Supporting information

One-pot microwave-assisted synthesis and antimalarial activity of ferrocenyl benzodiazepines

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Experimental

General experimental methods

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC300 spectrometer using tetramethylsilane (TMS) as the internal standard and $CDCl_3$ as the solvents. Mass spectra were recorded with a LCMS-MS triple-quadrupole system (Varian 1200ws). HPLC analysis were performed using a C18 TSK-GEL Super ODS 2 µm particle size column, dimensions 50 * 4.6 mm. LCMS gradient starting from 100% H_2O / 0.1% formic acid and reaching 20% H_2O / 80% CH_3CN / 0.08% formic acid within 10 min at a flow rate of 1 mL/min was used. Microwave reactions were performed on a CEM Discover. Thin layer chromatography (TLC) was carried out on aluminium-baked Macherey-Nagel silica gel 60. Column chromatography was performed on silica gel (230-400 mesh). Melting points were determined on a Kofler apparatus and are uncorrected. Elemental analyses were performed with a varioMICRO analyser.

General procedure for preparation of 7-9 by microwave-assisted method

Substituted 2-aminobenzophenone (0.126 mmol), DCC (0.126 mmol, 0.026 g) and *N*-Boc-glycine (0.126 mmol, 0.022 g) in toluene (400 µL) were placed in a 10 mL microwave tube equipped with a magnetic stirrer. The sealed tube was placed in the cavity of the microwave reactor and irradiated for 20 min at 150°C. Trifluoroacetic acid (600 µL, 7.9 mmol) was then added to the mixture and the reaction vessel was irradiated at 150°C for 20 min. The solution was neutralized by an aqueous 6*N* NaOH solution and extracted with dichloromethane (3×3 mL). The organic layers were dried over $MgSO_4$ and evaporated. The residue was purified using column chromatography (eluent: petroleum ether/diethyl ether: 7/3).

7-Chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (7)

Yield= 86%. M.p. 216°C. δ_H (300 MHz, $CDC1_3$) 9.20 (1H, s, NH), 7.47 (2H, m, ArH), 7.46–7.40 (2H, m, ArH), 7.39 (2H, m, ArH), 7.34 (1H, d, *J* 2.4 Hz, ArH), 7.10 (1H, d, *J* 8.6 Hz, ArH), 4.26 (2H, s, CH_2); δ_C (75.5 MHz, $CDC1_3$) 172.8, 167.8, 138.6, 137.1, 131.7, 130.5, 130.4, 129.7, 128.9, 128.6, 128.3, 122.6, 58.8.

7-Chloro-1,3-dihydro-5-(-2-fluorophenyl)-2H-1,4-benzodiazepin-2-one (8)

Yield= 80%. M.p. 208°C. δ_H (300 MHz, $CDC1_3$) 9.04 (1H, s, NH), 7.51 (1H, t, *J* 8 Hz, ArH), 7.51 (1H, t, *J* 8 Hz, ArH), 7.41–7.36 (2H, m, ArH), 7.19–7.18 (2H, m, ArH), 7.01–6.91 (2H, m, ArH), 4.30 (2H, s, CH_2); δ_C (75.5 MHz, $CDC1_3$) 172.1, 166.7, 162.1, 138.8, 136.2, 132.4, 132.0, 131.4, 130.5, 128.2, 127.2, 124.5, 124.4, 116.5, 56.5.

1,3-Dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (9)

Yield= 83%. M.p. 148°C. δ_H (300 MHz, $CDC1_3$) 9.78 (1H, s, NH), 7.44 (2H, d, *J* 7.4 Hz, ArH), 7.39 (2H, t, *J* 7.6 Hz, ArH), 7.29 (1H, t, *J* 8.0 Hz, ArH), 7.25 (1H, d, *J* 8.1 Hz,

ArH), 7.22 (1H, t, *J* 7.6 Hz, ArH), 7.15 (1H, d, *J* 8.1 Hz, ArH), 7.06 (1H, t, *J* 8.0 Hz, ArH), 4.26 (2H, s, CH_2); δ_C (75.5 MHz, $CDC1_3$) 172.4, 171.2, 139.4, 138.8, 131.7, 131.3, 130.3, 129.7, 128.2, 127.2, 123.3, 121.3, 56.7.

General procedure of ferrocenyl benzodiazepine synthesis

To a solution of benzodiazepines 7-**9** (0.636 mmol) in freshly distilled DMF (10 mL) was added *t*-BuOK (0.713 g, 0.636 mmol) at 0°C. After stirring for 30 min at 0°C, ferrocenylmethyltrimethyl ammonium iodide[1] **10** (0.169 g, 0.438 mmol) in DMF (5 mL) was added to the mixture at 20°C. The solution was heated at 150°C for 4h under N_2. 1M HCl solution was then added until neutralization and the mixture was extracted by CH_2Cl_2 (3×15 mL). The organic layers were dried over $MgSO_4$ and evaporated. The residue was purified using column chromatography (eluent: ethyl acetate/petroleum ether: 5/5).

7-Chloro-1,3-dihydro-1-ferrocenylmethyl-5-phenyl-2H-1,4-benzodiazepin-2-one (11)

Yield= 68%. Yellow solid. M.p. 184°C. δ_H (300 MHz, $CDC1_3$) 7.37 (3H, m, ArH), 7.27 (4H, m, ArH), 7.06 (1H, m, ArH), 5.12 (1H, d, *J* 14.0 Hz, CH_2Fc), 4.70 (1H, d, *J* 10.3 Hz, CH_2), 4.41 (1H, d, *J* 14.0 Hz, CH_2Fc), 4.09 (5H, s, Cp'), 3.97 (4H, m, Cp), 3.64 (1H, d, *J* 10.3 Hz, CH_2); δ_C (75.5 MHz, $CDC1_3$) 169.1, 168.2, 140.7, 138.2, 132.0, 131.0, 130.5, 129.7, 129.5, 129.4, 128.2, 124.4, 69.4, 69.2, 68.6, 67.9, 56.9, 46.2. rt_{LCMS} 3.58 MS 468.9 (M^+). Found: C, 66.35; H, 4.57, N, 5.96. $C_{26}H_{21}ClFeN_2O$ requires C, 66.62; H, 4.52; N, 5.98.

7-Chloro-1,3-dihydro-1-ferrocenylmethyl-5-(-2-fluorophenyl)-2H-1,4-benzodiazepin-2-one (12)

Yield= 78%. Yellow solid. M.p. 110°C. δ_H (300 MHz, $CDC1_3$) 7.37–7.30 (3H, m, ArH), 7.29 (1H, td, *J* 9.1 and 3.0 Hz, ArH), 7.10 (1H, td, *J* 7.1 and 3.0 Hz, ArH), 6.99 (1H, s, ArH), 6.97–6.95 (1H, m, ArH), 5.05 (1H, d, *J* 14.2 Hz, CH_2Fc), 4.78 (1H, d, *J* 10.5 Hz, CH_2), 4.61 (1H, d, *J* 14.1 Hz, CH_2Fc), 4.07–3.96 (3H, m, Cp), 4.05 (5H, s, Cp'), 3.98 (2H, m, Cp), 3.67 (1H, d, *J* 10.5 Hz, CH_2); δ_C (75.5 MHz, $CDC1_3$) 168.1, 165.8, 140.1, 132.2, 1322, 131.5, 131.1, 130.0, 128.4, 124.3, 124.1, 117.1, 116.4, 116.1, 69.7, 69.1, 68.7, 68.1, 57.0, 46.6. rt_{LCMS} 3.51 MS 486.9 (M^+). Found: C, 63.99; H, 4.13; N, 5.65. $C_{26}H_{20}ClFFeN_2O$ requires C, 64.16; H, 4.14; N, 5.76.

1,3-Dihydro-1-ferrocenylmethyl-5-phenyl-2H-1,4-benzodiazepin-2-one (13)

Yield= 84%. Yellow solid. M.p. 215°C. δ_H (300 MHz, $CDC1_3$) 7.49 (2H, m, ArH), 7.42–7.31 (5H, m, ArH); 7.28 (2H, m, ArH), 5.20 (1H, d, *J* 15.0 Hz, CH_2Fc), 4.70 (1H, d, *J* 10.1 Hz, CH_2), 4.53 (1H, d, *J* 15.0 Hz, CH_2Fc), 4.16 (5H, s, Cp'), 3.90 (4H, m, Cp), 3.67 (1H, d, *J* 10.2 Hz, CH_2); δ_C (75.5 MHz, $CDC1_3$) 172.2, 171.2, 139.4, 138.8, 131.7, 131.3, 130.3, 129.7, 128.7, 127.2, 123.3, 121.2, 69.7, 69.1, 68.7, 68.0, 60.7, 55.7. rt_{LCMS} 3.31 MS 434.9 (M^+). Found: C, 71.12; H, 5.23; N, 6.33. $C_{26}H_{22}FeN_2O$ requires C, 71.40; H, 5.11; N, 6.45.

In vitro *P. falciparum* culture and drug assays.

P. falciparum strains were maintained continuously in culture on human erythrocytes.[2] *In vitro* antiplasmodial activity[3] was determined by following [3H]hypoxanthine incorporation by the parasite.[3] *P. falciparum* CQ-sensitive (F32/Tanzania) and CQ-resistant (FcB1/Colombia and K1/Thailand) strains were used in sensitivity testing. Stock solutions of chloroquine diphosphate, artemisinine and test compounds were prepared in sterile distilled water and DMSO, respectively. Drug solutions were serially diluted with culture medium and introduced to asynchronous parasite cultures (1%parasitemia and 1% final hematocrit) in 96-well plates for 24 h at 37°C prior to the addition of [3H]hypoxanthine (0.5 μCi per well, 1–5 Ci $mmol^{-1}$) for 24 h. The growth inhibition for each drug concentration was determined by comparison of the radioactivity incorporated into the treated culture with that in the control culture (without drug) maintained on the

same plate. Concentrations causing 50% inhibition (IC_{50}) or 90% inhibition (IC_{90}) were obtained from the drug concentration–response curve, and the results are expressed as the mean determined from several experiments.

Clinical isolates.

Fresh clinical isolates were collected at May 2010, from patients with *P. falciparum* infection in "Hôpital de l'Amitié Sino Gabonais" of Franceville, South-East of Gabon. This study was approved by the Human Ethics Committee of the Centre International de Recherches Médicales de Franceville. Malaria diagnosis was based on blood smear according to Lambaréné method.[4] After obtaining informed consent from patients, venous blood samples were collected. Samples contained initial parasitaemia between 0.005 to 0.5% were included in the study. Three (3) mL of blood were washed three times with RPMI 1640 medium. Pellet was suspended at 1.5% of hematocrit, in RPMI 1640 medium supplemented in presence of different concentrations of each drug in wells of 96-well flat bottom plates (NUNC, VW International, Strasbourg, France). Then, plates were incubated at 37°C during 44h, as described previously.[5] After, this time, it was frozen at -20 °C for night to stop parasite growth. Isolates were also tested for their susceptibility to chloroquine.

Antiplasmodial activity was analyzed according to pLDH measurement by ELISA as previously described.[6] Briefly, the ELISA plates were coated with a monoclonal antibody (MAb) against pan-*Plasmodium* LDH. Lysing buffer (100 µl) and, subsequently, 1 to 200 µl of hemolyzed culture were transferred into 96-well plates, pre-coated with a capture monoclonal antibody 17E4. Plates were incubated for 1 h at 37°C with shaking and then washed 5 times. After the addition of 100 µl per well of a biotinylated MAb 19G7, the plates were incubated for 30 min at 37°C and then washed 5 times. A third incubation for 15 min at 37°C with 100 µl of a streptavidinhorseradish peroxidase solution was followed by a last washing step. Enzyme activity was revealed by incubation for 15 min at 37°C with 100 µl of tetramethylbenzidine, the reaction was stopped with sulfuric acid, and the absorbance was read with a microplate spectrophotometer (LP400; Bio-Rad, France) at 450 nm with a reference wavelength of 620 nm. The IC_{50} were calculated from mean of three experiments.

Cytotoxicity test

The cytotoxicity of the benzodiazepines was assessed with MRC-5 human diploid embryonic lung cells, using a tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma®) colorimetric method based on reagent cleavage by mitochondrial dehydrogenase in viable cells. Briefly, 5000 cells per well were seeded in 96-well microplates in culture medium (DMEM+ 10% inactivated SVF+2mM L-glutamine + penicillin/streptomycin/neomycin (0.5/0.5/1µg/ml)). After 24 h, the cells were washed and incubated with different concentrations of each benzodiazepine for 7 days at 37°C in 5% CO_2-air. Cytotoxicity was scored as the percentage reduction in absorbance at 540 nm versus untreated control cultures. CC_{50} values were obtained from the drug concentration–response curve. The results were expressed as the mean ± the standard deviations determined from several independent experiments.

References

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