Identification and optimization of an aminoalcohol-carbazole series with antimalarial properties.

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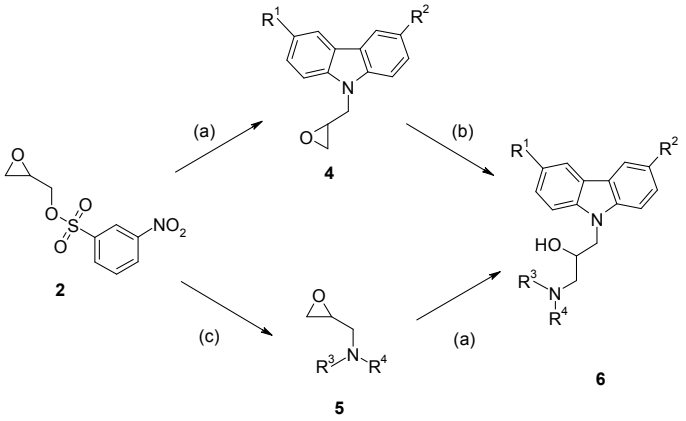
Experimental

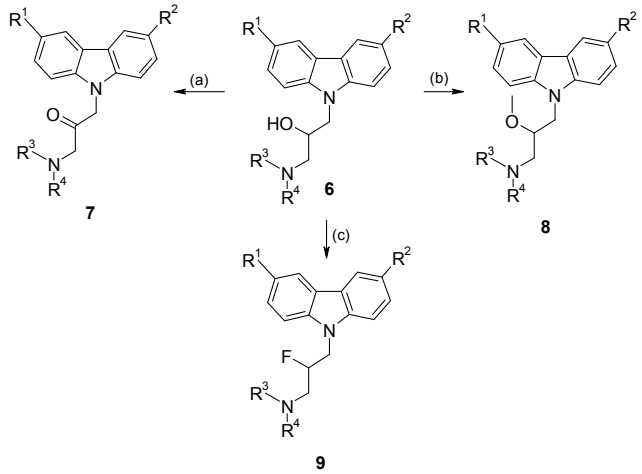
General synthetic pathway



Scheme 1. General synthesis of compounds **6**. Reagents and conditions: (a) 3,6-disubstituted carbazole **1**, NaH, THF, reflux, 18h, 60-80%; (b) secondary or primary amine **3**, K_2CO_3, THF, EtOH, MW, 65-99%; (c) secondary or primary amine **3**, K_2CO_3, MeCN, rt, 60-75%.

The general approach consisted in a two-step synthesis. The deprotonated carbazoles **1** reacted with glycidyl nosylate **2** to deliver the epoxide **4** in good yield. Subsequent opening of the epoxide **4** with diverse amines 3 afforded the desired amino alcohols **6**. In the case where R3 and R4 form a cyclic amide, the epoxide opening is performed in THF by using sodium hydride as deprotonating agent of the amide **3.** Alternatively, glycidyl nosylate **2** could be added to a solution of secondary amines **3** in acetonitrile in basic conditions to give epoxyde **5**, followed by epoxide-opening with carbazoles **1** to obtain compounds **6**.

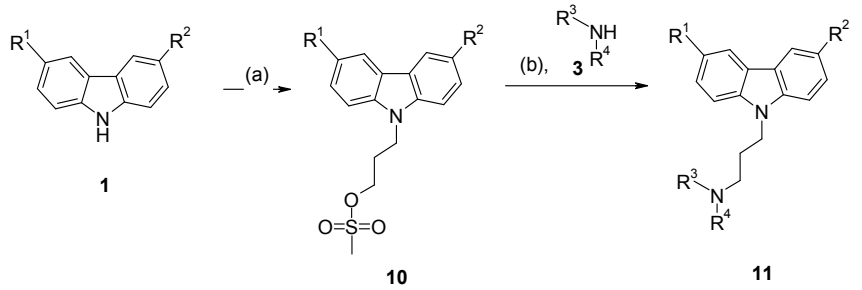
Classic transformation of the hydroxyl group of propyl linker allowed a first exploration around the linker. Indeed, alcohols **6** were easily transformed in ketone **7**, methyl ether **8**, and fluoro analog **9** by known chemistry (Scheme 2).



Scheme 2. Synthesis of non hydroxylated analogs. Reagents and conditions: (a) i) $(COCl)_2$, DMSO, DCM, -78°C, 20 min, ii) Et_3N, -78°C to -30°C, 10 min, 58%; (b) NaH, MeI, THF, rt, 1h, 60%; (c) DAST, DCM, 0°C, 18h, 52%.

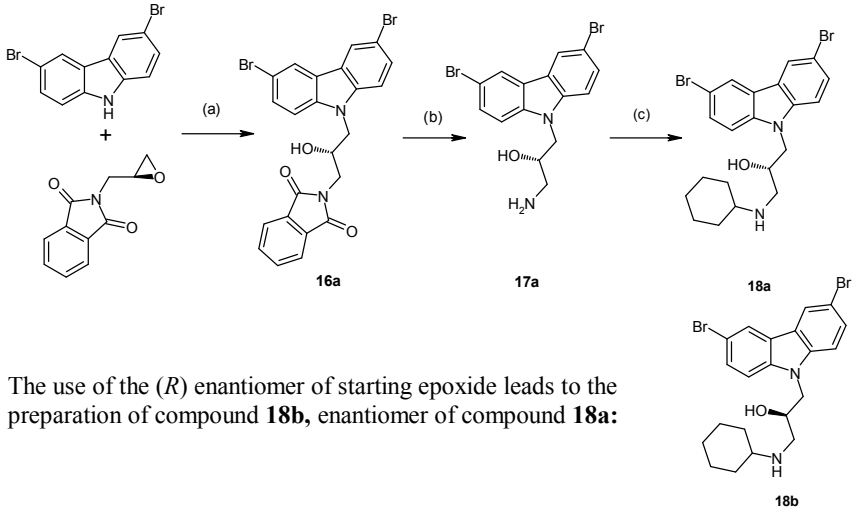
To access compounds which do not bear hydroxyl groups on the propyl linker, a second approach was used (Scheme 3). SN2 reaction between substituted carbazole **1** and 2-(3-bromopropoxy)tetrahydro-2H-

pyran allowed the introduction of the propyl chain. Cleavage of the tetrahydropyran protecting group followed by addition of mesyl chloride delivered the mesylate compound **10**. Subsequent displacement of the sulfonyl leaving group with adequate amine **3** yielded the desired compound **11**.



Scheme 3. Synthesis of compound **11**. Reagents and conditions: (a) i) $Br(CH_2)_3OTHP$, NaOH, DMF, 90°C, 5h; ii) pTsOH, MeOH, 0°C to rt, 2h, 83%; iii) MsCl, Et_3N, THF, rt, 1.5h. b) $K2CO_3$, MeCN, reflux, 18h, 65%.

The enantioselective synthesis of compounds **18a** and **18b** was performed in a 3 steps chemical scheme (Scheme 4).



The use of the (*R*) enantiomer of starting epoxide leads to the preparation of compound **18b,** enantiomer of compound **18a:**

Scheme 4. Enantioselective synthesis of compounds 18a and 18b. reagents and conditions: (a) Cs_2CO_3 5 equiv. DMF, 60%. (b) $N_2H_4.H_2O$, EtOH, 80%. (c) Cyclohexanone 1.1 equiv. $NaBH(OAc)_3$ 1.3 equiv., DCM, rt, 53%.

Chemistry

The HPLC data provided were obtained as follow:

Column Waters XbridgeTM C8 50 mm x 4.6 mm at a flow of 2 mL/min; 8 min gradient from 0.1 % TFA in H_2O to 0.07 % TFA in MeCN.

The MS data provided were obtained using a LC/MS Waters ZMD (ESI)

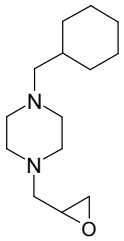
The NMR data were obtained on a Bruker DPX-300MHz.

Preparative HPLC purifications were performed with a mass directed autopurification

Fractionlynx from Waters equipped with a Sunfire Prep C18 OBD column 19x100 mm 5 µm, unless otherwise reported. All HPLC purifications were performed with a gradient of MeCN/H_2O or MeCN/H_2O/HCOOH (0.1%).
The microwave chemistry was performed on a single mode microwave reactor Emrys™ Optimiser from Personal Chemistry
cLogD and cLogP values were calculated using ACD/PhysChem Suite, version 12.01.

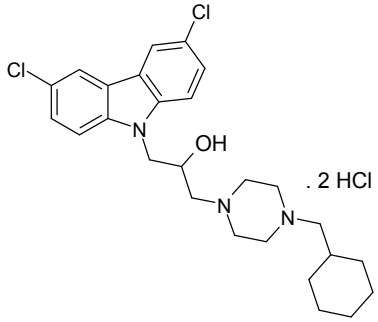
Compounds **12**, **14a**, **14b**, and **14c** are commercially available. Compound **14d** is described in the following patent: Halazy S., Church D., Antonsson B., Bombrun A., Gerber P., Martinou J.-C. 9-(Piperazinylalkyl)carbazoles as Bax-modulators, Eur. Pat. Appl. EP 1094063, **2001**.

1-(Cyclohexylmethyl)-4-(oxiran-2-ylmethyl)piperazine (5a)



To a solution of 1-cyclohexylpiperazine (1.0 g, 5.5 mmol, 1.0 equiv.) in MeCN (25 mL) were added successively potassium carbonate (760 mg, 5.5 mmol, 1.0 equiv.) and 3-oxiran-2-ylmethyl-3-nitrobenzenesulfonate (1.42 g, 5.5 mmol, 1.0 equiv.). The mixture was stirred at rt during 15 h, then the reaction was filtered and concentrated under reduced pressure. The resulting yellow oil was purified by chromatography on silica gel (MeOH 10% in DCM) to afford 1.12 g (85%) of 1-(cyclohexylmethyl)-4-(oxiran-2-ylmethyl)piperazine **(5a)** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 3.19 – 3.04 (m, 1H), 2.83 – 2.38 (m, 11H), 2.37 – 2.27 (m, 1H), 2.14 (d, J = 7.1 Hz, 2H), 1.83 – 1.61 (m, 5H), 1.58 – 1.39 (m, 1H), 1.35 – 1.08 (m, 3H), 0.97 – 0.76 (m, 2H).

1-[4-(cyclohexylmethyl)piperazin-1-yl]-3-(3,6-dichloro-9*H*-carbazol-9-yl)propan-2-ol dihydrochloride salt (13a)

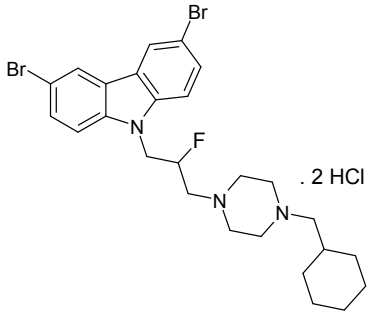


To a solution of 3,6-dichloro-9*H*-carbazole (3.84 g, 16.28 mmol, 2.0 equiv.) in THF (50 mL) at 0°C was added sodium hydride (0.65 g, 16.28 mmol, 2.0 equiv.). Then, the reaction was stirred at rt for 1h. A solution of 1-(cyclohexylmethyl)-4-(oxiran-2-ylmethyl)piperazine **(5a)** previously prepared (1.94 g, 8.14 mmol, 1.0 equiv.) in THF (50 mL) was added dropwise and the reaction was refluxed overnight. After completion of the reaction, the mixture was cooled to rt. Water (20 mL) was added and the reaction was

extracted 3 times with EtOAc (3*40 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated to give a yellow foam.

The crude mixture was purified by flash chromatography using a 9/1 mixture of DCM/MeOH to give 1-[4-(cyclohexylmethyl)piperazin-1-yl]-3-(3,6-dichloro-9*H*-carbazol-9-yl)propan-2-ol as a white foam (2.2 g, 57 %). The compound was stirred in a solution of HCl 1M in Et_2O, filtered and dried under reduced pressure to afford 2.3 g (97 %) of 1-[4-(cyclohexylmethyl)piperazin-1-yl]-3-(3,6-dichloro-9*H*-carbazol-9-yl)propan-2-ol dihydrochloride salt (**13a**) as a white powder. ^{1}H NMR (DMSO) δ: 8.34 (d, J = 2.1 Hz, 2H), 7.79 (d, J = 8.8 Hz, 2H), 7.53 – 7.39 (m, 2H), 5.94 (s, 1H), 4.45 (s, 3H), 3.70 (s, 6H), 3.40 (s, 4H), 2.95 (s, 2H), 2.00 – 1.48 (m, 6H), 1.39 – 1.02 (m, 3H), 1.03 – 0.64 (m, 2H). UPLC/MS 474 (M+H+). HPLC (purity) maxplot 98.7%.

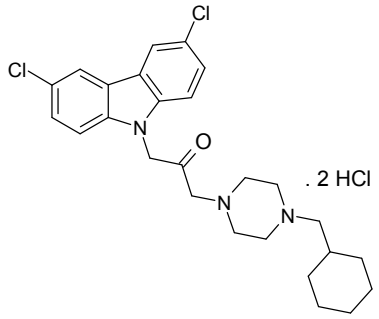
3,6-Dibromo-9-[3-(4-cyclohexylmethyl-piperazin-1-yl)-2-fluoro-propyl]-9*H*-carbazole dihydrochloride salt (13b)



To a solution of 3,6-dibromocarbazole (685 mg, 2.10 mmol, 1.25 equiv.) in THF (10 mL) was added NaH (60% in mineral oil, 92 mg, 2.10 mmol, 1.25 equiv.) and the mixture was stirred at rt. After 30 min, 1-(cyclohexylmethyl)-4-(oxiran-2-ylmethyl)piperazine **(5a)** (400 mg, 1.68 mmol, 1 equiv.) was added and the reaction was heated to 60°C and stirred for 15h. The reaction was quenched by adding MeOH (4 mL) and the solvents were removed under reduced pressure. The resulting yellow foam was purified by chromatography on silica gel (5% MeOH in DCM) to afford 745 mg (79 %) of 1-(4-cyclohexylmethyl-piperazin-1-yl)-3-(3,6-dibromo-carbazol-9-yl)-propan-2-ol as a light yellow powder. ^{1}H NMR ($CDCl_3$) δ: 8.01 (d, J = 1.9 Hz, 2H), 7.45 (dd, J = 8.7, 1.9 Hz, 2H), 7.26 (d, J = 8.7 Hz, 2H), 4.29 – 4.07 (m, 2H), 4.07 – 3.89 (m, 1H), 2.58 – 2.42 (m, 2H), 2.36 – 2.15 (m, 8H), 1.98 (d, J = 7.1 Hz, 2H), 1.73 – 1.47 (m, 5H), 1.46 – 1.25 (m, 1H), 1.21 – 0.97 (m, 3H), 0.85 – 0.61 (m, 2H).

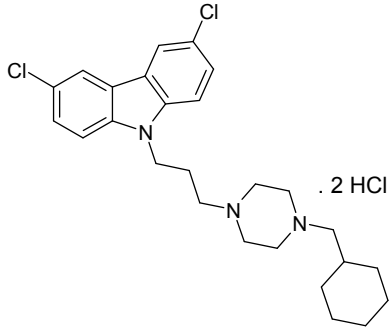
This powder (100 mg, 0.18 mmol, 1 equiv.) was then dissolved in DCM (4 mL) and cooled to 0°C. A solution of DAST (100 µL, 0.77 mmol, 4.3 equiv) was added and the mixture was stirred 15h at rt. The reaction was quenched by adding a saturated solution of K_2CO_3 (10 mL) and the organic layer was washed, separated and dried over $MgSO_4$. The solvents were evaporated under reduced pressure and the resulting yellow oil was purified by chromatography on silica gel (PE 8.5 /EtOAc 1.3 / MeOH 0.2) to afford 3,6-dibromo-9-[3-(4-cyclohexylmethyl-piperazin-1-yl)-2-fluoro-propyl]-9*H*-carbazole (53 mg, 52 %) as a white powder. The powder was stirred in a solution of HCl 1M in Et_2O, filtered and dried under reduced pressure to afford 49 mg (95%) of 3,6-dibromo-9-[3-(4-cyclohexylmethyl-piperazin-1-yl)-2-fluoro-propyl]-9*H*-carbazole dihydrochloride salt (**13b**) as a white powder. ^{1}H NMR (DMSO) δ: 8.34 (d, J = 2.1 Hz, 2H), 7.77 (d, J = 8.8 Hz, 2H), 7.60 – 7.39 (m, 2H), 4.43 (s, 3H), 3.70 (s, 11H), 2.95 (s, 1H), 1.97 – 1.47 (m, 6H), 1.39 – 1.09 (m, 3H), 0.93 (d, J = 11.3 Hz, 2H). HPLC (purity) maxplot 87.4% .

1-(4-Cyclohexylmethyl-piperazin-1-yl)-3-(3,6-dichloro-carbazol-9-yl)-propan-2-one dihydrochloride salt (13c)



To a solution of oxalyl chloride (28 μL, 0.328 mmol, 2 equiv.) in DCM (2 mL) at -78 °C, was added DMSO (43 μL, 0.613 mmol, 3.8 equiv.). The mixture was stirred at -78°C during 20 min, then a solution of 1-(4-cyclohexylmethyl-piperazin-1-yl)-3-(3,6-dichloro-carbazol-9-yl)-propan-2-ol **(13a)** (78 mg, 0.164 mmol, 1.0 equiv.) previously prepared, in DCM (500 μL) was added dropwise. After 1 h stirring at -78°C, Et_3N (125 μL) was added and the reaction was stirred at -78°C one additional hour and was warmed to -30 °C. After 2h stirring, the reaction was quenched by adding water (15 mL), and the organic materials were extracted with DCM (3*25 mL). The combined organic layers were dried over $MgSO_4$and concentrated under reduced pressure to give 115 mg of a brown solid, that was purified by chromatography on silica gel (5 % MeOH in DCM). The resulting light yellow solid was dissolved in MeOH (4 mL) and a solution of HCl 1M in Et_2O was added. The reaction was stirred at rt. Then the precipitate was filtrated and washed by Et_2O (3*5 mL), and finally dried under reduced pressure to give 1-(4-cyclohexylmethyl-piperazin-1-yl)-3-(3,6-dichloro-carbazol-9-yl)-propan-2-one dihydrochloride salt **(13c)** (40 mg, 50 %) as a white powder.^{1}H NMR (DMSO) *δ*: 8.29 (s, 1H), 7.54 (d, *J* = 8.9 Hz, 2H), 7.42 (d, *J* = 8.9 Hz, 2H), 5.49 (s, 2H), 4.17 (s, 2H), 3.51 (d, *J* = 11.6 Hz, 2H), 3.19 (s, 6H), 2.88 (s, 2H), 2.02 – 1.40 (m, 6H), 1.40 – 0.65 (m, 6H). HPLC (purity) maxplot 98.0 %.

3,6-Dichloro-9-{3-[4-(cyclohexylmethyl)piperazin-1-yl]propyl}-9*H*-carbazole dihydrochloride salt (13d)



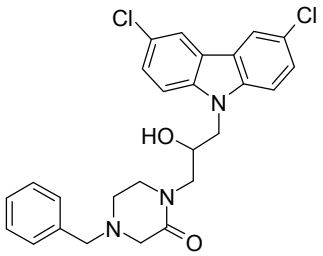
To a mixture of 2-(3-bromopropoxy)tetrahydro-2*H*-pyran (2.90 mL, 15.08 mmol, 2.20 equiv.) and 3,6-dichloro-9*H*-carbazole (1.78 g, 7.54 mmol, 1.00 equiv.) in DMF (9 mL) was added sodium hydroxide (0.60 g, 15.08 mmol, 2.20 equiv.). The reaction was heated at 90°C for 5 h and cooled to rt after completion. The precipitate was filtered and washed with DMF (3*5 mL). Methanol (8 mL) was added to the filtrate followed by the addition of *p*-toluene sulfonic acid monohydrate (0.72 g, 3.77 mmol, 0.50 equiv.) and the reaction was stirred at 50°C for 2h. The reaction was quenched by adding water (20 mL)

and the aqueous layer was extracted 3 times with toluene. The combined organic layers were washed with a saturated solution of sodium bicarbonate (2*10 mL), dried over $MgSO_4$, filtered and concentrated under redcued pressure to give 3-(3,6-dichloro-9*H*-carbazol-9-yl)propan-1-ol as a white solid (1.84 g, 83 %). ^{1}H NMR (DMSO) δ: 8.32 (d, *J* = 2.1 Hz, 2H), 7.65 (d, *J* = 9.0 Hz, 2H), 7.49 (dd, *J* = 9.0, 2.1 Hz, 2H), 4.66 (t, *J* = 4.5 Hz, 1H), 4.43 (t, *J* = 6.1 Hz, 2H), 3.36 (q, *J* = 5.7 Hz, 2H), 1.88 (p, *J* = 6.4 Hz, 2H). UPLC/MS 352 (M+(CH3CO2)$^-$). HPLC (purity) maxplot 95.5 %.

To a solution of 3-(3,6-dichloro-9*H*-carbazol-9-yl)propan-1-ol (200 mg, 0.68 mmol, 1.00 equiv.) previously prepared, and triethylamine (189 µL, 1.36 mmol, 2.00 equiv.) in THF (2 mL) at rt was added methanesulfonyl chloride (58 µL, 0.75 mmol, 1.10 equiv.). The reaction was quenched after 2 h, by adding a saturated solution of sodium bicarbonate and organic materials were extracted with DCM (2*10 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated under reduced pressure to give 3-(3,6-dichloro-9*H*-carbazol-9-yl)propyl methanesulfonate as a colorless oil (250 mg, 98%). ^{1}H NMR ($CDCl_3$) δ: 8.02 (d, *J* = 2.1 Hz, 2H), 7.55 – 7.42 (m, 2H), 7.42 – 7.31 (m, 2H), 4.48 (t, *J* = 6.6 Hz, 2H), 4.25 – 4.08 (m, 2H), 3.00 – 2.89 (m, 3H), 2.40 – 2.24 (m, 2H). UPLC/MS 395 (M+Na$^+$).

A solution of 3-(3,6-dichloro-9*H*-carbazol-9-yl)propyl methanesulfonate (125 mg, 0.34 mmol, 1.00 equiv.) previously prepared, 1-cyclohexylmethylpiperazine (73 mg, 0.40 mmol, 1.20 equiv.) and potassium carbonate (93 mg, 0.67 mmol, 2.00 equiv.) in MeCN (3 mL) was refluxed overnight. The reaction was cooled to rt and quenched by adding water (5 mL). The aqueous layer was washed with DCM (2*5 mL) and the combined organic extracts were dried over magnesium sulfate, filtered and concentrated to give a yellow oil. MeCN was added and heated until complete dissolution. The resulting solution was cooled to rt, while a prepcipitate was formed. The white powder was filtered and suspended in DCM (5 mL). A solution of HCl 1.25 M in MeOH was added and the reaction was stirred during 1 h. The reaction was concentrated under reducedpressure and the resulting white powder was dried under redcued pressure at 50 °C to afford 3,6-dichloro-9-{3-[4-(cyclohexylmethyl)piperazin-1-yl]propyl}-9*H*-carbazole dihydrochloride (**13d**) as a white powder (110 mg, 62 %). ^{1}H NMR (DMSO) δ: 8.36 (d, *J* = 2.1 Hz, 2H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.53 (d, *J* = 8.8 Hz, 2H), 4.50 (t, *J* = 8.0 Hz, 2H), 3.57 (d, *J* = 21.2 Hz, 3H), 3.34 – 2.82 (m, 7H), 2.15 (s, 2H), 1.84 – 1.47 (m, 7H), 1.33 – 1.02 (m, 4H), 0.92 (d, *J* = 11.7 Hz, 2H). UPLC/MS 458 (M+H)+. HPLC (purity) maxplot 96.4 %.

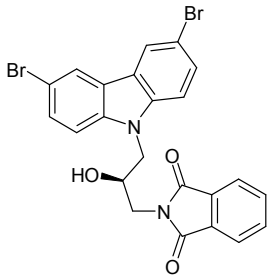
4-Benzyl-1-[3-(3,6-dichloro-carbazol-9-yl)-2-hydroxy-propyl]-piperazin-2-one (15)



To a solution of 1-benzyl-3-oxopiperazine (50 mg, 0.26 mmol, 1.00 equiv.) in THF (2 mL) at rt, was added sodium hydride 60% in mineral oil (10 mg, 0.26 mmol, 1.00 equiv.). The reaction was stirred at rt for 1h. 3,6-dichloro-9-(oxiran-2-ylmethyl)-9*H*-carbazole (76.79 mg, 0.26 mmol, 1.00 equiv.) was added and the vial was heated in the microwave at 120°C during 30 min. The reaction was concentrated and the

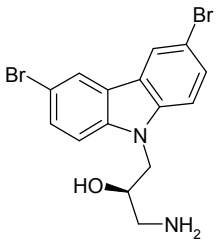
resulting crude mixture was purified by flash chromatography (MeOH 0 to 10% in DCM) to give 28 mg (22%) of 4-benzyl-1-[3-(3,6-dichloro-carbazol-9-yl)-2-hydroxy-propyl]-piperazin-2-one (**15**) as a colorless glass. ^{1}H NMR ($CDCl_3$) δ: 7.91 (d, *J* = 1.9 Hz, 2H), 7.45 – 7.04 (m, 9H), 4.37 – 4.16 (m, 3H), 3.90 (d, *J* = 3.2 Hz, 1H), 3.76 – 3.57 (m, 1H), 3.46 (s, 2H), 3.21 – 2.96 (m, 5H), 2.65 – 2.36 (m, 2H). UPLC/MS 482 (M+H+). HPLC (rt, purity) 4.0 min, maxplot 94.7 %.

2-[(*S*)-3-(3,6-Dibromo-carbazol-9-yl)-2-hydroxy-propyl]-isoindole-1,3-dione (16b)



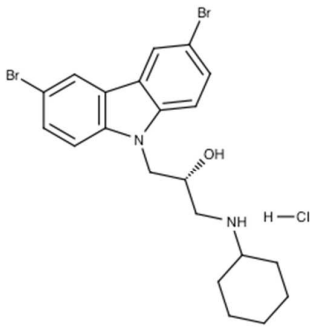
Cesium carbonate (5.51 g, 16.92 mmol, 1.10 equiv.) and 3,6-dibromocarbazole (5 g, 15.38 mmol, 1.00 equiv.) were stirred in DMF (31 mL) at 30°C during 1h. 2-(*R*)-1-oxiranylmethyl-isoindole-1,3-dione (3.13 g, 15.38 mmol, 1.00 equiv.) was then added and the mixture was stirred during 15 h at 30°C. The mixture was then diluted by EtOAc (20 mL) and organic layer was washed by brine (3*10 mL). The organic layer was separated and concentrated under vacuum. The resulting powder was washed by DCM then Et_2O to afford 2-[(*S*)-3-(3,6-dibromo-carbazol-9-yl)-2-hydroxy-propyl]-isoindole-1,3-dione **(16b)** (6 g, 72%) as a white powder. ^{1}H NMR (MeOD) δ: 8.24 (s, 2H), 7.86 – 7.71 (m, 1H), 7.68 – 7.54 (m, 4H), 7.53 – 7.37 (m, 3H), 4.61 – 4.26 (m, 3H), 3.74 – 3.55 (m, 1H), 3.55 – 3.40 (m, 1H). UPLC/MS 547 (M+H_2O+H)+. HPLC (purity) maxplot 88.0 %.

(2*R*)-1-Amino-3-(3,6-dibromo-9*H*-carbazol-9-yl)propan-2-ol (17b)



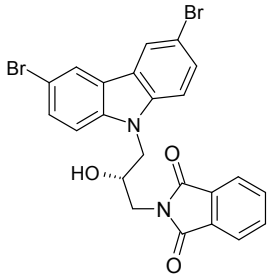
2-[(*S*)-3-(3,6-Dibromo-carbazol-9-yl)-2-hydroxy-propyl]-isoindole-1,3-dione **(16b)** (6.02 g, 10.98 mmol, 1.00 equiv.) and hydrazine hydrate (5.34 mL; 109.85 mmol; 10.00 equiv.) were stirred at 90°C during 15 h in EtOH (90 mL). The mixture was concentrated under reduced pressure then the resulting mixture was dissolved in DCM, washed by an aqueous solution of NaOH 1M. The organic layer was extracted, washed with brine and concentrated under reduced pressure to give (2*R*)-1-Amino-3-(3,6-dibromo-9*H*-carbazol-9-yl)propan-2-ol **(17b)** as a white powder(4.2 g, 80 %). ^{1}H NMR (300 MHz, DMSO) δ = 8.46 (d, *J* = 1.8 Hz, 2H), 7.70 - 7.51 (m, 4H), 4.95 (s, 1H), 4.44 (dd, *J* = 14.8, 4.4 Hz, 1H), 4.25 (dd, *J* = 14.8, 7.4 Hz, 1H), 3.77 (s, 1H), 3.33 (s, 1H), 2.71 - 2.51 (m, 2H), 1.55 (s, 2H).UPLC/MS 399.0 (M+H)+. HPLC (purity) maxplot 98.7 %.

(2*R*)-1-(Cyclohexylamino)-3-(3,6-dibromo-9*H*-carbazol-9-yl)propan-2-ol hydrochloride salt (18b)



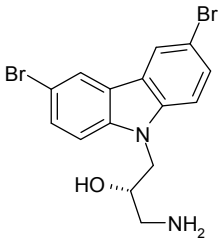
(2*R*)-1-Amino-3-(3,6-dibromo-9*H*-carbazol-9-yl)propan-2-ol **(17b)** (1.20 g, 3.01 mmol, 1.00 equiv.) was stirred during 3h at rt with sodium triacetoxyborohydride (0.83 g, 3.92 mmol, 1.30 equiv.) and cyclohexanone (0.34 mL, 3.32 mmol, 1.10 equiv.) in DCM (10 mL). The reaction was quenched by adding water (5 mL) then the organic materials were extracted with DCM (2*5 mL). The organic layers were combined, dried with $MgSO_4$ and concentrated under reduced pressure. The crude powder was purified by chromatography on silica gel (MeOH in DCM, 2 to 30%). The selected fractions were combined and concentrated under reduced pressure. The resulting powder was dissolved in MeOH (5 mL) and HCl 2N in Et_2O (3 mL) was added. After total precipitation of the white powder, the medium was filtered and the powder was washed with Et_2O (2*5 mL) to afford (2*R*)-1-(cyclohexylamino)-3-(3,6-dibromo-9*H*-carbazol-9-yl)propan-2-ol hydrochloride **(18b)** (825 mg, 53 %) as a white powder. ^{1}H NMR (DMSO) δ: 8.49 (d, *J* = 1.9 Hz, 3H), 7.76 – 7.68 (m, 2H), 7.68 – 7.58 (m, 2H), 5.83 (d, *J* = 4.9 Hz, 1H), 4.53 – 4.34 (m, 2H), 4.27 (s, 1H), 3.19 – 2.89 (m, 3H), 2.03 – 1.92 (m, 2H), 1.75 (d, *J* = 11.2 Hz, 2H), 1.60 (d, *J* = 12.0 Hz, 1H), 1.38 – 1.05 (m, 5H). UPLC/MS 481 (M+H)+. HPLC (purity) maxplot 98.8 %.

2-[(*R*)-3-(3,6-Dibromo-carbazol-9-yl)-2-hydroxy-propyl]-isoindole-1,3-dione (16a)



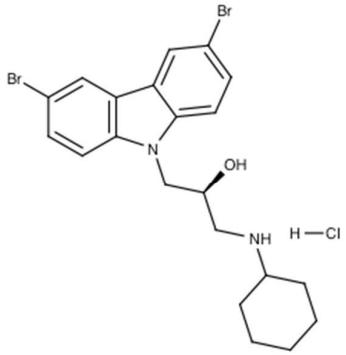
Following the same protocol than for compound (**16b**), using 2-(S)-1-oxiranylmethyl-isoindole-1,3-dione (1.9 g, 9.23 mmol, 1.00 equiv), 3,6-dibromocarbazole (3.0 g, 9.23 mmol, 1 equiv.) and cesium carbonate (3.3 g, 10.1 mmol, 1.1 equiv.) in DMF (30 mL), 2-[(R)-3-(3,6-dibromo-carbazol-9-yl)-2-hydroxy-propyl]-isoindole-1,3-dione (**16a**) was obtained as a white powder (3.42 g, 68%). 1H NMR (MeOD) δ: 8.24 (s, 2H), 7.86 – 7.71 (m, 1H), 7.68 – 7.54 (m, 4H), 7.53 – 7.37 (m, 3H), 4.61 – 4.26 (m, 3H), 3.74 – 3.55 (m, 1H), 3.55 – 3.40 (m, 1H). UPLC/MS 547 (M+H2O+H)+. HPLC (purity) maxplot 88.0 %.

(2*S*)-1-Amino-3-(3,6-dibromo-9*H*-carbazol-9-yl)propan-2-ol (17a)



Following the same protocol than for compound (17b), using 2-[(R)-3-(3,6-Dibromo-carbazol-9-yl)-2-hydroxy-propyl]-isoindole-1,3-dione (**16a**) (2.84 g, 5.2 mmol, 1 equiv.), hydrazine hydrate (2.15 mL, 52.0 mmol, 10 equiv.) in EtOH (15 mL), (2S)-1-amino-3-(3,6-dibromo-9H-carbazol-9-yl)propan-2-ol (**17a**) was obtained as a white powder (1.2 g, 58 %). 1H NMR (300 MHz, DMSO) δ = 8.46 (d, J = 1.8 Hz, 2H), 7.70 - 7.51 (m, 4H), 4.95 (s, 1H), 4.44 (dd, J = 14.8, 4.4 Hz, 1H), 4.25 (dd, J = 14.8, 7.4 Hz, 1H), 3.77 (s, 1H), 3.33 (s, 1H), 2.71 - 2.51 (m, 2H), 1.55 (s, 2H).UPLC/MS 399.0 (M+H)+. HPLC (purity) maxplot 98.7 %.

(2*S*)-1-(Cyclohexylamino)-3-(3,6-dibromo-9*H*-carbazol-9-yl)propan-2-ol hydrochloride salt (18a)



Following the same protocol than for compound **(18b)**, using (2*S*)-1-amino-3-(3,6-dibromo-9*H*-carbazol-9-yl)propan-2-ol **(17a)** (1.1 g, 2.8 mmol, 1 equiv.), cyclohexanone (300 mg, 3.04 mmol, 1.1 equiv.), sodium triacteoxyborohydride (750 mg, 3.6 mmol, 1.3 equiv.), (2*S*)-1-(cyclohexylamino)-3-(3,6-dibromo-9*H*-carbazol-9-yl)propan-2-ol hydrochloride **(18a)** was obtained (900 mg, 63 %) as a white powder. ^{1}H NMR (DMSO) *δ*: 8.49 (d, *J* = 1.9 Hz, 3H), 7.76 – 7.68 (m, 2H), 7.68 – 7.58 (m, 2H), 5.83 (d, *J* = 4.9 Hz, 1H), 4.53 – 4.34 (m, 2H), 4.27 (s, 1H), 3.19 – 2.89 (m, 3H), 2.03 – 1.92 (m, 2H), 1.75 (d, *J* = 11.2 Hz, 2H), 1.60 (d, *J* = 12.0 Hz, 1H), 1.38 – 1.05 (m, 5H). UPLC/MS 481 (M+H)+. HPLC (purity) maxplot 99.1 %.

Parasitemia determination in vitro and in vivo assays.

Compounds were screened against chloroquine-resistant (K1) strains of *P. falciparum in vitro* as described by Vennerstrom *et al*.

Reference: Vennerstrom, J. L., Arbe-Barnes, S., Brun, R., Charman, S. A., Chiu, F. C. K., Chollet, J., Dong, Y., Dorn, A., Hunziker, D., Matile, H., McIntosh, K., Padmanilayam, M., Tomas, J.S., Scheurer, C., Scorneaux, B., Tang, Y., Urwyler, H., Wittlin, S., Charman, W. N., *Nature* **2004**, *430*, 900-904.

In vivo anti-malarial activity of the test compounds was assessed using female NMRI mice (20-22g) infected with *Plasmodium berghei* (GFP ANKA strain). Chloroquine (Sigma C68) and Artemisinin (Sigma 36, 159-3) were used as positive controls.

Reference: B. Franke-Fayard *et al.*, *Mol.Biochem.Parasitol*. **2004**, *137*, 23-33.

On day 0 each mouse (n = 6 per group) was infected intravenously with 0.2 mL of 10^8 parasitized erythrocytes per mL. For this heparinized blood from a donor mouse with approx. 30% parasitemia was diluted in physiological saline. The mice were housed in standard macrolon type II cages at 22 °C, 60 – 70 % relative humidity and were fed with food pellets (PAB45 – NAFAG 9009, Provimi Kliba AG, CH-4303 Kaiseraugst, Switzerland) and water *ad libitum*.

The compounds were prepared at doses of 3, 10, 30, and 100 mg/kg in 10 % DMSO in water and the concentration adjusted so that 0.1 mL / 10 g body weight were administered. The experimental groups were treated orally 4 h, 24 h, 48 h and 72 h post infection with test compounds or vehicle, respectively. 24 h after the last treatment, 1 μL tail blood was collected and diluted with 1 mL PBS buffer. Parasitemia was determined with a FACScan (Becton Dickinson) by counting 100'000 red blood cells. The difference between the mean value of the control group and those of the experimental groups was then calculated and expressed as a percent relative to the control group (%activity). Three animals per group were euthanized 24 h after the last treatment and blood collected by cardiac puncture for the determination of plasma exposures. The survival of the animals was monitored up to 30 days and mice surviving for 30 days were checked for parasitemia and subsequently euthanized. A compound was considered curative if the animal survived to day 30 post-infection with no detectable parasitemia. The results are expressed as percent reduction of parasitemia on day 4 compared to the untreated control group, and mean survival of the animals.

hERG patchclamp assay

Test article and positive control concentrations are prepared fresh daily by diluting stock solutions into a HEPES-buffered physiological saline (HB-PS) solution (in mM): NaCl, 137; KCl, 4.0; $CaCl_2$, 1.8; $MgCl_2$, 1; HEPES, 10; Glucose, 10; pH adjusted to 7.4 with NaOH (prepared weekly and refrigerated until use). Since previous results have shown that ≤ 0.3% DMSO does not affect channel current, all tests and control solutions contain 0.3% DMSO. Each test article formulation is sonicated at ambient room temperature for at least 20 minutes to facilitate dissolution. A glass-lined 96-well compound plate will be loaded with the appropriate amounts of test and control solutions, and placed in the plate slot of QPatch HT (Sophion Bioscience A/S, Denmark).

In this study, *h*ERG channels are expressed in a Chinese hamster ovary (CHO) cell line that lacks endogenous I_{Kr}. CHO cells are stably transfected with hERG cDNA. Stable transfectants are selected by coexpression with an antibiotic-resistance gene incorporated into the expression plasmid. Selection pressure is maintained by including the selection antibiotic in the culture medium. Cells are cultured in Nutrient Mixture F-12 (F-12) supplemented with 10% fetal bovine serum, 100 U/mL penicillin G sodium, 100 μg/mL streptomycin sulfate. Before testing, cells in culture dishes are washed twice with Hank's Balanced Salt Solution, treated with Accutase and re-suspended in the CHO Serum Free Media (1-1.5 $x10^6$ cells/mL). Intracellular solution for whole cell recordings will consist of (in mM): potassium aspartate, 130; $MgCl_2$, 5; EGTA, 5; ATP, 4; HEPES, 10; pH adjusted to 7.2 with KOH. Intracellular solution is loaded into the intracellular compartments of the *QPlate*. Cell suspension is pipetted into the

extracellular compartments of the *QPlate*. After establishment of a whole-cell configuration, membrane currents will be recorded using QPatch HT® system. Before digitization, the current records will be low-pass filtered at one-fifth of the sampling frequency.

References :

Brown, A.M. and Rampe, D., Drug-induced long QT syndrome: is HERG the root of all evil? *Pharmaceutical News* **2000**, *7*, 15-20.

Weirich, J. and Antoni, H. , Rate-dependence of antiarrhythmic and proarrhythmic properties of class I and class III antiarrhythmic drugs. *Basic Res Cardiol 93 Suppl* **1998***, 1,* 125-132.

Yap, Y.G. and Camm, A.J.,Arrhythmogenic mechanisms of non-sedating antihistamines. *Clin. Exp. Allergy 29 Suppl 3*, **1999**, 174-181.

Cytochrome P450 inhibition in vitro assay.

Seven human recombinant cytochrome P450 isoenzymes were tested (CYP 1A2, CYP 2C9, CYP 2C19, CYP 2C8, CYP 2B6, CYP 2D6 and CYP 3A4). The aim of this method is to determine the concentration of a compound required to obtain 50% inhibition of the recombinant human cytochrome P450. The assay is based on the Promega P450-Glo™ Screening System, which includes, a luminogenic substrate, a NADPH regeneration system and a Luciferin Detection Reagent.

Upon CYP450 activity, the substrate *e.g*. Luciferin-ME EGE for 2B6 assay is converted to luciferin EGE, which is converted to D-luciferin by the Luciferin Detection Reagent. The D-luciferin reacts in turn with the Luciferin Detection Reagent to produce light. The CYP membranes are prepared from baculovirus-infected insect cells and contain human CYP450 and P450 reductase. In order not to be depleted in NADPH cofactor during the course of the reaction, the assay includes a NADPH Regeneration System.

Test compounds are pre-incubated for 15 min at room temperature with the CYP450 enzyme (and appropriate cofactors), in the absence of substrate. Then the enzymatic reaction is initiated by the addition of the substrate, followed by 30 min of incubation at 37°C. As controls, BLANK and NEUTRAL values are tested on each plate. In the NEUTRAL control, DMSO is added in place of the test compound: it defines the maximum achievable substrate conversion, reflected by the strongest luminescence signal. In the BLANK control, a membrane fraction devoid of cytochrome P450 activity (control membranes prepared from wild type baculovirus-infected cells), substitutes the membranes from CYP450 expressing cells: consequently, no substrate conversion is observed. The amount of control membranes in BLANK controls is calculated to match the amount of membranes proteins brought by the CYP450 positive membranes in the rest of the assay plate. The measured signal in BLANK wells describes the background value. The percentage of inhibition is determined for each inhibitor concentration and the corresponding IC50 value is calculated by non-linear curve fitting. The IC_{50} value is determined in triplicate, using 10 different concentrations of test compound. As additional control, the IC_{50} value of one well-characterized CYP inhibitor (called reference control) is determined.

Protein Binding assays

Protein binding of test compounds was determined by ultrafiltration using serum from various species (mouse, rat, human etc). The test items (final concentration 5 µM) were incubated in triplicate with three different serum dilutions (1:2, 1:5 and 1:10) for 30 min at 37 °C using slight agitation. After the incubation the 96-well filter plates were centrifuged for 45 min at 3500 rpm and 37 °C. 25 µL of filtrate

samples were treated with 50 µL of ethanol and 50 µL internal standard solution and analyzed by LC-MS/MS. The fraction unbound was calculated from the drug concentrations in the filtrate samples.

Microsomal stability

The stability of the compounds was studied *in vitro* in mouse and human liver microsomes. Microsomes (final concentration 0.5 mg/mL), 50 mM phosphate buffer pH 7.4, NADPH (final concentration 1.5 mM) and compound (final concentration 1 µM,) were added to the assay plate. The microsome suspension was added to initiate the reaction and the plate was incubated at 37°C. The reaction was stopped by the addition of cold acetonitrile at the appropriate time points (time 0, 5, 15 and 45 minutes). The samples were centrifuged at 4000 rpm for 30 minutes at 4 °C and analyzed by LC-MS/MS. The in vitro intrinsic clearance was calculated from the rate of compound disappearance.

In vivo Pharmacokinetic Evaluation in Mouse.

In order to study the pharmacokinetic (PK) profile of the test compounds *in vivo*, the test compounds were administered to NMRI female mice intravenously (1 mg/kg in solution in 10% ethanol, 10% *N, N*-dimethylacetamide, 30% propylene glycol, 50% water, v/v) and by oral gavage (5 mg/kg in suspension in 0.5% carboxymethylcellulose suspension containing 0.25% Tween 20 in water). The volume of administration was 2 mL/kg for i.v. dosing and 10 mL/kg for oral gavage. The PK profiles were determined from 3 animals for each time point. Blood samples (100 µL/time point) were collected from intracardiac puncture at sacrifice at the following time points: 0.083, 0.25, 0.5, 1, 4, 7 and 24 hours post-dose for i.v. dosing, and at 0.5, 1, 4, 7 and 24 h for oral dosing. For bioanalysis, samples were processed by protein precipitation and analysed using a sensitive and selective LC-MS/MS method.

Cytotoxicity assay.

Compounds were added to assay wells containing 3000 12 hour adherent cells/well (HEK293) in an assay volume of 45 µl. The plates were incubated for 72 hours at 37°C and 5% CO_2. After incubation the supernatant was removed and 40 µl of 10% Alamar blue added per well. Plates were incubated for a further 5-6 hours and measured for fluorescence. The % inhibition of HEK293 cell proliferation was calculated using DMSO and Puromycin control data.

Additional information

In vitro anti malarial activity (IC50 and IC90) for (12) against a panel of Plasmodium falciparum strains.

Unit : ng/mL	*Pf* K1[a)]		*Pf* NF54[b)]		*Pf* D6[c)]		*Pf* W2[d)]		*Pf* TM91C235[e)]		*Pf* 7G8[f)]		*Pf* V1S[g)]	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
12	4.0	5.7	9	16	6	13	1	5	8	30	2	5.7	3	8.1
Chloroquine	58	105	3	7	2	9.9	67	114	37	58	17	35	85	149
Artemisinin	1.2	2.5	2	4	3	5	1	2.5	4.2	6	1	1.7	2.1	3.7
Mefloquine	2.5	4.5	3.4	5	2	3.9	4.2	1.4	5.5	15	1.1	2.5	1.6	4.6
Pyrimethanine	4.4	9.2	4.7	9.7	0.9	2.2	7258	>1[e4]	3850	>5000	2862	4610	3730	>5000
Cycloguanil	4.2	6	4.3	6	4	6	71	*ND*	61	101	34	79	120	231
Atovaquone	0.6	1.5	0.1	1.5	0.1	1	1	2.4	0.1	0.5	0.6	0.8	0.3	1.8

a) from Thailand resistant to CQ and PYR b) from West Africa, sensitive to all known antimalarial drugs c) from Sierra Leone sensitive to chloroquine, pyrimethamine, sulfadoxine and quinine, less sensitive to mefloquine d) from Indochina resistant to CQ, QN, PYR, SDX e) Thailand resistant to MEF f) from Brazil resistant to CQ, PYR, CYC g) from Vietnam or Cambodia resistant to CQ ,PYR, QN. ND: not determined.

In vitro physicochemical and eADME properties and in vivo DMPK data of compounds 12, 13a 18a and 18b.

Both compounds **12** and **13a** showed a high lipophilicity (logD 7.4 >3), with **13a** being slightly less lipophilic than **12** (respectively measured LogD7.4 = 3.17 vs 4.20 and calculated LogP = 6.06 vs. 6.26). Consequently, both compounds **12** and **13a** exhibited a low solubility in PBS pH 7.4 (<0.001 mg/mL). Despite this and considering their good potency, preliminary DMPK studies were performed.

The compounds were shown to be stable in human and mouse plasma at pH 7.4. The serum protein binding was very high, showing a fraction unbound lower than 0.05% for both compounds, a consequence of the high affinity of the compounds for lipidic media. Another parameter to take into account for combination therapy was CYP inhibition (Table 1). Both compounds showed medium to low inhibition on all types of CYP isoforms tested, except for CYP 2D6 (IC50=40 nM and 770 nM for **12** and **13a**, respectively).

	CLint (µL/min/mg prot.)		**CYP inhibition (µM)**						
	h	**m**	**1A2**	**2B6**	**2C8**	**2C9**	**2C19**	**2D6**	**3A4**
12	27	<10	>20	>20	29	>20	>10	0.04	>20
13a	30	30	>20	>20	28	>20	>10	0.8	2.3

	PK mouse					
	CL (L/h/kg)	**Vss (L/kg)**	**Fz (%)**	***p.o.* $t_{1/2}$ (h)**	**AUC (h*ng/mL)**	**Cmax (ng/mL)**
12	0.8	13	51	15.4	3753	158
13a	1.7	15.2	51	8.1	1583	135

Table 1. Summary of in vitro and in vivo DMPK data. CLint: intrinsic clearance in human (h) and mouse (m) liver microsomes. PK: pharmacokinetic profile in mice. CL: intravenous clearance, Vss: volume of distribution at steady state, Fz: oral bioavailability. po t1/2: terminal half-life following oral administration.

	hERG Ki, μM	CYP inhibition (μM)						
		1A2	2B6	2C8	2C9	2C19	2D6	3A4
18a	0.79	>20	>20	18	>20	7.2	0.04	>20
18b	1.0	>20	18	>20	>20	9.2	0.29	>20

Table 2. hERG and CYP inhibition of separated enantiomers 18a and 18b

NB : Data from Table 1 CYP inhibitions were generated using a fluorimetric protocol, as compared to Table 2, for which luminescence protocol was used.

In vivo data in the P. berghei mouse model.

Compound	Dose (mg/kg/day)	% Activity	MSD
Vehicle	0	0	6
12	3	4.73 ± 11.4	6 ± 0
	10	38.1 ± 11.8	7.67 ± 1.15
	30	95.3 ± 2.96	15.0 ± 2.00
	100	99.97 ± 0.02	30.0 ± 0
13a	3	9.45 ± 12.7	6 ± 0
	10	0.041 ± 16.7	6 ± 0
	30	33.8 ± 11.1	6.67 ± 0.58
	100	99.93 ± 0.078	24.7 ± 7.57

Correlation study between IC90 measured on Pf-K1 and IC90 measured on P.berghei

The correlation study was performed using the IC50 and IC90 values of selected 62 compounds, generated by Swiss TPH, using the two different strains. For our series of compounds a correlation is seen between the IC50 values with both parasites strains, however a deviation between potency on Pf-K1 compared to P.berghei is apparent. The dataset shows that compounds are more potent on K1 strain than on P. berghei. Compounds are generally 4.7 fold more potent on K1.26 A similar difference is observed for the artemisinin control values.

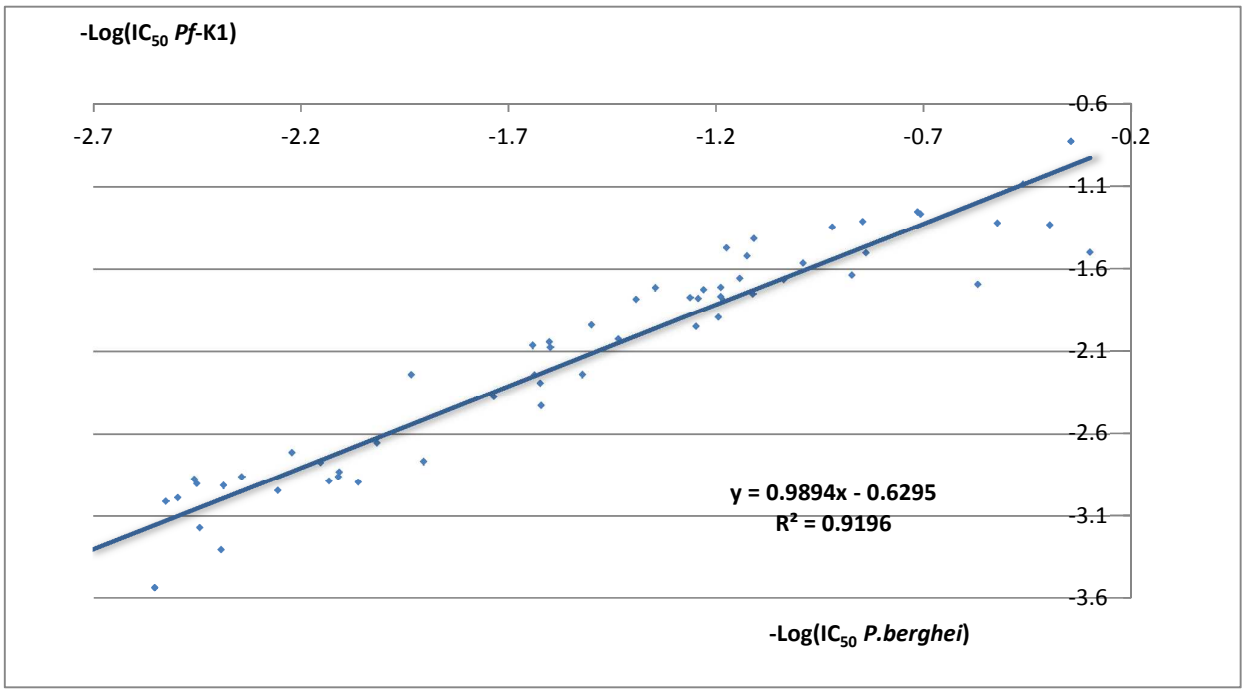


Figure 1.Comparison of *in vitro* IC_{50} values against *P.berghei* and *Pf*-K1 species.

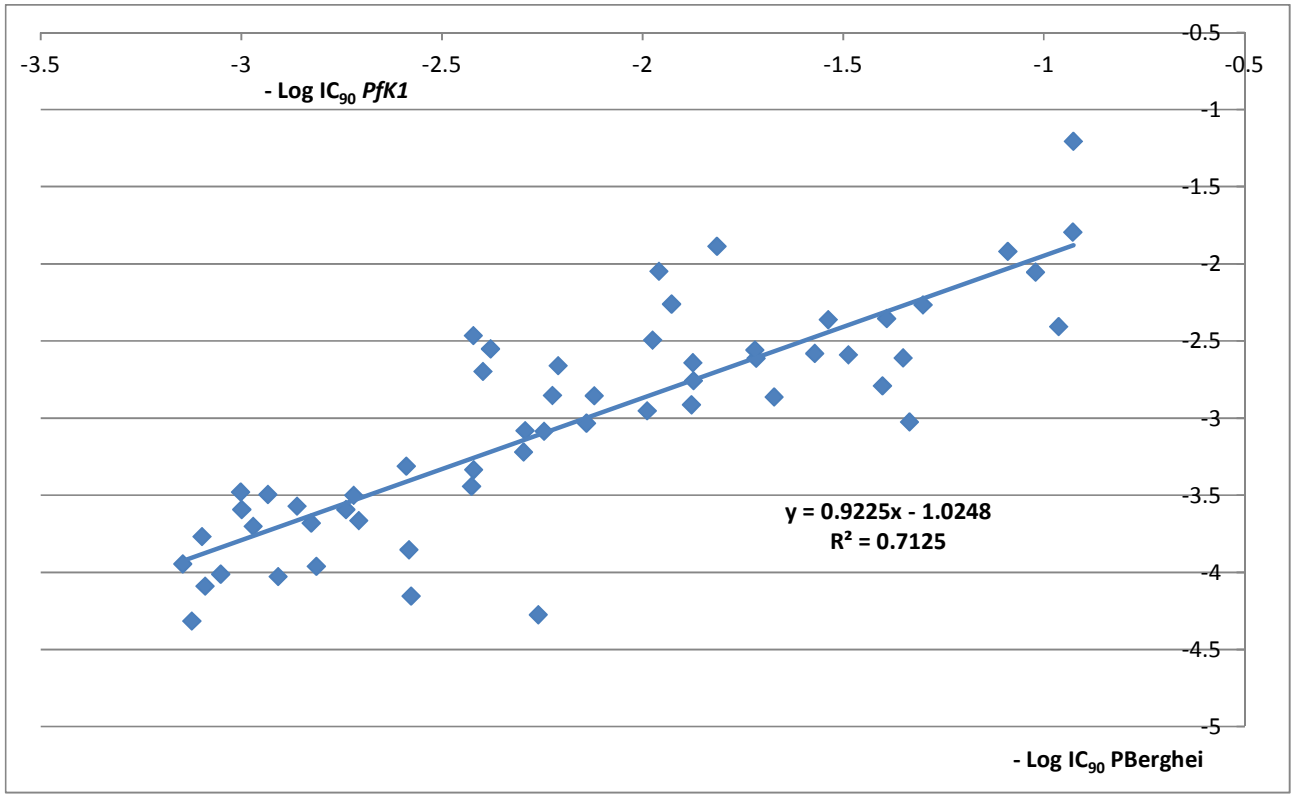


Figure 2. Comparison of *in vitro* IC_{90} values against *P.berghei* and *Pf*-K1 species.

The study was perfomed using a set of selected 56 compounds that have been tested on both strains.

The study highlights, as in the case of the IC_{50} study, a correlation between activities on both strains. Nevertheless, the compounds are generally 8 folds more potent on *Pf*-K1 strain. The studies were performed using a set of 56 selected compounds that have been tested on both strains.

Cardiac ion channel profiling of compounds (18a) and (18b) at 10μM.

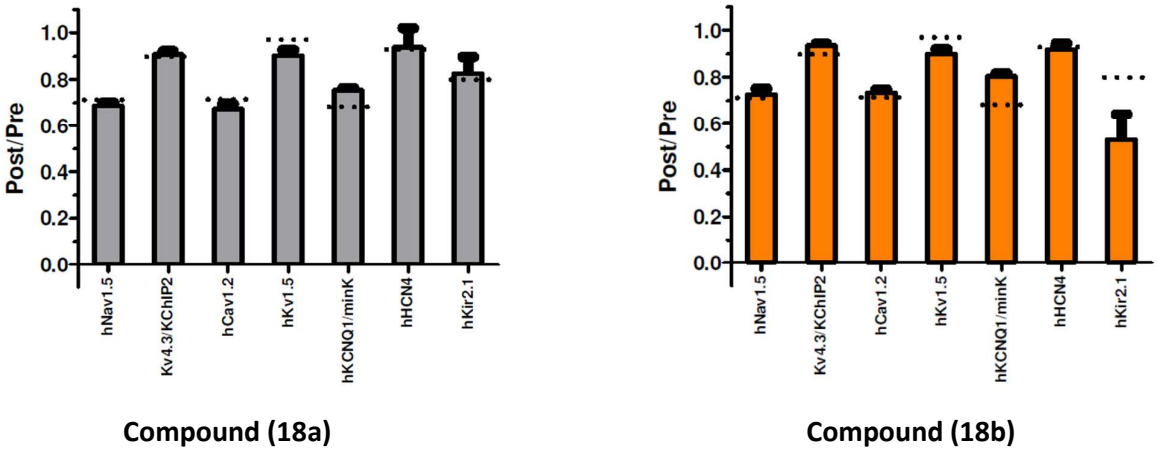


Figure 3: evaluation of the activity of enantiomers 18a and 18b on a panel of cardiac ion channels by patchclamp assay.

The dashed horizontal line associated with each bar indicates the mean effect of 0.3% DMSO controls. Automated whole cell patch clamp at nominal concentration of 10 µM. None of the compounds did inhibit any of the seven cardiac channel currents more than 50% at nominal 10 µM.