Electronic Supplementary Information

Mycobacterium tuberculosis histidinol dehydrogenase: biochemical characterization and inhibition studies

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***1**. Apparatus and analysis*

All common reactants and solvents were used as obtained from commercial suppliers and used without further purification. Melting points were determined using a Microquímica MQAPF-302 apparatus. ^{1}H NMR spectra were acquired on an Anasazi EFT-60 spectrometer (^{1}H at 60.13 MHz) at 30 °C or on a Varian (Federal University of Rio Grande do Sul, UFRGS/Brazil) spectrometer (^{1}H at 400.13 MHz) at 25 °C. High-resolution mass spectra (HRMS) were obtained for all compounds on an LTQ Orbitrap Discovery mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). This hybrid system combines the LTQ XL linear ion trap mass spectrometer with an Orbitrap mass analyzer. The experiments were performed using direct infusion of the sample in a solution of acetonitrile (50%), methanol (50%), and formic acid (0.1%), in positive-ion mode using electrospray ionization. Elemental composition calculations were performed using a specific tool included in the Qual Browser module of the Xcalibur (Thermo Fisher Scientific, release 2.0.7) software. Fourier transform infrared (FTIR) spectra were recorded using a universal attenuated total reflectance (UATR) attachment on a PerkinElmer Spectrum 100 spectrometer in the wavenumber range of 650-4000 cm^{-1} with a resolution of 4 cm^{-1}.

***2**. Procedure for the synthesis of L-histidine methyl ester dihydrochloride **2**.*

L-Histidine monohydrochloride monohydrate (2 mmol, 0.419 g) was reacted with a solution of thionyl chloride in CH_2Cl_2 (1 M, 10 mL) in refluxing methanol (15 mL) for 16 h. Subsequently, the solvent mixture was evaporated under reduce pressure. The solid obtained was washed with diethyl ether (3 x 15 mL). Finally, the solid was dried under reduce pressure. The product was used without further purification.

White powder; yield: 0.464 g (96%); m.p. 195-197 °C; ^{1}H NMR (60 MHz, DMSO-d_6): δ 3.36 (d, 2H, CH_2), 3.74 (s, 3H, OCH_3), 4.51 (t, 1H, CH), 7.57 (s, 1H, Im-H*), 9.14 (s, 1H, Im-H*), 10.83 (Br, 3H, NH, NH_2); IR (UATR) ν / cm^{-1} 1757 (C=O); HRMS (ESI) calcd for $C_7H_{11}N_3O_2$ + H: 170.0924, found: 170.0921 $(M + H)^{+}$; Im-H*: imidazole hydrogens.

***3**. Procedure for the synthesis of (S)-2-amino-3-(1H-imidazol-4-yl)propanehydrazide **3**.*

L-Histidine methyl ester dihydrochloride **2** (2.0 mmol, 0.338 g) was solubilized in dry methanol (10 mL). To this solution, hydrazine hydrate (4.1 mmol, 0.205 g) in dry methanol (5 mL) was added. After the addition, the reaction mixture was stirred for 16 h at 65 °C. Subsequently, the solvent was evaporated under reduce pressure and the oil obtained was dissolved with ethanol (15 mL). To this solution, 4-fluorobenzaldehyde (2.1 mmol, 0.260 g) was added and the reaction mixture was stirred for 1 h at 25 °C. The 4-(fluorobenzylidene)hydrazine was filtered off furnishing the analytically pure product **3**.

Yellow pale oil; yield: 0.271 g (80%); ^{1}H NMR (60 MHz, DMSO-d_6): δ 2.75-2.90 (m, 2H, CH_2), 3.67 (t, 1H, CH), 5.84 (Br, 6H, H-N), 6.86 (s, 1H, Im-H*), 7.58 (s, 1H, Im-H*); IR (UATR) ν / cm^{-1} 1678 (C=O); HRMS (ESI) calcd for $C_6H_{11}N_5O$ + H: 170.1036, found: 170.1028 $(M + H)^+$; Im-H*: imidazole hydrogens.

***4**. General procedure for the synthesis of hydrazones **4**.*

Benzaldehyde (1 mmol) was reacted with hydrazide **3** (1.0 mmol, 0.169 g) in refluxing ethanol (3 mL) for 4 h. After cooling to room temperature, the solvent was evaporated under reduced pressure. The obtained hydrazone was dissolved in dry CH_2Cl_2 (10 mL) and the mixture was washed with aqueous NaCl (5%, w/v, 2 x 8 mL) and saturated $NaHCO_3$ solution (2 x 8 mL). Finally, the organic layer was dried over anhydrous magnesium sulfate. The mixture was filtered, and the solvent was evaporated under reduce pressure. When necessary, the products were purified using silica gel chromatography or were recrystallized from hexane. As we sought to first evaluate any *Mt*HisD inhibitory activity by the synthesized compounds **4**, the reaction conditions were not completely optimized. In order to try improving the yields, protective groups can be used to reduce the possibility of side reactions involving other nucleophilic groups of compound **3**. All evaluated compounds were > 90% pure based on HPLC experiments. The stereochemistry of the double bond was assigned based on the observed imine proton chemical shifts. [1] It is noteworthy that some

signals were duplicated in ^{1}H NMR spectra. This effect was attributed to tautomeric equilibria between protonated and deprotonated forms as basic or acid media restored the ^{1}H NMR spectra of the compounds to one set of signals.

*3.1. (S,E)-2-amino-N'-benzylidene-3-(1H-imidazol-4-yl)propanehydrazide (**4a**)*

White powder; yield: 0.103 g (40%); m.p. 207-209 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.05-3.19 (m, 2H, CH_2), 4.16 (t, 0.5H, CH), 4.80 (t, 0.5H, CH), 6.97 (d, 1H, Im-H*), 7.43-7.44 (m, 3H, Ar), 7.63 (s, 0.5H, Im-H*), 7.67-7.68 (m, 2H, Ar), 8.09 (s, 0.5H, CH), 8.33 (s, 0.5H, CH); IR (UATR) ν / cm^{-1} 1686 (C=O); HRMS (ESI) calcd for $C_{13}H_{15}N_5O$ + H: 258.1349, found: 258.1348 $(M + H)^+$; Im-H*: imidazole hydrogens.

*3.2. (S,E)-2-amino-3-(1H-imidazol-4-yl)-N'-(4-methylbenzylidene)propanehydrazide (**4b**)*

White powder; yield: 0.171 g (63%); m.p. 236-238 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.37 (s, 1.5H, CH_3), 2.51 (s, 1.5H, CH_3), 3.03-3.20 (m, 2H, CH_2), 4.16 (t, 0.5H, CH), 4.80 (t, 0.5H, CH), 7.00 (d, 1H, Im-H*), 7.26 (d, 2H, Ar), 7.59 (d, 2H, Ar), 7.74 (d, 1H, Im-H*), 8.03 (s, 0.5H, CH), 8.26 (s, 0.5H, CH), 11.85 (br, 0.5H, NH); IR (UATR) ν / cm^{-1} 1679 (C=O); HRMS (ESI) calcd for $C_{14}H_{17}N_5O$ + H: 272.1506, found: 272.1482 $(M + H)^+$; Im-H*: imidazole hydrogens.

*3.3. (S,E)-2-amino-N'-(4-(dimethylamino)benzylidene)-3-(1H-imidazol-4-yl)propanehydrazide (**4c**)*

Yellow powder; yield: 0.183 g (61%); m.p. 218-220 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.97 (s, 6H, $N(CH_3)_2$), 3.05-3.20 (m, 2H, CH_2), 4.14 (t, 0.5H, CH), 4.76 (t, 0.5H, CH), 6.73 (d, 2H, Ar), 6.98 (d, 1H, Im-H*), 7.50 (d, 2H, Ar), 7.68 (d, 1H, Im-H*), 7.96 (s, 0.5H, CH), 8.16 (s, 0.5H, CH), 11.65 (br, 1H, NH); IR (UATR) ν / cm^{-1} 1683 (C=O); HRMS (ESI) calcd for $C_{15}H_{20}N_6O$ + H: 301.1771, found: 301.1770 $(M + H)^+$; Im-H*: imidazole hydrogens.

*3.4. (S,E)-2-amino-3-(1H-imidazol-4-yl)-N'-(4-methoxybenzylidene)propanehydrazide (**4d**)*

White powder; yield: 0.187 g (65%); m.p. 214-216 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.54-2.69 (m, 2H, CH_2), 3.30 (s, 3H, OCH_3), 3.65 (t, 0.5H, CH), 4.28 (t, 0.5H, CH), 6.46 (s,

0.5H, Im-H*), 6.50 (d, 2H, Ar), 7.12-7.15 (m, 2H, Ar), 7.19 (s, 0.5H, Im-H*), 7.53 (s, 0.5H, Im-H*), 7.76 (s, 0.5H, Im-H); IR (UATR) ν / cm^{-1} 1685 (C=O); HRMS (ESI) calcd for $C_{14}H_{17}N_5O_2$ + H: 288.1455, found: 288.1433 $(M + H)^+$; Im-H*: imidazole hydrogens.

*3.5. (S,E)-2-amino-N'-(4-(benzyloxy)benzylidene)-3-(1H-imidazol-4-yl)propanehydrazide (**4e**)*

Yellow powder; yield: 0.261 g (72%); m.p. 215-217 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.05-3.17 (m, 2H, CH_2), 4.14 (t, 0.5H, CH), 4.77 (t, 0.5H, CH), 5.15 (s, 2H, CH_2), 6.97 (d, 1H, Im-H*), 7.07-7.09 (m, 2H, Ar), 7.32-7.46 (m, 5H, Ar), 7.63-7.64 (m, 2H, Ar), 7.68 (s, 0.5H, Im-H*), 8.01 (s, 0.5H, CH), 8.24 (s, 0.5H, CH); IR (UATR) ν / cm^{-1} 1679 (C=O); HRMS (ESI) calcd for $C_{20}H_{21}N_5O_2$ + H: 364.1768, found: 364.1751 $(M + H)^+$; Im-H*: imidazole hydrogens.

*3.6. (S,E)-2-amino-N'-(2-hydroxybenzylidene)-3-(1H-imidazol-4-yl)propanehydrazide (**4f**)*

Yellow powder; yield: 0.087 g (32%); m.p. 202-204 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.07-3.18 (m, 2H, CH_2), 4.17 (t, 0.5H, CH), 4.80 (t, 0.5H, CH), 6.99 (d, 1H, Im-H*), 7.09-7.13 (m, 2H, Ar), 7.34-7.36 (m, 1H, Ar), 7.57-7.58 (m, 1H, Ar), 7.64 (d, 0.5H, Im-H*), 8.15 (s, 0.5H, CH), 8.43 (s, 0.5H, CH); IR (UATR) ν / cm^{-1} 1693 (C=O); HRMS (ESI) calcd for $C_{13}H_{15}N_5O_2$ + H: 274.1299, found: 274.1277 $(M + H)^+$; Im-H*: imidazole hydrogens.

*3.7. (S,E)-2-amino-N'-(4-fluorobenzylidene)-3-(1H-imidazol-4-yl)propanehydrazide (**4g**)*

White powder; yield: 0.138 g (50%); m.p. 221-223 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.05-3.18 (m, 2H, CH_2), 4.17 (t, 0.5H, CH), 4.80 (t, 0.5H, CH), 6.97 (d, 1H, Im-H*), 7.27-7.29 (m, 2H, Ar), 7.66 (d, 1H, Im-H*), 7.74-7.76 (m, 2H, Ar), 8.08 (s, 0.5H, CH), 8.33 (s, 0.5H, CH); IR (UATR) ν / cm^{-1} 1682 (C=O); HRMS (ESI) calcd for $C_{13}H_{14}FN_5O$ + H: 276.1255, found: 276.1254 $(M + H)^+$; Im-H*: imidazole hydrogens.

*3.8. (S,E)-2-amino-N'-(4-chlorobenzylidene)-3-(1H-imidazol-4-yl)propanehydrazide (**4h**)*

White powder; yield: 0.085 g (29%); m.p. 228-230 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.05-3.17 (m, 2H, CH_2), 4.17 (t, 0.5H, CH), 4.80 (t, 0.5H, CH), 6.97 (d, 1H, Im-H*), 7.49-7.51 (m, 2H, Ar), 7.64 (s, 0.5H, Im-H*), 7.69-7.73 (m, 2H, Ar), 8.07 (s, 0.5H, CH), 8.32 (s,

0.5H, CH); IR (UATR) ν / cm^{-1} 1680 (C=O); HRMS (ESI) calcd for $C_{13}H_{14}ClN_5O$ + H: 292.0960, found: 292.0959 $(M + H)^+$; Im-H*: imidazole hydrogens.

*3.9. (S,E)-2-amino-N'-(4-bromobenzylidene)-3-(1H-imidazol-4-yl)propanehydrazide (**4i**)*

White powder; yield: 0.202 g (60%); m.p. 228-229 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 3.08-3.17 (m, 2H, CH_2), 4.18 (t, 0.5H, CH), 4.81 (t, 0.5H, CH), 6.99 (d, 1H, Im-H*), 7.65 (s, 4H, Ar), 7.71 (s, 0.5H, Im-H*), 8.07 (s, 0.5H, CH), 8.32 (s, 0.5H, CH); IR (UATR) ν / cm^{-1} 1682 (C=O); HRMS (ESI) calcd for $C_{13}H_{14}BrN_5O$ + H: 336.0454, found: 336.0437 $(M + H)^+$; Im-H*: imidazole hydrogens.

*3.10. (S,E)-2-amino-3-(1H-imidazol-4-yl)-N'-(4-nitrobenzylidene)propanehydrazide (**4j**)*

Yellow powder; yield: 0.209 g (69%); m.p. 219-221 °C (Dec.); ^1H NMR (400 MHz, DMSO-d_6): δ 3.05-3.13 (m, 2H, CH_2), 4.20 (t, 0.5H, CH), 4.85 (t, 0.5H, CH), 6.98 (d, 1H, Im-H*), 7.66 (d, 1H, Im-H*), 7.94 (d, 2H, Ar), 8.17 (s, 0.5H, CH), 8.27 (d, 2H, Ar), 8.45 (s, 0.5H, CH); IR (UATR) ν / cm^{-1} 1680 (C=O); HRMS (ESI) calcd for $C_{13}H_{14}N_6O_3$ + H: 303.1200, found: 303.1209 $(M + H)^+$; Im-H*: imidazole hydrogens.

*3.11. (S,E)-2-amino-3-(1H-imidazol-4-yl)-N'-(naphthalen-2-ylmethylene)propanehydrazide (**4k**)*

White powder; yield: 0.105 g (34%); m.p. 121-123 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 3.15-3.26 (m, 2H, CH_2), 4.25 (t, 0.5H, CH), 4.90 (t, 0.5H, CH), 7.00 (d, 1H, Im-H*), 7.56 (s, 2H, Ar), 7.67 (d, 1H, Im-H*), 7.84 (s, 4H, Ar), 8.09-8.11 (m, 1H, Ar), 8.35 (s, 0.5H, CH), 8.61 (s, 0.5H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 25.6, 26.3, 49.1, 55.1, 115.5, 118.2, 122.5, 122.6, 126.8, 126.9, 127.4, 127.8, 128.4, 128.4, 128.5, 128.6, 129.4, 131.2, 132.8, 133.8, 133.9, 134.5, 145.8, 149.0, 163.9, 168.8; IR (UATR) ν / cm^{-1} 1682 (C=O); HRMS (ESI) calcd for $C_{17}H_{17}N_5O$ + H: 308.1506, found: 308.1492 $(M + H)^+$; Im-H*: imidazole hydrogens.

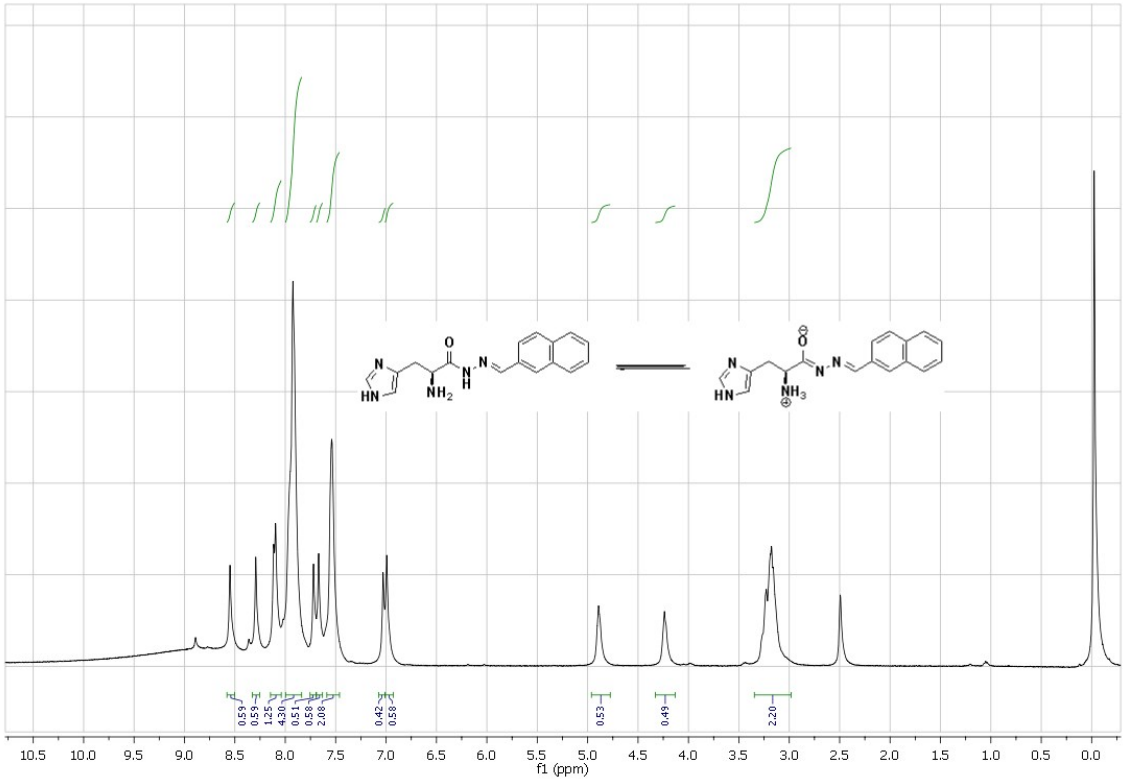

Figure ESI1. ^{1}H NMR (400 MHz, DMSO-d_6) spectra of compound **4k**.

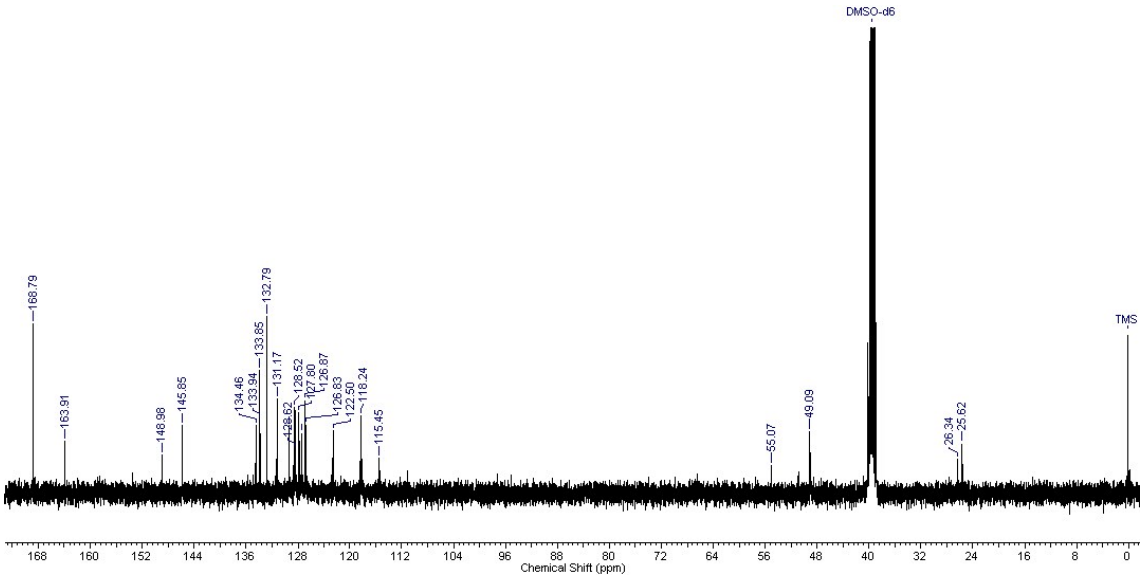

Figure ESI2. ^{13}C NMR (100 MHz, DMSO-d_6) spectra of compound **4k**.

6. Procedure for the IC_{50} measures

Briefly, the synthesized compounds (dissolved in DMSO) were added to the reaction mixture containing *Mt*HisD at concentrations ranging from 0 to 120 µM. The concentration of the substrates (L-Hol and NAD^+) was fixed in K_M values[2] in the presence of 3% DMSO (this concentration did not alter enzyme activity). The reactions were carried out in 50 mM PIPES pH 7.2 at 25 °C and measures were accomplished in spectrophotometer at 340 nm. IC_{50} values are defined as concentration of inhibitor required to reduce in 50% the initial enzyme activity. IC_{50} curves are showed in **Figure ESI3** and **Figure ESI4**.

References:

1. E. Pretsch, P. Bühlmann, C. Affolter, *Structure determination of organic compounds: tables of spectral data*, Springer: **2000**.
2. J. E. S. Nunes, R. G. Ducati, A. Breda, L. A. Rosado, B. M. de Souza, M. S. Palma, D. S. Santos, L. A. Basso, *Arch. Biochem. Biophys*.,2011, **512**, 143-153.

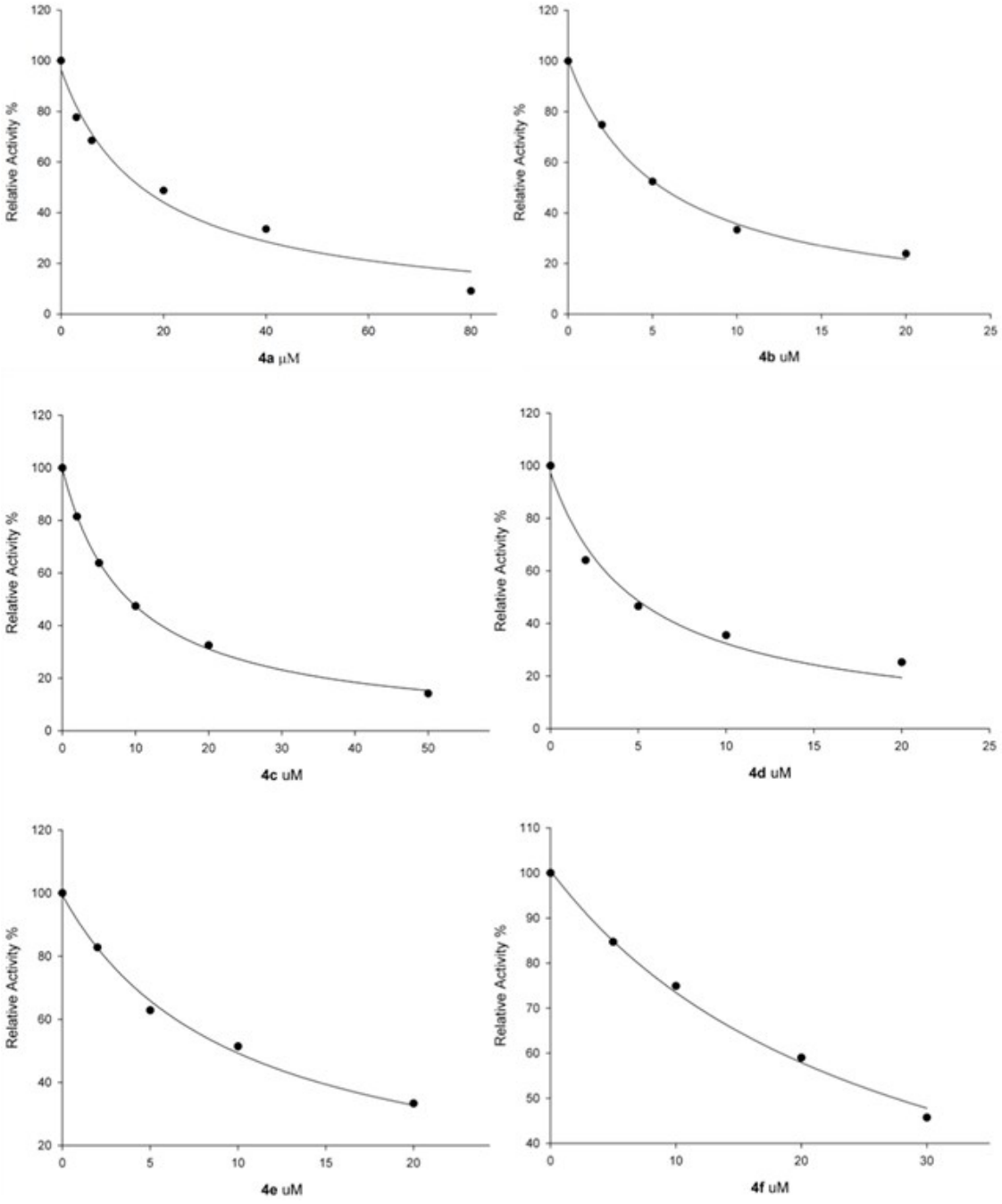

Figure ESI3. IC_{50} determination for the compounds **4a** - **f**.

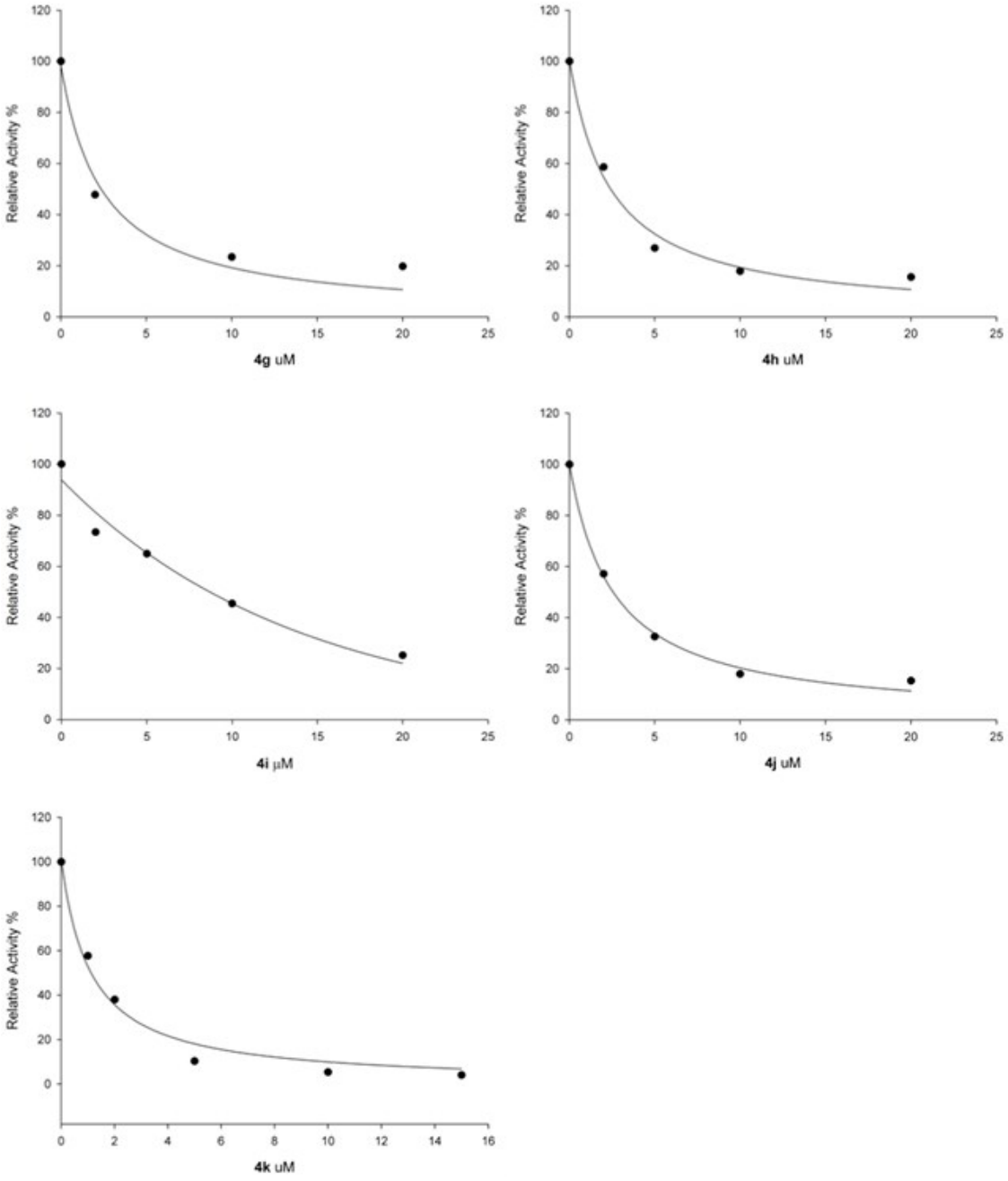

Figure ESI4. IC_{50} determination for the compounds **4g** - **k**.