Supplementary Material

Synthesis and Biological Evaluation of 1,4-Naphthoquinones and Quinoline-5,8-diones as Antimalarial and Schistosomicidal Agent

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15 Enzymatic studies

**Enzyme preparation.** Recombinant hGR was purified as previously reported.1 One unit of GR activity is defined as the consumption of 1 μmol NADPH per min under conditions of substrate saturation. The enzyme stock solution (266 μM) was used for kinetic determinations. The enzyme was stored at -20°C. The protein was judged to be > 98% pure as judged from silver stained SDS-PAGE and had specific activities of 200 U/mg (hGR). A hGR stock II solution was prepared by dilution (1/10) of the enzyme stock solution with hGR buffer (pH 6.9).2 The hGR buffer (pH 6.9) was prepared by dissolving 200 μM HClPO4 (2.79 g), K2HPO4 3 H2O (6.04 g), EDTA (0.372 g), and KCl (14.91 g) in 1 L water. The pH was adjusted by dropwise addition of 5 M KOH. Recombinant SmTGR was produced in Escherichia coli as previously described.3

**Pharmacokinetics**

**Solubility express.** Shake-flask method allowed determination of the apparent thermodynamic solubilities from DMSO stock solutions of compounds. Compound dilutions were prepared to 200 μM in an aqueous 7.4 HEPEs buffer. The mix was equilibrated at 20 °C during 24 hours. After centrifugation, the supernatant was injected in an HPLC column coupled to a LC-MS apparatus (with UV-Vis detection) in order to determine the compound concentration. A reference solution at 200 μM was made in CH3CN/water and a dilution at 100 μM allowed analysis of the linearity of the UV response. These solutions were also injected in order to calibrate the concentration determination. Comparison of the responses of the injections of three dilutions obtained from a DMSO reference solution allowed determination of the solubility if lower than 200 μM. Measurements were done in duplicate.

**Determination of logD values.** The partition coefficient between octanol and water is the most widely used physicochemical determination of lipophilicity. The "shake flask" method was used to determine the partition coefficients. A solution of 100 μM tested compound was prepared from a stock DMSO solution in a "mix" aqueous buffer / octanol. Three octanol / buffer volume ratios were used to cover partition coefficients ranging from -1.5 to +3.2. The partition experience with the best ratio was repeated twice. The mix was equilibrated at 20°C for one hour. Then, the aqueous phase and a reference solution at 100 μM were injected in an HPLC column coupled to a LC-MS apparatus (with UV-Vis detection) to determine compound concentration. The partition coefficient was calculated from the peak areas obtained in the chromatograms. Measurements were done in duplicate. For optimal oral absorption, logD values should be between 0.5 and 2.

**Determination of CHI values.** Compound lipophilicity influences permeability and propensity to nonspecific binding to plasma proteins. Although precise, the classical « shake flask » method is not always appropriate for determination of high hydrophobicity, which can be alternatively measured using the Chromatographic Hydrophobicity Index method. This HPLC-based technique leads to rapid and cheap estimates of the partition coefficients. The lipophilicity is determined by partition of the compound between a hydro-organic mobile phase and a reverse stationary phase, typically a C18 column, using a fast gradient. Compound solution samples (200 μM) were prepared in a 50/50 v/v mixture of water and acetonitrile. Five μL were injected onto the HPLC (with UV-Vis detection). CHI values were derived from the retention time and its comparison with 10 reference compounds of known CHI. Measurements were done in duplicate. In general, CHI values correlate satisfactorily with logD7.4 values. The CHI is approximately equal to the proportion of acetonitrile in the mobile phase when the compound is eluted from the column.

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NAME          bb48.1038
EXPNO         10
PROCNO        1
Date           20100920
Time           18.48
INSTRUM       spect
PROBHD        5 mm Dual 13C/
PULPROG       zgpq30
TD            65536
SOLVENT       CDC13
NS             1024
DS             4
SMH           17985.611 Hz
FIDRES        0.274439 Hz
AG            1.8219508 sec
RG             1024
dw            27.800 usec
dE            6.50 usec
dT            298.3 K
dl           2.00000000 sec
dl1        0.03000000 sec
td0          1

==== CHANNEL 11 ====
NUC1         13C
p1           5.50 usec
pL1         -6.00 dB
SF01       75.4752953 MHz

==== CHANNEL 22 =====
CPDPRG2    waltz16
NUC2         1H
PCPD2      80.00 usec
PL2         -3.00 dB
PL12       12.14 dB
PL13        20.50 dB
SF02   300.1312065 MHz
SI          32768
SF        75.4677490 MHz
WDW         EM
SSB         0
LB          1.00 Hz
GB           0
PC           1.40

200 180 160 140 120 100 80 60 40 20 0 ppm