.73	3.04	2	2	1	0.43	502.08	496.04	110.47	834.22	-3.21	0.30	-5.61	3
IIId	0	0	294.18	3.35	2	2	1	0.56	491.91	512.32	109.83	875.21	-3.74	0.41	-5.42	3
IIIe	0	0	270.29	1.69	3	2	2	-0.67	61.31	506.66	110.05	859.53	-2.68	0.18	-5.56	5
IIIf	0	0	255.32	2.52	3	2	2	0.21	493.67	501.58	201.08	870.15	-2.65	0.25	-5.30	5
IIIg	0	0	285.34	2.71	4	2	3	0.17	560.95	539.25	271.61	944.75	-2.94	0.29	-5.42	6
IIIh	0	0	285.34	2.63	4	2	3	0.11	496.58	543.12	292.28	943.14	-3.01	0.28	-5.42	6
IIIi	0	0	285.34	2.63	4	2	3	0.11	498.58	547.12	298.28	951.14	-2.94	0.28	-5.42	6
IIIj	0	0	218.15	2.62	1	2	1	0.15	458.68	537.76	215.53	925.08	-2.65	0.27	-5.13	5
IIIk	0	0	304.19	2.89	2	2	1	0.44	491.10	494.37	109.56	839.44	-3.02	0.32	-5.47	4
IIIl	0	0	293.29	3.36	2	2	1	0.53	493.13	520.84	110.25	889.70	-3.85	0.45	-5.58	3
IIIm	0	0	301.39	3.95	2	2	2	0.13	495.30	589.39	109.78	1022.46	-4.39	0.76	-6.90	3
IIIn	0	0	304.19	2.89	2	2	1	0.44	494.10	494.37	109.56	839.44	-3.20	0.32	-5.47	4
IIIo	1 ^a	0	243.28	2.58	2	2	1	0.38	494.01	474.61	109.70	803.45	-2.72	0.23	-5.36	4
IIIp	1 ^a	0	234.28	2.77	2	2	1	0.43	492.35	497.87	109.72	845.33	-3.29	0.34	-5.52	3
IIIq	0	0	243.28	2.59	2	2	1	0.38	493.27	474.89	109.62	803.69	-3.11	0.23	-5.36	3
IIIr	0	0	304.73	2.20	3	2	2	0.37	495.10	470.47	104.54	808.04	-2.73	0.24	-5.23	4
IIIs	0	0	334.21	3.10	3	2	2	-0.50	67.63	526.87	109.23	905.68	-3.20	0.30	-5.45	4
IIIt	0	0	286.29	1.07	4	3	3	0.36	495.03	535.65	197.04	926.14	-3.58	0.39	-5.46	5
IIItu	0	0	225.29	2.37	2	2	1	-1.06	25.05	511.41	103.30	884.99	-2.26	0.02	-5.28	6
								0.27	496.43	471.63	108.05	791.71	-2.47	0.34	-5.62	4

^a Property which falls outside the recommended range for 95% of known drugs. ^b Number of computed properties which fall outside the required range for 95% of known drugs. ^c Number of violations of Lipinski's "Rule of Five". ^d Molar weight (range for 95% of drugs: 130–725 Da). ^e Logarithm of partitioning coefficient between *n*-octanol and water phases (range for 95% of drugs: -2 to 6.5). ^f Number of hydrogen bonds accepted by the molecule (range for 95% of drugs: 2–20). ^g Number of hydrogen bonds donated by the molecule (range for 95% of drugs: 0–6). ^h Number of rotatable bonds (range for 95% of drugs: 0–15). ⁱ Logarithm of predicted blood-brain barrier partition coefficient (range for 95% of drugs: -3.0 to 1.0). ^j Predicted apparent Caco-2 cell membrane permeability in Boehringer-Ingelheim scale, in nm s⁻¹ (range for 95% of drugs: <5 low, >500 high). ^k Total solvent-accessible molecular surface, in Å² (probe radius 1.4 Å) (range for 95% of drugs: 300–1000 Å²). ^l Hydrophobic portion of the solvent-accessible molecular surface, in Å² (probe radius 1.4 Å) (range for 95% of drugs: 0–750 Å²). ^m Total volume of molecule enclosed by solvent-accessible molecular surface, in Å³ (probe radius 1.4 Å) (range for 95% of drugs: 500–2000 Å³). ⁿ Logarithm of aqueous solubility (recommended range is -6.0 to 0.5). ^o Logarithm of predicted binding constant to human serum albumin (range for 95% of drugs: -1.5 to 1.5). ^p Predicted IC₅₀ value for blockage of HERG K⁺ channels (concern < -5). ^q Number of likely metabolic reactions (range for 95% of drugs: 1–8).

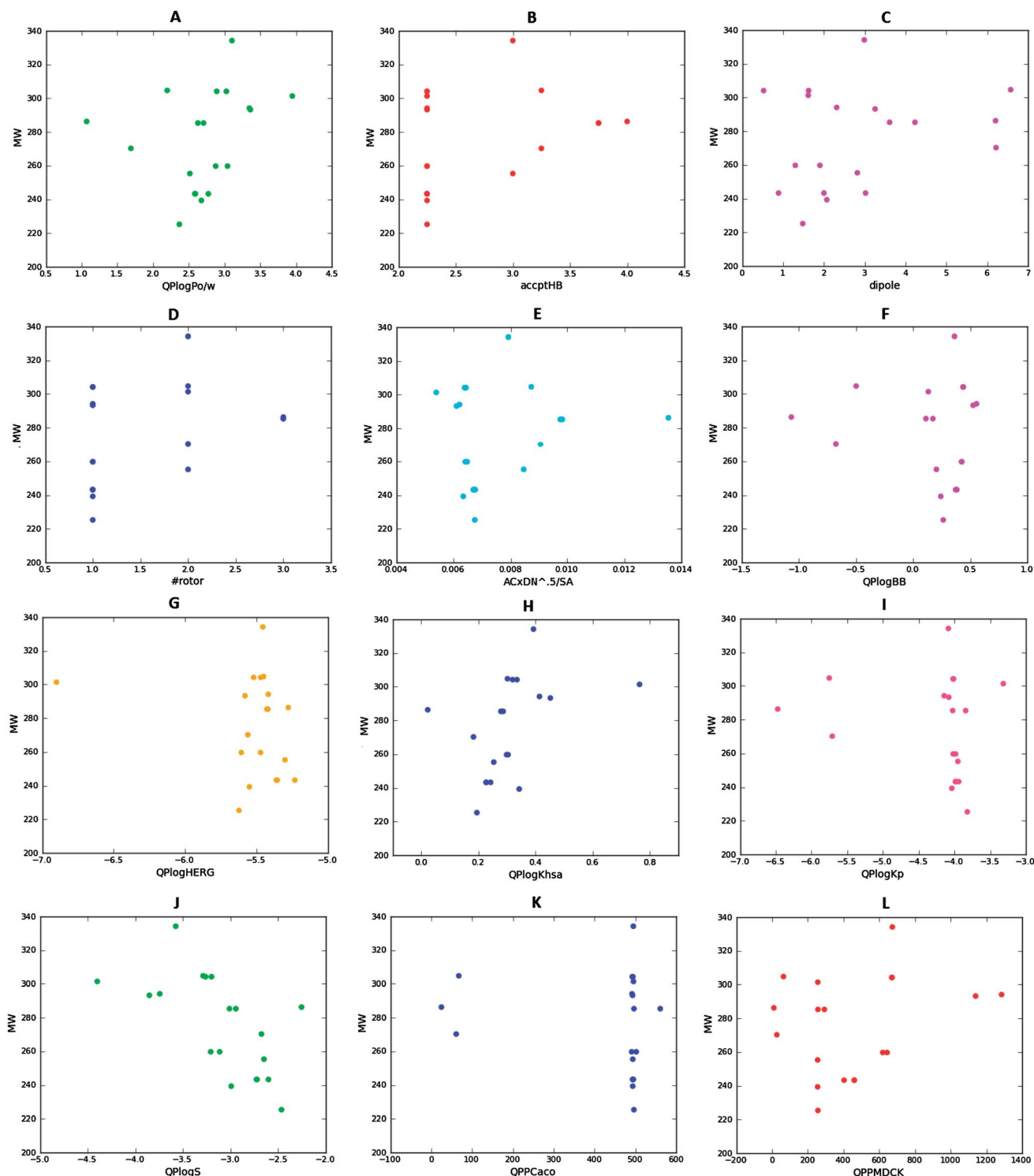
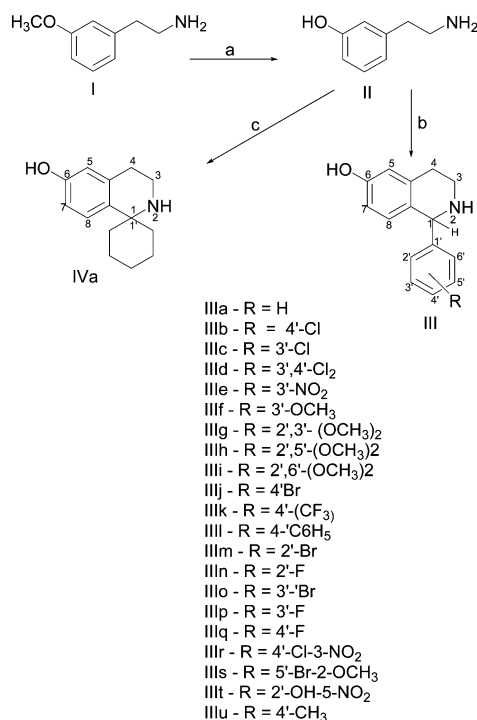


Fig. 4 2D pairwise scatter plots of selected ADMET properties: (A) molecular weight against lipophilicity, (B) molecular weight against number of hydrogen bond acceptors, (C) molecular weight against dipole moments, (D) molecular weight against number of rotatable bonds, (E) molecular weight against cohesion indices, (F) molecular weight against blood–brain barrier penetration coefficient, (G) molecular weight against predicted $\log IC_{50}$ of HERG channel blockage, (H) molecular weight against predicted binding coefficient to human serum albumin, (I) molecular weight against skin penetration parameter, (J) molecular weight against aqueous solubility, (K) molecular weight against Caco-2 penetration, (L) molecular weight against Madin–Darby canine kidney parameter.

(singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet). Mass spectra were recorded on a BIOTO FIIEST mass spectrometer. TLC analyses were carried out on

aluminum plates (Merck) precoated with silica gel 60 F254 (0.2 mm thickness). Visualization of spots was performed with UV light or by treatment with iodine.



Reagents:

a: acetic acid (HOAc) and HBr (1:1), reflux;

b: benzaldehyde or substituted benzaldehyde, triethylamine and ethanol, reflux;

c: cyclohexanone, triethylamine and ethanol, reflux.

Scheme 1 Synthesis of 6-hydroxy-1,2,3,4-tetrahydroisoquinoline analogues.

General procedure for the synthesis of 1-aryl-6-hydroxy-1,2,3,4-tetrahydroisoquinolines

6-Hydroxy-1-phenyl-1,2,3,4-tetrahydro-isoquinoline (IIIa).

The synthesis followed the procedure earlier described by Kame-tani *et al.*²¹ A mixture of 3-methoxyphenethylamine (2.0 g, 13 mmol), hydrobromic acid (10 mL) and acetic acid (10 mL) was stirred and heated under reflux for six (6) hours. The cooled mixture was concentrated under reduced pressure to obtain a residue. To this residue, benzaldehyde (1.96 g, 14.5 mmol), triethylamine (1 mL), and ethanol (10 mL) were added. The resulting mixture was stirred, heated under reflux for 10 hours and concentrated to remove the solvent. The residue obtained after evaporation was diluted with methylene chloride (100 mL) and water (100 mL) with resulting precipitation of solid. The solid was collected by filtration, washed with acetone (3 × 10 mL) and air dried to afford IIIa as a white solid (2.80 mg, 10%); mp 260 °C ¹H NMR (CD₃OD) 3.08–3.58 (m, 4, -(CH₂)₂), 4.923 (s, 1, OH), 5.07 (s, 1, -CH-N), 6.64–7.48 (m, 8, aryl), melting point. In subsequent runs, the Pictet-Spengler reaction was allowed to run for up to 24 hours.

1-(4-Chlorophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIIb). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 3-chlorobenzaldehyde (2.04 g, 14.5 mmol)], 2.70 g (79%); mp, 270–280 °C. ¹H NMR (CD₃OD) 3.08–3.53 (m, 4, -(CH₂)₂), 4.91 (s, 1, OH), 5.73 (s, 1, -CH-N), 6.64–7.51 (m, 7, aryl).

1-(3-Chlorophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIIc). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol)

and 3-chlorobenzaldehyde (2.04 g, 14.5 mmol)], 1.6 g, (47%); mp, 210 °C ¹H NMR (CD₃OD) 3.08–3.53 (m, 4, -(CH₂)₂), 4.91 (s, 1, OH), 5.73 (s, 1, -CH-N), 6.64–7.51 (m, 7, aryl).

1-(3,4-Dichlorophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIId). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 3,4-dichlorobenzaldehyde (2.54 g, 14.5 mmol)], 2.4 g (62%); mp, 240 °C. ¹H NMR (CD₃OD) 3.08–3.53 (m, 4, -(CH₂)₂), 4.92 (s, 1, OH), 5.77 (s, 1, -CH-N), 6.65–7.65 (m, 6, aryl).

6-Hydroxy-1-(3-nitrophenyl)-1,2,3,4-tetrahydroisoquinoline (IIIe). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 3-nitrobenzaldehyde (2.194 g, 14.5 mmol)] 2.90 g (84%). ¹H NMR (CD₃OD) 3.12–3.56 (m, 4, -(CH₂)₂), 4.98 (s, 1, OH), 5.93 (s, 1, -CH-N), 6.65–8.37 (m, 7, aryl), mp, 248–250 °C.

6-Hydroxy-1-(3-methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline (IIIff). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 3-methoxybenzaldehyde (1.98 g, 14.5 mmol)], 120 mg (4%); mp, 264–266 °C. ¹H NMR (CD₃OD) 3.07–3.55 (m, 4, -(CH₂)₂), 3.80 (s, 3, methoxyl), 4.89 (s, 1, OH), 5.65 (s, 1, -CH-N), 6.36–7.40 (m, 7, aryl). MS [M + H]⁺ 256.13 corresponding to molecular formula (C₁₆H₁₈NO₂).

1-(2,3-Dimethoxyphenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIIgg). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 2,3-dimethoxybenzaldehyde (2.41 g, 14.5 mmol)], 3.6 g (96%); mp, 240 °C. ¹H NMR (CD₃OD) 3.05–3.54 (m, 4, -(CH₂)₂), 3.52–3.89 (s, 6, methoxyl), 4.89 (s, 1, OH), 5.71 (s, 1, -CH-N), 6.63–7.19 (m, 6, aryl). MS [M + H]⁺ 286.146 corresponding to (C₁₇H₂₀NO₃).

1-(2,5-Dimethoxyphenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIIhh). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 2,5-dimethoxybenzaldehyde (2.4 g, 14.5 mmol)] (3.6 g, 98%); mp, 260 °C. ¹H NMR (CD₃OD) 3.06–3.46 (m, 4, -(CH₂)₂), 3.69–3.83 (s, 6, methoxyls), 4.89 (s, 1, OH), 5.82 (s, 1, -CH-N), 6.58–7.09 (m, 6, aryl). MS [M + H]⁺ 286.146 corresponding to (C₁₇H₂₀NO₃).

1-(2,6-Dimethoxyphenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIIi). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 2,6-dimethoxybenzaldehyde (2.81 g, 14.5 mmol)], (3.70 g, 99%); mp, 260–266 °C. ¹H NMR (CD₃OD) δ 2.99–3.69 (m, 4, -(CH₂)₂), 3.79 (s, 6, methoxyl), 4.89 (s, 1, OH), 6.05 (s, 1, -CH-N), 6.42–7.46 (m, 6, aryl). MS [M + H]⁺ 286.146 corresponding to C₁₇H₂₀NO₃.

6-Hydroxyspiro[1,2,3,4-tetrahydroisoquinoline-1:1'-cyclohexane] (IVa). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and cyclohexanone (4 g, 14.5 mmol)], 250 mg (10%); mp, 240 °C for the hydrochloride analogue. ¹H NMR (CD₃OD) 1.49–2.08 (m, 10, cyclohexyl), 3.05–3.47 (t, 4, -(CH₂)₂), 4.89 (s, 1, OH), 6.60–7.26 (m, 3, aromatic protons). MS [M + H]⁺ 218.154 corresponding to (C₁₄H₂₀NO).

1-(4-Bromophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIIj). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 4-bromobenzaldehyde (0.875 g, 4.73 mmol)], (0.68 g, 52%). ¹H NMR (CD₃OD) δ 3.09–3.52 (m, 4, -(CH₂)₂), 4.89 (s, 1, OH), 5.71 (s, 1, -CH-N), 6.20–7.59 (m, 7, aryl). MS [M + H]⁺ 304.0344 corresponding to C₁₅H₁₅NOBr.

1-(α,α,α-trifluoromethylphenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIIk). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and α,α,α-trifluorotolualdehyde (0.63 g, 3.63 mmol)], 0.12 g (16%). ¹H NMR (CD₃OD) δ 3.11–3.52 (m, 4,

–(CH₂)₂), 4.90 (s, 1, OH), 5.80 (s, 1, –CH–N), 6.62–7.81 (m, 7, aryl). MS [M + H]⁺ 295.1108 corresponding to C₁₆H₁₅NOF₃.

1-(4-Biphenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIIj). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 4-biphenylcarboxaldehyde (1.33 g, 7.27 mmol)], (1.40 g, 70%); mp, 260–266 °C. ¹H NMR (CD₃OD) δ 2.99–3.69 (m, 4, –(CH₂)₂), 3.79 (s, 6, methoxyl), 4.89 (s, 1, OH), 6.05 (s, 1, –CH–N), 6.42–7.46 (m, 6, aryl). MS [M + H]⁺ 286.146 corresponding to C₁₇H₂₀NO₃.

1-(2-Bromophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIIk). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 2-bromobenzaldehyde (1.35 g, 7.27 mmol)], 1.1 g (55%). ¹H NMR (CD₃OD) δ 3.11–3.58 (m, 4, –(CH₂)₂), 4.91 (s, 1, OH), 6.10 (s, 1, –CH–N), 6.57–7.81 (m, 7, aryl). MS [M + H]⁺ 304.0593 corresponding to C₁₅H₁₅NOBr.

1-(2-Fluorophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIIln). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 2-fluorobenzaldehyde (1 g, 7.27 mmol)], 1 g (63%). ¹H NMR (CD₃OD) δ 3.10–3.53 (m, 4, –(CH₂)₂), 4.90 (s, 1, OH), 5.97 (s, 1, –CH–N), 6.66–7.56 (m, 7, aryl). MS [M + H]⁺ 244.1361 corresponding to C₁₅H₁₅NOF.

1-(3-Bromophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIIlo). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 3-bromobenzaldehyde (1.35 g, 7.27 mmol)], 1.22 g (55%). ¹H NMR (CD₃OD) δ 3.08–3.53 (m, 4, –(CH₂)₂), 4.89 (s, 1, OH), 5.72 (s, 1, –CH–N), 6.64–7.66 (m, 7, aryl). MS [M + H]⁺ 304.0626 corresponding to C₁₅H₁₅NOBr.

1-(3-Fluorophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIIlp). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 3-fluorobenzaldehyde (1 g, 7.27 mmol)], 1.1 g (69%). ¹H NMR (CD₃OD) δ 3.09–3.54 (m, 4, –(CH₂)₂), 4.89 (s, 1, OH), 5.75 (s, 1, –CH–N), 6.64–7.54 (m, 7, aryl). MS [M + H]⁺ 244.1375 corresponding to C₁₅H₁₅NOF.

1-(4-Fluorophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIIq). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 4-fluorobenzaldehyde (1 g, 7.27 mmol)], (1.2 g, 75%). ¹H NMR (CD₃OD) δ 3.08–3.52 (m, 4, –(CH₂)₂), 4.92 (s, 1, OH), 5.73 (s, 1, –CH–N), 6.65–7.44 (m, 7, aryl). MS [M + H]⁺ 244.1385 corresponding to C₁₅H₁₅NOF.

1-(4-Chloro-3-nitrophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIIr). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 4-chloro-3-nitrobenzaldehyde (1.35 g, 7.27 mmol)], 0.7 g (35%). ¹H NMR (CD₃OD) δ 3.07–3.53 (m, 4, –(CH₂)₂), 4.91 (s, 1, OH), 5.88 (s, 1, –CH–N), 6.69–8.03 (m, 6, aryl).

1-(5-Bromo-2-methoxyphenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIIs). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 5-bromo-*o*-anisaldehyde (1.6 g, 7.27 mmol)], (1.0 g, 46%). ¹H NMR (CD₃OD) δ 3.10–3.45 (m, 4, –(CH₂)₂), 3.90 (s, 3, methoxyl), 4.90 (s, 1, OH), 5.88 (s, 1, –CH–N), 6.69–7.62 (m, 6, aryl).

1-(2-Hydroxy-5-nitrophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline (IIIu). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and 2-hydroxy-5-nitrobenzaldehyde (1.22 g, 7.27 mmol)], 0.32 g (17.78%). ¹H NMR (CD₃OD) δ 3.07–3.52 (m, 4, –(CH₂)₂), 4.90 (s, 2, OH), 5.96 (s, 1, –CH–N), 6.68–8.27 (m, 6, aryl).

Synthesis of 6-hydroxy-1-(4-methylphenyl)-1,2,3,4-tetrahydroisoquinoline (IIIu). Yield [from 3-methoxyphenethylamine (2.0 g, 13 mmol) and *p*-tolualdehyde (0.9 g, 7.27 mmol)], 1.2 g

(76%). ¹H NMR (CD₃OD) δ 2.37 (s, 3, methyl), 3.06–3.53 (m, 4, –(CH₂)₂), 4.92 (s, 1, OH), 5.63 (s, 1, –CH–N), 6.63–7.31 (m, 7, aryl).

In vitro sensitivity assays of *P. falciparum* ([³H]-hypoxanthine incorporation)

The test samples were assayed on a *P. falciparum* clone designated K1 which is chloroquine/pyrimethamine resistant. Test samples of 10 mg were prepared in dimethyl sulfoxide (DMSO). The testing procedure was as follows: a serial dilution factor of 1 : 2 was prepared with 100 μL of medium RPMI 1640 without hypoxanthine 10.44 g L^{−1}; HEPES 5.94 g L^{−1}; Albumax® 5 g L^{−1}; neomycin 10 mL L^{−1} (100 U mL^{−1}); NaHCO₃ 50 g L^{−1} stock 42 mL L^{−1} (2.1 g L^{−1}), the final concentration was distributed into 96 wells of the Costar TM 96 – wells microtitre plates. To these 96 wells, 100 μL of medium, washed human red blood cells A+ (RBC) and *P. falciparum* mix, were added. The plates were incubated for 48 hours at 37 °C in a chamber and gassed with a 4% CO₂, 3% O₂, 93% N₂. After 50 μL of medium and [³H]-hypoxanthine (0.5 μCi) were added to each well. The plates were then put back into the chamber and gassed with a 4% CO₂, 3% O₂, and 93% N₂ mix. The chamber was then placed in the incubator for 24 hours at 37 °C. The assay was terminated by harvesting the content of each microtiter plate. Data were transferred into a graphic program (Excel) and analyzed to determine the IC₅₀. The test scores were grouped as follows; inactive (no repeat) IC₅₀ > 5 μg mL^{−1}; moderate activity (repeat) 0.5 μg mL^{−1} < IC₅₀ < 5 μg mL^{−1}; high activity (repeat) IC₅₀ < 0.5 μg mL^{−1}.^{34,35}

In vitro sensitivity assays (cytotoxicity)

The test samples were submitted to L-6 (rat skeletal myoblast cells). Compounds were prepared in DMSO the testing procedure was as follows. A sample of 100 μL medium (RPMI + 10% FCS + 1.7 μM – glutamine (850 μL 200 mM for 100 mL)), was added to wells of a microtiter plate. Then 100 μL of a cell suspension of 4 × 10⁴ cells per mL was added. After a serial dilution factor of 1 : 3 was prepared into the wells. The plates were then incubated for 70 hours at 37 °C/5%CO₂.

In silico modelling

Each compound was sketched using the GaussView software and geometry optimisation was carried out using the Gaussian 09W package³⁶ with the density functional theory (DFT) using the B3LYP/6-31+G(d,p) approach until convergence was reached. This was necessary in order to generate good starting geometries for the 3D models. The low-energy 3D chemical structures were saved in mol2 format and initially treated with LigPrep,³⁷ distributed by Schrodinger, Inc. This implementation was carried out with the graphical user interface (GUI) of the Maestro software package,³⁸ using the OPLS force field.^{39–41} The treatment with LigPrep was necessary in order to correct protonation states and set the right parameters necessary for running QikProp.⁴² Protonation states at biologically relevant pH were correctly assigned (group I metals in simple salts were disconnected, strong acids were deprotonated and strong bases

protonated, while explicit hydrogens were added). A set of the ADMET-related properties (a total of 46 molecular descriptors) were calculated using the QikProp program⁴² running in normal mode. QikProp generates physically relevant descriptors and uses them to perform ADMET predictions. An overall ADMET-compliance score, drug-likeness parameter (indicated by #stars), was used to assess the pharmacokinetic profiles of the compounds. The #stars parameter indicates the number of property descriptors computed by QikProp, which fall outside the optimum range of values for 95% of known drugs. The methods implemented were developed by Jorgensen *et al.*^{43–45} All molecular modelling was carried out on a Linux workstation with a 3.5 GHz Intel Core2 Duo processor.

Conclusions

A series of 1-aryl-6-hydroxy-1,2,3,4-tetrahydroisoquinoline analogues was synthesized using simple methodology. To the best of our knowledge this is the first time the antiplasmodial of these analogues are reported. Following the criteria established by the WHO/TDR screening program,⁴⁶ a compound is considered active against *P. falciparum* when *in vitro* IC₅₀ is <1 µM (or 0.2 µg mL⁻¹) and SI > 100. In the present study, three compounds (**IIIb**, **IIIk** and **IVb**) fell within this category and could be described as antimalarial hits from which more potent THIQ analogues could be obtained by further investigation. Additionally, *in vivo* and mechanistic studies of these compounds are underway. The study clearly demonstrates that 1-aryl-6-hydroxy-1,2,3,4-tetrahydroisoquinolines display sufficient antiplasmodial activity and favourable predicted pharmacokinetic properties to warrant further investigation of their antiplasmodial activity.

Acknowledgements

The academic license is acknowledged from Schrodinger Inc., for running the QikProp software.

Notes and references

- 1 P. D. Hoeprich, in *Infectious Diseases*, J. B. Lippincott Company, Philadelphia, 4th edn, 1972, p. 26.
- 2 A. Fournet and V. Munoz, *Curr. Top. Med. Chem.*, 2002, **2**, 1915.
- 3 J. D. Phillipson and C. W. Wright, *J. Ethnopharmacol.*, 1991, **32**, 155.
- 4 S. Schwikkard and F. R. van Heerden, *Nat. Prod. Rep.*, 2002, **19**, 675.
- 5 A. Gringuaz, Antimalarial Drugs II, Chemotherapy of malaria, in *Introduction to Medicinal Chemistry*, Wiley-Vc Inc., New York, 1997, vol. 197, pp. 202–210.
- 6 N. J. White, *J. Clin. Invest.*, 2004, **113**, 1084.
- 7 S. I. Murahashi and T. Shiola, *Tetrahedron Lett.*, 1987, **28**(21), 2383.
- 8 N. J. O'reilly, W. S. Derwin and H. C. Lin, *Synthesis*, 1990, **7**, 550.
- 9 M. Strolin-Bendetti, P. Doistert and P. J. Cannitiati, *J. Neural Transm.*, 1989, **78**, 43.
- 10 P. Yakoyama, T. Ohwada and K. J. Shudo, *Org. Chem.*, 1999, **64**, 611.
- 11 G. Bringman, R. Brun, M. Kaiser and S. Neumann, *Eur. J. Med. Chem.*, 2008, **43**(1), 32.
- 12 G. François, G. Timperman, W. Eling, L. A. Assi, J. Hotemz and G. Bringmann, *Antimicrob. Agents Chemother.*, 1997, **41**, 2533.
- 13 G. Bringmann, K. Messer, K. Wolf, J. Mühlbacher, M. Grüne, R. Brun and A. M. Louis, *Phytochemistry*, 2002, **60**, 389.
- 14 G. Bringmann, C. Günthera, W. Saeb, J. Mies, R. Brun and L. Aké Assi, *Phytochemistry*, 2000, **54**, 337.
- 15 Y. Iwasawa and A. Kiyomoto, *Jpn. J. Pharmacol.*, 1967, **17**, 143.
- 16 P. Cheng, N. Huang, Z.-Y. Jiang, Q. Zhang, Y.-T. Zheng, J.-J. Chen, X.-M. Zhang and Y.-B. Ma, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 2475.
- 17 P. Manmi, M. d'Ischia and G. J. Prota, *Org. Chem.*, 2001, **66**, 5048.
- 18 Z. Z. Liu and V. F. Tang, *Chin. Lett.*, 2003, **12**, 947.
- 19 J. D. Scott and R. M. Williams, *Angew. Chem., Int. Ed.*, 2001, **40**, 1463.
- 20 A. Kumar, B. S. Katiyar, S. Gupta and M. S. Prem, *Eur. J. Med. Chem.*, 2002, **61**(2), 195.
- 21 T. Kametani, K. Kigasawa, M. Hiiragi and H. J. Ishimaru, *J. Chem. Soc. C*, 1971, 2632, DOI: 10.1039/J39710002632.
- 22 P. F. Moundipa, F. N. Njyou, S. Yantitoum, B. Sonke and F. M. Tchouanguep, *Cam. J. Biol. Biochem. Sci.*, 2002, **12**, 39.
- 23 K. Saidu, J. Onah, A. Orisadipe, A. Olusola, C. Wambebe and K. Gamaniel, *J. Ethnopharmacol.*, 2000, **71**, 275.
- 24 F. R. Irvine, *Woody Plants of Ghana*, University Press, London, Oxford, 1961, p. 358.
- 25 R. M. Soto-Hernández, R. García-Mateos, R. S. Miguel-Chávez, G. Kite, M. Martínez-Vázquez and A. C. Ramos-Valdivia, Erythrina, a Potential Source of Chemicals from the Neotropics, in *Bioactive Compounds in Phytomedicine*, Intech, 2012, pp. 163–184.
- 26 M. I. Aguilar, F. Giral and O. Espejo, *Phytochemistry*, 1981, **20**, 2061.
- 27 H. Van de Waterbeemd and E. Gifford, *Nat. Rev. Drug Discovery*, 2003, **2**, 192.
- 28 (a) C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Adv. Drug Delivery Rev.*, 1997, **23**, 3; (b) C. A. Lipinski, *J. Pharmacol. Toxicol. Methods*, 2000, **44**, 253.
- 29 D. F. Veber, S. R. Johnson, H. Y. Cheng, B. R. Smith, K. W. Ward and K. D. Kopple, *J. Med. Chem.*, 2002, **45**, 2615.
- 30 P. L. Hedley, P. Jørgensen, S. Schlamowitz, R. Wangari, J. Moolman-Smook, P. A. Brink, J. K. Kanters, V. A. Corfield and M. Christiansen, *Hum. Mutat.*, 2009, **30**, 1486.
- 31 J. I. Vandenberg, B. D. Walker and T. J. Campbell, *Trends Pharmacol. Sci.*, 2001, **22**, 240.
- 32 N. Chiesa, B. Rosati, A. Arcangeli, M. Olivotto and E. Wanke, *J. Physiol.*, 1997, **501**, 31.
- 33 A. M. Aronov, *Drug Discovery Today*, 2005, **10**, 149.
- 34 R. E. Desjardins, C. J. Canfield, J. D. Haynes and J. D. Chulay, *Antimicrob. Agents Chemother.*, 1979, **16**, 710.

- 35 J. D. Berman and J. V. Gallalee, *Antimicrob. Agents Chemother.*, 1985, **28**, 723.
- 36 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria and M. A. Robb, *et al.*, *Gaussian 09*, Gaussian, Inc., Pittsburgh, PA, 2009.
- 37 *Schrödinger: LigPrep software, version 2.5*, LLC, New York, NY, 2011.
- 38 *Schrödinger: Maestro, version 9.2*, LLC, New York, NY, 2011.
- 39 D. Shivakumar, J. Williams, Y. Wu, W. Damm, J. Shelley and W. Sherman, *J. Chem. Theory Comput.*, 2010, **6**, 1509.
- 40 W. L. Jorgensen, D. S. Maxwell and J. Tirado-Rives, *J. Am. Chem. Soc.*, 1996, **118**(45), 11225.
- 41 W. L. Jorgensen and J. Tirado-Rives, *J. Am. Chem. Soc.*, 1988, **110**(6), 1657.
- 42 *Schrödinger: QikProp, version 3.4*, LLC, New York, NY, 2011.
- 43 W. L. Jorgensen and E. M. Duffy, *Adv. Drug Delivery Rev.*, 2002, **54**, 355.
- 44 E. M. Duffy and W. L. Jorgensen, *J. Am. Chem. Soc.*, 2000, **122**, 2878.
- 45 W. L. Jorgensen and E. M. Duffy, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1155.
- 46 S. Nwaka, B. Ramirez, R. Brun, R. L. Maes, F. Douglas and R. Ridley, *PLoS Neglected Trop. Dis.*, 2009, **3**, e440.