Exploring the anti-cancer activity of novel thiosemicarbazones generated through the combination of retro-fragments: Dissection of critical structure-activity relationships

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S1 Chemistry

General reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA), ACROS Organics (Belgium) or Princeton Chemicals Ltd (Luton, Bedfordshire, UK). Silica gel 60 (0.040-0.063 mm; Merck, Darmstadt, Germany) was used for column chromatography. Thin layer chromatography (TLC) was performed on alumina-backed silica gel 40 F_{254} plates (Merck). The plates were illuminated under UV (254 nm) and evaluated in iodine vapor. The melting points were determined on Optimelt MPA100 instrument (SRS, USA) and are uncorrected. Syntheses were performed on a CEM-DISCOVERY microwave reactor (CEM Corporation, Matthews, NC, USA) with temperature and pressure control. High resolution-mass spectrometry (HRMS) analysis was performed for all new compounds on a Finnigan MAT95 spectrometer (Thermo Fisher Scientifc, Bremen, GmbH) or on Mariner ESI-TOF spectrometer (Applied Biosystems, USA). The purity of all novel compounds was assessed using a Gynkotek HPLC Modular System equipped with a DAAD UVD340U detector at 250 nm.

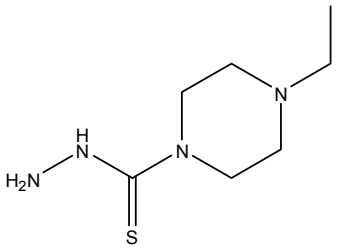
All ^{1}H NMR spectra were recorded on a Bruker AM-400 spectrometer (399.95 MHz for ^{1}H; 99.99 MHz for ^{13}C; BrukerBioSpin Corp., Germany). Chemical shifts are reported in ppm against the internal standard, $Si(CH_3)_4$. Easily exchangeable signals were omitted when diffuse.

LogP_{calc} values were calculated using ChemDraw 12 (Perkin-Elmer, Waltham, MA, USA) by using Crippen's fragmentation [1], Viswanadhan's fragmentation [2] and Broto's method [3] and then calculating the average logP_{calc}.

S1.1 Chemical Characterization of Thiosemicarbazides and Thiosemicarbazones

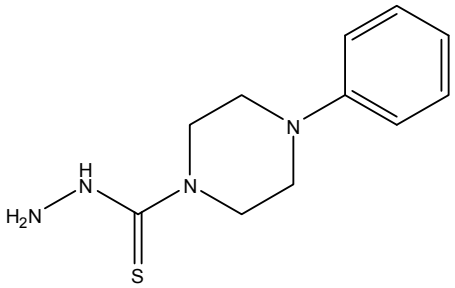
The thiosemicarbazides (**a**-**f**) and their thiosemicarbazones (Series **1**-**6**) were prepared according to Schemes 1-3. The characterization of all novel products is described below.

4-Ethylpiperazine-1-carbothiohydrazide **(a)**



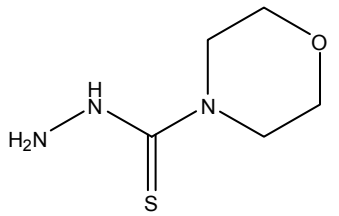
Yield: 47%. Purity: 98.67%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 3.71-3.68 (m, 4H, CH_2), 2.35-2.29 (m, 6H, CH_2), 1.00 (t, 3H, J= 7.2 Hz). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 183.0, 52.45, 51.86, 47.75, 12.35. MP: 137-138°C (ethanol). HRMS-ESI-TOF: 189.1171 [M + H]$^+$ ($C_7H_{17}N_4S$; Exact Mass: 189.1174). Log P_{calc}: 0.57.

4-Phenylpiperazine-1-carbothiohydrazide **(b)**



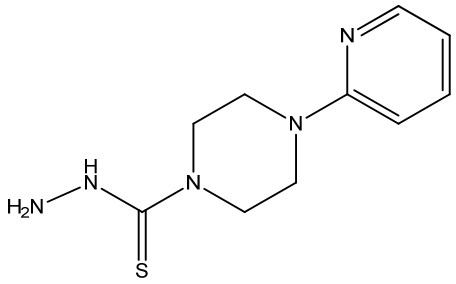
Yield: 58%. MP: 161-162°C (ethanol) [176-177°C (dioxane) [4]]. Log P_{calc}: 1.472.

Morpholine-4-carbothiohydrazide **(c)**



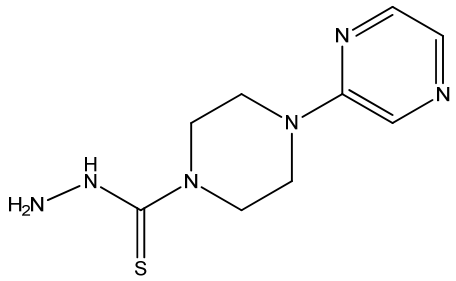
Yield: 76%. MP: 170-171°C (ethanol) [170-171°C; [5]] LogP_{calc}: -0.52.

4-(Pyridin-2-yl)piperazine-1-carbothiohydrazide **(d)**



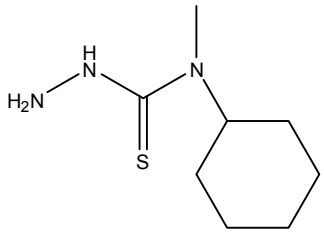
Yield: 95%. MP: 177-178°C (ethanol) [179-180°C; [4]]. Log P_{calc}: 0.524.

4-(Pyrazin-2-yl)piperazine-1-carbothiohydrazide **(e)**



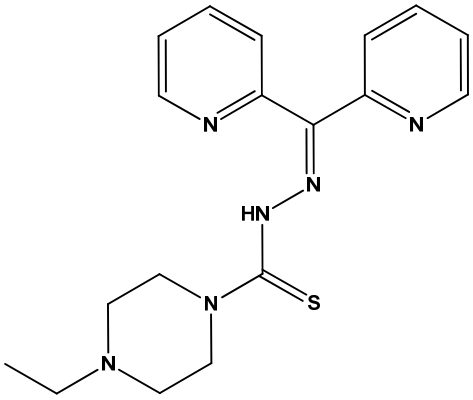
Yield: 95%. Purity: 96.98% (250 nm). ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 9.17 (bs, 1H, NH), 8.31 (bs, 1H, pyrazine), 8.09 (bs, 1H, pyrazine), 7.86 (bs, 1H, pyrazine), 4.83 (bs, 2H, NH), 3.89 (m, 4H, CH_2), 3.61 (m, 4H, CH_2). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 183.0, 154.7, 141.9, 133.1, 131.7, 47.0, 43.7. MP: 167-168°C (ethanol). HRMS-ESI-TOF: 239.1080 $[M + H]^+$ ($C_9H_{15}N_6S$; Exact Mass: 239.1079). Log P_{calc}: -0.24.

N-Cyclohexyl-N-methylhydrazinecarbothioamide **(f)**



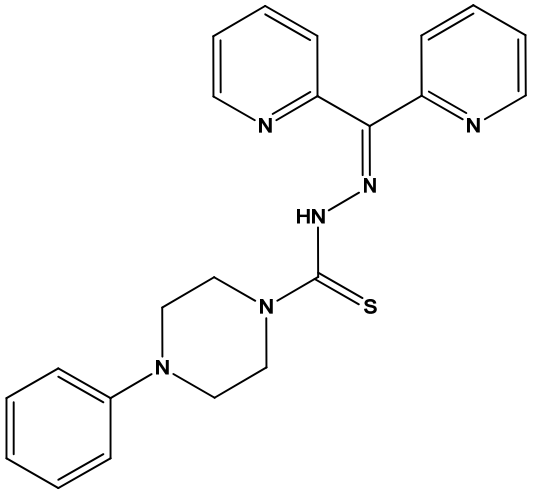
Yield: 66%. MP:138-139°C (ethanol) [140°C; [6]] Log P_{calc}: 1.733.

***(Z)-N'*-(Di(pyridin-2-yl)methylene)-4-ethylpiperazine-1-carbothiohydrazide (1a)**



Yield: 62%. Purity: 97.32%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 14.47 (bs, 1H, NH), 8.85 (d, 1H, *J*= 3,6 Hz), 8.60 (d, 1H, *J*= 3.6 Hz), 8.01-7.91 (m, 3H), 7.59 (m, 2H), 7.58 (m, 1H), 4.00 (m, 4H, CH_2, piperazine), 2.50 (m, 6H, CH_2, piperazine), 1.06 (t, 3H, *J*= 6.8 Hz, CH_3). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 180.8, 148.9, 148.3, 138.3, 127.4, 125.1, 124.2, 56.5, 52.4, 51.7, 12.4. MP: 144-145°C. R_f= 0.52 [dichloromethane:ethanol 16:1 (v/v)]. HRMS-EI: 354.1626 ($C_{18}H_{22}N_6S$; Exact Mass: 354.1627). Log P_{calc}: 1.842.

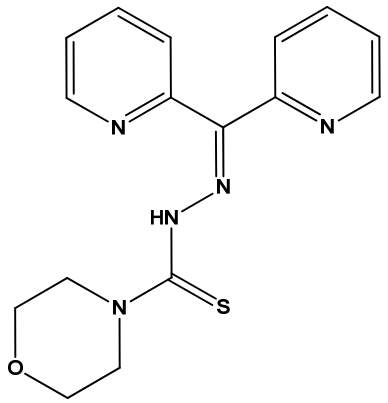
***N'*-(Di(pyridin-2-yl)methylene)-4-phenylpiperazine-1-carbothiohydrazide (1b)**



Yield: 78%. Purity: 98.65%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 14.59 (bs, 1H, NH), 8.89 (d, 1H, *J*= 3.9 Hz), 8.60 (d, 1H, *J*= 4.0 Hz, pyridine), 8.01-7.91 (m, 3H), 7.60 (m, 2H), 7.49 (m, 1H), 7.25 (t, 2H, *J*= 7.7 Hz), 6.96 (d, 2H, *J*= 8.2 Hz), 6.81 (t, 1H, *J*= 7.2 Hz), 4.17 (m, 4H, CH_2, piperazine), 3.34 (m, 4H, CH_2, piperazine). ^{13}C-NMR (d_6-DMSO, 100 MHz,

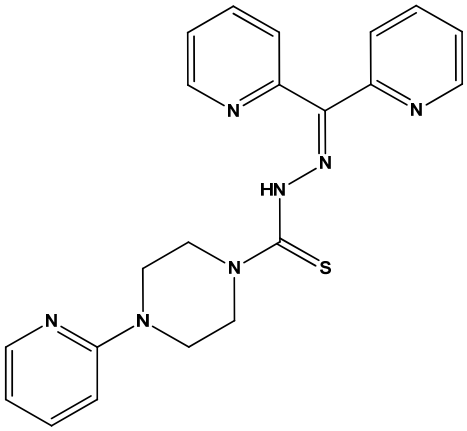
ppm):180.7, 150.8, 148.7, 148.4, 138.3, 137.7, 129.5, 127.3, 125.1, 119.4, 115.7, 49.6, 48.1. MP: 151-152°C (151-152°C, [7]). R_f= 0.28 [dichloromethane:methanol 40:1 (v/v)]. HRMS-EI: 402.1628 ($C_{22}H_{22}N_6S$; Exact Mass: 402.1627). LogP_{calc}: 2.744.

_N_'-(Di(pyridin-2-yl)methylene)morpholine-4-carbothiohydrazide (1c)



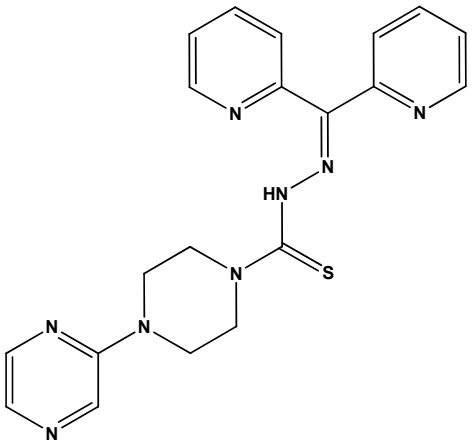
Yield: 78%. Purity: 96.32%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 14.50 (bs, 1H, NH), 8.85 (d, 1H, *J*= 3,7 Hz), 8.61 (d, 1H, *J*= 3.5 Hz), 8.01-7.91 (m, 3H), 7.58 (m, 2H), 7.49 (m, 1H), 4.00 (m, 4H, CH_2); 3.71 (m, 4H, CH_2). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm):180.2, 148.3, 148.4, 138.7, 127.5, 125.1, 124.0, 68.5, 52.6. MP: 124-125°C. R_f= 0.57 [dichloromethane:ethyl acetate 8:1 (v/v)]. HRMS-EI: 327.1158 ($C_{16}H_{17}N_5OS$; Exact Mass: 327.1154). Log P_{calc}: 0.752.

***(Z)*-_N_'-(Di(pyridin-2-yl)methylene)-4-(pyridin-2-yl)piperazine-1-carbothiohydrazide (1d)**



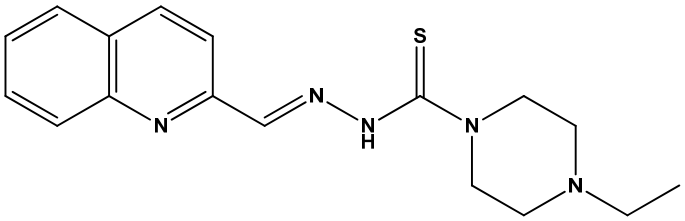
Yield: 74%. Purity: 95.62%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 14.65 (bs, 1H, NH), 8.90 (d, 1H, J= 5.2 Hz, pyridine), 8.62 (d, 1H, J= 4.4 Hz, pyridine), 8.15 (dd, 1H, J_1= 4.6 Hz, J_2= 1.5 Hz, pyridine), 8.05-7.93 (m, 3H, pyridine), 7.62-7.55 (m, 3H, pyridine), 7.49 (t, 1H, J= 7.2 Hz, pyridine), 6.82 (d, 1H, J= 8.6 Hz, pyridine), 6.68 (dd, 1H, J_1= 6.8 Hz, J_2= 5.2 Hz, pyridine), 4.15 (m, 4H, CH_2, piperazine), 3.72 (m, 4H, CH_2, piperazine). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 180.7, 158.9, 148.9, 148.4, 148.0, 138.3, 137.8, 127.3, 125.1, 124.2, 113.5, 107.3, 49.0, 44.2. MP: 143-144°C. HRMS-ESI-TOF: 404.1652 $[M + H]^+$ ($C_{21}H_{22}N_7S$; Exact Mass: 404.1657). Log P_{calc}: 1.796.

_N_'-(Di(pyridin-2-yl)methylene)-4-(pyrazin-2-yl)piperazine-1-carbothiohydrazide (1e)



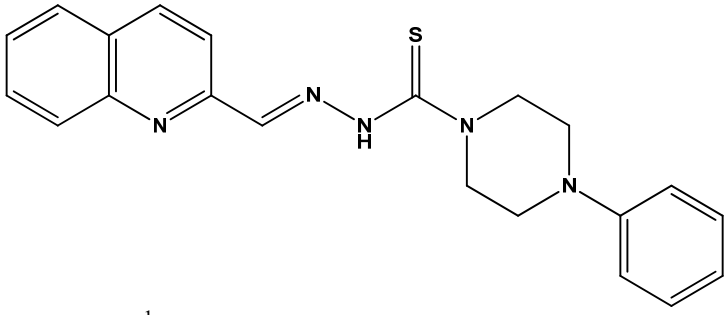
Yield: 66%. Purity: 95.45%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 14.66 (bs, 1H, NH), 8.89 (d, 1H, J = 4.7 Hz, pyridine), 8.62 (d, 1H, J = 4.0 Hz), 8.31 (bs, 1H, pyrazine), 8.12 (bs, 1H, pyrazine), 8.06 – 7.91 (m, 3H, pyridine), 7.88 (d, 1H, J = 2.2 Hz, pyrazine), 7.61 (t, 2H, J= 6.4 Hz, pyridine), 7.49 (t, 1H, J= 5.6 Hz, pyridine), 4.18 (m, 4H, CH_2, piperazine), 3.80 (m, 4H, CH_2, piperazine). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 180.7, 154.5, 149.4, 148.9, 148.4, 141.9, 138.2, 137.8, 127.3, 125.1, 124.2, 48.8, 43.5. MP: 175-176°C. HRMS-ESI-TOF: 405.1606 $[M + H]^+$ ($C_{20}H_{21}N_8S$; Exact Mass: 405.1610). Log P_{calc}: 1.031.

(*E*)-4-Ethyl-*N*'-(quinolin-2-ylmethylene)piperazine-1-carbothiohydrazide (2a)



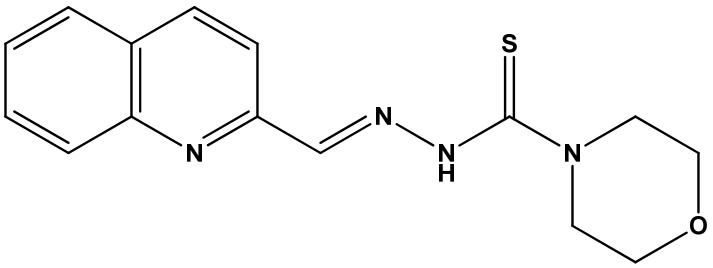
Yield: 78%. Purity: 96.67%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 8.39 (d, 1H, *J*= 8.7 Hz, quinoline), 8.32 (s, 1H, CH), 8.03-7.98 (m, 3H, quinoline), 7.77 (t, 1H, *J*= 8.4 Hz, quinoline), 7.62 (t, 1H, *J*= 7.8 Hz, quinoline), 3.97 (m, 4H, CH_2, piperazine), 2.51 (m, 6H, CH_2, piperazine, $\underline{CH_2}$-CH_3), 1.04 (t, 3H, *J*= 7.2 Hz, CH_2-$\underline{CH_3}$). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 181.1, 154.3, 147.8, 144.2, 137.1, 130.5, 129.2, 128.5, 128.2, 127.6, 117.7, 52.6, 51.8, 50.5, 12.3. MP: 168-169°C. R_f= 0.48 [dichloromethane:ethanol 10:1 (v/v)]. HRMS-ESI-TOF: 350.1414 $[M + Na]^+$ ($C_{17}H_{21}N_5SNa$; Exact Mass: 350.1415). Log P_{calc}: 2.883.

(*E*)-4-Phenyl-*N*'-(quinolin-2-ylmethylene)piperazine-1-carbothiohydrazide (2b)



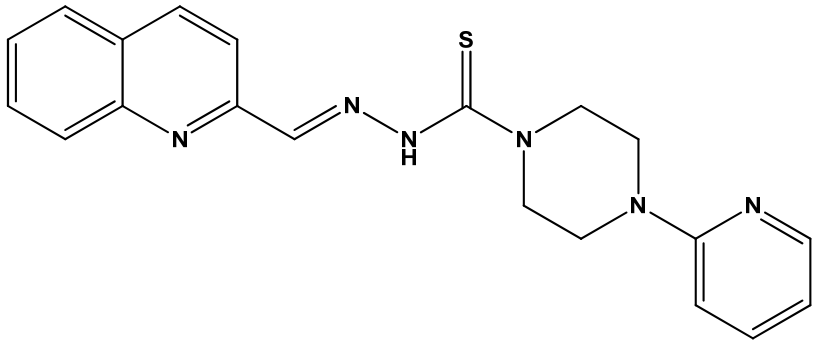
Yield: 81%. Purity: 98.00%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.66 (bs, 1H, NH), 8.39 (d, 1H, *J*= 8.7 Hz, quinoline), 8.35 (s, 1H, CH), 8.03 (d, 2H, *J*= 8.5 Hz, quinoline), 7.99 (d, 1H, *J*= 8.0 Hz, quinoline), 7.79 (t, 1H, *J*= 7.6 Hz, quinoline), 7.62 (t, 1H, *J*= 7.8 Hz, quinoline), 7.25 (t, 2H, *J* = 7.8 Hz, phenyl), 6.99 (d, 2H, *J* = 8.1 Hz, phenyl), 6.82 (t, 1H, *J* = 7.1 Hz, phenyl), 4.14 (m, 4H, CH_2, piperazine), 3.32 (m, 4H, CH_2, piperazine). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 181.2, 154.3, 150.9, 147.9, 144.4, 137.1, 130.5, 129.5, 129.3, 128.5, 128.2, 127.6, 119.6, 117.8, 116.0, 50.3, 48.5. MP: 162-163°C. HRMS-ESI-TOF: 376.1591 $[M + H]^+$ ($C_{21}H_{22}N_5S$; Exact Mass: 376.1596). Log P_{calc}: 3.785.

(*E*)-*N*'-(Quinolin-2-ylmethylene)morpholine-4-carbothiohydrazide (2c)



Yield: 85%. Purity: 97.42%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.61 (bs, 1H, NH), 8.38 (d, 1H, *J* = 8.7 Hz, quinoline), 8.32 (s, 1H, CH), 8.01 (m, 3H, quinoline), 7.80 (t, 1H, *J*= 8.0 Hz, quinoline), 7.64 (t, 1H, *J*= 6.4 Hz, quinoline), 3.99 (m, 4H, piperazine), 3.72 (m, 4H, piperazine). ^{13}C-NMR(d_6-DMSO, 100 MHz, ppm):181.4, 154.3, 147.8, 144.4, 137.1, 130.5, 129.3, 128.4, 128.2, 127.6, 117.7, 66.5, 51.2. MP: 145-146°C. HRMS-EI: 300.1034 ($C_{15}H_{16}N_4OS$; Exact Mass: 300.1045). Log P_{calc}: 1.793.

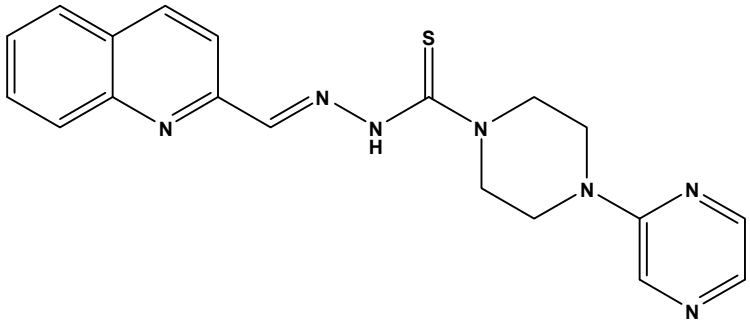
(*E*)-4-(Pyridin-2-yl)-*N*'-(quinolin-2-ylmethylene)piperazine-1-carbothiohydrazide (2d)



Yield: 69%. Purity: 97.87%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.50 (bs, 1H, NH), 8.38 (m, 2H, quinoline, pyridine), 8.16 (d, 1H, *J*= 4.0 Hz, pyridine), 8.03 (d, 2H, *J*= 8.6 Hz, quinoline), 7.98 (d, 1H, *J*= 8.0 Hz, quinoline), 7.79 (t, 1H, *J*= 7.2 Hz, quinoline), 7.64-7.56 (m, 2H, quinoline, pyridine), 6.85 (d, 1H, *J*= 8.8 Hz, pyridine), 6.68 (t, 1H, *J*= 5.2 Hz, pyridine), 4.12 (m, 4H, piperazine), 3.71 (m, 4H, piperazine). ^{13}C-NMR, (d_6-DMSO, 100 MHz, ppm):181.5, 159.0, 154.3, 148.0, 147.9, 144.5, 138.0, 137.1, 130.4, 129.3, 128.4, 128.2,

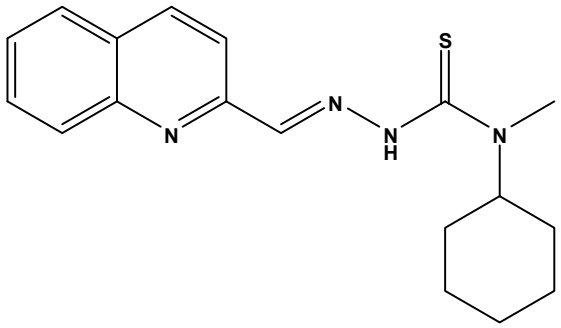
127.6, 117.8, 113.6, 107.5, 50.2, 44.7. MP: 133-134°C. HRMS-ESI-TOF: 377.1549 $[M + H]^+$ ($C_{20}H_{21}N_6S$; Exact Mass: 377.1548). Log P_{calc}: 2.837.

(*E*)-4-(Pyrazin-2-yl)-*N*'-(quinolin-2-ylmethylene)piperazine-1-carbothiohydrazide (2e)



Yield: 96%. Purity: 98.98%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.77 (bs, 1H, NH), 8.39 (d, 1H, *J*= 8.7 Hz), 8.35 (m, 2H, CH, pyrazine), 8.13 (m, 1H), 8.03 (m, 2H), 8.00 (d, 1H, *J*= 7.8 Hz), 7.89 (d, 1H, *J*= 2.4 Hz), 7.79 (t, 1H, *J*= 7.0 Hz), 7.63 (t, 1H, *J*= 7.9 Hz), 4.13 (m, 4H, piperazine), 3.78 (m, 4H, piperazine). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm):181.3, 154.7, 154.3, 147.9, 144.4, 141.9, 137.1, 133.1, 131.7, 130.5, 129.3, 128.5, 128.2, 127.6, 117.8, 49.9, 44.0. MP: 188-189° C. HRMS-ESI-TOF: 378.1498 $[M + H]^+$ ($C_{19}H_{20}N_7S$; Exact Mass: 378.1501). Log P_{calc}: 2.072.

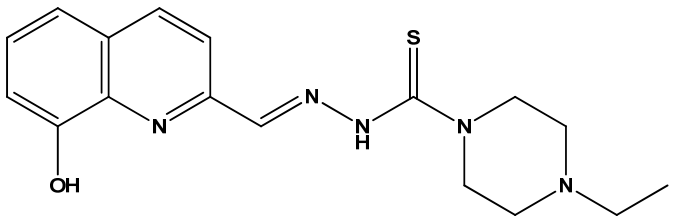
(*E*)-*N*-Cyclohexyl-*N*-methyl-2-(quinolin-2-ylmethylene)hydrazinecarbothioamide (2f)



Yield: 58%. Purity: 99.12%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.25 (bs, 1H, NH), 8.39 (m, 2H, CH, quinoline), 8.03-7.98 (m, 3H, quinoline), 7.78 (t, 1H, *J*= 8.0 Hz, quinoline), 7.62 (t, 1H, *J*= 7.6 Hz, quinoline), 4.74 (bs, 1H, C_1-cyclohexyl), 3.14 (s, 3H, CH_3), 1.80 (m, 4H,

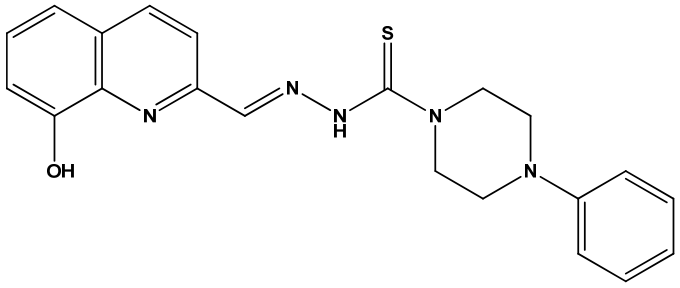
cyclohexyl), 1.58 (m, 3H, cyclohexyl), 1.30 (m, 2H, cyclohexyl), 1.15 (t, 1H, *J*= 12.0 Hz, cyclohexyl). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm):181.1, 154.5, 147.9, 144.2, 137.0, 130.5, 129.2, 128.4, 128.1, 127.5, 117.7, 61.2, 39.8, 34.9, 29.7, 25.8, 25.7, 25.4. MP: 125-126°C. HRMS-EI: 326.1564 ($C_{18}H_{22}N_4S$; Exact Mass: 326.1565). Log P_{calc}: 4.145.

(*Z*)-4-Ethyl-*N*'-((8-hydroxyquinolin-2-yl)methylene)piperazine-1-carbothiohydrazide (3a)



Yield: 74%. Purity: 95.03%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 15.29 (bs, 1H, NH), 8.33 (s,1H, CH), 8.30 (d, 1H, *J*= 8.4 Hz, quinoline), 7.97 (d, 1H, *J*= 8.4 Hz, quinoline), 7.44 (t, 1H *J* = 7.6 Hz), 7.38 (d, 1H, *J* = 7.2 Hz), 7.11 (d, 1H *J* = 7.4 Hz), 3.95 (m, 4H, piperazine), 2.50 (m, 6H, piperazine, CH_2), 1.04 (t, 3H, *J*= 6.8 Hz). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm):181.2, 153.8, 152.3, 144.0, 138.6, 136.9, 129.1, 128.5, 118.2, 117.9, 112.6, 52.7, 51.7, 50.5, 12.4. MP: 167-168°C. HRMS-ESI-TOF: 366.1362 $[M + Na]^+$ ($C_{17}H_{21}N_5OSNa$; Exact Mass: 366.1365). Log P_{calc}: 2.931.

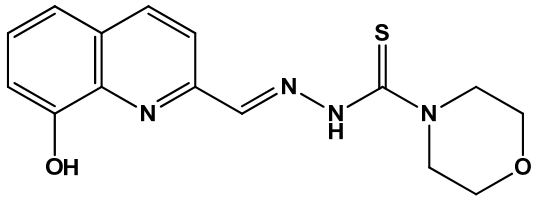
(*E*)-*N*'-((8-Hydroxyquinolin-2-yl)methylene)-4-phenylpiperazine-1-carbothio-hydrazide (3b)



Yield: 67%. Purity: 96.59%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.71 (bs, 1H, NH), 9.83 (bs, 1H, OH), 8.38 (bs, 1H, CH), 8.32 (d, 1H, *J*= 8.7 Hz, quinoline), 8.01 (d, 1H, *J*= 8.7 Hz,

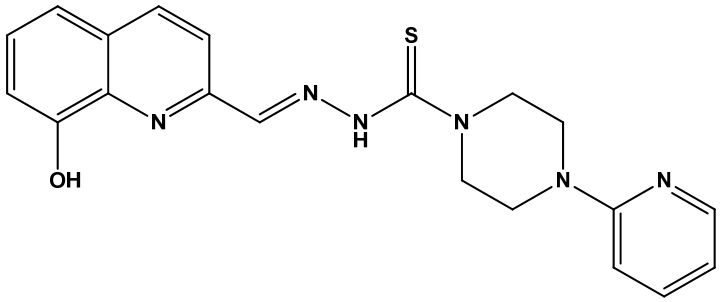
quinoline), 7.44 (m, 1H, quinoline), 7.39 (m, 1H), 7. 25 (t, 2H, *J*= 7.9 Hz, phenyl), 7.13 (d, 1H, *J*= 7.1 Hz), 6.99 (d, 2H, *J*= 8.2 Hz, phenyl), 6.82 (t, 1H, *J*= 7.1 Hz, phenyl), 4.13 (m, 4H, piperazine), 3.32 (m, 4H, piperazine). ^{13}C-NMR (*d*$_6$-DMSO, 100 MHz, ppm):181.3, 153.8, 152.3, 151.0, 144.2, 138.6, 137.0, 129.5, 129.2, 128.6, 119.6, 118.3, 117.9, 115.9, 112.7, 50.4, 48.6. MP: 175-176°C. HRMS-ESI-TOF: 392.1540 $[M + H]^+$ ($C_{21}H_{22}N_5OS$; Exact Mass: 392.1545). Log P_{calc}: 3.833.

(*E*)-*N*'-((8-Hydroxyquinolin-2-yl)methylene)morpholine-4-carbothiohydrazide (3c)



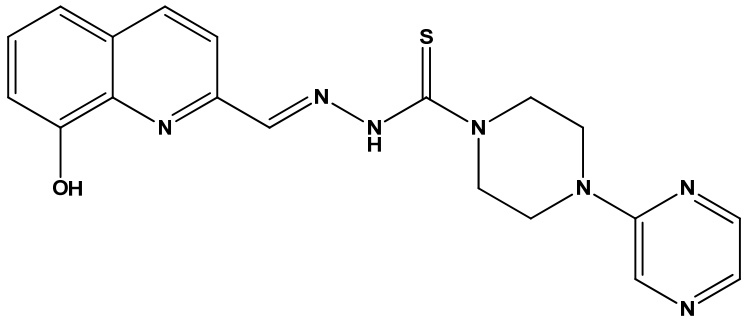
Yield: 76%. Purity: 95.77%. ^{1}H-NMR (*d*$_6$-DMSO, 400 MHz, ppm): 11.66 (bs, 1H, NH), 9.81 (bs, 1H, OH), 8.35 (s, 1H, CH), 8.30 (d, 1H, *J* = 8.7 Hz, quinoline), 7.97 (d, 1H, *J* = 8.7 Hz, quinoline), 7.45 (t, 1H, *J* = 7.7 Hz, quinoline), 7.39 (d, 1H, *J* = 7.6 Hz, quinoline), 7.12 (d, 1H, *J* = 7.1 Hz, quinoline), 3.98 (m, 4H, piperazine), 3.72 (m, 4H, piperazine). ^{13}C-NMR (*d*$_6$-DMSO, 100 MHz, ppm):181.4, 153.8, 152.2, 144.3, 138.6, 137.0, 129.1, 128.6, 118.3, 117.9, 112.6, 66.5, 51.2. MP: 181-182°C (181-182°C, [7]). HRMS-EI: 316.0984 ($C_{15}H_{16}N_4O_2S$; Exact Mass: 316.0994). Log P_{calc}: 1.841.

(*E*)-*N*'-((8-Hydroxyquinolin-2-yl)methylene)-4-(pyridin-2-yl)piperazine-1-carbothiohydrazide (3d)



Yield: 77%. Purity: 99.25%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.70 (bs, 1H, NH), 9.82 (bs, 1H, OH), 8.38 (s, 1H, CH), 8.31 (d, 1H, *J*= 8.7 Hz, quinoline), 8.16 (d, 1H, *J*= 4.8 Hz, pyridine), 8.02 (d, 1H, *J*= 8.7 Hz, quinoline), 7.58 (m, 1H, pyridine), 7.45 (t, 1H, *J*= 7.6 Hz, quinoline), 7.39 (d, 1H, *J*= 7.9 Hz, quinoline), 7.13 (d, 1H, *J*= 7.3 Hz, quinoline), 6.86 (d, 1H, *J*= 8.6 Hz, pyridine), 6.68 (m, 1H, pyridine), 4.11 (m, 4H, piperazine), 3.70 (m, 4H, piperazine). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm):181.3, 159.0, 153.8, 152.3, 148.0, 144.2, 138.6, 138.1, 137.0, 129.1, 128.6, 118.3, 118.0, 113.6, 112.6, 107.5, 50.2, 44.7. MP: 176-177°C. HRMS-ESI-TOF: 393.1490 $[M + H]^+$ ($C_{20}H_{21}N_6OS$; Exact Mass: 393.1498). Log P_{calc}: 2.885.

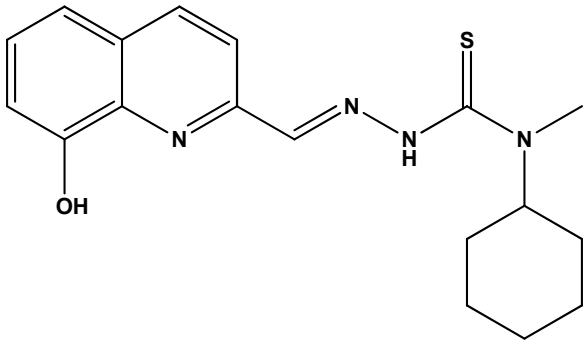
(*E*)-*N*'-((8-Hydroxyquinolin-2-yl)methylene)-4-(pyrazin-2-yl)piperazine-1-carbothiohydrazide (3e)



Yield: 86%. Purity: 99.8%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.72 (bs, 1H, NH), 9.82 (bs, 1H, OH), 8.38 (s, 1H, CH), 8.36 (m, 1H, pyrazine), 8.31 (d, 1H, *J*= 8.7 Hz, quinoline), 8.13 (m, 1H, pyrazine), 8.02 (d, 1H, *J*= 8.7 Hz, quinoline), 7.89 (m, 1H, pyrazine), 7.45 (t,

1H, *J* = 7.6 Hz, quinoline), 7.39 (d, 1H, *J*= 7.5 Hz, quinoline), 7.13 (d, 1H, *J*= 6.7 Hz, quinoline), 4.13 (m, 4H, piperazine), 3.78 (m, 4H, piperazine). ^{13}C-NMR (*d*$_6$-DMSO, 100 MHz, ppm):181.4, 154.7, 153.8, 152.2, 144.3, 141.9, 138.6, 137.0, 133.1, 131.7, 129.2, 128.6, 118.3, 118.0, 112.6, 49.9, 44.0. MP: 200-201°C. HRMS-ESI-TOF: 394.1448 $[M + H]^+$ ($C_{19}H_{20}N_7OS$; Exact Mass: 394.1450). Log P_{calc}: 2.121.

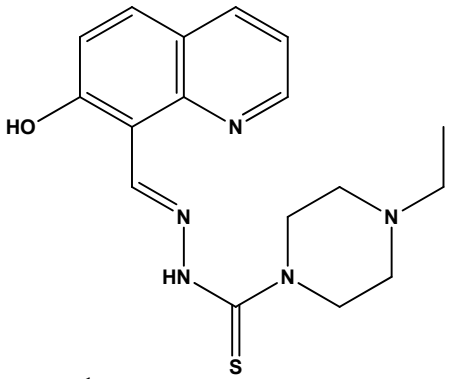
(*E*)-*N*-Cyclohexyl-2-((8-hydroxyquinolin-2-yl)methylene)-N-methylhydrazinecarbothioamide (3f)



Yield: 65%. Purity: 98.01%. ^{1}H-NMR (*d*$_6$-DMSO, 400 MHz, ppm): 11.33 (bs, 1H, NH), 9.77 (bs, 1H, OH), 8.39 (s, 1H, CH), 8.30 (d, 1H, *J*= 8.8 Hz, quinoline), 7.99 (d, 1H, *J*= 8.8 Hz, quinoline), 7.44 (t, 1H, *J*= 7.6 Hz, quinoline), 7.38 (dd, 1H, J_1= 8.0 Hz; J_2= 1.2 Hz, quinoline), 7.11 (dd, 1H, J_1= 7.2 Hz; 1.2 Hz, quinoline), 4.74 (bs, 1H, C_1-cyclohexyl), 3.14 (s, 3H, CH_3), 1.80 (m, 4H, cyclohexyl),1.58 (m, 3H, cyclohexyl), 1.32 (m, 2H, cyclohexyl), 1.16 (t, 1H, *J*= 11.4 Hz, cyclohexyl). ^{13}C-NMR (*d*$_6$-DMSO, 100 MHz, ppm):181.2, 153.8, 152.5, 144.1, 138.6, 136.9, 129.1, 128.4, 118.3, 117.90, 112.6, 61.2, 39.8, 35.0, 29.7, 25.7, 25.4. MP: 164-165°C. HRMS-EI: 342.1513 ($C_{18}H_{22}N_4OS$; Exact Mass: 342.1514). Log P_{calc}: 4.193.

(*E*)-4-Ethyl-*N*'-((7-hydroxyquinolin-8-yl)methylene)piperazine-1-carbothiohydrazide

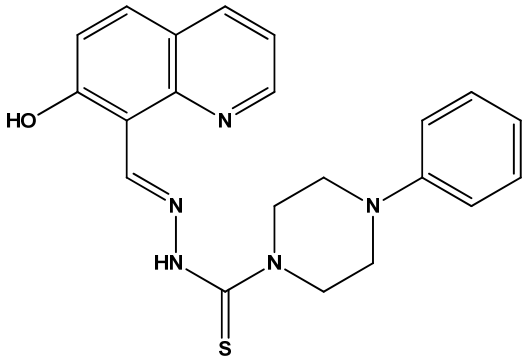
(4a)



Yield: 76.5%. Purity: 95.35%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 13.23 (bs, 1H, NH), 11.59 (bs, 1H, OH), 9.76 (s, 1H, CH), 8.84 (d, 1H; J = 2.8 Hz, quinoline), 8.30 (d, 1H; J = 8.1 Hz, quinoline), 7.92 (d, 1H, J = 9.0 Hz, quinoline), 7.42 (dd, 1H, J_1 = 8.1 Hz, J_2= 4.3 Hz, quinoline), 7.28 (d, 1H, J= 9.0 Hz, quinoline), 3.97 (m, 4H, piperazine), 2.51 (m, 6H, piperazine, CH_2), 1.05 (t, J= 7.2 Hz, 3H, CH_3). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm):179.2, 160.4, 150.6, 147.0, 144.9, 136.9, 131.6, 122.2, 120.8, 119.7, 111.0, 52.3, 51.7, 48.2, 12.1. MP: 145-146°C. HRMS-EI: 343.1464 ($C_{17}H_{21}N_5OS$; Exact Mass: 343.1467). Log P_{calc}: 2.931.

(*E*)-*N*'-((7-Hydroxyquinolin-8-yl)methylene)-4-phenylpiperazine-1-carbothio-hydrazide

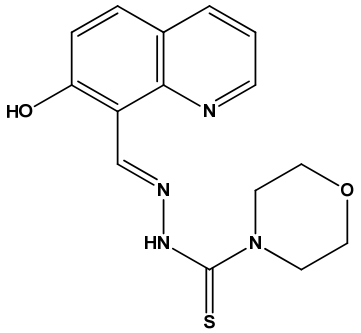
(4b)



Yield: 67%. Purity: 96.12%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 13.14 (bs, 1H, NH), 11.83 (bs, 1H, OH), 9.83 (s, 1H, CH), 8.87 (m, 1H, quinoline), 8.32 (d, 1H, J = 8.0 Hz, quinoline), 7.95 (d, 1H, J = 9.0 Hz, quinoline), 7.45 (dd, 1H, J_1 = 8.0 Hz, J_2= 4.2 Hz, quinoline), 7.31 (d, 1H, J = 8.9 Hz, quinoline), 7.25 (t, 2H, J = 7.7 Hz, phenyl), 7.00 (d, 2H, J

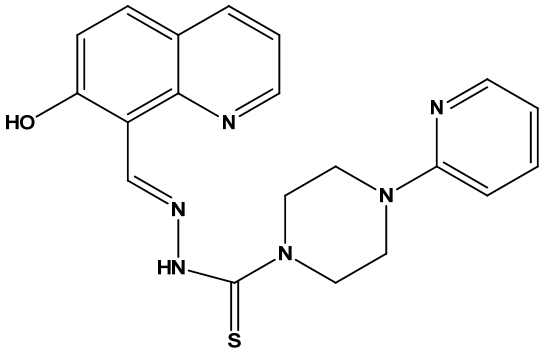
= 8.2 Hz, phenyl), 6.82 (t, 1H, *J* = 7.1 Hz, phenyl), 4.13 (m, 4H, piperazine), 3.29 (m, 4H, piperazine). ^{13}C-NMR (*d*$_6$-DMSO, 100 MHz, ppm):179.2, 159.8, 150.9, 150.7, 146.9, 145.2, 137.0, 131.6, 129.5, 122.4, 120.4, 119.9, 119.6, 115.9, 111.2, 48.3, 48.2. MP: 212-213°C. HRMS-EI: 391.1470 ($C_{21}H_{21}N_5OS$; Exact Mass: 391.1467). Log P_{calc}: 3.833.

(*E*)-*N*'-((7-Hydroxyquinolin-8-yl)methylene)morpholine-4-carbothiohydrazide (4c)



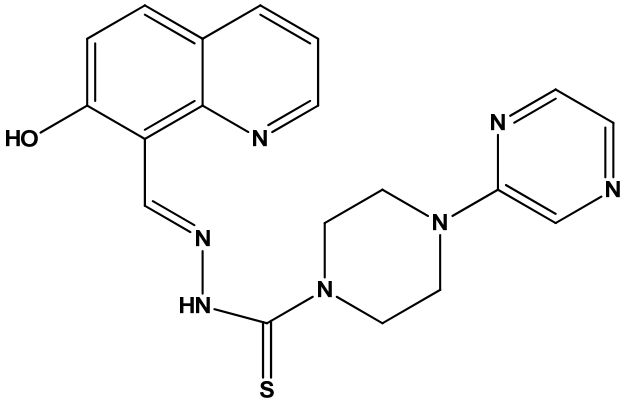
Yield: 76%. Purity: 96.22%. ^{1}H-NMR (*d*$_6$-DMSO, 400 MHz, ppm): 13.10 (bs, 1H, NH), 11.75 (bs, 1H, OH), 9.81 (s, 1H, CH), 8.87 (m, 1H, quinoline), 8.33 (d, 1H, *J* = 7.8 Hz, quinoline), 7.95 (d, 1H, *J* = 9.0 Hz, quinoline), 7.45 (dd, 1H, J_1 = 8.0 Hz, J_2= 4.3 Hz, quinoline), 7.31 (d, 1H, *J* = 8.9 Hz, quinoline), 3.97 (m, 4H, piperazine), 3.69 (m, 4H, piperazine). ^{13}C-NMR (*d*$_6$-DMSO, 100 MHz, ppm):179.5, 159.7, 150.7, 146.8, 145.3, 137.1, 131.6, 122.4, 120.4, 119.9, 111.2, 66.2, 49.1. MP: 198-199°C. HRMS-EI: 316.0982 ($C_{15}H_{16}N_4O_2S$; Exact Mass: 316.0994). Log P_{calc}: 1.841.

(*E*)-*N*'-((7-Hydroxyquinolin-8-yl)methylene)-4-(pyridin-2-yl)piperazine-1-carbothio-hydrazide (4d)



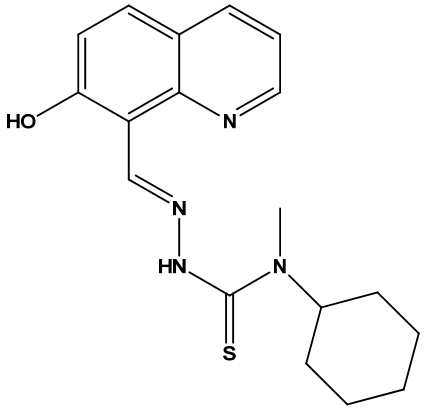
Yield: 72%. Purity: 95.29%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 13.14 (bs, 1H, NH), 11.80 (s, 1H, OH), 9.83 (s, 1H, CH), 8.86 (dd, 1H, J_1 = 4.2, J_2= 1.6 Hz, quinoline), 8.32 (dd, 1H, J_1 = 8.2, J_2= 1.7 Hz, quinoline), 8.15 (dd, 1H, J_1 = 4.9, J_2= 1.6 Hz, pyridine), 7.95 (d, 1H, J = 9.0 Hz, quinoline), 7.58 (ddd, 1H, J_1 = 8.8, J_2=7.2, J_3= 1.9 Hz, pyridine), 7.45 (dd, 1H, J_1=8.2 Hz, J_2= 4.3 Hz, quinoline), 7.32 (d, 1H, J= 8.6 Hz, quinoline), 6.87 (d, 1H, J = 8.6 Hz, pyridine), 6.68 (dd, 1H, J_1 = 7.0, J_2= 5.0 Hz, pyridine), 4.11 (m, 4H, piperazine), 3.66 (m, 4H, piperazine). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm):179.2, 159.3, 158.9,150.6,148.0, 146.9, 145.2, 138.1, 137.0, 131.6, 122.3, 120.4, 119.8, 113.7, 111.9, 107.5, 56.5, 48.1. MP: 186-187°C. HRMS-ESI-TOF: 393.1493 $[M + H]^+$ ($C_{20}H_{21}N_6OS$; Exact Mass: 393.1498). Log P_{calc}: 2.885.

(*E*)-*N*'-((7-Hydroxyquinolin-8-yl)methylene)-4-(pyrazin-2-yl)piperazine-1-carbothiohydrazide (4e)



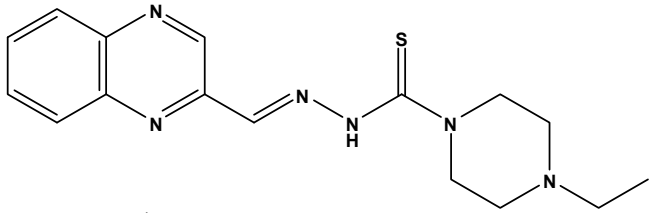
Yield: 76%. Purity: 98.37%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 13.13 (bs, 1H, NH), 11.82 (bs, 1H, OH), 9.84 (s, 1H, CH), 8.87 (d, 1H, *J*= 2.5 Hz, quinoline), 8.37 (s, 1H, pyrazine), 8.33 (d, 1H, *J* = 7.9 Hz, quinoline), 8.13 (s, 1H, pyrazine), 7.95 (d, 1H, *J* = 9.0 Hz, quinoline), 7.89 (d, 1H, *J* = 2.3 Hz, pyrazine), 7.45 (dd, 1H, J_1 = 8.0, J_2= 4.2 Hz, quinoline), 7.32 (d, 1H, *J*= 8.4 Hz, quinoline), 4.13 (m, 4H, piperazine), 3.74 (m, 4H, piperazine). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm):179.3, 159.8, 154.7, 150.7, 146.9, 145.3, 141.9, 137.0, 133.1, 131.8, 131.6, 122.4, 120.4, 119.9, 111.2, 56.5, 47.8. MP: 209-210°C. HRMS-ESI-TOF: 394.1446 $[M + H]^+$ ($C_{19}H_{20}N_7OS$; Exact Mass: 394.1450). Log P_{calc}: 2.121.

(*E*)-*N*-Cyclohexyl-2-((7-hydroxyquinolin-8-yl)methylene)-*N*-methylhydrazine carbothioamide (4f)



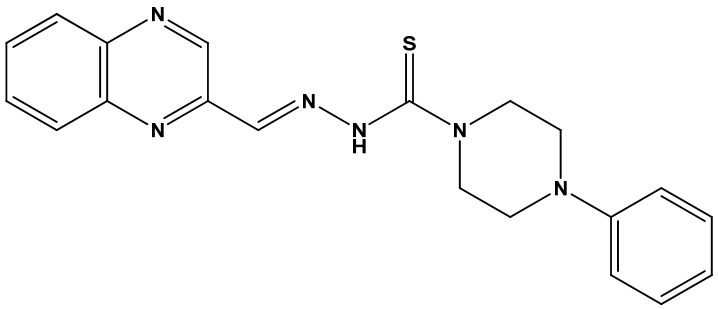
Yield: 76%. Purity: 95.46%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 13.26 (bs, 1H, NH), 11.20 (bs, 1H, OH), 9.82 (s, 1H, CH), 8.85 (dd, 1H, J_1= 4.0 Hz; J_2= 1.6 Hz, quinoline), 8.30 (dd, J_1= 8.0 Hz, J_2= 1.9 Hz, quinoline), 7.92 (d, 1H, *J*= 9.0 Hz, quinoline), 7.43 (dd, 1H, J_1= 8.1 Hz, J_2= 4.3 Hz, quinoline), 7.29 (d, 1H, *J*= 9.0 Hz, quinoline), 5.00 (bs,1H, C_1-cyclohexyl), 3.10 (s, 3H, CH_3), 1.79 (m, 4H, cyclohexyl), 1.50 (m, 3H, cyclohexyl), 1.35 (m, 2H, cyclohexyl), 1.06 (t, 1H, *J*= 6.8 Hz, cyclohexyl). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 179.0, 160.1, 150.5, 147.0, 144.8, 136.9, 131.4, 122.3, 120.6, 119.7, 111.2, 59.5, 32.7, 29.6, 25.7, 25.4, 24.2. MP: 184-185°C. HRMS-EI: 342.1512 ($C_{18}H_{22}N_4OS$; Exact Mass: 342.1514). Log P_{calc}: 4.1935.

(*E*)-4-Ethyl-*N*'-(quinoxalin-2-ylmethylene)piperazine-1-carbothiohydrazide (5a)



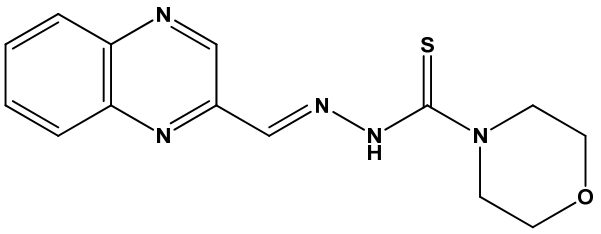
Yield: 86%. Purity: 96.00%. ^{1}H-NMR (d_6-DMSO, 500 MHz, ppm): 11.69 (bs, 1H, NH), 9.34 (s, 1H, quinoxaline), 8.32 (s, 1H, quinoxaline), 8.09 (t, 2H, J = 9.1 Hz, quinoxaline), 7.91 – 7.80 (m, 2H, quinoxaline), 3.98 (m, 4H, piperazine), 3.34 (m, 4H, piperazine), 2.39 (q, 2H, J = 7.1 Hz, CH_2), 1.04 (t, 3H, J = 7.2 Hz, CH_3). ^{13}C-NMR (d_6-DMSO, 125 MHz, ppm): 181.0, 149.1, 148.9, 143.2, 141.8, 141.8, 131.2, 130.9, 129.4, 129.4, 52.7, 51.8, 50.7, 12.4. MP: 150-151°C. HRMS-ESI-TOF: 329.1544 $[M + H]^+$ ($C_{16}H_{21}N_6S$; Exact Mass: 329.1548). Log P_{calc}: 2.136.

(*E*)-4-Phenyl-*N*'-(quinoxalin-2-ylmethylene)piperazine-1-carbothiohydrazide (5b)



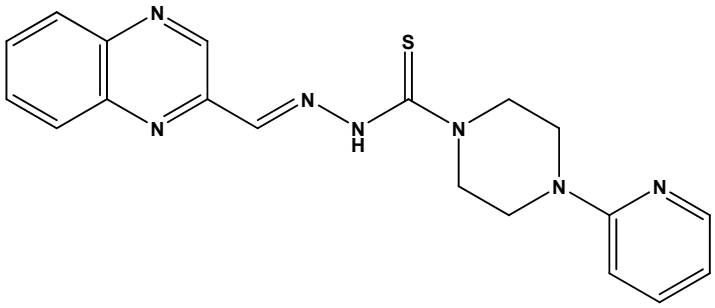
Yield: 69%. Purity: 99.10%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.82 (bs, 1H, NH), 9.39 (s, 1H, quinoxaline), 8.36 (s, 1H; CH), 8.10 (t, 2H, J= 7.2 Hz, quinoxaline), 7.87 (m, 2H, quinoxaline), 7.26 (t, 2H, J= 8.0 Hz, phenyl), 7.00 (d, 2H, J=8.1 Hz, phenyl), 6.82 (t, 1H, J= 7.2 Hz, phenyl), 4.15 (m, 4H, piperazine), 3.33 (m, 4H, piperazine). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 181.1; 150.9; 149.1; 143.3; 142.0; 141.9; 141.8; 131.2; 130.9; 129.5; 129.5; 119.6; 115.9; 50.4; 48.5. MP: 168-169°C. HRMS-ESI-TOF: 377.1546 $[M + H]^+$ ($C_{20}H_{21}N_6S$; Exact Mass: 377.1548). Log P_{calc}: 3.038.

(*E*)-*N*'-(Quinoxalin-2-ylmethylene)morpholine-4-carbothiohydrazide (5c)



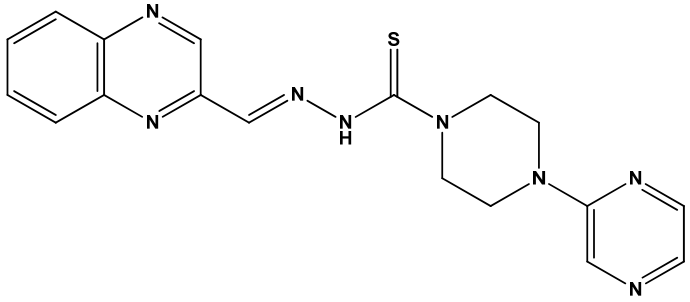
Yield: 83%. Purity: 99.00%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.56 (bs, 1H, NH), 9.34 (s, 1H, quinoxaline), 8.33 (s, 1H, CH), 8.09 (m, 2H, quinoxaline), 7.85 (m, 2H, quinoxaline), 4.00 (m, 4H, piperazine), 3.73 (s, 4H, piperazine). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 181.3, 149.0, 143.3, 142.0, 141.8, 141.7, 131.2, 130.9, 129.4, 66.5, 51.3. MP: 176-177°C. HRMS-ESI-TOF: 324.0892 $[M + Na]^+$ ($C_{14}H_{15}N_5OSNa$; Exact Mass: 324.0895). Log P_{calc}: 1.046.

(*E*)-4-(Pyridin-2-yl)-*N*'-(quinoxalin-2-ylmethylene)piperazine-1-carbothiohydrazide (5d)



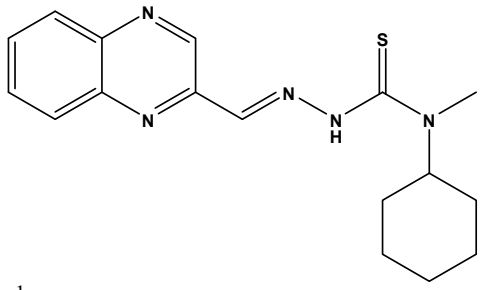
Yield: 69%. Purity: 98.45%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.79 (s, 1H, NH), 9.39 (s, 1H, quinoxaline), 8.36 (s, 1H, CH), 8.15 (d, 1H, *J*= 3.2 Hz, pyridine), 8.10 (m, 2H, quinoxaline), 7.87 (m, 2H, quinoxaline), 7.58 (t, 1H, *J*= 7.2 Hz, pyridine), 6.85 (d, 1H, *J*= 8.0 Hz, pyridine), 6.68 (t, 1H, *J*= 5.6 Hz, pyridine), 4.12 (m, 4H, piperazine), 3.70 (m, 4H, piperazine). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 181.2, 159.0, 149.1, 148.1, 143.3, 141.9, 141.9, 141.8, 138.1, 131.2, 130.9, 129.5, 113.7, 107.5, 50.3, 44.7. MP: 190-191°C. HRMS-EI: 377.1405($C_{19}H_{19}N_7S$; Exact Mass: 377.1423). Log P_{calc}: 2.09.

(*E*)-4-(Pyrazin-2-yl)-*N*'-(quinoxalin-2-ylmethylene)piperazine-1-carbothiohydrazide (5e)



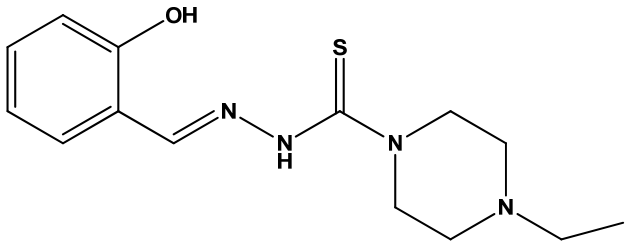
Yield: 79 %. Purity: 95.37%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.81 (bs, 1H, NH), 9.40 (s, 1H, quinoxaline), 8.36 (m, 2H, CH, pyrazine), 8.13-8.08 (m, 3H, quinoxaline, pyrazine), 7.89-7.84 (m, 3H, quinoxaline, pyrazine), 4.15 (m, 4H, piperazine), 3.79 (m, 4H, piperazine). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 181.2, 154.7, 149.1, 143.3, 142.0, 141.9, 141.9, 141.8, 133.1, 131.7, 131.2, 130.9, 129.4, 129.3, 50.01, 44.0. MP: 186-187°C. HRMS-ESI-TOF: 401.1270 $[M + Na]^+$ ($C_{18}H_{18}N_8SNa$; Exact Mass: 401.1273). Log P_{calc}: 1.325.

(*E*)-*N*-Cyclohexyl-*N*-methyl-2-(quinoxalin-2-ylmethylene)hydrazinecarbothioamide (5f)



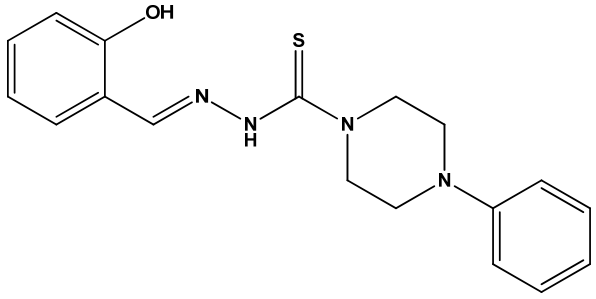
Yield: 78%. Purity: 98.70%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.44 (bs, 1H, NH), 9.36 (s, 1H, quinoxaline), 8.38 (s, 1H, CH), 8.12-8.07 (m, 2H, quinoxaline), 7.88-7.85 (m, 2H, quinoxaline), 4.74 (bs, 1H, C_1-cyclohexyl), 3.16 (s, 3H, CH_3), 1.80 (m, 4H, cyclohexyl), 1.58 (m, 3H, cyclohexyl), 1.32 (m, 2H, cyclohexyl), 1.16 (t, 1H, J= 12.8 Hz, cyclohexyl). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 181.0, 149.3, 143.2, 141.9, 141.8, 131.2, 130.8, 129.4, 129.4, 61.3, 35.1, 29.7, 25.7, 25.4. MP: 154-155°C. HRMS-EI: 327.1513 ($C_{17}H_{21}N_5S$; Exact Mass: 327.1518). Log P_{calc}: 3.398.

(*E*)-4-Ethyl-*N*'-(2-hydroxybenzylidene)piperazine-1-carbothiohydrazide (6a)



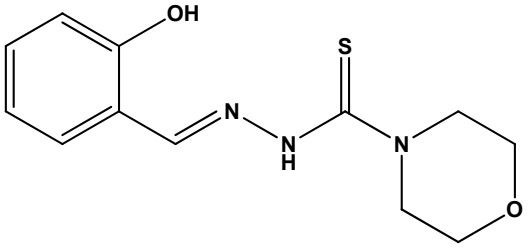
Yield: 86%. Purity: 95.2%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.60 (bs, 1H, NH), 8.46 (s, 1H, CH), 7.41 (dd, 1H, J_1 = 7.9 Hz, J_2= 1.6 Hz, phenyl), 7.27 (td, 1H, J_1 = 7.8 Hz, J_2= 7.4 Hz, J_3= 1.7 Hz, phenyl), 6.92-6.88 (m, 2H, phenyl), 3.92 (m, 4H, piperazine), 2.45 (m, 4H, piperazine), 2.35 (m, 2H, CH_2), 1.03 (t, 3H, J = 7.2 Hz, CH_3). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 183.0, 159.0, 148.0, 138.0, 128.5, 121.2, 118.6, 117.5, 57.2, 44.4, 12.5. MP: 160-161°C (160-161°C, [7]). HRMS-ESI-TOF: 293.1434 $[M + H]^+$ ($C_{14}H_{21}N_4OS$; Exact Mass: 293.1436). Log P_{calc}: 2.832.

(*E*)-*N*'-(2-Hydroxybenzylidene)-4-phenylpiperazine-1-carbothiohydrazide (6b)



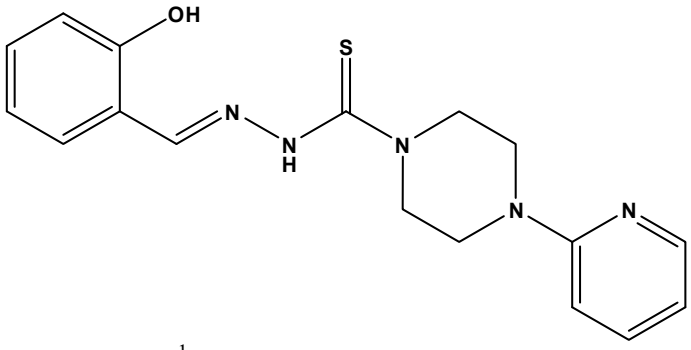
Yield: 89%. Purity: 96.12%. MP: 206-207°C [207-209°C; [4]]. Log P_{calc}: 3.231.

(*E*)-*N*'-(2-Hydroxybenzylidene)morpholine-4-carbothiohydrazide (6c)



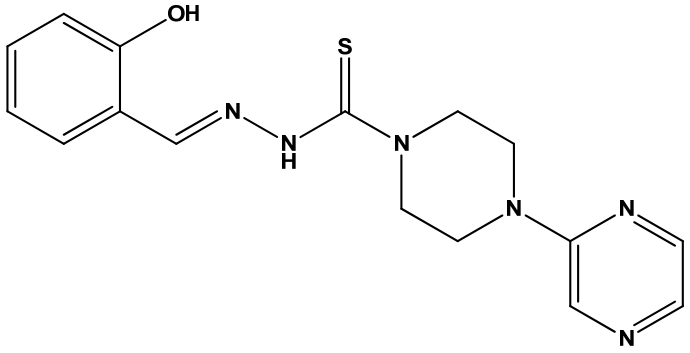
Yield: 91%. Purity: 95.96%. MP: 196-197°C [195°C; [4]]. Log P_{calc}: 1.239.

(*E*)-*N*'-(2-Hydroxybenzylidene)-4-(pyridin-2-yl)piperazine-1-carbothiohydrazide (6d)



Yield: 88%. Purity: 99.3%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.56 (bs, 1H, NH), 11. 54 (bs, 1H, OH), 8.50 (s, 1H, CH), 8.15 (dd, 1H, J_1 = 4.7 Hz, J_2= 1.4 Hz, pyridine), 7.58 (ddd, J_1 = 8.9 Hz, J_2= 7.2 Hz, J_3= 1.9 Hz, pyridine), 7.45–7.40 (m, 1H, phenyl), 7.32–7.24 (m, 2H, phenyl), 6.91 (dd, 1H, J_1= 7.7, J_2= 4.5 Hz, phenyl), 6.86 (d, 1H, J = 8.6 Hz, pyridine), 6.68 (dd, 1H, J_1 = 6.9 Hz, J_2= 5.0 Hz), 4.14–3.98 (m, 4H, piperazine), 3.67–3.59 (m, 4H, piperazine). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 179.8, 158.9, 157.6, 148.0, 146.8, 138.1, 131.3, 130.4, 119.5, 119.0, 117.0, 113.7, 107.5, 48.4, 44.4. MP: 191-192°C. HRMS-ESI-TOF: 342.1385 $[M+H]^+$ ($C_{17}H_{20}N_5OS$; Exact Mass: 342.1389). Log P_{calc}: 2.283.

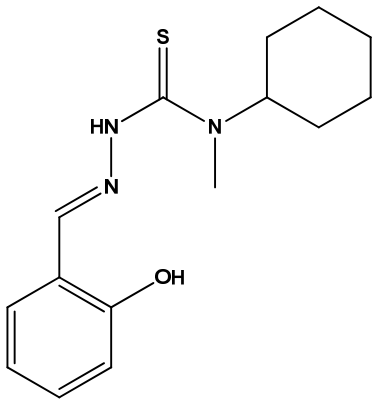
(*E*)-*N*'-(2-Hydroxybenzylidene)-4-(pyrazin-2-yl)piperazine-1-carbothiohydrazide (6e)



Yield: 89%. Purity: 98.9%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.56 (bs, 1H, NH), 11. 54 (bs, 1H, OH), 8.51 (s, 1H, CH), 8.35 (d, 1H, J = 1.6 Hz, pyrazine), 8.12 (s, 1H, pyrazine), 7.88 (d, 1H, J = 2.6 Hz, pyrazine), 7.43 (dd, 1H, J_1 = 7.8 Hz, J_2= 1.7 Hz, phenyl), 7.28 (t, 1H, J= 8.0 Hz, phenyl), 6.97–6.82 (m, 2H, phenyl), 4.09 (m, 4H, piperazine), 3.73 (m, 4H, piperazine). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 179.8, 157.6, 154.7, 141.9, 133.1, 131.8,

131.4, 130.4, 119.6, 119.0, 117.0, 48.1, 43.7. MP: 189-190°C. HRMS-ESI-TOF: 343.1343 ($C_{16}H_{19}N_6OS$; Exact Mass: 343.1341). Log P_{calc}: 1.518.

(*E*)-*N*-Cyclohexyl-2-(2-hydroxybenzylidene)-*N*-methylhydrazinecarbothioamide (6f)



Yield: 74%. Purity: 95.89%. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm): 11.73 (bs, 1H, NH), 11.13 (bs, 1H, OH), 8.52 (s, 1H, CH), 7.38 (dd, 1H, J_1 = 7.8, J_2= 1.4 Hz, 1H, phenyl), 7.27 (t, 1H, J= 7.6 Hz, phenyl), 6.94–6.85 (m, 2H, phenyl), 4.98 (bs, 1H, C_1-cyclohexyl), 3.07 (s, 3H, CH_3), 1.79 (m, 4H, cyclohexyl), 1.50 (m, 3H, cyclohexyl), 1.29 (m, 2H, cyclohexyl), 1.14 (t, 1H, J = 15.4 Hz, cyclohexyl). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm): 179.4, 157.6, 146.8, 131.2, 130.6, 119.5, 118.9, 117.0, 59.8, 32.9, 29.5, 25.7, 25.4. MP: 145-146°C. HRMS-EI: 291.1401 ($C_{15}H_{21}N_3OS$; Exact Mass: 291.1405). Log P_{calc}: 3.591.

S1.2 X-ray data for selected thiosemicarbazones and thiosemicarbazides

X-ray crystal data were collected on an Oxford Diffraction Gemini A Ultra diffractometer using graphite monochromated Mo Kα radiation at a temperature of 295.0(2) K with x scan mode. Polarization, lorentz and empirical absorption corrections were applied using spherical harmonics implemented in the SCALE3 ABSPACK scaling algorithm (CrysAlis RED, Oxford Diffraction Ltd., Version 1.171.29.2). The structures were solved by direct methods and subsequently completed from Fourier difference recycling. All the non-hydrogen atoms were refined anisotropically using full-matrix, least-squares analysis. All hydrogen atoms were located from the difference maps after four cycles of anisotropic refinement, and refined using a riding model. The OLEX2 [8], SHELXS97 and SHELXL97 [9] programs were used for all calculations. All data were deposited with the Cambridge Crystallographic Data Center (CCDC 918418 & 919683).

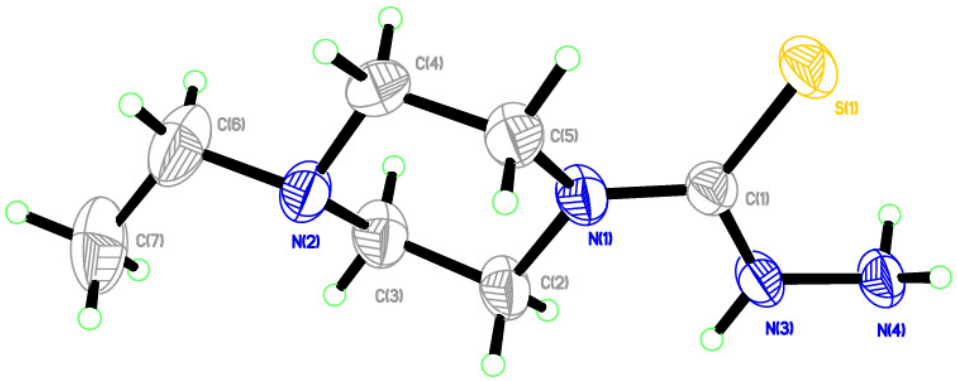


Figure S1. The crystal structure of 4-ethylpiperazine-1-carbothiohydrazide (**a**).

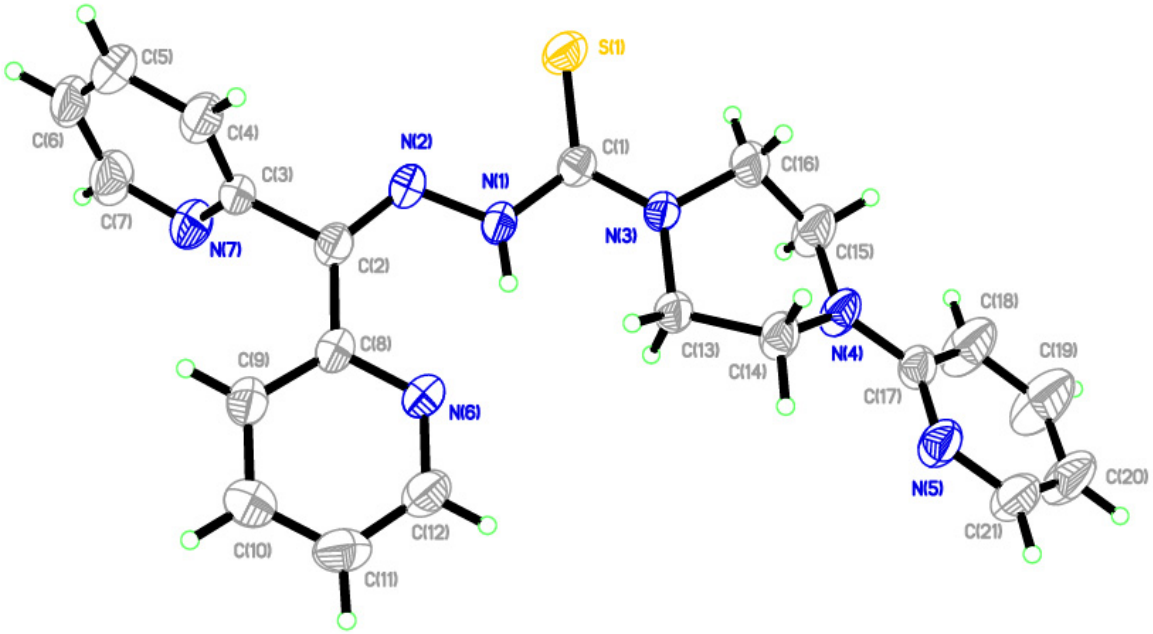


Figure S2. The crystal structure of *Z*-*N*'-(di(pyridin-2-yl)methylene)-4-(pyridin-2-yl)piperazine-1-carbothiohydrazide (**1d**).

Table S1. Crystal data of **a** and **1d**.

	4-Ethylpiperazine-1-carbothiohydrazide (**a**)	*Z*-*N*'-(Di(pyridin-2-yl)methylene)-4-(pyridin-2-yl)piperazine-1-carbothiohydrazide (**1d**)
Formula	$C_7H_{16}N_4S$	$C_{21}H_{21}N_7S$
Formula weight	188.3	403.51
Crystal system	Monoclinic	Triclinic
Space group	*C*2/*c*	$P\bar{1}$
Color	White	Yellow
a (Å)	12.3069(7)	9.2850(6)
b (Å)	8.0115(4)	9.5201(8)
c (Å)	21.0221(12)	11.6512(7)
α (°)	90	76.265(6)
β (°)	101.825(6)	88.780(5)
γ (°)	90	79.734(6)
V (Å^3)	2028.72(19)	984.19(12)
T (K)	295	295
D_{Calc} (mg/m^3)	1.233	1.362
Z	8	2
R_1 (obsd data)	0.0350(1490)	0.0444(2603)
wR_2 (all data)	0.0934(1789)	0.1174(3480)
CCDC no.	918418	919683

S1.3 HPLC purity data

Table S2. HPLC purity data for all chelators of series **1**-**6**.

Compound	t_R (min)	Purity (%)
1a	2.34	97.32
1b	2.07	98.65
1c	2.15	96.32
1d	2.15	95.62
1e	2.25	95.45
2a	1.98	96.67
2b	1.96	98.00
2c	2.0	97.42
2d	2.05	97.87
2e	2.02	98.98
2f	1.98	99.12
3a	2.4	95.03
3b	2.5	96.59
3c	2.52	95.77
3d	2.39	99.25
3e	2.53	99.8
3f	2.56	98.01
4a	2.0	95.35
4b	2.01	96.12
4c	2.07	96.22
4d	1.98	98.00
4e	2.1	98.37
4f	2.01	95.46
5a	1.99	96.00
5b	1.98	99.10
5c	2.0	99.00
5d	2.05	98.45
5e	2.11	95.37
5f	1.98	98.70
6a	2.54	95.2
6b	1.96	96.12
6c	2.00	95.96
6d	2.02	99.3
6e	2.06	98.9
6f	1.97	95.89

S1.4 Isosbestic curves

The ligand and complexes were prepared by dissolving the ligand (0.1 mM) in DMSO and various concentrations of $FeCl_3$ were added to obtain the following ligand:Fe^{3+} ratios: 1:1, 2:1, 4:1, 5:1, and 10:1. The collected spectra were overlaid to observe isosbestic points.

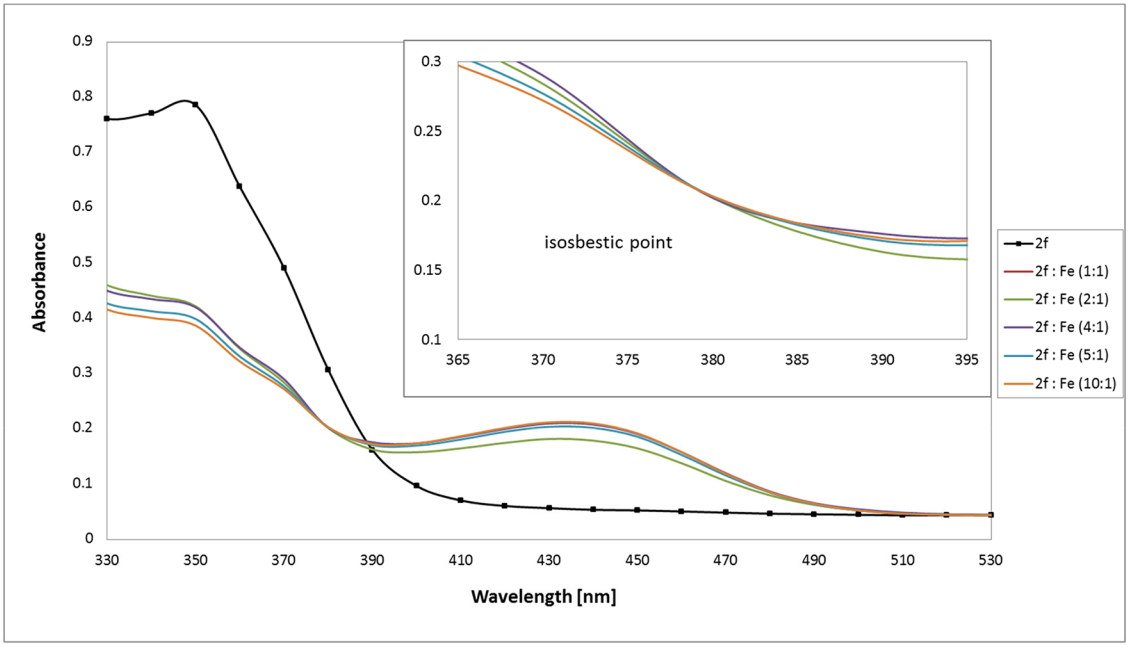


Figure S3. The absorbance spectrum of **2f** and its Fe^{3+} complexes prepared *in situ* to obtain 1:1, 2:1, 4:1, 5:1, and 10:1 ligand:Fe ratios.

S2 Anti-proliferative activity color maps

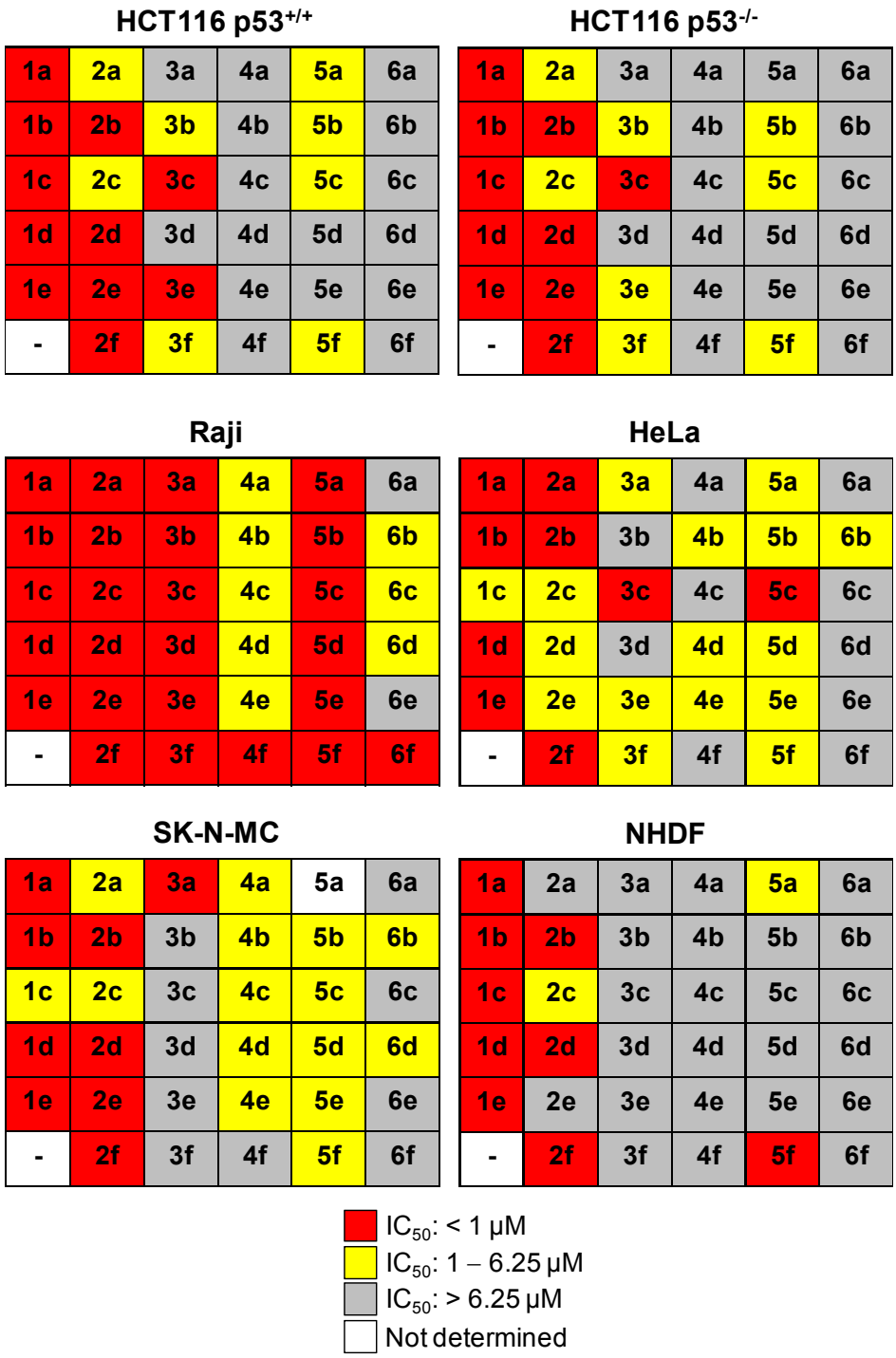


Figure S4. Color maps of the anti-proliferative activity of series **1-6** in several tumor cell-types and normal human dermal fibroblast (NHDF) cells. Red represents the thiosemicarbazones with the greatest anti-proliferative activity (IC_{50}: <1 µM), yellow represents the thiosemicarbazones with moderate activity (IC_{50}: 1 – 6.25 µM) and grey represents those analogs with poor anti-proliferative effects (IC_{50} > 6.25 µM).

S3 References

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