**SUPPLEMENTARY MATERIAL**

Simple dialkyl pyrazole-3,5-dicarboxylates show *in vitro* and *in vivo* activity against disease-causing trypanosomatids

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CHEMISTRY

*General methods*

Melting points (Mps) were determined in a Mettler Toledo Scientific MP70 apparatus. 1H (300 MHz) and 13C (75 MHz) NMR spectra were recorded at room temperature (~20 ºC) on a Varian Unity 300 spectrometer. Chemical shifts are reported in ppm from TMS (δ scale) but were measured against the solvent signal. Owing to the pyrazole ring tautomerism, very broad signals are observed for C-3 and -5, and for 3- and 5-CO of diesters. FAB mass spectra were obtained on a VG AutoSpec spectrometer using a *m*-nitrobenzyl alcohol (NBA) matrix. DC-Alufolien silica gel 60 PF254 (Merck, layer thickness 0.2 mm) was used for analytical thin layer chromatography (TLC). Microanalyses were performed on a Heraeus CHN-O-RAPID analyzer and were within ± 0.3% of the theoretical values.

*Preparation of dialkyl 1*H*-pyrazole-3,5-dicarboxylates* 2–6

These diesterswere prepared following a procedure closely related to those previously reported for dimethyl (*2*) (Askew et al., 1997) and diethyl (*3*) (Schenck et al., 1985) pyrazole-3,5-dicarboxylates (Fig. 1). A solution of the commercially available 1*H*-pyrazole-3,5-dicarboxylic acid monohydrate (*1*) (5.00 g, 28.7 mmol) in 100 mL of the appropriate alcohol was saturated at rt with gaseous hydrogen chloride. The reaction mixture was stirred at rt for 24 h; then, the solvent was evaporated to dryness and a 10% aqueous solution of NaHCO3 was added until pH was basic. The mixture was extracted with chloroform and the organic phase was dried with MgSO4. Removal of the solvent led to the desired products as chromatographically pure oils (TLC) which solidified on standing.

*Dipropyl 1*H*-pyrazole-3,5-dicarboxylate* (5). Yield: 6.35 g (92%). Mp 53−55 oC. 1H NMR [(CD3)2SO]: δ 14.64 (s, 1H, NH), 7.18 (s, 1H, 4-H), 4.21 (t, *J* = 6.6 Hz, 4H, Pr 1-CH2), 1.69 (m, 4H, Pr 2-CH2), 0.94 (t, *J* = 7.3 Hz, 6H, Pr CH3); 13C NMR [(CD3)2SO]: δ 161.2, 158.6 (3-, 5-CO), 143.8, 134.7 (C-3, -5), 110.8 (C-4), 66.2 (Pr 1-CH2), 21.5 (Pr 2-CH2), 10.2 (Pr CH3); FAB-MS: *m/z* 241 (MH+). Anal. calcd. for C11H16N2O4: C 54.99; H 6.71; N 11.66. Found: C 54.91; H 6.65; N 11.71.

*Diisopropyl 1*H*-pyrazole-3,5-dicarboxylate* (7). Yield: 6.21 g (90%). Mp 50−52 oC. 1H NMR [(CD3)2SO]: δ 14.58 (s, 1H, NH), 7.13 (s, 1H, 4-H), 5.11 (hept, *J* = 7.0 Hz, 2H, iPr CH), 1.28 (d, *J* = 7.0 Hz, 12H, iPr CH3); 13C NMR [(CD3)2SO] δ 160.8, 158.3 (3-, 5-CO), 144.2, 135.2 (C-3, -5), 110.7 (C-4), 68.7 (iPr CH), 21.6 (iPr CH3); FAB-MS: *m/z* 241 (MH+). Anal. calcd. for C11H16N2O4: C 54.99; H 6.71; N 11.66. Found: C 55.07; H 7.00; N 11.62.

*Dibutyl 1*H*-pyrazole-3,5-dicarboxylate* (8). Yield: 6.78 g (88%). Mp 46−47 oC. 1H NMR [(CD3)2SO]: δ 14.66 (s, 1H, NH), 7.17 (s, 1H, 4-H), 4.25 (t, *J* = 6.5 Hz, 4H, Bu 1-CH2), 1.66 (m, 4H, Bu 2-CH2), 1.39 (m, 4H, Bu 3-CH2), 0.91 (t, *J* = 7.4 Hz, 6H, Bu CH3); 13C NMR [(CD3)2SO]: δ 159.7 (3-, 5-CO), 143.4, 134.9 (C-3, -5), 110.7 (C-4), 64.5 (Bu 1-CH2), 30.1 (Bu 2-CH2), 18.6 (Bu 3-CH2), 13.6 (Bu CH3); FAB-MS: *m/z*: 269 (MH+). Anal. calcd. for C13H20N2O4: C 58.19; H 7.51; N 10.44. Found: C 58.17; H 7.42; N 10.40.

*Preparation of sodium 3,5-bis(alkoxycarbonyl)pyrazolates* 7–9

These salts were prepared following the procedure reported for the corresponding bis(ethoxycarbonyl) derivative *8* (Reviriego et al., 2006) (Fig. 1). To a solution of the corresponding dialkyl 1*H*-pyrazole-3,5-dicarboxylate (5.0 mmol) in 30 mL of the corresponding alcohol, an equimolar amount of sodium hydroxide (0.20 g, 5.0 mmol) dissolved in 30 mL of the same alcohol was slowly added. The reaction mixture was stirred at rt overnight, then the solution was concentrated and the obtained solid collected by filtration and dried *in vacuo*.

*Sodium 3,5-bis(methoxycarbonyl)pyrazolate* (7). Yield: 0.98 g (95%). Mp > 215 oC. 1H NMR [(CD3)2SO]: δ 6.97 (s, 1H, 4-H), 3.68 (s, 6H, CH3); 13C NMR [(CD3)2SO]: δ 163.3 (CO), 142.5 (C-3, -5), 111.0 (C-4), 50.3 (CH3); FAB-MS: *m/z* 207 (MH+). Anal. calcd. for C7H7N2O4Na: C 40.79; H 3.42; N 13.59. Found: C 40.52; H 3.55; N 13.42.

*Sodium 3,5-bis(propoxycarbonyl)pyrazolate* (9). Yield: 1.16 g (97%). Mp > 300 oC. 1H NMR [(CD3)2SO]: δ 6.93 (s, 1H, 4-H), 4.05 (t, *J* = 7.3 Hz, 4H, Pr 1-CH2), 1.64 (m, 4H, Pr 2-CH2), 0.92 (t, *J* = 7.3 Hz, 6H, Pr CH3);13C NMR [(CD3)2SO]: δ 163.9 (CO), 142.5 (C-3, -5), 110.9 (C-4), 63.9 (Pr 1-CH2), 21.7 (Pr 2-CH2), 10.3 (Pr CH3); FAB-MS: *m/z* 263 (MH+). Anal. calcd. for C11H15N2O4Na: C 50.38; H 5.77; N 10.68. Found: C 50.60; H 5.68; N 10.48.

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