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Supporting Information

Novel Lipophilic Acetohydroxamic Acid Derivatives Based on Conformationally Constrained Spiro Carbocyclic 2,6-Diketopiperazine Scaffolds with Potent Trypanocidal Activity

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Experimental

Chemistry

General. Melting points were determined using a Büchi capillary apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were obtained on either a Bruker MSL 400 (400 MHz ^1H ; 100 MHz ^{13}C) or Bruker 600 (600 MHz ^1H) spectrometer, using CDCl_3 or $\text{DMSO-}d_6$ as solvent. Chemical shifts are reported in δ (ppm) with the tetramethylsilane or solvent ($\text{DMSO-}d_6$) as internal standard. Splitting patterns are designated as s, singlet; d, doublet; dd, doublet of doublets; t, triplet; td, triplet of doublets; q, quartet; m, multiplet; br, broad; v br, very broad; sym, symmetrical. Coupling constants (J) are expressed in units of hertz (Hz). The spectra were recorded at 293 K (20 °C) unless otherwise specified. Carbon multiplicities were established by DEPT experiments. The 2D NMR experiments (HMQC and COSY) were performed for the elucidation of the structures of the newly synthesized compounds. Low resolution mass spectra (MS) were measured either in chemical ionization (CI) in positive mode using methane as CI reagent gas or in electron impact (EI) on a Thermo Electron Corporation DSQ mass spectrometer. High resolution mass spectra (HRMS) were performed on a hybrid LTQ-Orbitrap Discovery spectrometer under electrospray ionization (ESI) in positive mode. Optical rotations were measured on a Perkin Elmer 341 polarimeter at the sodium D line (589). Analytical thin-layer chromatography (TLC) was conducted on precoated Merck silica gel 60 F_{254} plates (layer thickness 0.2 mm) with the spots visualized by iodine vapors and/or UV light. Column chromatography purification was carried out on silica gel 60 (70-230 mesh). Elemental analyses (C, H, N) were performed by the Service Central de Microanalyse at CNRS (France), and were within $\pm 0.4\%$ of the theoretical values except where noted (compounds **10b.HCl** and **38.HCl**). Elemental analysis results for the tested compounds correspond to $>95\%$ purity. The commercial reagents were purchased from Alfa Aesar, Sigma-Aldrich, and Merck, and were used without further purification except for the benzyl bromoacetate. This reagent was purified by fractional distillation in vacuo prior to use. Organic solvents used were in the highest purity, and when necessary, were dried by the standard methods. Solvent abbreviations: THF, tetrahydrofuran; DMF, dimethylformamide; Et_2O , ethyl ether; MeOH, methanol; EtOH, ethanol; AcOEt, ethyl acetate; DMSO, dimethylsulfoxide.

The ^1H and ^{13}C NMR spectra for all hydroxamate derivatives described in this report (compounds **7a-e**, **8**, **9a-d**, **10a**, **10b**, **32-36**, **42**, **60-63**, **72**, **73**, and **45**) and hydrazide **44**, are consistent with a *Z/E* conformational behavior of these molecules in solution. The detected double set of characteristic peaks in ^1H and ^{13}C NMR spectra confirm the occurrence of the two carbonyl conformers. The assignment of the *Z* or *E* conformers was based on literature data concerning *Z/E* conformational isomerism studies in simple hydroxamate and hydrazide structures,²⁴ and 2D NMR experiments.

(24) (a) Brown, D. A.; Glass, W. K.; Mageswaran, R.; Mohammed, S. A. ^1H and ^{13}C NMR studies of isomerism in hydroxamic acids. *Magn. Reson. Chem.* **1991**, 29, 40-45, and references therein. (b) Bouchet, P.; Jacquier, R.; Pereillo, J. M.; Elguero, J. NMR study of configuration of hydrazides. *Bull. Soc. Chim. Fr.* **1972**, 6, 2264-2271.

***N*-Hydroxy-3,5-dioxospiro[piperazine-2,2'-tricyclo[3.3.1.1^{3,7}]decane]-4-acetamide (7a)**

Following the general hydrogenolysis procedure described in the main manuscript, *O*-benzyl hydroxamate **32** gave the title compound **7a** as a white crystalline solid (96%): mp 193-195 °C (dec) ($\text{EtOH-Et}_2\text{O}$); ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 1.43 (d, 2H, $J=12.0$ Hz, 4'e, 9'e-H), 1.55-1.68 (m, 4H, 6', 8'e, 10'e-H), 1.75 (s, 1H, 7'-H), 1.79 (s, 1H, 5'-H), 1.94 (s, 2H, 1', 3'-H), 2.25 (t, 4H, $J=10.6$ Hz, 4'a, 8'a, 9'a, 10'a-H), 3.13 (t, 1H, $J=7.8, 8.4$ Hz, 1-H), 3.56 (d, 2H, $J=8.4$ Hz, 6-H),

4.14 (s, 1.5H, CH₂CONHOH, Z-isomer), 4.44 (s, 0.4H, CH₂CONHOH, E-isomer), 8.87 (s, 0.7H, CONHOH₂ Z- isomer), 9.28 (s, 0.2H, CONHOH₂ E- isomer), 10.15 (s, 0.2H, CONHOH, E-isomer), 10.55 (s, 0.7H, CONHOH, Z- isomer); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 26.7 (5'-C), 26.9 (7'-C), 31.9 (1', 3'-C), 32.0 (4', 9'-C), 32.8 (8', 10'-C), 37.8 (6'-C), 39.2 (CH₂CONHOH, Z-isomer), 39.6 (CH₂CONHOH, E-isomer), 44.0 (6-C), 59.5 (2,2'-C), 164.2 (CONHOH, Z-isomer), 169.6 (CONHOH, E-isomer), 172.3, 174.6 (3, 5-C). Anal. (C₁₅H₂₁N₃O₄) C, H, N. The hydrochloride salt (**7a.HCl**) was prepared by treating an ethanolic solution of **7a** with ethereal HCl under ice cooling, and was fully precipitated by adding ether. The white solid was collected by filtration, triturated with ether, and dried in vacuo. Mp 219-222 °C (dec); Anal. (C₁₅H₂₂ClN₃O₄) C, H, N.

***N*-Hydroxy-1-methyl-3,5-dioxospiro[piperazine-2,2'-tricyclo[3.3.1.1^{3,7}]decane]-4-acetamide (7b)**

Following the general hydrogenolysis procedure described in the main manuscript, *O*-benzyl hydroxamate **42** gave the title compound **7b** as a white crystalline solid (80%): mp 190-192 °C (EtOH-Et₂O); ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.42 (d, 1H, *J*=10.4 Hz, 4'e-H), 1.49 (d, 1H, *J*=10.6 Hz, 9'e-H), 1.55-1.74 (m, 5H, 6', 8', 10'e-H), 1.78 (s, 2H, 5', 7'-H), 2.02-2.20 (m, 4H, 1', 3', 4'a, 9'a-H), 2.31, 2.33 (s+s, 3H, CH₃), 2.68 (d, 1H, *J*=12.0 Hz, 10'a-H), 3.44-3.97 (q, 2H, AB, *J*_{AB}=19.1 Hz, 6-H), 4.07-4.25 (q, 1.5H, AB, *J*_{AB}=15.5 Hz, CH₂CONHOH, Z-isomer), 4.39-4.53 (q, 0.5H, AB, *J*_{AB}=16.9 Hz, CH₂CONHOH, E-isomer), 8.87 (s, 0.7H, CONHOH, Z-isomer), 9.30 (s, 0.2H, CONHOH₂ E-isomer), 10.17 (s, 0.2H, CONHOH₂ E-isomer), 10.63 (s, 0.7H, CONHOH₂ Z-isomer); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 25.9 (5'-C), 26.7 (7'-C), 30.0 (1'-C), 31.6 (3'-C), 31.7 (4'-C), 31.8 (9'-C), 32.7 (8'-C), 33.4 (10'-C), 36.7 (CH₃), 37.3 (6'-C), 38.9 (CH₂CONHOH, Z-isomer), 39.7 (CH₂CONHOH, E-isomer), 52.6 (6-C), 64.8 (2,2'-C), 164.1 (CONHOH, Z-isomer), 169.5 (CONHOH, E-isomer), 170.7, 173.8 (3, 5-C); Anal. (C₁₆H₂₃N₃O₄) C, H, N. The hydrochloride salt (**7b.HCl**) was prepared as described for **7a.HCl**. Mp 215-218 °C (dec). Anal. (C₁₆H₂₄ClN₃O₄) C, H, N.

***(S)*-N-Hydroxy-6-methyl-3,5-dioxospiro[piperazine-2,2'-tricyclo[3.3.1.1^{3,7}]decane]-4-acetamide (7c)**

Following the general hydrogenolysis procedure described in the main manuscript, *O*-benzyl hydroxamate **33** provided the title compound **7c** as a white foamy solid, which strongly binds the eluting solvent (AcOEt). Removal of the entrapped solvent upon drying at 62-64 °C under vacuum (10⁻³ mmHg) in an Abderhalden apparatus gave **7c** as a light yellow crystalline solid (91%): mp 88-91 °C; ¹H NMR (600 MHz, DMSO-*d*₆) δ 1.25 (d, 3H, *J*=13.8 Hz, CH₃), 1.38 (d, 1H, *J*=12.1 Hz, 9'e-H), 1.47 (d, 1H, *J*=11.7 Hz, 4'e-H), 1.55-1.72 (m, 5H, 6', 8', 10'e-H), 1.76 (s, 1H, 7'-H), 1.79 (s, 1H, 5'-H), 1.85 (s, 1H, 3'-H), 2.08 (s, 1H, 1'-H), 2.16 (d, 1H, *J*=11.8 Hz, 4'a-H), 2.44 (d, 1H, *J*=12.0 Hz, 9'a-H), 2.84 (d, 2H, *J*=11.2 Hz, 1, 10'a -H), 3.55-3.66 (sym m, 1H, 6-H), 4.09-4.16 (q, 1.5H, AB, *J*_{AB}=15.4 Hz, CH₂CONHOH, Z-isomer), 4.38-4.48 (q, 0.5H, AB, *J*_{AB}=16.4 Hz, CH₂CONHOH, E-isomer), 8.82 (s, 0.6H, CONHOH₂ Z-isomer), 9.24 (s, 0.2H, CONHOH₂ E-isomer), 10.10 (s, 0.2H, CONHOH, E-isomer), 10.50 (s, 0.5H, CONHOH₂ Z-isomer); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 17.9, 18.0 (CH₃), 26.6 (5'-C), 26.8 (7'-C), 30.1, 30.3 (1'-C), 31.1 (4'-C), 32.1 (8'-C), 32.6 (9'-C), 33.4 (10'-C), 33.7, 33.8 (3'-C), 37.7 (6'-C), 39.4 (CH₂CONHOH, Z-isomer), 39.8 (CH₂CONHOH, E-isomer), 48.6 (6-C), 60.0 (2,2'-C), 164.1 (CONHOH, Z-isomer), 169.5 (CONHOH, E-isomer), 174.6, 174.8 (3, 5-C); [α]_D²³₅₈₉ -20 (c 0.2, CHCl₃); CI⁺ MS: *m/z*: 322.2 ([M+H]⁺, 7), 306.2 ([M-CH₃]⁺, 37), 278.2 (100); HRMS (ESI): [M+H]⁺ calcd for C₁₆H₂₄N₃O₄, 322.1761, found 322.1739. The hydrochloride salt (**7c.HCl**) was prepared by treating an ether solution of **7c** with ethereal HCl under ice cooling. The white precipitate was collected by

filtration, triturated with ether, and dried in vacuo. Mp 178-180 °C (dec). Anal. (C₁₆H₂₄ClN₃O₄) C, H, N.

(S)-N-Hydroxy-3,5-dioxo-6-(phenylmethyl)spiro[piperazine-2,2'-tricyclo[3.3.1.1^{3,7}]decane]-4-acetamide (7d)

Following the general hydrogenolysis procedure described in the main manuscript, *O*-benzyl hydroxamate **34** provided the title compound **7d** as a white foamy solid, which strongly binds the eluting solvent (AcOEt). Removal of the entrapped solvent as described for **7c** gave **7d** as a pale yellow crystalline solid (95%): mp 97 – 100 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.29 (d, 2H, *J*=11.5 Hz, 4'e, 9'e-H), 1.41-1.73 (complex m, 7H, 4'a, 6', 7', 8', 10'e-H), 1.76 (s, 1H, 5'-H), 1.82 (s, 1H, 3'-H), 2.05 (s, 1H, 1'-H), 2.17 (d, 1H, *J*=11.4 Hz, 9'a-H), 2.66-2.90 (complex m, 3H, CH_AH_MPh, 1, 10'a-H), 3.32 (dd, 1H, AMX, M region, *J*_{AM}=13.8 Hz, *J*_{MX}= 2.8 Hz, CH_AH_MPh), 3.73 (td, 1H, *J*=3.3 Hz, 10.7 Hz, 6-H_x), 4.17 (s, 1.6H, CH₂CONHOH, *Z*-isomer), 4.42-4.52 (q, AB, 0,4 H, *J*_{AB}=16.8 Hz, CH₂CONHOH, *E*- isomer), 7.16-7.23 (m, 1H, 4-aromatic H), 7.24-7.36 (m, 4H, 2, 3, 5, 6-aromatic H), 8.88 (s, 0,7H, CONHOH, *Z*- isomer), 9.30 (s, 0,2H, CONHOH, *E*-isomer), 10.16 (s, 0,2H, CONHOH, *E*- isomer), 10.56 (s, 0,7H, CONHOH, *Z*- isomer); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 26.5 (5'-C), 26.7 (7'-C), 29.9, 30.0 (1'-C), 30.8 (4'-C), 32.0 (9'-C), 32.7 (8'-C), 33.3 (10'-C), 34.0, 34.1 (3'-C), 37.3 (CH₂Ph), 37.7 (6'-C), 39.6 (CH₂CONHOH, *Z*-isomer), 39.9 (CH₂CONHOH, *E*-isomer), 54.26, 54.33 (6-C), 59.85, 59.93 (2,2'-C), 126.2 (4-aromatic C), 128.0, 129.0 (2, 3, 5, 6- aromatic C), 138.6 (1- aromatic C), 164.1 (CONHOH, *Z*-isomer), 169.5 (CONHOH, *E*-isomer), 173.7, 174.5 (3, 5-C); [α]_D²⁵ -60 (c 0,2, CHCl₃); CI⁺ MS: *m/z* 398.2 ([*M*+H]⁺, 100), 306.1 ([*M*- CH₂Ph]⁺, 57); HRMS (ESI): [*M*+H]⁺ calcd. for C₂₂H₂₈N₃O₄, 398.2074, found 398.2059. The hydrochloride salt (**7d.HCl**) was prepared as described for **7c.HCl**. Mp 143-146 °C (dec); Anal. (C₂₂H₂₈ClN₃O₄) C, H, N.

(R)-N-Hydroxy-3,5-dioxo-6-(phenylmethyl)spiro[piperazine-2,2'-tricyclo[3.3.1.1^{3,7}]decane]-4-acetamide (7e)

Following the general hydrogenolysis procedure described in the main manuscript, *O*-benzyl hydroxamate **35** provided the title compound **7e** as a white foamy solid, which strongly binds the eluting solvent (AcOEt). Removal of the entrapped solvent as described for **7c** gave **7e** as a light yellow crystalline solid (95%): mp 96–98 °C; NMR spectroscopic data of this compound are identical to that of **7d**; [α]_D²⁵ +59 (c 0.2, CHCl₃); EI MS: *m/z* 397.2 ([*M*]⁺, 7), 307,2 ([*M*+H-CH₂Ph]⁺, 17), 306 ([*M*-CH₂Ph]⁺, 100); HRMS (ESI): [*M*+H]⁺ calcd for C₂₂H₂₈N₃O₄, 398.2074, found 398.2064. The hydrochloride salt (**7e.HCl**) was prepared as described for **7c.HCl**. Mp 142-145 °C (dec). Anal. (C₂₂H₂₈ClN₃O₄) C, H, N.

(R,S)-N-Hydroxy-3,5-dioxo-6-(phenylmethyl)spiro[piperazine-2,2'-tricyclo[3.3.1.1^{3,7}]decane]-4-acetamide (8)

Following the general hydrogenolysis procedure described in the main manuscript, *O*-benzyl hydroxamate **36** provided the title compound **8** as a white foamy solid, which strongly binds the eluting solvent (AcOEt). Removal of the entrapped solvent as described for **7c** gave **8** as a light yellow crystalline solid (96%): mp 90–92 °C. NMR spectroscopic data of this compound are identical to that of **7d**; CI⁺ MS: *m/z* 398.3 ([*M*+H]⁺, 100), 306,2 ([*M*-CH₂Ph]⁺, 61); HRMS (ESI): [*M*+H]⁺ calcd for C₂₂H₂₈N₃O₄, 398.2074, found 398.2025. The hydrochloride salt (**8.HCl**) was prepared as described for **7c.HCl**. Mp 135-138 °C (dec). Anal. (C₂₂H₂₈ClN₃O₄) C, H, N.

N-Hydroxy-3,5-dioxo-1,4-diazaspiro[5.7]tridecane-4-acetamide (9a)

Following the general hydrogenolysis procedure described in the main manuscript, *O*-benzyl hydroxamate **60** provided the title compound **9a** as a white foamy solid, which strongly binds the

eluting solvent (AcOEt, MeOH). Removal of the entrapped solvents as described for **7c** gave **9a** as a pale yellow solid (90%): mp 148–151 °C; ¹H NMR (600 MHz, DMSO-*d*₆) δ 1.50 (br s, 8H, 8, 9, 10, 11, 12-H), 1.58-1.70 (m, 4H, 7, 9, 11, 13-H), 1.95-1.99 (q, 2H, *J*=6.7, 9.2 Hz, 7, 13-H), 2.98 (t, 1H, *J*=8.5 Hz, 1-H) 3.57 (d, 2H, *J*=7.9 Hz, 2-H), 4.13 (s, 1.5H, CH₂CONHOH, Z-isomer), 4.42 (s, 0.4H, CH₂CONHOH, E-isomer), 8.86 (s, 0.7H, CONHOH, Z-isomer), 9.27 (s, 0.2H, CONHOH, E-isomer), 10.15 (s, 0.2H, CONHOH, E-isomer), 10.52 (s, 0.7H, CONHOH, Z-isomer); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 20.9 (9, 11-C), 24.4 (10-C), 27.7 (8, 12-C), 30.2 (7, 13-C), 38.6 (CH₂CONHOH, Z-isomer), 38.8 (CH₂CONHOH, E-isomer), 44.7 (2-C), 58.9 (6-C), 163.9 (CONHOH, Z-isomer), 169.3 (CONHOH, E-isomer), 171.5, 176.3 (3, 5-C); CI⁺ MS: *m/z*: 284.2 ([M+H]⁺, 11), 268.2 (46), 240.2(100), 222.2 (72), 195 (70); HRMS (ESI): [M+H]⁺ calcd for C₁₃H₂₂N₃O₄, 284.1605, found 284.1586. The hydrochloride salt (**9a.HCl**) was prepared as described for **7a.HCl**. Mp 179-182 °C. Anal. (C₁₃H₂₂ClN₃O₄) C, H, N.

***N*-Hydroxy-1-methyl-3,5-dioxo-1,4-diazaspiro[5.7]tridecane-4-acetamide (9b)**

Following the general hydrogenolysis procedure described in the main manuscript, *O*-benzyl hydroxamate **72** provided the title compound **9b** as a white foamy solid, which strongly binds the eluting solvent (AcOEt). Removal of the entrapped solvent as described for **7c** gave **9b** as a white crystalline solid (90%): mp 160–163 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.33-1.63 (m, 8H, 8, 9, 10, 11, 12-H), 1.65-1.87 (m, 4H, 7, 9, 11, 13-H), 1.89-2.04 (m, 2H, 7, 13-H), 2.36 (s, 2.2H, CH₃, Z-isomer), 2.38 (s, 0.9H, CH₃, E-isomer), 3.73 (s, 2H, 2-H), 4.16 (s, 1.5H, CH₂CONHOH, Z-isomer), 4.46 (s, 0.5H, CH₂CONHOH, E-isomer), 8.88 (s, 0.8H, CONHOH, Z-isomer), 9.30 (br s, 0.3H, CONHOH, E-isomer), 10.18 (s, 0.3H, CONHOH, E-isomer), 10.63 (s, 0.7H, CONHOH, Z-isomer); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 20.4 (9, 11-C), 24.3 (10-C), 27.5 (8, 12-C), 29.3 (7, 13-C), 38.1 (CH₂CONHOH, Z-isomer), 38.5, 38.6 (CH₃), 38.9 (CH₂CONHOH, E-isomer), 53.7 (2-C), 63.7 (6-C), 164.0 (CONHOH, Z-isomer), 169.3 (CONHOH, E-isomer), 170.0, 175.5 (3, 5-C); CI⁺ MS: *m/z*: 298.2 ([M+H]⁺, 7), 282.2 ([M-CH₃]⁺, 77), 281.2(26), 254.2(100), 236.2(89), 209.2(100); HRMS (ESI): [M+H]⁺ calcd for C₁₄H₂₄N₃O₄, 298.1761, found 298.1738. The hydrochloride salt (**9b.HCl**) was prepared as described for **7a.HCl**. Mp 163-165 °C (dec). Anal. (C₁₄H₂₄ClN₃O₄) C, H, N.

***(S)*-*N*-Hydroxy-2-methyl-3,5-dioxo-1,4-diazaspiro[5.7]tridecane-4-acetamide (9c)**

Following the general hydrogenolysis procedure described in the main manuscript, *O*-benzyl hydroxamate **61** provided the title compound **9c** as a white foamy solid, which strongly binds the eluting solvent (AcOEt). Removal of the entrapped solvent as described for **7c** gave **9c** as a white crystalline solid (90%): mp 82–85 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ (ppm): 1.28 (d, 3H, *J*=6.8 Hz, CH₃), 1.35-1.95 (complex m, 13H, 7, 8, 9, 10, 11, 12, 13-H), 2.07-2.13 (sym q, 1H, *J*=9.2 Hz, 13-H), 2.72 (br d, 1H, *J*=8.3 Hz, 1-H), 3.66 (~br t, 1H, *J*=6-7 Hz, 2-H), 4.07-4.16 (q, AB, 1.5H, *J*_{AB}=15.2 Hz, CH₂CONHOH, Z-isomer), 4.41 (s, 0.4H, CH₂CONHOH, E-isomer), 8.85 (s, 0.8H, CONHOH, Z-isomer), 9.26 (s, 0.2H, CONHOH, E-isomer), 10.13 (s, 0.2H, CONHOH, E-isomer), 10.51 (s, 0.8H, CONHOH, Z-isomer); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 17.0 (CH₃), 20.8 (9-C), 21.2 (11-C), 24.5 (10-C), 26.9 (8-C), 27.8 (7-C), 28.5 (12-C), 33.3 (13-C), 39.1 (CH₂CONHOH, Z-isomer), 39.2 (CH₂CONHOH, E-isomer), 48.8 (2-C), 59.8 (6-C), 163.9 (CONHOH, Z-isomer), 169.3 (CONHOH, E-isomer), 173.8, 176.6 (3, 5-C); [α]_D²⁶₅₈₉ +12 (c 0.1, CHCl₃); EI MS: *m/z*: 297.2([M]⁺, 11), 269.1([M-H₂O]⁺, 61), 264.1(52), 209.2(53); HRMS (ESI): [M+H]⁺ calcd for C₁₄H₂₄N₃O₄, 298.1767, found 298.1744. The hydrochloride salt (**9c.HCl**) was prepared as described for **7c.HCl**. Mp 170-172 °C (dec). Anal. (C₁₄H₂₄ClN₃O₄) C, H, N.

***(S)*-*N*-Hydroxy-3,5-dioxo-2-(phenylmethyl)-1,4-diazaspiro[5.7]tridecane-4-acetamide (9d)**

Following the general hydrogenolysis procedure described in the main manuscript, *O*-benzyl hydroxamate **62** provided the title compound **9d** as a white foamy solid, which strongly binds the eluting solvent (AcOEt). Removal of the entrapped solvent upon drying at 50-55 °C under vacuum (10⁻³ mmHg) in an Abderhalden apparatus gave **9d** as a white crystalline solid (98%): mp 65–68 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ1.18-1.67 (m, 11H, 7, 8, 9, 10, 11, 12, 13-H), 1.69-1.82 (m, 2H, 7, 12-H), 2.0-2.12 (m, 1H, 13-H), 2.56-2.65 (m, 1H, 1-H), 2.68-2.82 (m, 1H, AMX, A region, CH_AH_MPh), 3.32 (dd under DMSO water peak, 1H, AMX, M region, *J*_{MX}=3.6 Hz, CH_AH_MPh), 3.75-3.86 (m, 1H, AMX, X region, 2-Hx), 4.09-4.18 (q, AB, 1.6H, *J*_{AB}=15.6 Hz, CH₂CONHOH, *Z*-isomer), 4.42 (s, 0.4H, CH₂CONHOH, *E*-isomer), 7.14-7.22 (m, 1H, 4-aromatic H), 7.23-7.33 (m, 4H, 2,3,5,6-aromatic H), 8.85(s, 0.7H, CONHOH, *Z*-isomer), 9.27 (s, 0.2H, CONHOH, *E*-isomer), 10.15(s, 0.2H, CONHOH, *E*-isomer), 10.51 (s, 0.7H, CONHOH, *Z*-isomer); ¹³C NMR (100 MHz, DMSO-*d*₆) δ20.3 (9-C), 21.1 (11-C), 24.2 (10-C), 26.7 (8-C), 27.5 (7-C), 28.3 (12-C), 33.8 (13-C), 36.2 (CH₂Ph), 39.2 (CH₂CONHOH), 54.3 (2-C), 59.6 (6-C), 126.0 (4-aromatic C), 127.9, 129.0 (2, 3, 5, 6-aromatic C), 138.7 (1-aromatic C), 163.9 (CONHOH, *Z*-isomer), 169.3 (CONHOH, *E*-isomer), 172.9, 176.6 (3, 5-C); [α]²¹₅₈₉ -16 (C 0.2, CHCl₃); EI MS: *m/z* 373.1([M]⁺, 4), 282.1([M-CH₂Ph]⁺, 100); HRMS (ESI): [M+H]⁺ calcd for C₂₀H₂₈N₃O₄, 374.2069, found 374.2047. The hydrochloride salt (**9d.HCl**) was prepared as described for **7c.HCl**. Mp 202-205 °C (dec). Anal. (C₂₀H₂₈Cl N₃O₄) C, H, N.

***N*-Hydroxy-3,5-dioxo-1,4-diazaspiro[5.6]dodecane-4-acetamide (10a)**

Following the general hydrogenolysis procedure described in the main manuscript, *O*-benzyl hydroxamate **63** provided the title compound **10a** as a white foamy solid, which strongly binds the eluting solvent (AcOEt). Removal of the entrapped solvent as described for **7c** gave **10a** as a pale yellow crystalline solid (90%): mp 142–144 °C (dec); ¹H NMR (400 MHz, DMSO-*d*₆) δ1.40-1.76 (m, 10H, 7, 8, 9, 10, 11, 12-H), 1.82-1.97 (m, 2H, 7, 12-H), 3.01 (br s, 1H, 1-H), 3.56 (d, 2H, *J*=6.4 Hz, 2-H), 4.13 (s, 1.6H, CH₂CONHOH, *Z*-isomer), 4.42 (s, 0.4H, CH₂CONHOH, *E*-isomer), 8.87 (s, 0.7H, CONHOH, *Z*-isomer), 9.27 (s, 0.2H, CONHOH, *E*-isomer), 10.15 (s, 0.2H, CONHOH, *E*-isomer), 10.53 (s, 0.7H, CONHOH, *Z*-isomer); ¹³C NMR (100 MHz, , DMSO-*d*₆) δ21.7(8, 11-C), 29.4, 29.5 (9, 10-C), 35.1 (7, 12-C), 38.7(CH₂CONHOH, *Z*-isomer), 38.9 (CH₂CONHOH, *E*-isomer) 44.7 (2-C), 59.6, 59.7 (6-C), 163.9 (CONHOH, *Z*-isomer), 169.3 (CONHOH, *E*-isomer), 171.5, 176.9 (3, 5-C); Cl⁺ MS: *m/z* 270.2([M+H]⁺, 18), 252.1([M+H-H₂O]⁺, 24), 226.2(86), 209.1(96), 208.1(100); HRMS (ESI): [M+H]⁺ calcd for C₁₂H₂₀N₃O₄, 270.1448, found 270.1434. The hydrochloride salt (**10a.HCl**) was prepared as described for **7a.HCl**. Mp 190-192 °C (dec). Anal. (C₁₂H₂₀ClN₃O₄) C, H, N.

***N*-Hydroxy-1-methyl-3,5-dioxo-1,4-diazaspiro[5.6]dodecane-4-acetamide (10b)**

Following the general hydrogenolysis procedure described in the main manuscript, *O*-benzyl hydroxamate **73** provided the title compound **10b** as a white crystalline solid, which strongly binds the eluting solvent (AcOEt). Removal of the entrapped solvent upon drying at 40 °C under vacuum (10⁻³ mmHg) in an Abderhalden apparatus gave **10b** as a glass solid (93%): ¹H NMR (400 MHz, DMSO-*d*₆) δ1.46-1.65 (br s, 8H, 8, 9, 10, 11-H), 1.78-2.0 (m, 4H, 7, 12-H), 2.37 (s, 2.2H, CH₃, *Z*-isomer), 2.39 (s, 0.8H, CH₃, *E*-isomer), 3.72 (s, 2H, 2-H), 4.16 (s, 1.4H, CH₂CONHOH, *Z*-isomer), 4.46 (s, 0.5H, CH₂CONHOH, *E*-isomer), 8.87 (s, 0.7H, CONHOH, *Z*-isomer), 9.29 (s, 0.2H, CONHOH, *E*-isomer), 10.17 (s, 0.2H, CONHOH, *E*-isomer), 10.62 (s, 0.7H, CONHOH, *Z*-isomer); ¹³C NMR (100 MHz, , DMSO-*d*₆) δ21.6 (8, 11-C), 28.7 (9, 10-C), 33.4 (7, 12-C), 38.16, 38.21 (CH₃), 38.3 (CH₂CONHOH, *Z*-isomer), 39.0 (CH₂CONHOH, *E*-isomer), 53.7 (2-C), 64.6 (6-C), 163.9 (CONHOH, *Z*-isomer), 169.3 (CONHOH, *E*-isomer), 170.0, 175.4, 175.5 (3, 5-C); Cl⁺ MS: *m/z*: 284.2 ([M+H]⁺, 100), 283.2 ([M]⁺, 21), 256.2(86); HRMS (ESI): [M+H]⁺ calcd for C₁₃H₂₂N₃O₄, 284.1610, found 284.1573. The hydrochloride salt (**10b.HCl**) was prepared as described for **7c.HCl**. Mp 132-136 °C (slightly hygroscopic). Anal. (C₁₃H₂₂ClN₃O₄) C, H, N.

3,5-Dioxospiro[piperazine-2,2'-tricyclo[3.3.1.1^{3,7}]decane]-4-acetamide (43)

Using a procedure essentially similar to that described earlier for the preparation of compound **22**,⁹ the amide ester precursor **17** (365 mg, 1.3 mmol) was treated with (CH₃)₃SiNK (259 mg, 1.3 mmol), and the 2,6-DKP imidic potassium salt formed was subsequently reacted with bromoacetamide (190 mg, 1.37 mmol). The reaction mixture was then poured onto cold brine and quenched as in **22**. The resulting residue was chromatographed on a silica gel column eluting first with AcOEt-Et₂O 1:1 and then AcOEt to give the title compound **43** as a white solid (191 mg, 50%): mp 168–169 °C (EtOH – Et₂O); ¹H NMR (600 MHz, DMSO-*d*₆) δ 1.43 (d, 2H, *J*=12.0 Hz, 4'e, 9'e-H), 1.57-1.69 (m, 4H, 6', 8'e, 10'e-H), 1.75 (s, 1H, 7'-H), 1.79 (s, 1H, 5'-H), 1.95 (s, 2H, 1', 3'-H), 2.17-2.32 (m, 4H, 4'a, 8'a, 9'a, 10'a-H), 3.09 (br s, 1H, 1-H), 3.56 (d, 2H, *J*=6.5 Hz, 6-H), 4.17 (s, 2H, CH₂CONH₂), 6.99 (s, 1H, CONHH), 7.38 (s, 1H, CONHH); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 26.6 (5'-C), 26.8 (7'-C), 31.8 (1', 3'-C), 31.9 (4', 9'-C), 32.7 (8', 10'-C), 37.7 (6'-C), 41.4 (CH₂CONH₂), 43.9 (6-C), 59.3 (2, 2'-C), 168.5 (CONH₂), 172.1, 174.5 (3, 5-C). Anal. (C₁₅H₂₁N₃O₃) C, H, N.

3,5-Dioxo-*N*-(phenylmethoxy)spiro[piperazine-2,2'-tricyclo[3.3.1.1^{3,7}]decane]-4-acetamide (32)

To a solution of the carboxylic acid **27**⁹ (320 mg, 1.1 mmol) in dry THF (15 mL) was added 1,1'-carbonyldiimidazole (214 mg, 1.32 mmol), and the mixture was stirred at 28 °C for 1h under argon. Then, *O*-benzylhydroxylamine hydrochloride (210 mg, 1.32 mmol) and triethylamine (202 mg, 2 mmol) were added successively, and the stirring was continued at 28 °C for 24h and then at 45 °C for 1h under argon. After removal of the solvent in vacuo, water (20 mL) was added, and the mixture was extracted with ethyl acetate (3x20 mL). The combined extracts were washed with brine (2x20 mL) dried (Na₂SO₄) and evaporated in vacuo. The viscous oily residue was purified by column chromatography on silica gel eluting first with Et₂O-*n*-hexane 2:1 and then AcOEt to give the corresponding *O*-benzyl hydroxamate **32** as a white solid (312 mg, 72%): mp 162-164 °C (AcOEt); ¹H NMR (400 MHz, CDCl₃, 270K) δ 1.50 (d, 2H, *J*=12.5 Hz, 4'e, 9'e-H), 1.62-1.75 (m, 4H, 6', 8'e, 10'e-H), 1.82 (s, 1H, 7'-H), 1.86 (s, 1H, 5'-H), 1.94 (s, 1H, 1'-H), 1.99 (s, 1H, 3'-H), 1.90-2.08 (br s, 1H, 1-H), 2.23 (br s, 4H, 4'a, 8'a, 9'a, 10'a-H), 3.68, 3.72 (s+s, 2H, 6-H), 4.23 (s, 0.99H, CH₂CONHOCH₂Ph, *Z*-isomer), 4.59 (s, 0.94H, CH₂CONHOCH₂Ph, *E*-isomer), 4.84, 4.88 (s+s, 2H, CONHOCH₂Ph), 7.37 (br s, 5H, aromatic-H), 8.34 (s, 0.4H, CONHOCH₂Ph, *E*-isomer), 8.89 (s, 0.5H, CONHOCH₂Ph, *Z*-isomer); ¹³C NMR (100 MHz, CDCl₃, 270K) δ 26.8 (5'-C), 27.0 (7'-C), 32.1 (4', 9'-C), 32.3 (1', 3'-C), 33.0 (8', 10'-C), 37.7 (6'-C), 39.8, 39.9 (CH₂CONHOCH₂Ph), 44.2 (6-C), 60.3, 60.4 (2,2'-C), 78.1, 79.4 (CONHOCH₂Ph), 128.5, 128.8, 129.1, 129.3, 129.4 (2, 3, 4, 5, 6-aromatic C), 133.9, 134.9 (1-aromatic C), 165.4 (CONHOCH₂Ph, *Z*-isomer), 170.5 (CONHOCH₂Ph, *E*-isomer), 172.4, 172.5, 174.5, 174.6 (3, 5-C). Anal. (C₂₂H₂₇N₃O₄) C, H, N.

3,5-Dioxospiro[piperazine-2,2'-tricyclo[3.3.1.1^{3,7}]decane]-4-acetohydrazide (44)

Carboxylic acid **27**⁹ (585 mg, 2.0 mmol) was treated with 1,1'-carbonyldiimidazole (390 mg, 2.4 mmol) in dry THF (26 mL) as described in **32**. To the solution was then added benzylcarbazate (399 mg, 2.4 mmol), and the reaction mixture was stirred at 28 °C for 25h under argon. After removal of the solvent in vacuo, the residue was dissolved in chloroform, and the resulting solution washed with brine (2x30 mL), dried (Na₂SO₄), and evaporated. The residue was purified by column chromatography on silica gel eluting first with AcOEt-*n*-hexane 1:1 and then AcOEt to give a white foamy solid (700 mg): EI MS *m/z* 440.4 ([M]⁺, 7). This was then dissolved in EtOH (55 mL), and the solution was hydrogenated as described for the preparation of **7a-e**, **8**, **9a-d**, **10a**, and **10b** to yield the acetohydrazide **44** as a TLC and ¹H NMR pure white solid (466 mg, 76% from **27**), which was recrystallized from EtOH-Et₂O: mp 197 – 200 °C (dec); ¹H NMR (600 MHz, DMSO-*d*₆) δ 1.43 (d, 2H, *J*=11.9 Hz, 4'e, 9'e-H), 1.58-1.72 (m, 4H, 6', 8'e, 10'e-H), 1.75 (s, 1H,

7'-H), 1.79 (s, 1H, 5'-H), 1.90-2.0 (s, 2H, 1', 3'-H), 2.26 (~t, 4H, $J=11.9, 12.8$ Hz, 4'a, 8'a, 9'a, 10'a-H), 3.13 (~br s, 1H, 1-H), 3.56 (d, 2H, $J=7.0$ Hz, 6-H), 3.92-4.75 (v br s, 2H, CONHNH₂), 4.18 (s, 1.7H, CH₂CONHNH₂, Z-isomer), 4.52 (s, 0.2H, CH₂CONHNH₂, E-isomer), 8.46 (s, 0.1H, CONHNH₂, E-isomer), 9.06 (s, 0.8H, CONHNH₂, Z-isomer); ¹³C NMR (100 MHz, DMSO-*d*₆) δ26.6 (5'-C), 26.9 (7'-C), 31.8 (1', 3'-C), 31.9 (4', 9'-C), 32.7 (8', 10'-C), 37.7 (6'-C), 39.95, 40.02 (CH₂CONHNH₂), 44.0 (6-C), 59.4 (2, 2'-C), 166.5 (CONHNH₂), 172.2, 172.3, 174.5, 174.7 (3, 5-C). Anal. (C₁₅H₂₂N₄O₃) C, H, N.

***N*-Methoxy-3,5-dioxospiro[piperazine-2,2'-tricyclo[3.3.1.1^{3,7}]decane]-4-acetamide (45)**

Prepared from carboxylic acid **27**⁹ in exactly the same procedure described in **32** except that *O*-methyl hydroxylamine hydrochloride was used. The resulting oily residue was chromatographed on silica gel column using AcOEt-Et₂O 1:1 to afford colourless viscous oil which strongly binds the aforementioned solvents. Removal of the entrapped solvents upon drying at 50 °C under vacuum (10⁻³ mmHg) in an Abderhalden apparatus gave the title compound **45** as a glass solid: (82%); ¹H NMR (400 MHz, CDCl₃) δ1.51 (d, 2H, $J=12.3$ Hz, 4'e, 9'e-H), 1.63-1.77 (m, 4H, 6', 8'e, 10'e-H), 1.82 (s, 1H, 7'-H), 1.86 (s, 1H, 5'-H), 1.92-2.10 (br s, 3H, 1, 1', 3'-H), 2.27 (d, 4H, $J=11.8$ Hz, 4'a, 8'a, 9'a, 10'a-H), 3.60-3.88 (br s, 5H, CONHOCH₃, 6-H), 4.27 (br s, 1.1H, CH₂CONHOCH₃, Z-isomer), 4.63 (br s, 0.7H, CH₂CONHOCH₃, E-isomer), 8.97 (br s, 0.3H, CONHOCH₃, E-isomer), 9.67 (br s, 0.6H, CONHOCH₃, Z-isomer); ¹³C NMR (100 MHz, CDCl₃) δ27.0 (5'-C), 27.2 (7'-C), 32.2 (4', 9'-C), 32.5 (1', 3'-C), 33.1 (8', 10'-C), 37.9 (6'-C), 39.8 (CH₂CONHOCH₃), 44.4 (6-C), 60.4 (2,2'-C), 64.3, 65.1 (CONHOCH₃), 165.6 (CONHOCH₃, Z-isomer), 170.6 (CONHOCH₃, E-isomer), 172.6, 174.6 (3, 5-C); CI⁺ MS: *m/z* 322.2 ([M+H]⁺, 24), 290.2 ([M-OCH₃]⁺, 15), 294.2 (64), 219.1 (79). The hydrochloride salt (**45.HCl**) was prepared by treating a solution of **45** in Et₂O-AcOEt 3:2 with ethereal HCl under ice cooling. The white solid was collected by filtration, triturated with Et₂O and dried at 62-64 °C under vacuum (10⁻³ mmHg). Mp 193-195 °C; Anal. (C₁₆H₂₄ClN₃O₄·0.4Et₂O) C, H, N.

***N*-[2-Cyano(tricyclo[3.3.1.1^{3,7}]dec-2-yl)]-L-phenylalanine methyl ester (14)**

Prepared by employing the Strecker reaction on the adamantanone **11** in exactly the same procedure described earlier for the preparation of compound **12**,⁹ except that methyl *L*-phenylalaninate hydrochloride was used. The resulting off-white solid was purified by column chromatography on silica gel eluting first with Et₂O-*n*-hexane 1:2 and then 1:1 to afford the title compound **14** as a white crystalline solid (89%): mp 78-80 °C (Et₂O-*n*-pentane); ¹H NMR (400 MHz, CDCl₃) δ1.15 (dd, 1H, $J=2.5, 12.4$ Hz, 4e-Ad H), 1.40 (dd, 1H, $J=2.4, 12.7$ Hz, 9e-Ad H), 1.48-1.62 (complex m, 4H, 4a, 5, 6-Ad H), 1.64-1.88 (complex m, 6H, 1, 3, 7, 8e, 10e-Ad H, NH), 1.98 (d, 1H, $J=13.4$ Hz, 8a-Ad H), 2.03-2.13 (m, 2H, 9a, 10a-Ad H), 2.76-2.81 (q, 1H, AMX, A region, $J_{AM}=13.4$ Hz, $J_{AX}=8.0$ Hz, NH-CH_X-CH_AH_MPh), 2.92-2.97 (q, 1H, AMX, M region, $J_{AM}=13.4$ Hz, $J_{MX}=5.6$ Hz, NH-CH_X-CH_AH_MPh), 3.58-3.62 (q, 1H, AMX, X region, $J_{AX}=8.0$ Hz, $J_{MX}=5.6$ Hz, NH-CH_X-CH_AH_MPh), 3.64 (s, 3H, CH₃), 7.09-7.24 (m, 5H, aromatic H); ¹³C NMR (100 MHz, CDCl₃) δ(ppm):26.4 (5-Ad C), 26.6 (7-Ad C), 29.6 (4-Ad C), 30.4 (9-Ad C), 33.4 (1-Ad C), 34.1 (8-Ad C), 34.7 (10-Ad C), 36.5 (3-Ad C), 37.4 (6-Ad C), 40.8 (CH₂Ph), 51.8 (CH₃), 57.7 (HN-CH), 60.7 (2-Ad C), 121.9 (CN), 126.7, 128.2, 129.5 (2, 3, 4, 5, 6-aromatic C), 137.1 (1-aromatic C), 174.8 (CO₂CH₃); [α]_D²⁵₅₈₉ = -6 (c 0.2, AcOEt). Anal. (C₂₁H₂₆N₂O₂) C, H, N.

***N*-[2-Aminocarbonyl(tricyclo[3.3.1.1^{3,7}]dec-2-yl)]-L-phenylalanine methyl ester (19)**

To a vigorous stirred solution of the aminonitrile **14** (1.93 g, 5.7 mmol) in CH₂Cl₂ (24 mL) was added dropwise H₂SO₄ 97% (9.5 mL), and the mixture was vigorously stirred at room temperature for 48 h. The reaction mixture was then poured onto ice (30 g) and neutralized with aqueous NH₃ 26 % under ice cooling. The organic layer was separated, and the aqueous layer was extracted with CH₂Cl₂ (3x30 mL). The combined organic phase was washed with water (30

mL) and brine (30 mL), dried (Na₂SO₄), and evaporated. The resulted coloured residue was purified by column chromatography on silica gel using AcOEt-Et₂O 1:1 to afford the title compound **19** as a white crystalline solid (1.04 g, 51%): mp 141-143 °C (CH₂Cl₂-Et₂O); ¹H NMR (400 MHz, CDCl₃) δ1.27 (~d, 1H, *J*=11.7 Hz, 4e-Ad H), 1.39 (~d, 1H, *J*=11.7 Hz, 9e-Ad H), 1.47-1.73 (complex m, 8H, 4a, 5, 6, 7, 8, 10e-Ad H), 1.74-1.92 (m, 3H, NH, 3, 10a-Ad H), 1.95 (s, 1H, 1-Ad H), 2.12 (d, 1H, *J*=12.6 Hz, 9a-Ad H), 2.67-2.72 (q, 1H, AMX, A region, *J*_{AM}=13.2 Hz, *J*_{AX}=7.8 Hz, NH-CH_X-CH_AH_MPh), 2.80-2.85 (q, 1H, AMX, M region, *J*_{AM}=13.2 Hz, *J*_{MX}=5.7 Hz, NH-CH_X-CH_AH_MPh), 3.53 (br s, 4H, CH₃, NH-CH_X-CH_AH_MPh), 5.50 (br s, 2H, CONH₂), 7.05-7.22 (m, 5H, aromatic H); ¹³C NMR (100 MHz, CDCl₃) δ26.7 (5-Ad C), 26.9 (7-Ad C), 31.4 (4-Ad C), 31.7 (1-Ad C), 32.1 (9-Ad C), 34.2 (3-Ad C), 34.4 (8-Ad C), 34.6 (10-Ad C), 37.6 (6-Ad C), 41.5 (CH₂Ph), 51.4 (CH₃), 56.3 (NHCH), 64.2 (2-Ad C), 126.5, 128.1, 129.6 (2, 3, 4, 5, 6-aromatic C), 137.5 (1-aromatic C), 175.4 (CO₂CH₃), 176.9 (CONH₂); [α]₅₈₉²⁵ +12 (c 0.2, MeOH). Anal. (C₂₁H₂₈N₂O₃) C, H, N.

(S)-3,5-Dioxo-6-(phenylmethyl)spiro[piperazine-2,2'-tricyclo[3.3.1.1^{3,7}]decane]-4-acetic acid benzyl ester (24)

Prepared from the above amide-ester precursor **19** in exactly the same procedure described earlier for the preparation of compound **22**.⁹ The resulted oily residue was purified by column chromatography on silica gel using Et₂O-*n*-hexane 1:1 to afford the title compound **24** as a colourless viscous oil (86%): ¹H NMR (400 MHz, CDCl₃) δ1.38 (d, 2H, *J*=12.9 Hz, 4'e, 9'e-H), 1.29-1.48 (v br s, 1H, 1-H), 1.51-1.70 (complex m, 7H, 4'a, 6', 7', 8', 10'e-H), 1.75 (br s, 2H, 3', 5'-H), 1.95 (d, 1H, *J*=12.8 Hz, 9'a-H), 2.02 (s, 1H, 1'-H), 2.82 (d, 1H, *J*=12.6 Hz, 10'a-H), 2.92-2.97 (q, 1H, AMX, A region, *J*_{AM}=13.8 Hz, *J*_{AX}=8.1 Hz, 6-CH_AH_MPh), 3.30 (dd, 1H, AMX, M region, *J*_{AM}=13.8 Hz, *J*_{MX}=4.0 Hz, 6-CH_AH_MPh), 3.77-3.87 (m, 1H, AMX, X region, 6-Hx), 4.33-4.53 (q, AB, 2H, *J*=16.8 Hz, CH₂CO₂CH₂Ph), 5.07 (s, 2H, CO₂CH₂Ph), 7.10-7.35 (m, 10H, aromatic H); ¹³C NMR (100 MHz, CDCl₃) δ26.9 (5'-C), 27.0 (7'-C), 30.5 (1'-C), 31.3 (4'-C), 32.4 (9'-C), 33.2 (8'-C), 33.9 (10'-C), 34.8 (3'-C), 37.8 (CH₂Ph), 37.9 (6'-C), 40.9 (CH₂CO₂CH₂Ph), 54.1 (6-C), 60.8 (2,2'-C), 67.2 (CO₂CH₂Ph), 127.1, 128.3, 128.4, 128.6, 128.7, 129.2, 135.1, 136.6 (aromatic C), 168.0 (CO₂CH₂Ph) 173.5, 174.1 (3, 5-C); [α]₅₈₉²⁴ -34 (c 0.2, CHCl₃); CI⁺ MS: *m/z* 473.3 ([M+H]⁺, 50), 472.3 ([M]⁺, 13), 382.2 ([M+H-CH₂Ph]⁺, 23), 381.2 ([M-CH₂Ph]⁺, 100); HRMS (ESI): [M+H]⁺ calcd for C₂₉H₃₃N₂O₄, 473.2440, found 473.2440.

(S)-3,5-Dioxo-6-(phenylmethyl)spiro[piperazine-2,2'-tricyclo[3.3.1.1^{3,7}]decane]-4-acetic acid (29)

Prepared from the above benzyl ester **24** by catalytic hydrogenolysis in EtOH-AcOEt 3:2 following the procedure described earlier for the preparation of carboxylic acid **27**.⁹ White foamy solid which strongly binds the aforementioned solvents. Removal of the entrapped solvents upon drying at 62-64 °C under vacuum (10⁻³ mmHg) in an Abderhalden apparatus gave the title compound **29** as a glass solid (almost quantitative yield): ¹H NMR (400 MHz, CDCl₃) δ1.35-1.47 (m, 2H, 4'e, 9'e-H), 1.52-1.73 (m, 7H, 4'a, 6', 7', 8', 10'e-H), 1.79 (s, 2H, 3', 5'-H), 1.95 (d, 1H, *J*=12.5 Hz, 9'a-H), 2.05 (s, 1H, 1'-H), 2.84 (d, 1H, *J*=12.8 Hz, 10'a-H), 2.99-3.05 (q, 1H, AMX, A region, *J*_{AM}=13.8 Hz, *J*_{AX}=8.0 Hz, CH_AH_MPh), 3.31 (dd, 1H, AMX, M region, *J*_{AM}=13.8 Hz, *J*_{MX}=4.1 Hz, CH_AH_MPh), 3.82-3.85 (q, 1H, AMX, X region, *J*_{AX}=8.0 Hz, *J*_{MX}=4.1 Hz, 6-Hx), 4.35-4.51 (q, AB, 2H, *J*_{AB}=17.1 Hz, CH₂CO₂H), 4.20-6.80 (v br s, 2H, COOH, 1-H), 7.13-7.30 (m, 5H, aromatic H); ¹³C NMR (100 MHz, CDCl₃) δ26.8 (5'-C), 26.9 (7'-C), 30.4 (1'-C), 31.3 (4'-C), 32.4 (9'-C), 33.2 (8'-C), 33.8 (10'-C), 34.7 (3'-C), 37.5 (CH₂Ph), 37.9 (6'-C), 40.7 (CH₂CO₂H), 54.0 (6-C), 61.1 (2,2'-C), 127.2, 128.8, 129.3 (2, 3, 4, 5, 6-aromatic C), 136.3 (1-aromatic C), 173.0 (CO₂H), 173.5 (3, 5-C). The hydrochloride salt (**29.HCl**) was prepared as described for **7c.HCl**. Mp 182-184 °C (dec); [α]₅₈₉²⁵ -24 (c 0.2, MeOH). Anal. (C₂₂H₂₇Cl N₂O₄) C, H, N.

(S)-6-(Phenylmethyl)spiro[piperazine-2,2'-tricyclo[3.3.1.1^{3,7}]decane]-3,5-dione (37)

Prepared from the above amide-ester precursor **19** in exactly the same procedure described earlier for the preparation of the diketopiperazine **6a**.⁹ White foamy solid which strongly binds the eluting solvents. Removal of the entrapped solvents upon drying at 62-64 °C under vacuum (10⁻³ mmHg) gave the title compound **37** as a glass solid (96%): ¹H NMR (400 MHz, CDCl₃) δ 1.39 (d, 2H, *J*=12.4 Hz, 4'e, 9'e-H), 1.37-1.50 (v br s, 1H, 1-H), 1.53-1.70 (complex m, 7H, 4'a, 6', 7', 8', 10'e-H), 1.74 (s, 1H, 3'-H), 1.80 (s, 1H, 5'-H), 1.92-2.05 (m, 2H, 1', 9'a-H), 2.86 (d, 1H, *J*=12.9 Hz, 10'a-H), 2.97-3.02 (q, 1H, AMX, A region, *J*_{AM}=13.8 Hz, *J*_{AX}=7.6 Hz, CH_AH_MPh), 3.28 (dd, 1H, AMX, M region, *J*_{AM}=13.8 Hz, *J*_{MX}=4.0 Hz, CH_AH_MPh), 3.68-3.71 (q, 1H, AMX, X region, *J*_{AX}=7.6 Hz, *J*_{MX}=4.0 Hz, 6-Hx), 7.16-7.28 (m, 5H, aromatic H), 7.89 (s, 1H, 4-H); ¹³C NMR (100 MHz, CDCl₃) δ 26.9 (5'-C), 27.0 (7'-C), 30.3 (1'-C), 31.2 (4'-C), 32.5 (9'-C), 33.2 (8'-C), 33.8 (10'-C), 34.6 (3'-C), 36.7 (CH₂Ph), 37.9 (6'-C), 53.6 (6-C), 61.1 (2,2'-C), 127.1, 128.7, 129.3 (2, 3, 4, 5, 6-aromatic C), 136.6 (1-aromatic C), 174.1, 174.8 (3, 5-C); Cl⁺ MS: *m/z* 325.2 ([M+H]⁺, 88), 324.2 ([M]⁺, 21), 233.1 ([M-CH₂Ph]⁺, 66). The hydrochloride salt (**37.HCl**) was prepared as described for **7c.HCl**. Mp 185-187 °C (dec). [α]₅₈₉²⁵ -38 (c 0.2, MeOH). Anal. (C₂₀H₂₅Cl N₂O₂) C, H, N.

1-Methylspiro[piperazine-2,2'-tricyclo[3.3.1.1^{3,7}]decane]-3,5-dione (6b)

A solution of diketopiperazine **6a**⁹ (500 mg, 2.13 mmol) in THF-MeOH 1:1 (14 mL), aqueous formaldehyde 37 % (1 mL, 12.5 mmol) was stirred for 3 h at room temperature, and NaCNBH₃ (217 mg, 3.45 mmol) was then added in one portion. After stirring for 20 min the pH of the reaction mixture was adjusted to 6-7 by dropwise addition of acetic acid. Stirring was continued for 4 h at ambient temperature with the occasional addition of acetic acid to maintain the pH at 6-7. Solvents were evaporated in vacuo and the residue was treated with water (20 mL), and then NaOH 1N and solid Na₂CO₃ until the pH was adjusted to 8. The off-white solid formed was isolated by vacuum filtration, washed with water (2 x 5 mL), and dried. Subsequent purification by column chromatography on silica gel using Et₂O-*n*-hexane 4:1 afforded the title compound **6b** as a white crystalline solid (490 mg, 92%): mp 191-192 °C (CH₂Cl₂-*n*-pentane); ¹H NMR (400 MHz, CDCl₃) δ 1.47 (d, 1H, *J*=12.0 Hz, 4'e-H), 1.54 (d, 1H, *J*=12.3 Hz, 9'e-H), 1.61-1.91 (complex m, 7H, 3', 5', 6', 7', 8'e, 10'e-H), 2.03-2.25 (complex m, 4H, 1'-H, 4a, 8a, 9a-H), 2.32 (s, 3H, CH₃), 2.76 (d, 1H, *J*=12.9 Hz, 10'a-H), 3.25-3.89 (q, 2H, AB, *J*_{AB}=19.0 Hz, 6-H), 7.87 (br, s, 1H, 4-H); ¹³C NMR (100 MHz, CDCl₃) δ 26.3 (5'-C), 27.1 (7'-C), 30.2 (1'-C), 31.8 (4'-C), 32.1 (3', 9'-C), 33.2 (8'-C), 33.7 (10'-C), 37.4 (CH₃), 37.5 (6'-C), 53.0 (6-C), 65.7 (2, 2'-C), 172.0, 174.1 (3, 5-C). Anal. (C₁₄H₂₀N₂O₂) C, H, N.

1-Methyl-3,5-dioxospiro[piperazine-2,2'-tricyclo[3.3.1.1^{3,7}]decane]-4-acetic benzyl ester (40)

Sodium hydride (47 mg, 1.94 mmol) was added portionwise to a stirred solution of diketopiperazine **6b** (400 mg, 1.61 mmol) in dry DMF (12 mL). After stirring at room temperature for 1 h under argon, benzyl bromoacetate (387 mg, 1.69 mmol), dissolved in dry DMF (4 mL) was added dropwise. Stirring was continued at rt for 48 h under argon, and the reaction mixture was then poured onto ice/water mixture (35 mL), and extracted with Et₂O (4x25 mL). The combined organic extracts were washed with brine (25 mL), dried (Na₂SO₄), and evaporated in vacuo. The oily residue was purified by column chromatography on silica gel using Et₂O-*n*-hexane 1:1 to afford the title compound **40** as a colourless viscous oil (530 mg, 83%): ¹H NMR (400 MHz, CDCl₃) δ 1.49 (dd, 1H, *J*=2.1, 12.2 Hz, 4'e-H), 1.55 (dd, 1H, *J*=2.2, 12.1 Hz, 9'e-H), 1.58-1.77 (complex m, 5H, 6', 8', 10'e-H), 1.85 (br s, 2H, 5', 7'-H), 2.06-2.28 (m, 4H, 1', 3', 4'a, 9'a-H), 2.36 (s, 3H, CH₃), 2.76 (d, 1H, *J*=13.0 Hz, 10'a-H), 3.36-4.03 (q, 2H, AB, *J*_{AB}=19.1 Hz, 6-H), 4.40-4.70 (q, 2H, AB, *J*_{AB}=16.8 Hz, CH₂CO₂CH₂Ph), 5.15 (s, 2H, CO₂CH₂Ph), 7.28-7.40 (m, 5H, aromatic H); ¹³C NMR (100 MHz, CDCl₃) δ 26.3 (5'-C),

27.1 (7'-C), 30.5 (1'-C), 31.9 (4'-C) 32.1 (9'-C), 32.2 (3'-C), 33.1 (8'-C), 33.7 (10'-C), 37.3 (CH₃), 37.6 (6'-C), 40.4 (CH₂CO₂CH₂Ph), 53.3 (6-C), 65.5 (2,2'-C), 67.3 (CO₂CH₂Ph), 128.3, 128.4, 128.5 (2, 3, 4, 5, 6-aromatic C), 135.1 (1-aromatic C), 168.0 (CO₂CH₂Ph), 171.2, 174.0 (3, 5-C); Cl⁺ MS: m/z 397.2 ([M+H]⁺, 23), 396.2 ([M]⁺, 18), 369.2 (100), 305.1 ([M-CH₂Ph]⁺, 12).

1-Methyl-3,5-dioxospiro[piperazine-2,2'-tricyclo[3.3.1.1^{3,7}]decane]-4-acetic acid (41)

Prepared from the above benzyl ester **40** in an almost quantitative yield by catalytic hydrogenolysis in exactly the same procedure described earlier for the preparation of carboxylic acid **27**.⁹ White solid: mp 210-212 °C (EtOH-Et₂O); ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.42 (d, 2H, *J*=11.9 Hz, 4'e-H), 1.52 (td, 2H, *J*=13.8, 16.8 Hz, 8'e, 9'e-H), 1.59-1.75 (m, 4H, 6', 8'a, 10'e-H), 1.78 (s, 2H, 5', 7'-H), 2.03-2.18 (m, 4H, 1', 3', 4'a, 9'a-H), 2.31 (s, 3H, CH₃), 2.69 (d, 1H, *J*=12.2 Hz, 10'a-H), 3.0-3.85 (v br s, 1H, CO₂H), 3.47-3.99 (q, 2H, AB, *J*_{AB}=19.2 Hz, 6-H), 4.19-4.39 (q, 2H, AB, *J*_{AB}=16.9 Hz, CH₂CO₂H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 25.9 (5'-C), 26.7 (7'-C), 30.0 (1'-C), 31.5 (3'-C), 31.7 (4'-C), 31.8 (9'-C), 32.7 (8'-C), 33.4 (10'-C), 36.6 (CH₃), 37.3 (6'-C), 40.2 (CH₂CO₂H), 52.5 (6-C), 64.8 (2,2'-C), 169.3 (CO₂H), 170.7, 173.7 (3, 5-C). Anal. (C₁₆H₂₂N₂O₄) C, H, N.

1-Methyl-1,4-diazaspiro[5,7]tridecane-3,5-dione (66)

Prepared by reductive methylation of diketopiperazine **64**⁹ (650 mg, 3.09 mmol) in MeOH-THF 1:3 (20 mL) as described above for the preparation of compound **6b**. The resulting solid residue was purified by column chromatography on silica gel using AcOEt-Et₂O 1:1 to afford the title compound as a white crystalline solid **66** (600 mg, 87%): mp 134-136 °C (CH₂Cl₂-*n*-pentane); ¹H NMR (400 MHz, CDCl₃) δ 1.36-1.68 (complex m, 8H, 8, 9, 10, 11, 12-H) 1.70-1.87 (m, 4H, 7, 9, 11, 13-H), 2.02-2.08 (~q, 2H, *J*=8.0, 9.9 Hz, 7, 13-H), 2.37 (s, 3H, CH₃), 3.61 (s, 2H, 2-H), 8.24 (br s, 1H, 4-H); ¹³C NMR (100 MHz, CDCl₃) δ 20.7 (9, 11-C), 24.5 (10-C), 27.9 (8, 12-C), 29.3 (7, 13-C), 39.3 (CH₃), 54.3 (2-C), 64.4 (6-C), 171.3, 176.4 (3, 5-C). Anal. (C₁₂H₂₀N₂O₂).

1-Methyl-3,5-dioxo-1,4-diazaspiro[5.7]tridecane-4-acetic acid benzyl ester (68)

Prepared from the diketopiperazine **66** in exactly the same procedure described above for the preparation of compound **40**. The resulting oily residue was purified by column chromatography on silica gel eluting first with Et₂O-*n*-hexane 1:1 and then AcOEt-*n*-hexane 1:1 to afford the title compound **68** as a colourless viscous oil (92%): ¹H NMR (400 MHz, CDCl₃) δ 1.38-1.67 (complex m, 8H, 8, 9, 10, 11, 12-H), 1.70-1.87 (m, 4H, 7, 9, 11, 13-H), 2.0-2.07 (~q, 2H, *J*=9.3, 9.6 Hz, 7, 13-H), 2.39 (s, 3H, CH₃), 3.72 (s, 2H, 2-H), 4.54 (s, 2H, CH₂CO₂CH₂Ph), 5.12 (s, 2H, CO₂CH₂Ph), 7.28-7.43 (m, 5H, aromatic H); ¹³C NMR (100 MHz, CDCl₃) δ 20.7 (9, 11-C), 24.5 (10-C), 27.9 (8, 12-C), 29.8 (7, 13-C), 39.2 (CH₃) 39.8 (CH₂CO₂CH₂Ph), 54.5 (2-C), 64.5 (6-C), 67.3 (CO₂CH₂Ph), 128.4, 128.5, 128.6 (2, 3, 4, 5, 6-aromatic C), 135.0 (1-aromatic C), 167.8 (CO₂CH₂Ph), 170.3, 175.9 (3, 5-C); Cl⁺ MS: m/z 372.3 ([M]⁺, 34), 373,3 ([M+H]⁺, 100), 281.1 ([M-CH₂Ph]⁺, 41).

1-Methyl-3,5-dioxo-1,4-diazaspiro[5.7]tridecane-4-acetic acid (70)

Prepared from the above benzyl ester **68** in an almost quantitative yield by catalytic hydrogenolysis in exactly the same procedure described earlier for the preparation of carboxylic acid **27**.⁹ White solid: mp 178-180 °C (EtOH-Et₂O); ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.20 - 1.57 (complex m, 8H, 8, 9, 10, 11, 12-H), 1.61-1.83 (complex m, 4H, 7, 9, 11, 13-H), 1.87-2.01 (m, 2H, 7, 13-H), 2.33 (s, 3H, CH₃), 3.72 (s, 2H, 2-H), 3.30-4.90 (v br s, 1H, CO₂H), 4.27 (s, 2H,

CH₂CO₂H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 20.7 (9, 11-C), 24.6 (10-C), 27.8 (8, 12-C), 29.5 (7, 13-C), 38.8 (CH₃), 39.8 (CH₂CO₂H), 53.9 (2-C), 64.1 (6-C), 169.5 (CO₂H), 170.4, 175.8 (3, 5-C). Anal. (C₁₄H₂₂N₂O₄) C, H, N.

Table 2. Antitrypanosome action of acetamide **43**, acetohydrazide **44**, O-methyl hydroxamate **45**, carboxylic acids **27-31**, **41**, **56**, **59**, and **71**, 2,6-DKP scaffold molecules **6a**, **6b**, **37-39**, and **64-67**, and acetohydroxamic acid tested against cultured blood stream form *T. brucei* (pH=7.4).

Compound ^(a)	IC ₅₀ (μM)	IC ₉₀ (μM)	Compound	IC ₅₀ (μM)	IC ₉₀ (μM)
43	200		71	>300	
44	118		6a	na ^b	
45	542		6b	na	
27	469		37	63.2	79.2
28	203	255	38	38.6	57.2
29	111	203	39	18.5	27.1
30	171	259	64	na	
31	103	208	65	>400	
41	na		66	na	
56	760		67	>400	
59	>400		Acetohydroxamic acid	680	

^aCompounds **45**, **29-31**, **37**, and **38** were tested as hydrochloride salts.

^bData not available.

Experimental. Biological Assays.

Trypanocidal assays

Cultured bloodstream form *T. brucei* (strain 427) were maintained at 37 °C in modified Iscove's medium (pH 7.4). Trypanocidal activity was assessed by growing parasites for 48 h in the presence of various drug concentrations to determine the levels which inhibited growth by 50% (IC₅₀) and 90% (IC₉₀). In the case of untreated cultures (volume 4 ml), cell densities increased from 0.5×10⁴ to 1×10⁶ cells ml⁻¹ over this period. Experiments were performed in triplicate. Cell densities at each drug concentration were determined using a hemocytometer (Weber Scientific International Ltd), and drug sensitivity was expressed as a percentage of growth of control cells. For *T. cruzi* epimastigotes (CL Brener clone), inhibition experiments were carried out in Nunclon 24 well plates in 2 ml of growth medium at an initial inoculum of 2 x 10⁵ epimastigotes ml⁻¹. After 5 days incubation at 28°C in RPMI medium (Kendall *et al.* 1990) in the absence of drug, cell density reached 2 x 10⁷ parasites ml⁻¹.

(25) Kendall, G.; Wilderspin, A. F.; Ashall, F.; Miles, M. A. and Kelly, J. M. *Trypanosoma cruzi* glycosomal glyceraldehyde-3-phosphate dehydrogenase does not conform to the "hotspot" topogenic signal model. *EMBO J.* **1990**, *9*, 2751-2758

Cytotoxicity tests

Cytotoxicity against mammalian cells was assessed using microtitre plates as described in Bot *et al.* (2010). Briefly, L6 cells (a rat skeletal muscle line) were seeded at $1 \times 10^4 \text{ ml}^{-1}$ in 200 μl of growth medium containing different compound concentrations. The plates were incubated for 6 days at 37°C and 20 μl Alamar Blue (Biosource UK Ltd.) was then added to each well. After further 8 h incubation, the fluorescence was determined using a Gemini fluorescent plate reader (Molecular Devices).

(26) Bot, C.; Hall, B. S.; Bashir, N.; Taylor, M. C.; Helsby, N. A.; Wilkinson, S. R. Trypanocidal activity of aziridinyl nitrobenzamide prodrugs. *Antimicrob. Agents Chemother.* **2010**, *54*, 4246-4252.

Abbreviation used: RPMI, Roswell Park Memorial Institute.

Table 3. Elemental analysis data for the tested compounds synthesized in this study.

Compounds	Molecular Formula	Calculated %			Found %		
		C	H	N	C	H	N
7a	C ₁₅ H ₂₁ N ₃ O ₄	58.62	6.89	13.67	58.62	6.98	13.71
7a.HCl	C ₁₅ H ₂₂ ClN ₃ O ₄	52.40	6.45	12.22	52.64	6.61	11.95
7b	C ₁₆ H ₂₃ N ₃ O ₄	59.79	7.21	13.08	59.52	7.30	13.37
7b.HCl	C ₁₆ H ₂₄ ClN ₃ O ₄	53.70	6.76	11.74	53.37	7.06	12.02
7c.HCl	C ₁₆ H ₂₄ ClN ₃ O ₄	53.70	6.76	11.74	53.58	7.16	11.61
7d.HCl	C ₂₂ H ₂₈ ClN ₃ O ₄	60.89	6.50	9.68	61.28	6.71	9.86
7e.HCl	C ₂₂ H ₂₈ ClN ₃ O ₄	60.89	6.50	9.68	60.52	6.77	10.02
8.HCl	C ₂₂ H ₂₈ ClN ₃ O ₄	60.89	6.50	9.68	61.26	6.83	9.91
9a.HCl	C ₁₃ H ₂₂ ClN ₃ O ₄	48.83	6.93	13.14	48.88	7.01	13.07

9b.HCl	$C_{14}H_{24}ClN_3O_4$	50.37	7.25	12.59	50.75	7.60	12.28
9c.HCl	$C_{14}H_{24}ClN_3O_4$	50.37	7.25	12.59	50.37	7.60	12.21
9d.HCl	$C_{20}H_{28}ClN_3O_4$	58.60	6.89	10.25	58.63	6.99	10.20
10a.HCl	$C_{12}H_{20}ClN_3O_4$	47.14	6.59	13.74	46.95	6.72	13.61
10b.HCl	$C_{13}H_{22}ClN_3O_4$	48.83	6.93	13.14	48.40	7.28	12.72
43	$C_{15}H_{21}N_3O_3$	61.83	7.27	14.42	61.46	6.89	14.78
44	$C_{15}H_{22}N_4O_3$	58.80	7.24	18.29	58.55	7.13	17.91
45.HCl	$C_{16}H_{24}ClN_3O_4 \cdot 0.4 Et_2O$	54.63	7.29	10.86	54.59	7.19	11.09
29.HCl	$C_{22}H_{27}ClN_2O_4$	63.07	6.50	6.69	63.08	6.57	6.43
30.HCl	$C_{22}H_{27}ClN_2O_4$	63.07	6.50	6.69	62.69	6.73	6.39
31.HCl	$C_{22}H_{27}ClN_2O_4$	63.07	6.50	6.69	63.41	6.81	6.31
41	$C_{16}H_{22}N_2O_4$	62.73	7.24	9.15	62.61	7.14	9.23
59	$C_{12}H_{18}N_2O_4$	56.68	7.14	11.02	57.07	7.16	11.38
71	$C_{13}H_{20}N_2O_4$	58.19	7.51	10.44	58.44	7.90	10.18
6b	$C_{14}H_{20}N_2O_2$	67.71	8.12	11.28	67.71	8.14	11.25
37.HCl	$C_{20}H_{25}ClN_2O_2$	66.56	6.98	7.76	66.95	7.04	7.36
38.HCl	$C_{20}H_{25}ClN_2O_2$	66.56	6.98	7.76	66.19	7.22	8.20
39	$C_{20}H_{24}N_2O_2$	74.04	7.46	8.64	74.11	7.44	8.57
65	$C_{10}H_{16}N_2O_2$	61.20	8.22	14.28	61.19	8.52	14.05
66	$C_{12}H_{20}N_2O_2$	64.25	8.99	12.49	64.10	8.99	12.65
67	$C_{11}H_{18}N_2O_2$	62.83	8.63	13.32	63.01	9.02	12.97