Supplementary Information

Supplementary Table S1

IC_{50} values of mefloquine and the two lead compounds WR7930, *threo*- enpiroline and it's enantiomers (+/- *threo*) evaluated with L6-rat skeletal myoblast cells after 72 h exposure.

Compound	Mefloquine	WR7930	Enpiroline	(+)-*threo*	(-)-*threo*
	IC_{50} [uM]				
AVERAGE [μM]	11.8	16.5	9.7	13.2	11.8
SD	7.2	9.8	4.7	6.1	8.0

The determination of the cytotoxicity was performed with L-6 rat skeletal myoblast cells according to a previously reported procedure (**1**). Briefly, L-6 cells were seeded in 96-well microtiter plates at a density of $4*10^4$ cells/mL in RPMI 1640 medium supplemented with 10 % fetal bovine serum and L-glutamine (2 mM). A three-fold serial dilution ranging from 30 to 0.04 μg/mL in test medium was added. Podophyllotoxin (concentration range: 0.1 - $4*10^{-4}$ μg/mL) served as positive control, with IC_{50} values of podophyllotoxin showing an average of 0.006 μg/mL in all experiments . The plates were incubated at 37°C ,5 % CO_2 atmosphere. After 70 hours, Alamar Blue (10 μL) was added to each well and incubation was continued for a further 2 - 3 hours. The plate was then read with a SpectraMax M2 (MolecularDevices) instrument by the use of an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Fluorescence development was expressed as percentage of the control and the IC_{50} values were determined. Experiments were repeated at least three times and IC_{50} values calculated as averages.

Supplementary Table S2

Percentage of HepG2-viability evaluated with MTT 24h post exposure with mefloquine, WR7930, enpiroline and its enantiomers.

Compound	Concentration [μM]	**Viability [%] (SD)**
Mefloquine	72.3	1.9 (1.6)
	7.2	90.3 (5.0)
	0.7	99.3 (0.9)
WR7930	69.4	0.8 (0.1)
	6.9	91.1 (0.4)
	0.7	96.9 (10.8)
Enpiroline	59.7	0.9 (0.1)
	6.0	98.1 (4.2)
	0.6	107.2.4 (9.0)
(+)-*threo*	59.7	0.8 (0.1)
	6.0	84.3 (9.2)
	0.6	94.4 (21.2)
(-)-*threo*	59.7	0.9 (0.2)
	6.0	89.5 (19.5)
	0.6	80.9 (9.5)

The viability of HepG2 cells was evaluated 24 h post exposure using colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) readout. For that purpose HepG2 cells were seeded at a density of $5x10^4$ cells/well in DMEM low glucose medium supplemented with 10% FCS, 1% Penicillin-Streptomycin and 1% non-essential amino acids in 96 well-plates. Plates were incubated 24 h at 37°C, 5% CO_2 to allow attaching of cells. Then the medium was removed and 100 µl supplemented DMEM l low glucode medium, spiked with test compounds, was added to each well reaching final test drug concentrations of 30, 3 or 0.3 µg/mL. Each concentration was performed at least in quadruplicate. Control wells

with blank medium were included for each drug and terfenadin (10 and 15 µM) served as positive control. Plates were incubated for 24 h. Next, medium was replaced with 100 µl of blank culture medium containing 10% MTT solution (5 mg MTT/ml PBS) and incubation was continued for 2 h. Finally, the medium was discarded and the water-insoluble crystals were dissolved by adding 20µl of 3% SDS and 100 µl of 10M isopropanol-HCl. When crystals were completely dissolved, optical density (OD) was measured at 550 nm using, Spectramax M2 (Molecular devices). OD development was expressed as percentage of the control.

Results

The lead compounds (WR7930, enpiroline and both enantiomers) showed similar patterns of cytotoxicity when compared to the parent drug mefloquine. Hepatic HepG2- cells were strongly affected after treatment with the highest concentration of 30 µg/ml, whereas the other tested concentrations (3, 0.3 µg/ml) did not reduce the cell viability. After a 72h- treatment of L6- rat skeleton cells IC_{50} values ranging from 9.7 – 16.5 µM were observed.

References

1. Sperandeo NR, Brun R. Synthesis and biological evaluation of pyrazolylnaphthoquinones as new potential antiprotozoal and cytotoxic agents. *Chembiochem* 2003; **4**: 69-72