Supporting Information

*In Vitro and In Vivo Activity of Benzo[c]phenanthridines against* *Leishmania amazonensis*

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Detailed procedure for the isolation of avicine (1), nitidine (2), fagaridine (4), and chelerythrine (5) from *Z. rhoifolium* root bark

NMR spectra were recorded on Bruker Avance 300 spectrometer. Mass spectra were recorded on a Thermo Electron LCQ DECA XP MAX.

500 g of powdered *Z. rhoifolium* root bark were wetted with 500 mL of an aqueous solution of ammonium hydroxide (14%). 3 L of dichloromethane were added, and the mixture was left 24 h in the dark at ambient temperature.

The plant residue was filtered off and the solution was extracted four times with 700 mL of 0.1 M aqueous HCl. For the first extraction, the pH was kept acidic with the addition of concentrated hydrochloric acid. Then the aqueous acidic layers were pooled together and basified with 100 mL of aqueous solution of ammonium hydroxide (28%). This solution was extracted three times with 1 L of dichloromethane. The organic phases were pooled together, dried over anhydrous magnesium sulfate, and filtered. The solvent was removed at 40°C under reduced pressure, and 2.1 g of alkaloid extract were obtained.

The plant residue was submitted to a new extraction with 1.5 L of dichloromethane, which, after the treatment described above, gave 0.46 g of alkaloid extract.

As the TLC profiles of the two extracts were similar, they were pooled together to give 2.56 g of alkaloid extract.

2.56 g of this alkaloid extract were submitted to flash column chromatography. The diameter of the column was 4 cm. The column was filled with 80 g of silica gel and eluted with 300 mL of CH$_2$Cl$_2$, 300 mL of CH$_2$Cl$_2$ with 1% MeOH, 600 mL of CH$_2$Cl$_2$ with 5% MeOH, 300 mL of CH$_2$Cl$_2$ with 8% MeOH, 500 mL of CH$_2$Cl$_2$ with 10% MeOH, and 1000 mL of CH$_2$Cl$_2$ with 20% MeOH. The volume of fractions (f$_1$ to f$_{111}$) was 28 mL.

These fractions were pooled according to their TLC profiles and seven fractions were obtained: F1 (68 mg, f$_7$ to f$_9$), F2 (195 mg, f$_{15}$ to f$_{23}$), F3 (453 mg, f$_{31}$ to f$_{33}$), F4 (376 mg, f$_{34}$ to f$_{39}$), F5 (400 mg f$_{41}$ to f$_{59}$), F6 (249 mg f$_{61}$ to f$_{79}$), and F7 (216 mg f$_{80}$ to f$_{111}$).
**Isolation of avicine (1):**

F5 was dissolved in 20 mL of CHCl₃ with 20% MeOH and cooled to 0°C. HCl 1M was added drop wise. The precipitate was filtered off and dried to give 310 mg of a yellow powder, which was recrystallized in boiling water and dried to give 180 mg of avicine chloride.

Avicine chloride: C₂₀H₁₄NO₄Cl, yellow solid, ¹H-NMR (300 MHz, DMSO-ᴅ₆) 4.81 (3H, s), 6.28 (2H, s), 6.42 (2H, s), 7.69 (1H, s), 7.80 (1H, s), 8.18 (1H, d, 8.9 Hz), 8.24 (1H, s), 8.53 (1H, s), 8.67 (1H, d, 8.9 Hz), 9.80 (1H, s). ESI-MS: m/z = 332 [M]+.

**Isolation of nitidine (2):**

F7 was dissolved in 20 mL of CHCl₃ with 20% MeOH and cooled to 0°C. HCl 1M was added drop wise. The precipitate was filtered off and dried to give 170 mg of a yellow powder, which was recrystallized in boiling water and dried to give 120 mg of nitidine chloride.

Nitidine chloride: C₂₁H₁₈NO₄Cl, yellow solid, ¹H-NMR (300 MHz, DMSO-ᴅ₆) 3.97 (3H, s), 4.16 (3H, s), 4.83 (3H, s), 6.28 (2H, s), 7.71 (1H, s), 7.83 (1H, s), 8.21 (1H, d, 9.0 Hz), 8.24 (1H, s), 8.30 (1H, s), 8.84 (1H, d, 9.0 Hz), 9.81 (1H, s). ESI-MS: m/z = 348 [M]+.

**Isolation of fagaridine (4):**

F4 was subjected to flash column chromatography. The diameter of the column was 2 cm. The column was filled with 16 g of silica gel, eluted with 100 mL of CH₂Cl₂ with 1% MeOH, 100 mL of CH₂Cl₂ with 2% MeOH, and 100 mL of CH₂Cl₂ with 3% MeOH.

The fraction size was 10 mL. Fractions were pooled according to their TLC profiles and a fraction of 118 mg was obtained. It was dissolved in 8 mL of CHCl₃ with 20% MeOH, and cooled to 0°C. HCl 1M was added drop wise. The precipitate was filtered off and dried to give 102 mg of an orange powder, which was recrystallized in boiling water and dried to give 72 mg of fagaridine chloride.

Fagaridine chloride: C₂₀H₁₆NO₄Cl, orange solid, ¹H-NMR (300 MHz, CDCl₃-CD₃OD 50-50) 4.31 (3H, s), 5.04 (3H, s), 6.23 (2H, s), 7.56 (1H, s), 7.98 (1H, d, 8.9 Hz), 8.16 (1H, s), 8.22 (1H, d, 8.9 Hz), 8.56 (1H, d, 9.1 Hz), 8.62 (1H, d, 9.1 Hz), 9.92 (1H, s). ESI-MS: m/z = 334 [M]+.
Isolation of chelerythrine (5):
F3 was subjected to flash column chromatography. The diameter of the column was 2 cm. The
column was filled with 16 g of silica gel, eluted with 100 mL of ethyl acetate-cyclohexane
50/50, 100 mL of ethyl acetate-cyclohexane 80/20, 100 mL of ethyl acetate, 100 mL of ethyl
acetate with 0.5% MeOH, 100 mL of ethyl acetate with 1% MeOH, and 100 mL of ethyl
acetate with 2% MeOH. The fraction size was 10 mL. Fractions were pooled according to their
TLC profiles and 2 fractions were obtained: F3-1 (140 mg, f_{3-12}) and F3-2 (15 mg, f_{38-41}), both
containing chelerythrine. To F3-1, ethyl acetate was added. A precipitate was discarded and the
solvent was removed under reduced pressure from the supernatant to give 117 mg of an oil,
which was pooled with F3-2.
This mixture was subjected to flash column chromatography. The diameter of the column was 1
cm. The column was filled with 8 g of silica gel, eluted with 100 mL CH$_2$Cl$_2$, 100 mL of CH$_2$Cl$_2$
with 0.25% MeOH, 100 mL of CH$_2$Cl$_2$ with 0.5% MeOH, 100 mL of CH$_2$Cl$_2$ with 1% MeOH,
and 100 mL of CH$_2$Cl$_2$ with 2% MeOH. The fraction size was 8 mL. Fractions 27 to 51 were
pooled to give 57 mg of a brown solid. This solid was dissolved in 4 mL of CHCl$_3$ and cooled to
0°C. HCl 1M was added drop wise. The precipitate was filtered off and dried to give 35 mg of a
yellow powder, which was recrystallized in boiling water and dried to give 25 mg of
chelerythrine chloride.
Chelerythrine chloride: C$_{21}$H$_{18}$NO$_4$Cl, yellow solid, $^1$H-NMR (300 MHz, DMSO-$d_6$) 4.04 (3H, s),
4.09 (3H, s), 4.91 (3H, s), 6.27 (2H, s), 7.71 (1H, s), 8.21 (1H, d, 9.0 Hz), 8.23 (1H, s), 8.24
(1H, d, 9.0 Hz), 8.77 (1H, d, 9.0 Hz), 8.77 (1H, d, 9.0 Hz), 10.03 (1H, s). ESI-MS: m/z = 348
[M]$^+$. 
Spectra of isolated or synthesized compounds

$^1$H avicine (1) DMSO-$d_6$. 
$^1$H nitidine (2) DMSO-$d_6$. 

$^1$H fagaronine (3) CDCl$_3$-CD$_3$OD 50-50.
$^1$H fagaridine (4) CDCl$_3$-CD$_3$OD 50-50.
$^1$H chelerythrine (5) DMSO-$d_6$. 
$^1$H normitidine (6) DMSO-$d_6$. 

\(^1H\) norfagaronine (7) DMSO-\(d_6\).