Screening and phenotypical characterization of *Schistosoma mansoni* histone deacetylase 8 (*Sm*HDAC8) inhibitors as multi-stage antischistosomal agents

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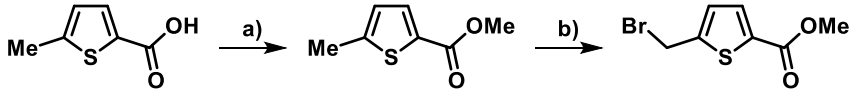
Scheme S1: Synthesis of S3



S1 **S2** **S3**

Reagents and conditions: a) $SOCl_2$, MeOH, 0 to 25 °C, 12 h; b) NBS, AIBN, CCl_4, 80 °C, 3 h.

Figure S1: Dose response curves of the indicated compounds on schistosomula. The y-axis indicates the percentage of ATP reduction (death of schistosomula) normalized against DMSO (0%) and gambogic acid 50 µM (100%). Each point represents the average and SEM values.

Figure S2: Confocal microscopy of carmine red-stained adult worm couples treated with compound **7** (10 µM) and **10** (20 µM). The images are representative of three-five worm pairs. The ootype, ovary, vitellarium and testis are shown. Abbreviations stand for: io, immature oocytes; mo, mature oocytes; ut, uterus; ot, ootype; sv, seminal vesicle; vt, vitellarium; star: egg; red arrows: cell degeneration. Scale-bar: 50 µm.

Figure S3: Confocal microscopy of carmine red-stained adult worm couples treated with DMSO and compound **9**, at 20 µM (9. a) and 30 µM (9. b) concentrations. The images are representative of three-five worm pairs. The ootype, ovary, vitellarium, testis and parenchyma are shown. Abbreviations stand for: io, immature oocytes; mo, mature oocytes; ut, uterus; ot, ootype; sv, seminal vesicle; vt, vitellarium; star: egg; red arrow: cell degeneration; red triangle: unstained, hole-like areas within vitellarium; asterisk: sperm cells; white arrows: unstained, hole-like areas buried into parenchyma. Scale-bar: 50 µm.

Molecular Docking of compounds 7 and 8

As explained in the main text for every compound the potential coordination bond is established by the carbonyl moiety of the hydroxamic portion of the molecule through a monodentate coordination.

R-enantiomer of compound **7** (Figure S4, panel A) was differently accommodated with respect to the corresponding *S*-enantiomer. In particular, the *R*-enantiomer can form two different π-π stackings with the central phenyl ring close to the metal binding group (MBG) with F151 and H188, and, differently from *R*-enantiomer of compound **6**, it formed only one additional stacking, established by the chlorophenyl with H292. In terms of polar contacts *R*-enantiomer of compound **7** can form an H-bond with G150, as depicted in Figure S1 and a salt bridge through the protonated methylpiperidine with D100. The main difference in the binding mode of the *S*-enantiomer of compound **7** (Figure S4, panel B) is represented by the different accommodation of the cap group in the binding site, this led to the lack of the stackings established by the two phenyl rings present in the cap, and the formation of a new cation-π stacking with H292 by the

protonated N atom of the piperidine ring, while the central portion of the molecule maintains the same contacts previously described.

For compound **8** the two enantiomers were similarly accommodated into the binding site. The MBG of the *R*-enantiomer can form, by the NH and OH portions, two different H-bonds with G150 (Figure S4 panel C), while the *S*-enantiomer (Figure S4 panel D) was able to form only an H-bond by the NH moiety. Differently from compound **10** with a central thiophene ring, (Figure 6 of the main text) the central benzene ring determined a different accommodation of the whole molecule. Because of that, the benzene ring cannot establish the two π-π stackings with F151 and H188, while for *R*-enantiomer the phenyl ring of the cap group can establish a π-π stacking with Y341 and a cation- π stacking with K20 (Figure S4 panel C). The *S*-enantiomer lacks the stacking with Y341, due to its different conformation with respect to *R*-enantiomer (Figure S4 panel D). The chlorine substituent of the phenyl ring of both enantiomers resulted more exposed to the solvent, due to its bigger size, compared to the fluorine of compound **9**. The exposure to the solvent of the two different halogen atoms of compound **8** and **9** represented the main difference between the mentioned compounds. In fact, the remaining part of the compound **9** established the same contacts for both *R*- and *S*-enantiomers as described for compound **8** (Figure S4 panels E and F).

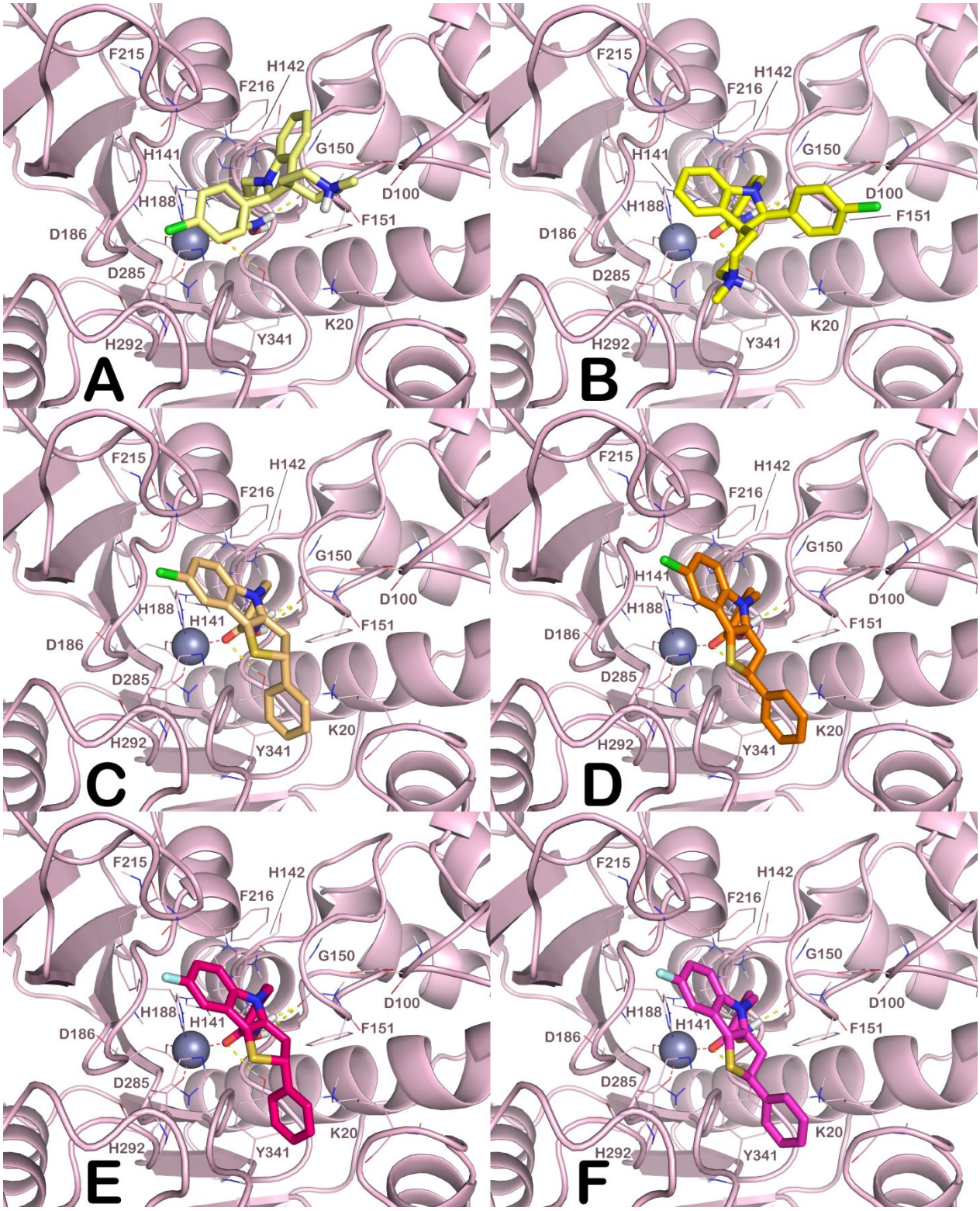


Figure S4. Docked poses for compounds **7** (*R*-enantiomer pose represented as pale yellow sticks, panel A; *S*-enantiomer pose represented as yellow sticks, panel B); **8** (*R*-enantiomer pose represented as wheat sticks, panel C; *S*-enantiomer pose represented as orange sticks, panel D) and **9** (*R*-enantiomer pose represented as raspberry sticks, panel E; *S*-enantiomer pose represented as magenta sticks, panel F) into *Sm*HDAC8 enzyme (light pink cartoon PDB ID: 4BZ7). The residues of the active site are represented as line and the Zn^{2+} is represented as a gray sphere. H-bonds are represented as yellow dotted lines, while red and blue dotted lines represent the metal coordination bonds. Figures were prepared using PyMOL (The PyMOL Molecular Graphics System, v1.8.4.0, Schrödinger LLC, New York, 2015).

Chemistry

4-Methyl-*N*'-(1-methylpiperidin-4-ylidene)benzenesulfonohydrazide (14). 1-Methyl-4-piperidone **2** (1.09 mL, 8.83 mmol) was added to a solution of tosylhydrazide **13** (1.64 g, 8.83 mmol) in MeOH (41 mL). The reaction mixture was stirred at 25 °C until complete conversion was observed by TLC (MeOH/ DCM 1:10). Solvent was removed *in vacuo* to give the title compound **14** as a white solid (quantitative yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.1 Hz, 2H), 7.26 (d, *J* = 7.9 Hz, 2H), 3.42 (s, 1H), 2.68 (t, *J* = 6.1, 1H), 2.51 – 2.29 (m, 10H), 2.25 (s, 3H); ESI MS *m/z*: $[M+H]^+$ 282.

(3-Chlorophenyl)(1-methylpiperidin-4-yl)methanone (15a). Tosylhydrazone **14** (1.00 g, 3.56 mmol) and Cs_2CO_3 (1.74 g, 5.34 mmol) were placed in a sealed tube. The tube was filled with nitrogen, the before addition of 1,4-dioxane (14 mL) under nitrogen atmosphere, followed by the addition of 3-chlorobenzaldehyde (0.50 g, 3.56 mmol). The tube was heated at 110 °C for 12 h. After this, the mixture was cooled down to 25 °C, quenched with a saturated solution of NH_4Cl (20 mL) and extracted with DCM (3 × 20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo* to give a residue that was purified by flash column chromatography on silica gel (MeOH/ DCM 1:15) to give title compound **15a** as white solid. (yield 75%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 – 7.69 (m, 2H), 7.44 (s, 1H), 7.27 – 7.17 (m, 1H), 3.12 (m, 1H), 2.88 (m, 2H), 2.40 (t, *J* = 5.0 Hz, 2H), 2.25 (s, 3H), 1.88 – 1.70 (m, 4H); ESI MS *m/z*: $[M+H]^+$ 238.

(4-Chlorophenyl)(1-methylpiperidin-4-yl)methanone (15b). Starting from **14** (1.0 g, 3.56 mmol) and 4-chlorobenzaldehyde (0.50 g, 3.56 mmol) compound **15b** was obtained following the procedure described for the preparation of **15a**. Purification by column chromatography on silica gel (MeOH/DCM 1:15) afforded the title compound **15b** as a white solid (yield 51%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, *J* = 8.6 Hz, 2H), 7.43 (d, *J* = 8.8 Hz, 2H), 3.19 – 3.10 (m, 1H), 2.96 – 2.94 (m, 2H), 2.32 (s, 3H), 2.17 – 2.03 (m, 2H), 1.91 – 1.80 (m, 4H); ESI MS *m/z*: $[M+H]^+$ 239.

2-(3-Chlorophenyl)-1'-methylspiro[indoline-3,4'-piperidine] (16a). Phenylhydrazine (197 µL, 2.0 mmol) and **15a** (0.48 g, 2.0 mmol) were dissolved in 1,4-dioxane (8 mL) and cooled to 0 °C. Concentrated sulfuric acid (9.5 mL) was added dropwise to the reaction at 0 °C. The reaction was then heated at 60 °C for 2 h, then it was then cooled to 25 °C and the pH was adjusted to approximately 12 by the addition of saturated aqueous $NaHCO_3$ solution followed by small portions of solid NaOH. The organic products were extracted with DCM (3 x 20 mL) and the combined organic layers were washed with brine (15 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography on silica gel (MeOH/DCM 1:20) afforded 2-(3-chlorophenyl)-1'-methylspiro[indole-3,4'-piperidine] as a yellow solid (yield 30%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.97 (d, *J* = 7.1 Hz, 1H), 7.85 (d, *J* = 7.7 Hz, 1H), 7.74 (d, *J* = 7.7 Hz, 1H), 7.50 – 7.36 (m, 3H), 7.30 – 7.20 (m, 1H), 3.00 – 2.92 (m, 2H), 2.81 – 2.59 (m, 4H), 2.51 (s, 3H), 1.50 – 1.42 (m, 2H); ESI MS *m/z*: $[M+H]^+$ 311. To a solution of the above compound (0.10 g, 0.33 mmol) compound in MeOH (5 mL), $NaBH_4$ (0.06 g, 1.65 mmol) was added. The reaction was kept stirring for 12 h at 50 °C. Then it was quenched with a saturated solution of $NaHCO_3$ (10 mL) and MeOH was removed under reduced pressure. The residue was dissolved in EtOAc and washed with water (3 × 10 mL). The organic phase was dried over Na_2SO_4 and solvent was removed *in vacuo* to give a crude, which was purified by flash column chromatography on silica gel (MeOH/DCM 1:20) affording the title compound **16a** in quantitative yield without any further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 – 7.17 (m, 5H), 7.10 (t, *J* = 8.4 Hz, 1H), 6.77 (t, *J* = 7.4 Hz, 1H), 6.68 (d, *J* = 7.8 Hz, 1H), 4.56 (s, 1H), 4.09 (br, 1H), 2.80 – 2.71 (m, 1H), 2.67 – 2.52 (m, 1H), 2.51 – 2.36 (m, 1H), 2.31 (s, 3H), 2.19 – 1.85 (m, 4H), 1.83 – 1.65 (m, 1H); ESI MS *m/z*: $[M+H]^+$ 313.

2-(4-Chlorophenyl)-1'-methylspiro[indoline-3,4'-piperidine] (16b).

Starting from **15b** (0.11 g, 0.35 mmol) the title compound **16b** was obtained following the procedure described for the preparation of **16a**, to give a crude which was purified by flash column chromatography on silica gel (MeOH/DCM 1:20) affording the title compound **16b** (yield

40%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 – 7.17 (m, 5H), 7.11 (t, *J* = 7.6 Hz, 1H), 6.78 (t, *J* = 7.5 Hz, 1H), 6.68 (d, *J* = 7.7 Hz, 1H), 4.07 (s, 1H), 2.82 (br, 1H), 2.65 – 2.52 (m, 2H), 2.35 (s, 3H), 2.19 – 1.90 (m, 4H), 1.41 (t, *J* = 9.8 Hz, 2H); ESI MS *m/z*: $[M+H]^+$ 313.

4-((2-(3-Chlorophenyl)-1'-methylspiro[indoline-3,4'-piperidin]-1-yl)methyl)-*N*-hydroxybenzamide (6). To a solution of **16a** (0.100 g, 0.32 mmol) and methyl 4-formylbenzoate (0.05 g, 0.32 mmol) in EtOH (5 mL), acetic acid (0.5 mL) and $NaBH_3CN$ (0.04 g, 0.64 mmol) were added. The reaction was then heated to 70 °C for 2 h and stirred for additional 12 h at 25 °C. The mixture was then treated with a saturated solution of $NaHCO_3$ (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over Na_2SO_4 and solvents removed *in vacuo* to give a crude, which was purified by flash column chromatography on silica gel to afford methyl 4-((2-(3-chlorophenyl)-1'-methylspiro[indoline-3,4'-piperidin]-1-yl)methyl)benzoate (MeOH/DCM 1:20) (yield 32%); ^{1}H NMR (300 MHz, CD_3OD) δ 7.94 (d, *J* = 7.5 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.32 – 7.17 (m, 4H), 7.14 – 7.00 (m, 2H), 6.73 (t, *J* = 7.5 Hz, 1H), 6.50 – 6.35 (m, 1H), 4.47 (s, 1H), 4.40 (d, *J* = 16.3 Hz, 1H), 3.93 (d, *J* = 16.8 Hz, 1H), 3.88 (s, 3H), 2.96 – 2.81 (m, 1H), 2.73 – 2.52 (m, 2H), 2.34 (s, 3H), 2.18 – 1.98 (m, 2H), 1.97 – 1.82 (m, 2H), 1.39 (d, *J* = 30.0 Hz, 1H); ESI MS *m/z*: $[M+H]^+$ 461. To a solution of the above compound (0.037 g, 0.08 mmol) in a mixture of DCM (2 mL) and MeOH (1 mL), a 50% solution of NH_2OH (0.53 mL, 8.00 mmol) in water and a 4 N solution of KOH in methanol (1.00 mL, 4.00 mmol) were added. Reaction was stirred at 25 °C for 4 h, then pH was adjusted to 7 with a 1 M solution of HCl and the solvent was removed under reduced pressure. Purification of the crude by column chromatography on silica gel (NH_4OH/MeOH/DCM 0.1:1:10) afforded the title compound in 57% yield. ^{1}H NMR (300 MHz, DMSO) δ 11.15 (br, 1H), 8.99 (br, 1H), 7.67 (d, *J* = 8.2 Hz, 2H), 7.34 (s, 1H), 7.29 (d, *J* = 6.5 Hz, 2H), 7.20 (d, *J* = 7.4 Hz, 2H), 7.00 (t, *J* = 7.6 Hz, 2H), 6.64 (t, *J* = 8.1 Hz, 1H), 6.43 – 6.22 (m, 2H), 4.54 (s, 1H), 4.32 (d, *J* = 14.0 Hz, 1H), 3.86 (d, *J* = 16.3 Hz, 1H), 2.74 – 2.58 (m, 1H), 2.49 – 2.32 (m, 1H), 2.17 (s, 3H), 2.00 – 1.82 (m, 2H), 1.79 – 1.57 (m, 2H), 1.29 – 1.09 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 164.9, 149.5, 139.6, 138.3, 137.7, 132.9, 130.6, 128.9, 128.5, 127.8, 127.7, 126.5, 124.2, 121.3, 110.3, 84.4, 52.1, 51.3, 50.6, 46.0, 34.7; ESI MS *m/z*: $[M+H]^+$ 462; HRMS (ESI) *m/z* calcd for $C_{27}H_{29}ClN_3O_2$: 462.1943; found, 462.1944 (M + 1).

4-((2-(4-Chlorophenyl)-1'-methylspiro[indoline-3,4'-piperidin]-1-yl)methyl)-*N*-hydroxybenzamide (7). Methyl 4-((2-(4-chlorophenyl)-1'-methylspiro[indoline-3,4'-piperidin]-1-yl)methyl)benzoate was prepared starting from **16b** (0.04 g, 0.13 mmol) as described above in 35% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.0 Hz, 2H), 7.34 – 7.20 (m, 5H), 7.10 (t, *J* = 7.6 Hz, 3H), 6.75 (t, *J* = 7.4 Hz, 1H), 6.38 (d, *J* = 7.8 Hz, 1H), 4.43 – 4.31 (m, 2H), 3.91 (s, 3H), 3.85 (d, *J* = 16.1 Hz, 1H), 2.89 (q, *J* = 5.8, 5.4 Hz, 2H), 2.68 – 2.54 (m, 2H), 2.38 (s, 3H), 2.18 – 1.91 (m, 2H), 1.58 – 1.39 (m, 2H); ESI MS *m/z*: $[M+H]^+$ 461. Starting from the above methyl ester (0.03 g, 0.06 mmol), the title compound **7** was prepared as described for the preparation of **6** in 33% yield. ^{1}H NMR (300 MHz, DMSO) δ 11.13 (s, 1H), 8.98 (s, 1H), 7.67 (d, *J* = 8.3 Hz, 2H), 7.44 – 7.30 (m, 2H), 7.28 (d, *J* = 8.3 Hz, 2H), 7.19 (d, *J* = 7.2 Hz, 2H), 6.99 (t, *J* = 7.7 Hz, 1H), 6.63 (t, *J* = 6.9 Hz, 1H), 6.34 (d, *J* = 6.9 Hz, 2H), 4.51 (s, 1H), 4.31 (d, *J* = 16.3 Hz, 1H), 3.83 (d, *J* = 16.3 Hz, 1H), 2.68 – 2.53 (m, 2H), 2.14 (s, 3H), 1.76 – 1.63 (m, 4H), 1.22 – 1.08 (m, 2H); ^{13}C NMR (75 MHz, DMSO) δ 164.7, 151.1, 142.3, 137.2, 136.9, 133.0, 132.2, 129.0, 128.4, 127.8, 127.7, 124.1, 118.1, 106.8, 76.2, 52.4, 52.1, 49.9, 49.3, 46.6, 46.2, 37.6, 31.6, 22.1, 15.2.ESI MS *m/z*: $[M+H]^+$ 462; HRMS (ESI) *m/z* calcd for $C_{27}H_{29}ClN_3O_2$: 462.1943; found, 462.1945 (M + 1).

5-Phenyldihydrothiophen-3(2*H*)-one (18). To a stirred solution of mercaptoacetic acid (3.0 g, 20.27 mmol) and cinnamic acid **17** (1.86 g, 20.27 mmol) in 1,4-dioxane (7 mL) triethylamine (2.5 g, 25,33 mmol) (7 mL) was added dropwise. The mixture was heated under reflux for 8 h. The mixture was cooled down to 25 °C, poured on ice and acidified with 1 N HCl (10 mL). The mixture was extracted with diethyl ether (3x20 mL) and the combined organic layers were washed with brine (15 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The resulting 3-

((carboxymethyl)thio)-3-phenylpropanoic acid was obtained without any further purification (quantitative yield); ^{1}H NMR (400 MHz, DMSO) δ 7.39 – 7.21 (m, 5H), 4.32 (m, 1H), 3.52 (s, 2H), 3.11 (d, *J* = 15.1 Hz, 1H), 2.91 (d, *J* = 15.1 Hz, 1H); ESI MS *m/z*: $[M+H]^+$ 241. A mixture of the above compound (3.0 g, 12,5 mmol), acetic anhydride (10 ml) and sodium acetate (0.64 g) was heated under reflux for 12 h. Then the reaction was cooled town to 25 °C and poured on ice containing H_2SO_4 (5 mL). The mixture was treated with a saturated solution of $NaHCO_3$ (20 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over Na_2SO_4 and solvent was removed *in vacuo* to give a crude which was purified by flash column chromatography on silica gel (EtOAc/ *n*-hexane 1:10) to afford **18** in yield 50%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 – 7.21 (m, 5H), 4.62 (dd, *J* = 9.0, 6.8 Hz, 1H), 3.47 (s, 2H), 3.00 (dd, *J* = 17.6, 6.7 Hz, 1H), 2.80 (dd, *J* = 17.6, 9.2 Hz, 1H); ESI MS *m/z*: $[M+H]^+$ 179

7-Chloro-2-phenyl-3,4-dihydro-2*H*-thieno[3,2-*b*]indole (19a). To a solution of **18** (1.0 g, 5.62 mmol) in EtOH (38 mL), H_2SO_4 (1 mL) and 4-chlorophenylhydrazine hydrochloride (1.3 g, 7.3 mmol) were added. The mixture was subjected to MW (150 W) irradiation (90 °C, 29 psi) for 12 min. The progress of the reaction was monitored by thin-layer chromatography. After completion of the reaction, the reaction mixture was poured on ice. pH was adjusted to 9 with a saturated solution of $NaHCO_3$ and the crude was extracted with DCM (3 × 20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo* to give a residue which was purified by flash column chromatography on silica gel (PetEt/EtOAc 6:1) to afford the title compound **19a** in 74% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (br, 1H), 7.48 (d, *J* = 6.3 Hz, 1H), 7.39 – 7.21 (m, 5H), 7.15 – 6.96 (m, 2H), 5.53 (t, *J* = 8.5 Hz, 1H), 3.56 (dd, *J* = 15.6, 8.6 Hz, 1H), 3.38 (dd, *J* = 15.6, 8.4 Hz, 1H); ESI MS *m/z*: $[M+H]^+$ 286

7-Fluoro-2-phenyl-3,4-dihydro-2*H*-thieno[3,2-*b*]indole (19b). Starting from **18** (0.06 g, 0.28 mmol) and 4-fluorophenylhydrazine hydrochloride (0.04 g, 0.37 mmol) compound **19b** was obtained following the procedure described for the preparation of **19a**. Purification by column chromatography on silica gel (EtOAc/ *n*-hexane 1:5) afforded the title compound in 47% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (br, 1H), 7.48 (d, *J* = 7.7 Hz, 2H), 7.41 – 7.15 (m, 4H), 7.03 (d, *J* = 9.4 Hz, 1H), 6.94 – 6.77 (m, 1H), 5.52 (t, *J* = 8.5 Hz, 1H), 3.53 (dd, *J* = 15.6, 8.5 Hz, 1H), 3.36 (dd, *J* = 15.6, 8.4 Hz, 1H); ESI MS *m/z*: $[M+H]^+$ 269.

Methyl 4-((7-chloro-2-phenyl-2,3-dihydro-4*H*-thieno[3,2-*b*]indol-4-yl)methyl)benzoate (20a). Compound **19a** (0.50 g, 1.74 mmol) was placed under argon atmosphere and dissolved in 2 mL of anhydrous DMF. Potassium *tert*-butoxide (0.21 g, 1.91 mmol) was dissolved in 2 mL of anhydrous DMF and added slowly to the reaction at 25 °C. The reaction turned from orange to dark brown. After 15 min, methyl 4-(bromomethyl)benzoate (0.40 g, 1.74 mmol) and a catalytic amount of potassium iodide were added to the reaction at 25 °C. The reaction was heated at 80 °C for 2 h, and at 25 °C for 12 h. Then it was diluted with 30 mL of EtOAc and 30 mL of water. The crude was extracted with EtOAc (2 x 10 mL) and the combined organic layers were washed with water (2 x 20 mL), brine (15 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography on silica gel (EtOAc/PetEt 1:9) afforded the title compound **20a** in 18% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.0 Hz, 2H), 7.50 – 7.36 (m, 3H), 7.36 – 7.25 (m, 3H), 7.14 – 6.97 (m, 4H), 5.49 (t, *J* = 8.4 Hz, 1H), 5.22 (s, 2H), 3.90 (s, 3H), 3.41 (dd, *J* = 15.6, 8.5 Hz, 1H), 3.23 (dd, *J* = 15.6, 8.3 Hz, 1H); ESI MS *m/z*: $[M+H]^+$ 434.

Methyl 4-((7-fluoro-2-phenyl-2,3-dihydro-4*H*-thieno[3,2-*b*]indol-4-yl)methyl)benzoate (20b). Compound **20b** was obtained from **19b** (0.50 g, 1.84 mmol) following the procedure described for the preparation of **20a**. Purification by column chromatography on silica gel (EtOAc/PetEt 1:9) afforded the title compound **20b** (yield 21%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.1 Hz, 2H), 7.44 (d, *J* = 6.1 Hz, 2H), 7.38 – 7.16 (m, 3H), 7.16 – 6.96 (m, 4H), 6.92 – 6.81 (m, 1H), 5.49 (t, *J* = 8.4

Hz, 1H), 5.27 (s, 2H), 3.90 (s, 3H), 3.44 (dd, *J* = 15.6, 8.5 Hz, 1H), 3.25 (dd, *J* = 15.6, 8.3 Hz, 1H); ESI MS *m/z*: $[M+H]^+$ 418.

Methyl 5-((7-fluoro-2-phenyl-2,3-dihydro-4*H*-thieno[3,2-*b*]indol-4-yl)methyl)thiophene -2-carboxylate (21)

Compound **21** was obtained from **19b** (0.07 g, 0.26 mmol) and methyl 5-(bromomethyl)thiophene-2-carboxylate (0.07 g, 0.31 mmol) following the procedure described for the preparation of **20a**. Purification by column chromatography on silica gel (EtOAc/PetEt 1:5) afforded the title compound **21** (yield 20%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (dd, *J* = 3.8, 0.7 Hz, 1H), 7.54 – 7.40 (m, 2H), 7.40 – 7.23 (m, 3H), 7.23 – 7.12 (m, 1H), 7.05 (dd, *J* = 9.3, 2.5 Hz, 1H), 6.99 – 6.85 (m, 1H), 6.85 – 6.78 (m, 1H), 5.51 (t, *J* = 8.4 Hz, 1H), 5.34 (s, 2H), 3.83 (s, 3H), 3.63 – 3.44 (m, 1H), 3.44 – 3.21 (m, 1H).

4-((7-Chloro-2-phenyl-2,3-dihydro-4*H*-thieno[3,2-*b*]indol-4-yl)methyl)-*N*-hydroxybenzamide (8). Compound **8** was obtained from **20a** (0.070 g, 0.16 mmol) following the procedure described for the preparation of **6**. Purification by column chromatography on silica gel (MeOH/DCM 1:30) afforded the title compound (yield 93%); ^{1}H NMR (300 MHz, DMSO) δ 11.13 (br, 1H), 8.99 (br, 1H), 7.66 (d, *J* = 8.2 Hz, 2H), 7.51 – 7.39 (m, 3H), 7.35 – 7.24 (m, 4H), 7.17 (d, *J* = 8.1 Hz, 2H), 7.06 (dd, *J* = 8.8, 2.1 Hz, 1H), 5.60 (t, *J* = 8.2 Hz, 1H), 5.44 (s, 2H), 3.60 (dd, *J* = 16.0, 8.4 Hz, 1H), 3.30 (s, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 166.5, 142.1, 141.6, 139.5, 139.0, 131.7, 130.1, 129.0, 128.4, 127.6, 127.4, 127.0, 126.6, 125.3, 123.8, 121.0, 117.5, 111.4, 110.6, 58.6, 36.0, 29.5; ESI MS *m/z*: $[M+H]^+$ 435; HRMS (ESI) *m/z* calcd for $C_{24}H_{20}ClN_2O_2S$: 435.0929; found, 435.0932 (M + 1)

4-((7-Fluoro-2-phenyl-2,3-dihydro-4*H*-thieno[3,2-*b*]indol-4-yl)methyl)-*N*-hydroxybenzamide (9). Compound **9** was obtained from **20b** (0.10 g, 0.21 mmol) following the procedure described for the preparation of **6**. Purification by column chromatography on silica gel (MeOH/DCM 1:30) afforded the title compound (yield 90%); ^{1}H NMR (300 MHz, DMSO) δ 11.14 (br, 1H), 9.00 (br, 1H), 7.66 (d, *J* = 7.8 Hz, 2H), 7.50 – 7.38 (m, 3H), 7.29 (q, *J* = 7.2, 6.7 Hz, 3H), 7.18 (d, *J* = 7.9 Hz, 2H), 7.13 – 7.02 (m, 1H), 6.97 – 6.83 (m, 1H), 5.59 (t, *J* = 8.0 Hz, 1H), 5.43 (s, 2H), 3.60 (dd, *J* = 16.0, 8.3 Hz, 1H), 3.33 (dd, *J* = 16.0, 8.3 Hz, 1H); ^{13}C NMR (75 MHz, DMSO) δ 164.5, 159.3, 156.2, 142.6, 141.5, 141.1, 137.3, 132.7, 129.2, 128.3, 127.9, 127.8, 127.5, 122.7, 112.6, 109.9, 109.5, 109.2, 103.9, 58.4, 48.4, 36.1; ^{19}F NMR (282 MHz, DMSO) δ -124.2; ESI MS *m/z*: $[M+H]^+$ 419; HRMS (ESI) *m/z* calcd for $C_{24}H_{20}FN_2O_2S$: 419.1224; found, 419.1222 (M + 1)

5-((7-Fluoro-2-phenyl-2,3-dihydro-4*H*-thieno[3,2-*b*]indol-4-yl)methyl)-*N*-hydroxythiophene-2-carboxamide (10). Compound **10** was obtained from **21** (0.02 g, 0.04 mmol) following the procedure described for the preparation of **6**. Purification by column chromatography on silica gel (NH_4OH/MeOH/DCM 0.1:1:10) afforded the title compound (yield 30%); ^{1}H NMR (300 MHz, Methanol-d_6) δ 7.53 – 7.35 (m, 4H), 7.35 – 7.17 (m, 3H), 7.00 – 6.77 (m, 3H), 5.61 – 5.42 (m, 3H), 3.72 – 3.51 (m, 1H), 3.44 – 3.25 (m, 1H). HRMS (ESI) *m/z* calcd for $C_{22}H_{18}FN_2O_2S_2$: 425.0788; found, 425.0787 (M + 1)

4-Methyl-*N*'-(propan-2-ylidene)benzenesulfonohydrazide (22). Starting from acetone (1.26 mL, 17.22 mmol) and **13** the title compound **22** was obtained following the procedure described for the preparation of **14** (quantitative yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (d, *J* = 8.3 Hz, 2H), 7.60 (s, 1H), 7.30 (d, *J* = 8.5 Hz, 2H), 2.42 (s, 3H), 1.91 (s, 3H), 1.78 (s, 3H); ESI MS *m/z*: $[M+H]^+$ 227.

2-Methyl-1-(pyridin-3-yl)propan-1-one (23). Starting from **22** (0.51 g, 2.27 mmol) and 3-pyridinecarboxaldehyde (0.21 mL , 2.27 mmol) the title compound **23** was obtained following the procedure described for the preparation of **15a**. Purification by column chromatography on silica gel (EtOAc/PetEt 1:2) afforded the title compound (yield 89%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.11 (s, 1H), 8.72 (d, *J* = 4.8 Hz, 1H), 8.23 – 8.13 (m, 1H), 7.44 – 7.33 (m, 1H), 3.60 – 3.36 (m, 1H), 1.20 (s, 3H), 1.18 (s, 3H); ESI MS *m/z*: $[M+H]^+$ 150.

5-Methoxy-3,3-dimethyl-2-(pyridin-3-yl)indoline (24). Starting from **23** (0.50 g, 3.36 mmol**)** and 4-methoxyphenylhydrazine (0.58, 3.36 mmol) the title compound **24** was obtained following the procedure described for the preparation of **16a**. Purification of the crude by column chromatography on silica gel (MeOH/DCM 1:30) afforded the title compound **16** in 32% yield over two steps; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.56 (d, *J* = 5.5 Hz, 2H), 7.85 (d, *J* = 7.9 Hz, 1H), 7.44 – 7.31 (m, 1H), 6.90 – 6.80 (m, 1H), 6.73 – 6.52 (m, 2H), 4.59 (s, 1H), 3.87 (s, 3H), 1.42 (s, 3H), 0.74 (s, 3H); **ESI MS** *m/z*: $[M+H]^+$ 255.

***N*-Hydroxy-4-((5-methoxy-3,3-dimethyl-2-(pyridin-3-yl)indolin-1-yl)methyl)benzamide (11).** Compound **11** was obtained from **24** following the procedure described for the preparation of **6**. Purification by column chromatography on silica gel (NH_4OH/MeOH/DCM 0.1:1:10) afforded the title compound in 11% yield over two steps; ^{1}H NMR (300 MHz, DMSO) δ 11.12 (br, 1H), 8.98 (br, 1H), 8.61 – 8.44 (m, 2H), 7.67 (d, *J* = 8.1 Hz, 2H), 7.43 – 7.33 (m, 1H), 7.32 (d, *J* = 8.1 Hz, 2H), 6.75 (d, *J* = 2.5 Hz, 1H), 6.62 – 6.51 (m, 2H), 6.30 (d, *J* = 8.4 Hz, 1H), 4.32 – 4.21 (m, 2H), 3.90 (d, *J* = 16.3 Hz, 1H), 3.64 (s, 3H), 1.30 (s, 3H), 0.71 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 166.7, 154.4, 148.9, 148.3, 144.6, 142.7, 139.6, 137.2, 134.5, 131.1, 127.6 (2C), 127.1 (2C), 123.8, 112.0, 109.4, 109.2, 78.6, 55.1, 52.3, 44.7, 25.2, 24.3; ESI MS *m/z*: $[M+H]^+$ 404; HRMS (ESI) *m/z* calcd for $C_{24}H_{26}N_3O_3$: 404.1969; found, 404.1970 (M + 1).

Methyl 5-methylthiophene-2-carboxylate (S2)

To a solution of 5-methyl-2-thiophenecarboxylic acid (1.0 g, 7.0 mmol) in methanol (15 mL), $SOCl_2$ (2 mL) was added dropwise at 0 °C. The reaction was allowed to reach 25 °C and then it was left stirring for 12 h at the same temperature. The solvent was removed *in vacuo*, the residue was dissolved in EtOAc and washed with a saturated solution of $NaHCO_3$. The title compound **S2**was obtained without any of further purification (quantitative yield). ^{1}H NMR (300 MHz $CDCl_3$) δ 7.60 (d, *J* = 3.7 Hz, 1H), 6.76 (d, *J* = 3.7 Hz, 1H), 3.85 (s, 3H), 2.51 (s, 3H). ESI MS *m/z*: $[M+H]^+$ 157.

Methyl 5-(bromomethyl)thiophene-2-carboxylate (S3)

To a solution of **S2** (0.57 g, 3.65 mmol) in CCl_4 (8 mL), NBS (0.59 g, 3.29 mmol) and AIBN (0.06 g, 0.37 mmol) were added and reaction mixture was stirred at 80 °C for 4 h. The solvent was removed under reduced pressure, the residue was diluted with DCM and purified by column chromatography on silica gel (EtOAc/*n*-hexane 1:30) giving **S3** (yield 60%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (d, *J* = 3.8 Hz, 1H), 7.08 (d, *J* = 3.8 Hz, 1H), 4.66 (s, 2H), 3.87 (s, 3H); ESI MS *m/z*: $[M+H]^+$ 235.