Supporting Information

1-(2-Hydroxybenzoyl)-thiosemicarbazides are promising antimicrobial agents targeting D-alanine-D-alanine ligase in bacterio.

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Optimization of the colorimetric malachite green assay.

The optimization of the enzymatic assay began with the validation of the linearity zone of the absorbance as a function of phosphate quantity (Figure 2a). The loss of linearity was observed at phosphate concentrations higher than 40 µM.

Then, optimal working conditions were determined by varying parameters like the incubation time with D-Ala and the enzyme concentration. The obtained results are depicted in Figure 2b. D-Ala and ATP concentrations were fixed respectively at 1 mM and 500 µM according to the data reported in the literature for determination of Ddl activity with colorimetric malachite green assay.1-5 An incubation time of 20 minutes and an enzyme concentration of 20 µg/mL were adopted.

Considering the relatively poor aqueous solubility of most thiosemicarbazides, the next step was to study the tolerance of the enzyme to DMSO. It was observed that a working concentration of 10% does not significantly impact the enzyme activity (Figure 2c).

Finally, Km for D-Ala of our purified His-tagged DdlB was determined by fitting data to the Michaelis-Menten equation. Kinetic analysis of Ddl ligase is not straightforward. As known, Ddl binds two molecules of D-Ala in two distinct binding sites.6 The Michaelis constants observed for the first D-Ala (Km1) are usually way lower than those for binding of the second substrate (Km2). The overall rate expression that reflects this process is given in Equation 1. For kinetic analysis of inhibitors, at concentrations of D-Ala high enough to occupy both sites, we will postulate that Km1 is negligible compared to Km2 as well depicted in the literature.7-8

\[
\frac{d[P]}{dt} = V = \frac{V_{\text{max}}[S]^2}{K_{m1}K_{m2} + K_{m2}[S] + [S]^2}
\]

Equation 1: Overall rate expression reflecting the two-step substrate binding process. Km1: Michaelis constant for the first D-Ala site, Km2: Michaelis constant for the second D-Ala site (mmol/L). [P]: concentration in product, [S]: concentration in substrate (mmol/L). V: reaction
rate, $V_{\max}$: maximal rate of the enzymatic reaction at saturating substrate concentration ($\mu$M/min).

In the context of our work, the initial velocity of the reaction for the second $\alpha$-Ala site was measured at several substrate concentrations between 0.45-30 fold the theoretical $K_m$ (value reported in the literature)$^7,^9$ with saturating concentration of 500 $\mu$M for ATP (Figure 2d). The inversion of Equation 1 predicts parabolic Lineweaver-Burk plot (Equation 2), from which $1/V_{\max}$ can be measured as the y intercept. The nonlinear regression of $1/V$ against $1/[S]$ plot gave us coefficients which allowed the calculation of the two $K_m$ values ($K_{m1} = 487.2$ $\mu$M and $K_{m2} = 10.5$ mM). These values are higher than the ones reported in the literature.$^9$-$^10$

$$\frac{1}{V} = \frac{1}{V_{\max}} + \frac{K_{m2}}{V_{\max}} \frac{1}{[S]} + \frac{K_{m1}K_{m2}}{V_{\max}} \frac{1}{[S]^2}$$

Equation 2: Second order polynomial (quadratic) equation. $K_{m1}$: Michaelis constant for the first $\alpha$-Ala site, $K_{m2}$: Michaelis constant for the second $\alpha$-Ala site (mmol/L). $[S]$: concentration in substrate (mmol/L). $V$: reaction rate, $V_{\max}$: maximal rate of the enzymatic reaction at saturating substrate concentration ($\mu$M/min).

### $V_{\max}$, $K_m$ and $V_{\max}/K_m$ values of competition studies against $\alpha$-Ala and ATP.

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MIC values of derivatives 3, 14-48 and 51-54 against Gram-positive sensitive strains.
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MIC values of active compounds on Ddl 3, 22-42 and 51 against Gram-positive resistant strains.

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<td>Cip&lt;sup&gt;c&lt;/sup&gt;</td>
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$^1$H and $^{13}$C NMR spectrum of compound 14 bearing no hydroxyl substituent in the 2-position. Three labile protons are visible around 9.7 to 10.7 ppm and each singlet integrates for 1H.
$^1$H and $^{13}$C NMR spectrum of compound 3 bearing a hydroxyl substituent in the 2-position. The labile proton of the -OH is visible at 11.9 ppm integrating for 1H. Two labile protons signals are duplicated and integrate for 0.3H and 0.7H due to the likely tautomerism (the two -NH- become a -SH and a -OH in the enol form). A last labile proton is visible around 9.9 ppm integrating for 1H (-NH- from the thio-urea function).
Experimental data of benzohydrazides precursors (4-13 and 50).

2-Hydroxybenzohydrazide (5). This compound was synthesized according to the general procedure described above using commercial methyl-2-hydroxybenzoate (1.52 g, 10 mmol), hydrazine hydrate (2.5 g, 50 mmol) in ethanol (10 mL) except that no precipitate was formed after cooling the reaction mixture. After 22 h this mixture was evaporated under reduce pressure to afford a brown oil which precipitates after trituration with brine. A white solid was collected after filtration (1.02 g, 67 %). The title compound was used for the following syntheses without any further purification. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ (ppm) 4.68 (brs, 2H, NH$_2$), 6.83-6.93 (m, 2H, ArH), 7.34-7.41 (m, 1H, ArH), 7.81 (dd, $J = 1.6$ Hz, $J = 8$ Hz, 1H, ArH), 10.10 (s, 1H, NH), 12.48 (s, 1H, OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C$ (ppm) 115.63 (Ar), 117.99 (Ar), 118.26 (Ar), 128.14 (Ar), 133.51 (Ar), 160.72 (Ar), 168.03 (C=O). HRMS (APCI⁺): $m/z$ calcd for C$_7$H$_9$N$_2$O$_2$ (M+H)$^+$ 153.06585, found 153.06545.

3-Hydroxybenzohydrazide (6). This compound was synthesized according to the general procedure described above using commercial methyl-3-hydroxybenzoate (1.52 g, 10 mmol), hydrazine hydrate (2.5 g, 50 mmol) in ethanol (10 mL) except that no precipitate was formed after cooling the reaction mixture. After 19 h this mixture was evaporated under reduce pressure to afford a brown oil which precipitates after trituration with brine. A white solid was collected after filtration (0.73 g, 47 %). The title compound was used for the following syntheses without any further purification. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ (ppm) 4.50 (brs, 2H, NH$_2$), 6.99-6.88 (m, 1H, ArH), 7.33-7.21 (m, 3H, ArH), 9.68 (s, 1H, NH), 9.71 (brs, 1H, OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C$ (ppm) 114.02 (Ar), 117.32 (Ar), 117.88 (Ar), 129.25 (Ar), 134.74 (Ar), 157.24 (Ar), 165.92 (C=O). HRMS (APCI⁺): $m/z$ calcd for C$_7$H$_9$N$_2$O$_2$ (M+H)$^+$ 153.06585, found 153.06569.

4-Hydroxybenzohydrazide (7). This compound was synthesized according to the general procedure described above using commercial methyl-4-hydroxybenzoate (1.52 g, 10 mmol),
hydrazine hydrate (2.5 g, 50 mmol) in ethanol (10 mL). After 21 h of reaction, a white solid was collected by filtration (1.20 g, 78 %). The title compound was used for the following syntheses without any further purification. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H (ppm)$ 5.12 (brs, 2H, NH$_2$), 6.75 (d, $J = 8.7$ Hz, 2H, ArH), 7.66 (d, $J = 8.7$ Hz, 2H, ArH), 9.47 (brs, 1H, OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C (ppm)$ 114.88 (Ar), 123.59 (Ar), 128.75 (Ar), 160.40 (Ar), 165.96 (C=O). HRMS (APCI$^+$): $m/z$ calcd for C$_7$H$_9$N$_2$O$_2$ (M+H)$^+$ 153.06585, found 153.06498.

3-Fluorobenzohydrazide (9). This compound was synthesized according to the general procedure described above using commercial methyl-3-fluorobenzoate (1.54 g, 10 mmol), hydrazine hydrate (2.5 g, 50 mmol) in ethanol (7 mL). After 24 h of reaction, white crystals (needles) were collected by filtration (0.83 g, 53 %). The title compound was used for the following syntheses without any further purification. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H (ppm)$ 4.56 (s, 2H, NH$_2$), 7.34-7.40 (m, 1H, ArH), 7.48-7.55 (m, 1H, ArH), 7.61 (dq, $J = 7.4$ Hz, 3H, ArH), 7.69 (dt, $J = 8.4$ Hz, 1H, ArH), 9.88 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C (ppm)$ 113.70 (d, $J = 23$ Hz, Ar), 117.94 (d, $J = 21$ Hz, Ar), 123.07 (d, $J = 3$ Hz, Ar), 130.48 (d, $J = 8$ Hz, Ar), 135.60 (d, $J = 7$ Hz, Ar), 161.91 (d, $J = 243$ Hz, Ar), 168.03 (C=O).

4-Fluorobenzohydrazide (10). This compound was synthesized according to the general procedure described above using commercial methyl-3-fluorobenzoate (1.54 g, 10 mmol), hydrazine hydrate (2.5 g, 50 mmol) in ethanol (7 mL). After 24 h of reaction, white crystals were collected by filtration (0.77 g, 50 %). The title compound was used for the following syntheses without any further purification. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H (ppm)$ 4.49 (s, 2H, NH$_2$), 7.29 (t, $J = 8.8$ Hz, 2H, ArH), 7.87-7.92 (m, 2H, ArH), 9.80 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C (ppm)$ 112.91 (d, $J = 21.5$ Hz, Ar), 125.76 (Ar), 127.20 (d, $J = 8.6$ Hz, Ar), 161.43 (d, $J = 246$ Hz, Ar), 162.59 (C=O).

2-Hydroxy-4-iodobenzohydrazide (11). This compound was synthesized according to the general procedure described above using commercial methyl-2-hydroxy-4-iodobenzoate (1 g,
3.6 mmol), hydrazine hydrate (0.9 g, 17.58 mmol) in ethanol (10 mL) except that no precipitate was formed after cooling the reaction mixture. After 5 h this mixture was evaporated under reduce pressure to afford an oil which precipitates after trituration with cold water. A white solid was collected by filtration after a night at 4°C and washing with cold EtOH (0.48 g, 48 %). The title compound was used for the following syntheses without any further purification.

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ (ppm) 4.71 (brs, 2H, NH$_2$), ArH), 7.22 (dd, $J = 8.3$, 0.9 Hz, 1H, ArH), 7.28 (s, 1H, ArH), 7.53 (d, $J = 8.3$ Hz, 1H, ArH), 10.08 (brs, 1H, NH), 12.59 (brs, 1H, OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C$ (ppm) 100.03 (Ar-I), 114.56 (Ar), 125.77 (Ar), 127.54 (Ar), 128.81 (Ar), 159.67 (Ar-OH), 167.11 (C=O).

2-Hydroxy-4-aminobenzohydrazide (12). This compound was synthesized according to the general procedure described above using commercial methyl-2-hydroxy-4-aminobenzoate (2 g, 11.96 mmol), hydrazine hydrate (2.99 g, 59.82 mmol) in ethanol (20 mL). After 18 h of reaction, a white solid was collected by filtration (0.92 g, 46 %). The title compound was used for the following syntheses without any further purification. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ (ppm) 4.37 (brs, 2H, NH$_2$), 5.68 (s, 2H, ArNH$_2$), 5.93 (d, $J = 2.1$ Hz, 1H, ArH), 6.00 (dd, $J = 8.6$, 2.1 Hz, 1H, ArH), 7.42 (d, $J = 8.6$ Hz, 1H, ArH), 9.50 (s, 1H, NH), 12.59 (brs, 1H, OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C$ (ppm) 99.40 (Ar), 101.68 (Ar), 105.49 (Ar), 127.72 (Ar), 153.75 (Ar-NH$_2$), 162.14 (Ar-OH), 169.38 (C=O).

2-Methoxybenzohydrazide (13). This compound was synthesized according to the general procedure described above using commercial methyl-2-methoxybenzoate (5 g, 30 mmol), hydrazine hydrate (7.53 g, 150 mmol) in ethanol (11 mL) except that no precipitate was formed after cooling the reaction mixture. After 2.5 h this mixture was evaporated under reduce pressure to afford a yellow oil which was extracted 3 times with Et$_2$O/AcOEt. The organic layers were dried over Na$_2$SO$_4$ and evaporated under reduce pressure. White crystals were obtained overnight from the resulting oil (4.03 g, 81 %). The title compound was used for the
following syntheses without any further purification. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ (ppm) 3.86 (s, 3H, OCH$_3$), 4.51 (brs, 2H, NH$_2$), 7.02 (td, $J = 0.8$ Hz, $J = 7.5$ Hz, 1H, ArH), 7.11 (d, $J = 8.3$ Hz, 1H, ArH), 7.45 (ddd, $J = 1$ Hz, $J = 1.8$ Hz, $J = 8.5$ Hz, 1H, ArH), 7.68 (dd, $J = 1.8$ Hz, $J = 7.6$ Hz, 1H, ArH), 9.20 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C$ (ppm) 55.72 (OCH$_3$), 111.83 (Ar), 120.41 (Ar), 122.33 (Ar), 130.10 (Ar), 131.95 (Ar), 156.78 (Ar), 164.73 (C=O).

3-Hydroxy-2-naphthohydrazide (50). This compound was synthesized according to the general procedure described above using commercial methyl-2-hydroxy-2-naphtoate (2 g, 9.89 mmol), hydrazine hydrate (2.47 g, 49.45 mmol) in ethanol (7 mL). After 1 h of reaction, the solvent was evaporated under reduced pressure. The solid was washed with cold water and a pale yellow powder was collected by filtration (1.5 g, 75 %). The title compound was used for the following syntheses without any further purification. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ (ppm) 4.80 (brs, 2H, NH$_2$), 7.31 (dd, $J = 7.4$ Hz, 1H, ArH), 7.47 (dd, $J = 7.5$ Hz, 1H, ArH), 7.71 (d, $J = 8.3$ Hz, 1H, ArH), 7.80 (d, $J = 8.2$ Hz, 1H, ArH), 10.24 (s, 1H, ArH), 8.43 (s, 1H, NH), 11.47 (s, 1H, OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta_C$ 108.70 (Ar), 117.15 (Ar), 120.93 (Ar), 123.54 (Ar), 124.14 (Ar), 125.81 (Ar), 126.73 (Ar), 127.24 (Ar), 134.10 (Ar), 154.77 (Ar), 164.86 (Ar).

Experimental data of thiosemicarbazides 15-28, 30-49 and 51-52.
1-Benzoyl-4-(2-chlorophenyl)-3-thiosemicarbazide (15). This compound was synthesized according to the general procedure described above using commercial benzohydrazide 4 (0.25 g, 1.837 mmol) and 2-chlorophenyl isothiocyanate (0.31 g, 1.837 mmol) in methanol (20 mL). After 24 h of reaction, the pure product was collected as white powder without recrystallization (0.23 g, 34 %). Rf 0.31 (PE/EtOAc 5:5). Mp: 149.3-151.6°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$H (ppm) 7.28 (ddd, $J$ = 7.6 Hz, $J$ = 1.6 Hz, 1H, ArH), 7.36 (dd, $J$ = 7 Hz, 1H, ArH), 7.45 (m, 1H, ArH), 7.51 (m, 3H, ArH), 7.60 (dd, $J$ = 7.2 Hz, 1H, ArH), 7.98 (d, $J$ = 7.2 Hz, 2H, ArH), 9.67 (s, 1H, NH), 9.88 (s, 1H, NH), 10.64 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$C (ppm) 124.56 (Ar), 125.50 (Ar), 125.75 (Ar), 126.80 (Ar), 128.36 (Ar), 128.81 (Ar), 129.40 (Ar), 129.97 (Ar), 134.36 (Ar), 163.66 (C=O), 179.42 (C=S). HRMS (ESI$^+$): m/z calcd for C$_{14}$H$_{13}$ClN$_3$OS (M+H)$^+$ 306.04624, found 306.04584.

1-Benzoyl-4-(3-chlorophenyl)-3-thiosemicarbazide (16). This compound was synthesized according to the general procedure described above using commercial benzohydrazide 4 (0.30 g, 2.2 mmol) and 3-chlorophenyl isothiocyanate (0.37 g, 2.2 mmol) in methanol (10 mL). After 24 h of reaction, the pure product was collected as white powder without recrystallization (0.57 g, 85 %). Rf 0.26 (PE/EtOAc 5:5). Mp: 170.6-175.4°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$H (ppm) 7.21 (d, $J$ = 7.8 Hz, 1H, ArH), 7.37 (dd, $J$ = 8.0 Hz, 1H, ArH), 7.47 (d, $J$ = 8.0 Hz, 1H, ArH), 7.52 (dd, $J$ = 7.2 Hz, 2H, ArH), 7.60 (ddddd, $J$ = 7.3 Hz, $J$ = 1.3 Hz, 2H, ArH), 7.97 (d, $J$ = 7.32 Hz, 2H, ArH), 9.90 (m, 2H, NH), 10.59 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$C (ppm) 124.22 (Ar), 124.73 (Ar), 125.28 (Ar), 127.91 (Ar), 128.28 (Ar), 129.52 (Ar), 131.94 (Ar), 132.37 (Ar), 140.77 (Ar), 165.98 (C=O), 180.96 (C=S). HRMS (ESI$^+$): m/z calcd for C$_{14}$H$_{13}$ClN$_3$OS (M+H)$^+$ 306.04624, found 306.04581.

1-Benzoyl-4-(4-chlorophenyl)-3-thiosemicarbazide (17). This compound was synthesized according to the general procedure described above using commercial benzohydrazide 4 (0.25 g, 1.837 mmol) and 3-chlorophenyl isothiocyanate (0.31 g, 1.837 mmol) in methanol (20 mL).
After 24 h of reaction, the pure product was collected as white powder without recrystallization (0.23 g, 35 %). Rf 0.21 (PE/EtOAc 6:4). Mp: 169.1-170.5°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ (ppm) 7.39 (d, $J = 8.4$ Hz, 2H, ArH), 7.52 (dd, $J = 7.6$ Hz, 4H, ArH), 7.60 (dd, $J = 7.6$ Hz, 1H, ArH), 7.97 (d, $J = 7.6$ Hz, ArH), 9.85 (m, 2H, NH), 10.58 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C$ (ppm) 127.65 (Ar), 127.89 (Ar), 128.25 (Ar), 129.05 (Ar), 131.90 (Ar), 132.40 (Ar), 138.23 (Ar), 165.94 (C=O), 181.07 (C=S). HRMS (ESI$^+$): m/z calcd for C$_{14}$H$_{13}$ClN$_3$OS (M+H)$^+$ 306.04624, found 306.04547.

1-Benzoyl-4-(2-fluorophenyl)-3-thiosemicarbazide (18).$^{11}$ This compound was synthesized according to the general procedure described above using commercial benzohydrazide 4 (0.30 g, 2.2 mmol) and 2-fluorophenyl isothiocyanate (0.34 g, 2.2 mmol) in methanol (20 mL). After 24 h of reaction, the pure product was collected as white powder without recrystallization (0.28 g, 44 %). Rf 0.25 (PE/EtOAc 5:5). Mp: 155.0-156.0°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ (ppm) 7.28 (dt, $J = 7.6$ Hz, $J = 1.2$ Hz, 1H, ArH), 7.36 (dd, $J = 6.8$ Hz, 1H, ArH), 7.44 (m, 1H, ArH), 7.51 (m, 3H, ArH), 7.60 (dd, $J = 7.2$ Hz, 1H, ArH), 7.98 (d, $J = 7.2$ Hz, 2H, ArH), 9.67 (s, 1H, NH), 9.88 (s, 1H, NH), 10.63 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C$ (ppm) 115.57 (Ar), 115.78 (Ar), 123.89 (Ar), 127.20 (Ar), 127.97 (Ar), 128.21 (Ar), 130.64 (Ar), 131.89 (Ar), 132.39 (Ar), 157.38 (d, $J = 247.6$ Hz, Ar), 166.05 (C=O), 182.20 (C=S). HRMS (ESI$^+$): m/z calcd for C$_{14}$H$_{13}$FN$_3$OS (M+H)$^+$ 290.07579, found 290.07532.

1-Benzoyl-4-(3-fluorophenyl)-3-thiosemicarbazide (19).$^{11}$ This compound was synthesized according to the general procedure described above using commercial benzohydrazide 4 (0.22 g, 1.6 mmol) and 3-fluorophenyl isothiocyanate (0.25 g, 1.6 mmol) in methanol (20 mL). After 24 h of reaction, the pure product was collected as white powder without recrystallization (0.20 g, 31 %). Rf 0.21 (PE/EtOAc 6:4). Mp: 168.1-169.5°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ (ppm) 7.00 (dd, $J = 7.84$ Hz, 1H, ArH), 7.29 - 7.42 (m, 2H, ArH), 7.46 - 7.57 (m, 3H, ArH), 7.60 (dd, $J = 7.0$ Hz, 2H, ArH), 7.98 (d, $J = 7.6$ Hz, 2H, ArH), 9.90 (m, 2H, NH), 10.60 (s, 1H,
13C NMR (100 MHz, DMSO-d6): δC (ppm) 115.51 (Ar), 112.45 (Ar), 121.55 (Ar), 127.88 (Ar), 128.26 (Ar), 129.41 (Ar), 131.90 (Ar), 132.40 (Ar), 141.03 (Ar), 161.45 (d, J = 238 Hz, Ar), 165.96 (C=O), 180.93 (C=S). HRMS (ESI+): m/z calcd for C14H13FN3OS (M+H)+ 290.07579, found 290.07513.

1-Benzoyl-4-(4-fluorophenyl)-3-thiosemicarbazide (20). This compound was synthesized according to the general procedure described above using commercial benzohydrazide 4 (0.25 g, 1.837 mmol) and 3-fluorophenyl isothiocyanate (0.28 g, 1.837 mmol) in methanol (20 mL). After 24 h of reaction, the pure product was collected as white powder without recrystallization (0.078 g, 13 %). Rf 0.21 (PE/EtOAc 4:6). Mp: 165.0-169.1°C. 1H NMR (400 MHz, DMSO-d6): δH (ppm) 7.17 (dd, J = 8.8 Hz, 2H, ArH), 7.39-7.48 (m, 2H, ArH), 7.51 (dd, J = 8 Hz, 2H, ArH), 7.60 (dddd, J = 7.4 Hz, J = 1.2 Hz, 1H, ArH), 7.97 (d, J = 7.2 Hz, 2H, ArH), 9.77 (s, 1H, NH), 9.83 (brs, 1H, NH), 10.56 (s, 1H, NH). 13C NMR (100 MHz, DMSO-d6): δC (ppm) 113.12 (Ar), 113.33 (Ar), 126.50 (Ar), 126.83 (Ar), 130.47 (Ar), 131.04 (Ar), 133.16 (Ar), 158.06 (d, J = 240 Hz, Ar), 164.56 (C=O), 180.00 (C=S). HRMS (ESI+): m/z calcd for C14H13FN3OS (M+H)+ 290.07579, found 290.07532.

1-Benzoyl-4-(3,4-dichlorophenyl)-3-thiosemicarbazide (21).11 This compound was synthesized according to the general procedure described above using commercial benzohydrazide 4 (0.48 g, 3.5 mmol) and 3,4-dichlorophenyl isothiocyanate (0.72 g, 3.5 mmol) in methanol (30 mL). After 4 h of reaction, the pure product was collected as white powder (0.88 g, 73 %). Rf 0.62 (PE/EtOAc 5:5). Mp: 198.5-199.0°C. 1H NMR (400 MHz, DMSO-d6): δH (ppm) 7.48-7.56 (m, 3H, ArH), 7.57-7.62 (m, 2H, ArH), 7.82 (s, 1H), 7.96 (d, J = 7.6 Hz, 2H), 9.92 (brs, 1H, NH), 9.99 (brs, 1H, NH), 10.60 (s, 1H, NH). 13C NMR (100 MHz, DMSO-d6): δC (ppm) 125.75 (Ar), 127.00 (Ar), 127.99 (Ar), 128.40 (Ar), 129.82 (Ar), 130.07 (Ar), 132.09 (Ar), 132.38 (Ar), 139.51 (Ar), 166.08 (C=O), 181.02 (C=S). HRMS (ESI+): m/z calcd for C14H12Cl2N3OS (M+H)+ 340.00726, found 340.00731.
4-(2-Chlorophenyl)-1-(2-hydroxybenzoyl)-3-thiosemicarbazide (22). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxybenzohydrazide 5 (0.4 g, 2.38 mmol) and 2-chlorophenyl isothiocyanate (0.4 g, 2.38 mmol) in methanol (10 mL). After 21 h of reaction, the pure product was collected as white needles (0.27 g, 35 %). Rf 0.38 (PE/EtOAc 5:5). Mp: 175.4-178.2°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ (ppm) 6.91-7.02 (m, 2H, Ar), 7.28 (dd, $J = 7.2$ Hz, 1H, ArH), 7.36 (dd, $J = 7.4$ Hz, 1H, ArH), 7.40-7.58 (m, 3H, ArH), 7.92 (m, 1H, ArH), 9.60-9.85 (brs, 0.3H and 0.7H, NH), 9.96 (s, 0.35H, NH), 10.7-11.05 (brs, 0.7H and 0.3H, NH), 11.38 (s, 0.35H, NH), 11.90 (brs, 0.3H and 0.7H, OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C$ (ppm) 114.82 (Ar), 117.14 (Ar), 118.72 (Ar), 127.08 (Ar), 128.07 (Ar), 128.73 (Ar), 129.33 (Ar), 130.90 (Ar), 131.27 (Ar), 134.17 (Ar), 136.67 (Ar), 159.62 (Ar), 168.90 (C=O), 182.00 (C=S). HRMS (ESI$^+$): m/z calcd for C$_{14}$H$_{13}$ClN$_3$O$_2$S (M+H)$^+$ 322.04282, found 322.04047.

4-(3-Chlorophenyl)-1-(2-hydroxybenzoyl)-3-thiosemicarbazide (23). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxybenzohydrazide 5 (0.4 g, 2.38 mmol) and 3-chlorophenyl isothiocyanate (0.403 g, 2.38 mmol) in methanol (10 mL). After 18 h of reaction, the pure product was collected as white needles (0.20 g, 26 %). Rf 0.19 (PE/EtOAc 5:5). Mp: 175.8-180.5°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ (ppm) 6.93-7.04 (m, 2H, ArH), 7.22 (d, $J = 7.2$ Hz, 1H, ArH), 7.37 (dd, $J = 7.8$ Hz, 1H, ArH), 7.48 (m, 2H, ArH), 7.72 (m, 1H, ArH), 9.97 (m, 1H, ArH), 10.74 (brs, 0.8H, NH), 11.26 (brs, 0.2H, NH), 11.90 (s, 1H, OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C$ (ppm) 115.02 (Ar), 117.16 (Ar), 118.94 (Ar), 124.05 (Ar), 124.68 (Ar), 125.04 (Ar), 128.88 (Ar), 129.69 (Ar), 132.13 (Ar), 134.09 (Ar), 140.63 (Ar), 159.45 (Ar), 168.89 (C=O), 180.87 (C=S). HRMS (ESI$^+$): m/z calcd for C$_{14}$H$_{13}$ClN$_3$O$_2$S (M+H)$^+$ 322.04282, found 322.04111.
4-(4-Chlorophenyl)-1-(2-hydroxybenzoyl)-3-thiosemicarbazide (24). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxybenzohydrazide 5 (0.39 g, 2.3 mmol) and 4-chlorophenyl isothiocyanate (0.38 g, 2.26 mmol) in methanol (10 mL). After 24 h of reaction, the pure product was collected as white powder (0.15 g, 20 %). Rf 0.21 (PE/EtOAc 5:5). Mp: 195.0-205.0°C. 1H NMR (400 MHz, DMSO-d6): δH (ppm) 6.92-7.01 (m, 2H, ArH), 7.40 (d, J = 8.4 Hz, 2H, ArH), 7.46 (dd, J = 7.7 Hz, J = 1.3 Hz, 1H, ArH), 7.52 (m, 2H, ArH), 7.90 (d, J = 5.4 Hz, 1H, ArH), 9.94 (m, 2H, NH), 10.74 (brs, 1H, NH), 11.90 (s, 1H, OH). 13C NMR (100 MHz, DMSO-d6): δC (ppm) 115.00 (Ar), 117.17 (Ar), 118.89 (Ar), 127.47 (Ar), 128.00 (Ar), 128.80 (Ar), 134.11 (Ar), 138.09 (Ar), 159.62 (Ar), 168.99 (C=O), 180.98 (C=S). HRMS (ESI+): m/z calcd for C14H13ClN3O2S (M+H)+ 322.04282, found 322.04105.

4-(2-Fluorophenyl)-1-(2-hydroxybenzoyl)-3-thiosemicarbazide (25). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxybenzohydrazide 5 (0.4 g, 2.38 mmol) and 2-fluorophenyl isothiocyanate (0.364 g, 2.38 mmol) in methanol (10 mL). After 5 h of reaction, the pure product was collected as white needles (0.12 g, 17 %). Rf 0.4 (PE/EtOAc 5:5). Mp: 178.2-180.2°C. 1H NMR (400 MHz, DMSO-d6): δH (ppm) 6.91-7.11 (m, 2H, ArH), 7.19 (dd, J = 7.8 Hz, 1H, ArH), 7.23-7.42 (m, 3H, Ar), 7.46 (dd, J = 7.7 Hz, 1H, ArH), 7.92 (brs, 1H, ArH), 9.72 (s, 1H, NH), 9.98 (brs, 0.7H, NH), 10.6-10.9 (m, 1H, NH), 11.3 (brs, 0.3H, NH), 11.96 (brs, 1H, OH). 13C NMR (100 MHz, DMSO-d6): δC (ppm) 114.66 (Ar), 115.57 (Ar), 115.76 (Ar), 117.16 (Ar), 118.71 (Ar), 123.94 (Ar), 127.00 (Ar), 128.13 (Ar), 128.68 (Ar), 130.45 (Ar), 134.19 (Ar), 159.71 (Ar), 168.98 (C=O), 182.26 (C=S). HRMS (ESI+): m/z calcd for C14H13FN3O2S (M+H)+ 306.07070, found 306.07074.

4-(3-Fluorophenyl)-1-(2-hydroxybenzoyl)-3-thiosemicarbazide (26). This compound was synthesized according to the general procedure described above using synthesized 2-
hydroxybenzohydrazide 5 (0.5 g, 2.97 mmol) and 3-fluorophenyl isothiocyanate (0.455 g, 2.97 mmol) in methanol (10 mL). After 5 h 30 of reaction, the pure product was collected as white powder without recrystallization (0.29 g, 32%). Rf 0.25 (PE/EtOAc 5:5). Mp: 174.9-179.1°C. 

\[^1\text{H} \text{NMR (400 MHz, DMSO-d}_6\): } \delta \text{H (ppm) 6.93-7.03 (m, 3H, ArH), 7.31 (dt, } J = 7 \text{ Hz, } J = 1.5 \text{ Hz, 1H, ArH), 7.37 (qd, } J = 7.7 \text{ Hz, } J = 1.02 \text{ Hz, 1H, ArH), 7.48 (ddd, } J = 7.8 \text{ Hz, } J = 1.5 \text{ Hz, 1H, ArH), 7.51 (brs, 1H, ArH), 7.89 (brs, 1H, ArH), 9.79-10.60 (brs, 1H, NH), 10.60-11.50 (brs, 1H, NH), 11.88 (s, 1H, OH).} \]

\[^{13}\text{C NMR (100 MHz, DMSO-d}_6\): } \delta \text{C (ppm) 108.98 (Ar), 111.48 (Ar), 112.11 (Ar), 115.10 (Ar), 117.16 (Ar), 118.93 (Ar), 121.18 (Ar), 128.82 (Ar), 129.51 (Ar), 134.08 (Ar), 140.90 (d, } J = 10.8 \text{ Hz, Ar), 159.39 (Ar), 161.51 (d, } J = 242 \text{ Hz, Ar), 168.84 (C=O), 180.75 (C=S).} \]

HRMS (ESI\(^+\)): m/z calcd for C\(_{14}\)H\(_{13}\)FN\(_3\)O\(_2\)S (M+H\(^+\)) 306.07070, found 306.07071.

4-(4-Fluorophenyl)-1-(2-hydroxybenzoyl)-3-thiosemicarbazide (27). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxybenzohydrazide 5 (0.4 g, 2.38 mmol) and 4-fluorophenyl isothiocyanate (0.364 g, 2.38 mmol) in methanol (10 mL). After 5 h 30 of reaction, the pure product was collected as white powder without recrystallization (0.46 g, 63%). Rf 0.25 (PE/EtOAc 5:5). Mp: 184.6-185.1°C. 

\[^1\text{H NMR (400 MHz, DMSO-d}_6\): } \delta \text{H (ppm) 6.92-7.01 (m, 2H, ArH), 7.18 (dd, } J = 8.8 \text{ Hz, 2H, ArH), 7.37-7.53 (m, 3H, ArH), 7.91 (d, } J = 8.4 \text{ Hz, 1H, ArH), 9.89 (m, 2H, NH), 10.6-11.1 (m, 1H, NH), 11.92 (s, 1H, OH).} \]

\[^{13}\text{C NMR (100 MHz, DMSO-d}_6\): } \delta \text{C (ppm) 114.74 (d, } J = 25 \text{ Hz, Ar), 117.16 (Ar), 118.85 (Ar), 127.15 (Ar), 127.90 (Ar), 128.76 (Ar), 134.08 (Ar), 135.40 (Ar), 159.43 (d, } J = 242 \text{ Hz, Ar), 159.50 (Ar), 168.94 (C=O), 181.26 (C=S).} \]

HRMS (ESI\(^+\)): m/z calcd for C\(_{14}\)H\(_{13}\)FN\(_3\)O\(_2\)S (M+H\(^+\)) 306.07070, found 306.07074.

4-(2,4-Dichlorophenyl)-1-(2-hydroxybenzoyl)-3-thiosemicarbazide (28). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxybenzohydrazide 5 (0.4 g, 2.38 mmol) and 2,4-dichlorophenyl isothiocyanate (0.51 g,
2.26 mmol) in methanol (10 mL). After 24 h of reaction, the pure product was collected as white powder (0.25 g, 29%). Rf 0.45 (PE/EtOAc 5:5). Mp: 188.0-191.0°C. 1H NMR (400 MHz, DMSO-d6): δH (ppm) 6.90-7.00 (m, 2H, ArH), 7.35-7.49 (m, 3H, ArH), 7.67 (s, 1H, ArH), 7.74-8.00 (m, 1H, ArH), 9.65 (s, 0.3H, NH), 9.76 (s, 0.7H, NH), 10.03 (s, 0.7H, NH), 10.78 (s, 0.7H, NH), 10.95 (s, 0.3H, NH), 11.34 (s, 0.3H, NH), 11.83 (s, 0.3H, OH), 11.91 (s, 0.7H, OH). 13C NMR (100 MHz, DMSO-d6): δC (ppm) 114.64 (Ar), 117.20 (Ar), 118.72 (Ar), 127.27 (Ar), 128.78 (Ar), 129.56 (Ar), 131.54 (Ar), 132.20 (Ar), 132.47 (Ar), 134.24 (Ar), 135.99 (Ar), 159.61 (Ar), 168.88 (C=O), 182.04 (C=S). HRMS (ESI+): m/z calcd for C14H12Cl2N3O2S (M+H)+ 356.00218, found 356.00137.

1-(2-Hydroxybenzoyl)-4-(2-methoxyphenyl)-3-thiosemicarbazide (30). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxybenzohydrazide 5 (0.30 g, 1.93 mmol) and 2-methoxyphenyl isothiocyanate (0.32 g, 1.93 mmol) in methanol (20 mL). After 18 h of reaction, the pure product was collected as white powder without recrystallization (0.53 g, 86%). Rf 0.41 (PE/EtOAc 5:5). Mp: 162.9-164°C. 1H NMR (400 MHz, DMSO-d6): δH (ppm) 3.75 (s, 3H, OCH3), 6.82-7.02 (m, 3H, ArH), 7.05 (d, J = 7.84 Hz, 1H, ArH), 7.16 (dd, J = 7.6 Hz, 1H, ArH), 7.44 (ddd, J = 7.74 Hz, J = 1.36 Hz, 1H, ArH), 7.60-8.20 (m, 2H, ArH), 9.21-9.53 (m, 1H, NH), 9.84 (brs, 0.5 H, NH), 10.53-10.99 (m, 1H, NH), 11.37 (brs, 0.5H, NH), 11.79 (s, 1H, OH). 13C NMR (100 MHz, DMSO-d6): δC (ppm) 55.66 (OCH3), 111.43 (Ar), 115.48 (Ar), 117.01 (Ar), 119.22 (Ar), 119.78 (Ar), 125.37 (Ar), 126.95 (Ar), 127.64 (Ar), 129.41 (Ar), 133.90 (Ar), 152.71 (Ar), 159.18 (Ar), 168.64 (C=O), 180.97 (C=S). HRMS (ESI+): m/z calcd for C15H16N3O3S (M+H)+ 318.09069, found 318.09015.

1-(2-Hydroxybenzoyl)-4-(4-methoxyphenyl)-3-thiosemicarbazide (31). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxybenzohydrazide 5 (0.30 g, 1.97 mmol) and 4-methoxyphenyl isothiocyanate (0.33 g, 1.97 mmol) in methanol (15 mL). After 24 h of reaction, the pure product was collected as white powder (0.39 g, 62%). Rf 0.47 (PE/EtOAc 5:5). Mp: 183.7-185°C. 1H NMR (400 MHz, DMSO-d6): δH (ppm) 3.80 (s, 3H, OCH3), 6.81-7.05 (m, 4H, ArH), 7.02 (d, J = 7.74 Hz, 1H, ArH), 7.14 (dd, J = 7.6 Hz, 1H, ArH), 7.43 (ddd, J = 7.74 Hz, J = 1.36 Hz, 1H, ArH), 7.60-8.20 (m, 2H, ArH), 9.16-9.53 (m, 1H, NH), 9.85 (d, J = 6.0 Hz, 1H, NH), 10.53-10.99 (m, 1H, NH), 11.38 (brs, 0.5H, NH), 11.95 (s, 1H, OH). 13C NMR (100 MHz, DMSO-d6): δC (ppm) 55.66 (OCH3), 110.36 (Ar), 114.98 (Ar), 116.61 (Ar), 118.72 (Ar), 124.88 (Ar), 126.95 (Ar), 127.64 (Ar), 129.41 (Ar), 133.90 (Ar), 152.71 (Ar), 159.18 (Ar), 168.64 (C=O), 180.97 (C=S). HRMS (ESI+): m/z calcd for C15H16N3O3S (M+H)+ 318.09069, found 318.09015.
powder without recrystallization (0.49 g, 78%). R_f 0.33 (PE/EtOAc 5:5). Mp: 199.9-200.8°C.

1H NMR (400 MHz, DMSO-d6): \( \delta_H \) (ppm) 3.75 (s, 3H, OCH3), 6.86-7.02 (m, 4H, ArH), 7.31 (d, \( J = 7.04 \) Hz, 2H, ArH), 7.45 (ddd, \( J = 7.72 \) Hz, \( J = 1.52 \) Hz, 1H, ArH), 7.90 (d, \( J = 7.36 \) Hz, 1H, ArH), 9.63-9.97 (m, 2H, NH), 10.75 (brs, 1H, NH), 11.95 (s, 1H, OH). 13C NMR (100 MHz, DMSO-d6): \( \delta_C \) (ppm) 55.12 (OCH3), 113.21 (OCH3), 115.04 (Ar), 117.22 (Ar), 118.92 (Ar), 127.34 (Ar), 128.83 (Ar), 131.80 (Ar), 134.08 (Ar), 156.67 (Ar), 159.39 (C=O), 180.99 (C=S). HRMS (ESI+): m/z calcd for C15H16N3O3S (M+H)+ 318.09069, found 318.09019.

4-(4-Cyanophenyl)-1-(2-hydroxybenzoyl)-3-thiosemicarbazide (32). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxybenzohydrazide 5 (0.31 g, 2.01 mmol) and 4-cyanophenyl isothiocyanate (0.33 g, 2.01 mmol) in methanol (15 mL). After 24 h of reaction, the pure product was collected as white powder without recrystallization (0.60 g, 96%). R_f 0.28 (PE/EtOAc 5:5). Mp: 187.6-188.0°C.

1H NMR (400 MHz, DMSO-d6): \( \delta_H \) (ppm) 6.85 (d, \( J = 8.8 \) Hz, 2H, Ar), 7.75-7.90 (m, 6H, Ar), 9.99 (m, 2H, NH), 10.14 (s, 1H, NH), 10.36 (s, 1H, OH). 13C NMR (100 MHz, DMSO-d6): \( \delta_C \) (ppm) 114.76 (Ar), 118.77 (Ar), 119.02 (CN), 121.46 (Ar), 122.98 (Ar), 125.22 (Ar), 129.94 (Ar), 132.06 (Ar), 133.05 (Ar), 143.89 (Ar), 160.75 (C=O), 165.71 (Ar), 180.86 (C=S). HRMS (ESI+): m/z calcd for C15H13N4O2S (M+H)+ 313.07537, found 313.07534.

1-(2-Hydroxybenzoyl)-4-pentyl-3-thiosemicarbazide (33). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxybenzohydrazide 5 (0.40 g, 2.38 mmol) and pentyl isothiocyanate (0.31 g, 2.38 mmol) in methanol (10 mL). The reaction was stopped after 24 h. The pure product was collected as white powder after evaporation of the solvent under reduced pressure and recrystallization from ethanol (0.19 g, 29 %). R_f 0.85 (EtOAc). Mp: 183.4-188.8°C. 1H NMR (400 MHz, DMSO-d6): \( \delta_H \) (ppm) 0.87 (t, \( J = 7 \) Hz, 3H, CH3), 1.20-1.35 (m, 4H, CH2), 1.49 (qt, \( J = 7.6 \) Hz, 2H, CH2), 3.42 (q, \( J = 6.6 \) Hz, 2H, CH2), 6.90-6.98 (m, 2H, ArH), 7.45 (ddd, \( J = 7.6 \) Hz, \( J = 1.6 \) Hz 1H,
ArH), 7.86 (d, J = 7.6 Hz, 1H, Ar), 8.15 (s, 1H, NH), 9.39 (brs, 1H, NH), 10.54 (brs, 1H, NH), 11.94 (s, 1H, OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C$ (ppm) 13.9 (CH$_3$), 21.9 (CH$_2$), 28.3 (CH$_2$), 28.4 (CH$_2$), 43.7 (CH$_2$-N), 99.5 (Ar), 114.8 (Ar), 117.2 (Ar), 118.8 (Ar), 128.5 (Ar), 134.0 (Ar), 159.63 (C=O), 181.2 (C=S). HRMS (ESI$^+$): m/z calcd for C$_{13}$H$_{20}$N$_3$O$_2$S (M+H)$^+$ 282.12707, found 282.12732.

1-(2-Hydroxybenzoyl)-4-(3-morpholinopropyl)-3-thiosemicarbazide (34). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxybenzohydrazide 5 (0.5 g, 2.97 mmol), 3-(4-morpholinopropyl) isothiocyanate (0.55 g, 2.97 mmol) in methanol (10 mL). The reaction was stopped after 24 h. The pure product was collected as white solid after evaporation of the solvent under reduced pressure, precipitation of the compound in EtOAc and filtration (0.083 g, 8 %). R$_f$ 0.15 (EtOAc). Mp: 119.2-126.1°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ (ppm) 1.64 (qt, J = 6.6 Hz, 2H, CH$_2$), 2.00-2.18 (m, 6H, CH$_2$), 3.40 (m, 4H, CH$_2$), 3.86-3.94 (dd, J = 8.0 Hz, 2H, CH$_2$), 6.95 (dd, J = 7.4 Hz, 1H, ArH), 7.02 (d, J = 8.0 Hz, 1H, ArH), 7.33 (dd, J = 7.6 Hz, J = 1.2 Hz, 1H, ArH), 7.42 (ddd, J = 1.2 Hz, J = 7.8 Hz, 1H, ArH) 10.35 (s, 1H, OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C$ (ppm) 25.48 (CH$_2$), 43.21 (CH$_2$), 54.08 (CH$_2$), 55.95 (CH$_2$), 67.40 (CH$_2$), 114.65 (Ar), 117.30 (Ar), 120.65 (Ar), 132.84 (Ar), 133.75 (Ar), 151.23 (Ar), 157.20 (C=O), 167.66 (C=S). HRMS (ESI$^+$): m/z calcd for C$_{15}$H$_{23}$N$_4$O$_3$S (M+H)$^+$ 339.14854, found 339.14881.

1-(2-Hydroxybenzoyl)-4-(1-naphtyl)-3-thiosemicarbazide (35). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxybenzohydrazide 5 (0.5 g, 2.97 mmol) and naphtyl isothiocyanate (0.55 g, 2.97 mmol) in methanol (10 mL) at reflux. After 1 h 30 of reaction, the pure product was collected as white crystals (0.30 g, 31 %). R$_f$ 0.26 (PE/EtOAc 4:6). Mp: 194.3-198.9°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ (ppm) 6.91-7.00 (m, 2H, ArH), 7.39 (brs, 1H, ArH), 7.46 (ddd, J = 1.06 Hz, J = 7.8 Hz, 1H, ArH), 7.54 (m, 3H, ArH), 7.87 (d, J = 8.4 Hz, 1H, ArH), 7.91-8.03 (m, 3H, ArH), 8.06 (s, 1H, NH), 10.54 (brs, 1H, NH), 11.94 (s, 1H, OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C$ (ppm) 13.9 (CH$_3$), 21.9 (CH$_2$), 28.3 (CH$_2$), 28.4 (CH$_2$), 43.7 (CH$_2$-N), 99.5 (Ar), 114.8 (Ar), 117.2 (Ar), 118.8 (Ar), 128.5 (Ar), 134.0 (Ar), 159.63 (C=O), 181.2 (C=S). HRMS (ESI$^+$): m/z calcd for C$_{13}$H$_{20}$N$_3$O$_2$S (M+H)$^+$ 282.12707, found 282.12732.
9.92 (s, 1H, NH), 10.12 (s, 1H, NH), 10.92 (brs, 0.7H, NH), 11.22 (brs, 0.3H, NH), 12.00 (brs, 1H, OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$C (ppm) 99.59 (Ar), 114.89 (Ar), 117.24 (Ar), 118.80 (Ar), 123.76 (Ar), 125.51 (Ar), 125.94 (Ar), 126.42 (Ar), 126.94 (Ar), 127.97 (Ar), 128.70 (Ar), 130.67 (Ar), 133.76 (Ar), 134.18 (Ar), 135.58 (Ar), 159.70 (Ar), 169.10 (C=O), 182.63 (C=S). HRMS (ESI$^+$): $m/z$ calcd for C$_{18}$H$_{16}$N$_3$O$_2$S (M+H)$^+$ 338.09577, found 338.09609.

1-(2-Hydroxybenzoyl)-4-(3-iodophenyl)-3-thiosemicarbazide (36). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxybenzohydrazide 5 (1 g, 6.58 mmol) and 3-iodophenyl isothiocyanate (1.72 g, 6.58 mmol) in methanol (15 mL). After 30 min of reaction, the pure product was collected by filtration as white powder without recrystallization (2.18 g, 83 %). R$_f$ 0.68 (PE/EtOAc 1:1). Mp: 184.8-188.8°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$H (ppm) 6.92 (d, $J$ = 7.5 Hz, 1H, ArH), 6.95 (d, $J$ = 8.0 Hz, 1H, ArH), 7.12 (dd, $J$ = 8.0 Hz, 1H, ArH), 7.44 (dd, $J$ = 8.0 Hz, 1H, ArH), 7.51 (m, 2H, ArH), 7.70-8.21 (m, 2H), 9.62-10.24 (m, 1.75H), 10.50 (s, 0.25H), 10.69 (s, 0.75H), 11.21 (s, 0.25H), 11.87 (s, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$C (ppm) 93.28 (Ar-I), 114.95 (Ar), 117.19 (Ar), 118.89 (Ar), 122.14 (Ar), 125.04 (Ar), 128.79 (Ar), 129.96 (Ar), 133.67 (Ar), 134.14 (Ar), 140.54 (Ar), 159.60 (Ar-OH), 168.92 (C=O), 180.85 (C=S). HRMS (ESI$^+$): $m/z$ calcd for C$_{14}$H$_{13}$IN$_3$O$_2$S (M+H)$^+$ 413.97677, found 413.97610.

1-(2-Hydroxybenzoyl)-4-(4-acetylphenyl)-3-thiosemicarbazide (37). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxybenzohydrazide 5 (1 g, 6.58 mmol) and 4-acetylphenyl isothiocyanate (1.16 g, 6.58 mmol) in methanol (20 mL). After 1 h 30 of reaction, the pure product was collected by filtration as white powder after washing with EtOH (1.82 g, 84%). R$_f$ 0.22 (PE/EtOAc 1:2). Mp: 190.6-193.8°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$H (ppm) 2.55 (s, 3H, CH$_3$), 6.95 (d, $J$ = 7.5 Hz, 1H, ArH), 6.98 (d, $J$ = 7.9 Hz, 1H, ArH), 7.45 (ddd, $J$ = 7.7, 1.2 Hz, 1H, ArH), 7.62-7.83 (m, 2H, ArH), 7.84-7.91 (m, 1H, ArH), 7.94 (d, $J$ = 8.6 Hz, 2H, ArH), 9.72-10.50 (m, 2H, NH), 10.74
(s, 0.7H, NH), 11.09-11.54 (m, 0.3H, NH), 11.87 (s, 1H, OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$C (ppm) 26.63 (CH$_3$), 115.29 (Ar), 117.36 (2 Ar), 119.00 (Ar), 120.83 (Ar), 124.68 (Ar), 128.46 (Ar), 128.94 (Ar), 134.15 (Ar), 143.75 (2 Ar), 159.51 (Ar-OH), 168.95 (C=O), 180.90 (C=S), 196.93 (C=S). HRMS (ESI$^+$): $m/z$ calcd for C$_{16}$H$_{16}$N$_3$O$_3$S (M+H)$^+$ 330.09069, found 330.09028.

1-(2-Hydroxybenzoyl)-4-(4-trifluoromethoxyphenyl)-3-thiosemicarbazide (38). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxy-benzohydrazide 5 (0.51 g, 3.37 mmol) and 4-trifluoromethoxy isothiocyanate (0.88 g, 4.04 mmol) in methanol (15 mL) at reflux. After 4 h 30 of reaction, pure product was collected by filtration as white solid without recrystallization after washing with cold methanol (0.77 g, 62%). R$_f$ 0.51 (PE/EtOAc 2:3). Mp: 187.7-188.9°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$H (ppm) 6.90-7.00 (m, 2H, ArH), 7.34 (d, $J = 8.6$ Hz, 2H, ArH), 7.45 (ddd, $J = 7.7$, 0.9 Hz, 1H, ArH), 7.51-7.73 (m, 2H, ArH), 7.90 (s, 1H, ArH), 9.68-10.26 (m, 1.7H, NH), 10.50 (brs, 0.3H, NH), 10.74 (brs, 0.7H, NH), 11.08 (brs, 0.3H, NH), 11.89 (s, 1H, OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$C (ppm) 115.24 (Ar), 117.45 (Ar), 120.38 (q, $J = 255.9$ Hz, CF$_3$), 121.05 (Ar), 124.34 (Ar), 127.67 (Ar), 129.07 (Ar), 134.40 (Ar), 138.60 (Ar), 145.39 (Ar), 159.87 (Ar-OH), 169.17 (C=O), 181.42 (C=S). HRMS (ESI$^+$): $m/z$ calcd for C$_{15}$H$_{13}$F$_3$N$_3$O$_3$S (M+H)$^+$ 372.06242, found 372.06203.

1-(2-Hydroxy)-4-(4-(benzyloxy)phenyl)-3-thiosemicarbazide (39). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxy-benzohydrazide 5 (0.63 g, 4.15 mmol) and 4-(benzyloxy)phenyl isothiocyanate (1 g, 4.15 mmol) in methanol (15 mL) at reflux. After 1 h 30 of reaction, pure product was collected by filtration as white solid without recrystallization (1.51 g, 92%). R$_f$ 0.52 (PE/EtOAc 1:1). Mp: 207.7-209.1°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$H (ppm) 5.09 (s, 2H, CH$_2$), 6.90-7.03 (m, 4H, ArH), 7.22-7.36 (m, 3H, ArH), 7.39 (dd, $J = 7.3$ Hz, 2H, ArH), 7.42-7.48 (m, 3H, ArH), 7.89...
(d, J = 7.2 Hz, 1H, ArH), 9.56-10.34 (m, 2H, NH), 10.72 (brs, 0.7H, NH), 11.06 (br, 0.3H, NH), 11.94 (brs, 1H, OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_c$ (ppm) 69.30 (CH$_2$), 114.19 (Ar), 114.98 (Ar), 117.12 (Ar), 118.83 (Ar), 127.24 (Ar), 127.68 (Ar), 127.81 (Ar), 128.41 (Ar), 128.77 (Ar), 132.08 (Ar), 134.00 (Ar), 137.06 (Ar), 155.77 (Ar), 159.27 (Ar-OH), 168.88 (C=O), 180.93 (C=S). HRMS (ESI$^+$): m/z calc for C$_{21}$H$_{20}$N$_3$O$_3$S (M+H)$^+$ 394.12199, found 394.12132.

1-(2-Hydroxybenzoyl)-4-(pyridin-3-yl)-3-thiosemicarbazide (40). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxy-benzohydrazide 5 (0.51 g, 3.35 mmol) and pyridin-3-yl isothiocyanate (0.55 g, 4.02 mmol) in methanol (15 mL) at reflux. After 2 h 45 of reaction, pure product was collected by filtration as white solid without recrystallization after washing with cold methanol (0.88 g, 92%). $R_f$ 0.34 (PE/EtOAc 2:3). Mp: 205.7-208.8$^\circ$C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_h$ (ppm) 6.87-7.09 (m, 2H, ArH), 7.40 (dd, J = 7.9, 4.8 Hz, 1H, ArH), 7.47 (dd, J = 7.7 Hz, 1H, ArH), 7.79-8.17 (m, 2H, ArH), 8.37 (d, J = 4.6 Hz, 1H, ArH), 8.62 (s, 1H, ArH), 9.83 -10.42 (m, 2H, NH), 10.42 -12.80 (m, 2H, NH and OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_c$ (ppm) 114.92 (ArH), 117.20 (ArH), 118.93 (ArH), 123.01 (ArH), 128.81 (ArH), 133.43 (ArH), 134.20 (ArH), 135.94 (ArH), 145.87 (ArH), 146.88 (ArH), 159.40 (Ar-OH), 168.79 (C=O), 181.48 (C=S). HRMS (ESI$^+$): m/z calc for C$_{13}$H$_{13}$N$_4$O$_2$S (M+H)$^+$ 289.07537, found 289.07532.

1-(2-Hydroxy-4-iodobenzoyl)-4-(3,4-dichlorophenyl)-3-thiosemicarbazide (41). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxy-4-iodobenzohydrazide 11 (0.4 g, 1.45 mmol) and 3,4-dichlorophenyl isothiocyanate (0.35 g, 1.75 mmol) in methanol (20 mL). After 5 h of reaction, pure product was collected by filtration as white solid without recrystallization (0.29 g, 43%). $R_f$ 0.39 (PE/EtOAc 1:1). Mp: 228.1-230.0$^\circ$C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_h$ (ppm) 7.35 (dd, J = 8.3, 1.4 Hz, 1H, ArH), 7.39 (s, 1H, ArH), 7.53 (dd, J = 8.7, 1.7 Hz, 1H, ArH), 7.60 (d, J = 8.7
Hz, 1H, ArH), 7.62-7.73 (m, 1H, ArH), 7.75-8.10 (m, 1H, ArH), 9.77-10.32 (m, 1.7H, NH), 10.98 – 10.49 (m, 1H, NH), 11.23 (brs, 0.3H, NH), 12.00 (brs, 1H, OH). 13C NMR (100 MHz, DMSO-d6): δC (ppm) 101.12 (Ar-I), 115.29 (Ar), 125.70 (Ar), 126.82 (Ar), 128.00 (Ar), 129.87 (Ar), 129.98 (Ar), 130.14 (Ar), 130.52 (Ar), 130.63 (Ar), 139.31 (Ar), 159.31 (Ar), 167.95 (C=O), 180.76 (C=S). HRMS (ESI+): m/z calcd for C14H11Cl2IN3O2S (M+H)+ 481.89882, found 481.89792.

1-(2-Hydroxy-4-aminobenzoyl)-4-(3,4-dichlorophenyl)-3-thiosemicarbazide (42). This compound was synthesized according to the general procedure described above using synthesized 2-hydroxy-4-aminobenzohydrazide 12 (0.92 g, 5.51 mmol) and 3,4-dichlorophenyl isothiocyanate (1.35 g, 6.61 mmol) in methanol (15 mL). After 1 h 30 of reaction, pure product was collected by filtration as white solid from recrystallization in EtOH (0.31 g, 15%). Rf 0.28 (PE/EtOAc 1:1). Mp: 235.2-237.9°C. 1H NMR (400 MHz, DMSO-d6): δH (ppm) 7.32 (d, J = 8.4 Hz, 1H, ArH), 7.36 (s, 1H, ArH), 7.51 (d, J = 8.9 Hz, 1H, ArH), 7.56 (d, J = 8.6 Hz, 1H, ArH), 7.64 (d, J = 6.3 Hz, 1H, ArH), 8.12 – 7.71 (m, 1H, ArH), 9.62-10.22 (m, 1.6H, NH), 10.70 (brs, 1H, NH), 11.27 (brs, 0.4H, NH), 12.01 (brs, 1H, OH). 13C NMR (100 MHz, DMSO-d6): δC (ppm) 101.34 (Ar), 115.68 (Ar), 125.89 (Ar), 126.10 (Ar), 127.15 (Ar), 128.45 (Ar), 130.28 (Ar), 130.59 (Ar), 130.98 (Ar), 139.61 (Ar), 139.74 (Ar), 159.62 (Ar), 168.31 (C=O), 181.14 (C=S).

4-(3,4-Dichlorophenyl)-1-(3-hydroxybenzoyl)-3-thiosemicarbazide (43).14 This compound was synthesized according to the general procedure described above using synthesized 3-hydroxybenzohydrazide 6 (0.5 g, 3.29 mmol) and 3,4-dichlorophenyl isothiocyanate (0.67 g, 3.29 mmol) in methanol (13 mL). After 24 h of reaction, the pure product was collected as white powder (0.55 g, 47 %). Rf 0.48 (PE/EtOAc 4:6). Mp: 197.8-199.8°C. 1H NMR (400 MHz, DMSO- d6): δH (ppm) 6.96 (dd, J = 8.0 Hz, J = 1.7 Hz, 1H, ArH), 7.28 (dd, J = 7.8 Hz, 1H, ArH), 7.40-7.31 (m, 2H, ArH), 7.52 (dd, J = 8.8 Hz, J = 2.28 Hz, 1H, ArH), 7.57 (d, J = 8.72
Hz, 1H, ArH), 7.80 (brs, 1H, ArH), 9.72 (s, 1H, NH), 9.87 (brs, 1H, NH), 9.94 (s, 1H, NH), 10.47 (s, 1H, OH). \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta c\) (ppm) 99.49 (Ar), 114.87 (Ar), 118.37 (Ar), 118.83 (Ar), 125.58 (Ar), 126.79 (Ar), 129.28 (Ar), 129.69 (Ar), 129.97 (Ar), 133.73 (Ar), 139.48 (Ar), 157.27 (Ar), 166.02 (C=O), 180.84 (C=S). HRMS (ESI\(^+\)): \(m/z\) calcd for C\(_{14}\)H\(_{12}\)Cl\(_2\)N\(_3\)O\(_2\)S (M+H\(^+\)) 356.00218, found 356.00118.

4-(3,4-Dichlorophenyl)-1-(4-hydroxybenzoyl)-3-thiosemicarbazide (44).\(^{14}\) This compound was synthesized according to the general procedure described above using synthesized 4-hydroxybenzohydrazide 7 (0.5 g, 3.29 mmol) and 3,4-dichlorophenyl isothiocyanate (0.67 g, 3.29 mmol) in methanol (20 mL) at reflux. The reaction was stopped after 24 h. The pure product was collected as pale yellow powder after evaporation of the solvent under reduced pressure and trituration with cold ethanol (0.33 g, 28 %). \(R_f\) 0.75 (EtOAc). Mp: 188.1-190.4\(^\circ\)C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta h\) (ppm) 6.86 (d, \(J = 8.72\) Hz, 2H, ArH), 7.49-7.63 (m, 2H, ArH), 7.76-7.92 (m, 3H, ArH), 9.69-10.03 (m, 2H, NH), 10.19 (s, 1H, NH), 10.36 (s, 1H, OH). \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta c\) (ppm) 114.72 (Ar), 114.86 (Ar), 122.92 (Ar), 125.57 (Ar), 126.76 (Ar), 128.96 (Ar), 129.65 (Ar), 129.96 (Ar), 139.51 (Ar), 160.89 (Ar), 165.77 (C=O), 180.91 (C=S). HRMS (ESI\(^+\)): \(m/z\) calcd for C\(_{14}\)H\(_{12}\)Cl\(_2\)N\(_3\)O\(_2\)S (M+H\(^+\)) 356.00218, found 356.00156.

1-(4-Hydroxybenzoyl)-4-(4-nitrophenyl)-3-thiosemicarbazide (45).\(^{16}\) This compound was synthesized according to the general procedure described above using synthesized 4-hydroxybenzohydrazide 7 (0.25 g, 1.67 mmol) and 4-nitrophenyl isothiocyanate (0.29 g, 1.67 mmol) in methanol (15 mL). After 24 h of reaction, the pure product was collected as yellow powder without recrystallization (0.38 g, 68 %). \(R_f\) 0.43 (PE/EtOAc 4:6). Mp: 207.0-209.5\(^\circ\)C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta h\) (ppm) 6.85 (ddd, \(J = 8.7\) Hz, \(J = 2.7\) Hz 2H, ArH), 7.83 (d, \(J = 8.6\) Hz, 2H, ArH), 7.92 (d, \(J = 8.6\) Hz, 2H, ArH), 8.21 (d, \(J = 9.1\) Hz, 2H, ArH), 10.01-10.12 (m, 2H, NH), 10.14 (s, 1H, NH), 10.38 (brs, 1H, OH). \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta c\)
(ppm) 114.93 (Ar), 122.90 (Ar), 123.59 (Ar), 124.77 (Ar), 130.06 (Ar), 143.26 (Ar), 145.81 (Ar), 160.91 (Ar), 165.72 (C=O), 180.77 (C=S). HRMS (ESI⁺): m/z calcd for C₁₄H₁₃N₄O₄S (M+H)⁺ 333.06520, found 333.06515.

4-(3,4-Dichlorophenyl)-1-(2-fluorobenzoyl)-3-thiosemicarbazide (46). This compound was synthesized using commercial 2-fluorobenzohydrazide 8 (0.26 g, 1.69 mmol) and 3,4-dichlorophenyl isothiocyanate (0.34 g, 1.69 mmol) in methanol (10 mL). The reaction was stopped after 24 h. The pure product was collected as pale yellow powder after evaporation of the solvent under reduced pressure and trituration with cold ethanol (0.16 g, 25 %). Rf 0.56 (PE/EtOAc 4:6). Mp: 180.8-182.1°C. ¹H NMR (400 MHz, DMSO- d₆): δH (ppm) 7.31-7.38 (m, 2H, ArH), 7.53 (dd, J = 8.8 Hz, J = 2.28 Hz, 1H, ArH), 7.58-7.65 (m, 2H, ArH), 7.76-7.96 (m, 2H, ArH), 9.86 (brs, 1H, NH), 10.09 (s, 1H, NH), 10.35 (brs, 1H, NH). ¹³C NMR (100 MHz, DMSO-d₆): δC (ppm) 116.29 (d, J = 22 Hz, Ar), 124.37 (Ar), 124.48 (Ar), 125.60 (Ar), 126.86 (Ar), 130.10 (Ar), 130.83 (Ar), 133.46 (Ar), 133.61 (Ar), 139.37 (Ar), 158.50 (d, J = 32 Hz, Ar), 162.19 (d, J = 259 Hz, Ar), 165.00 (C=O), 180.91 (C=S). HRMS (ESI⁺): m/z calcd for C₁₄H₁₁Cl₂FN₃OS (M+H)⁺ 357.99784, found 357.99661.

4-(3,4-Dichlorophenyl)-1-(3-fluorobenzoyl)-3-thiosemicarbazide (47). This compound was synthesized according to the general procedure described above using synthesized 3-fluorobenzohydrazide 9 (0.3 g, 1.93 mmol) and 3,4-dichlorophenyl isothiocyanate (0.395 g, 1.93 mmol) in methanol (15 mL) at reflux. After 24 h of reaction, pure product was collected as white solid without recrystallization (0.25 g, 48 %). Rf 0.13 (PE/EtOAc 4:6). Mp: 181.9-188.9°C. ¹H NMR (400 MHz, DMSO- d₆): δH (ppm) 7.47 (ddd, J = 8.5 Hz, J = 2.2 Hz, 1H, ArH), 7.54 (dd, J = 8.6 Hz, J = 2.2 Hz, 1H, ArH), 7.56-7.63 (m, 2H, ArH), 7.77 (d, J = 9.9 Hz, 1H, ArH), 7.81 (d, J = 7.8 Hz, 1H, ArH), 9.93 (brs, 1H, NH), 10.03 (s, 1H, NH), 10.71 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-d₆): δC (ppm) 114.30 (d, J = 22 Hz, Ar), 118.87 (d, J = 21 Hz, Ar), 124.07 (Ar), 129.05 (Ar), 125.68 (Ar), 126.95 (Ar), 129.76 (Ar), 130.04 (Ar), 130.51
4-(3,4-Dichlorophenyl)-1-(4-fluorobenzoyl)-3-thiosemicarbazide (48). This compound was synthesized according to the general procedure described above using synthesized 4-fluorobenzohydrazide (0.3 g, 1.93 mmol) and 3,4-dichlorophenyl isothiocyanate (0.39 g, 1.93 mmol) in methanol (15 mL) at reflux. After 24 h of reaction, pure product was collected as white solid without recrystallization (0.21 g, 30%). Rf 0.23 (PE/EtOAc 4:6). Mp: 199.0-199.6°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ (ppm) 7.37 (ddd, $J = 8.8$ Hz, $J = 2.8$ Hz, 2H, ArH), 7.54 (dd, $J = 8.6$ Hz, $J = 2.2$ Hz, 1H, ArH), 7.60 (d, $J = 8.7$ Hz, 1H, ArH), 7.83 (brs, 1H, ArH), 8.03 (dd, $J = 8.7$ Hz, $J = 5.6$ Hz, 1H, ArH), 9.93 (brs, 1H, NH), 10.00 (s, 1H, NH), 10.64 (s, 1H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C$ (ppm) 115.19 (Ar), 115.41 (Ar), 125.65 (Ar), 126.88 (Ar), 128.82 (Ar), 129.74 (Ar), 130.69 (Ar), 164.29 (d, $J = 248.2$ Hz, Ar), 165.01 (C=O), 180.88 (C=S). HRMS (ESI$^+$): m/z calcd for C$\text{$_{14}$}$H$\text{$_{11}$}$Cl$\text{$_2$}$F$\text{$_3$}$N$_3$OS (M+H)$^+$ 357.99784, found 357.99677.

4-(3,4-Dichlorophenyl)-1-(2-methoxybenzoyl)-3-thiosemicarbazide (49). This compound was synthesized according to the general procedure described above using synthesized 2-methoxybenzohydrazide (3.06 g, 18.4 mmol) and 3,4-dichlorophenyl isothiocyanate (3.76 g, 18.4 mmol) in ethanol (10 mL) at reflux. After 1 h of reaction, pure product was collected by filtration as a white solid after washing with hot ethanol (4.75 g, 70%). Rf 0.27 (PE/EtOAc 1:3). Mp: 197.2-198.3°C. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta_H$ (ppm) 3.93 (s, 3H, OCH$_3$), 7.09 (dd, $J = 7.4$ Hz, 1H, ArH), 7.2 (d, $J = 8.2$ Hz, 1H, ArH), 7.47-7.64 (m, 3H, ArH), 7.79-8.12 (m, 2H, ArH), 9.71 (brs, 0.7H, NH), 9.78-10.59 (m, 2H, NH), 11.00 (brs, 0.3H, NH). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta_C$ (ppm) 56.54 (OCH$_3$), 112.65 (Ar), 121.12 (Ar), 121.17 (Ar), 125.85 (Ar), 127.08 (Ar), 130.54 (Ar), 131.48 (Ar), 133.76 (Ar), 139.85 (Ar), 157.77 (Ar-OCH$_3$),
165.95 (C=O), 181.40 (C=S). HRMS (ESI⁺): m/z calcd for C₁₅H₁₄Cl₂N₃O₂S (M+H)⁺ 370.01783, found 370.01758.

1-(3-Hydroxy-2-naphthoyl)-4-(3,4-dichlorophenyl)-3-thiosemicarbazide (51). This compound was synthesized according to the general procedure described above using synthesized 3-hydroxy-2-naphthohydrazide 50 (0.51 g, 1.74 mmol) and 3,4-dichlorophenyl isothiocyanate (0.43 g, 2.08 mmol) in methanol (15 mL) at reflux. After 20 h of reaction, pure product was collected by filtration as yellowish solid after washing with cold methanol (0.58 g, 82%). Rf 0.21 (PE/EtOAc 2:3). Mp: 214.6-218.7°C. ¹H NMR (400 MHz, DMSO-d₆): δH (ppm) 7.33 (s, 1H, ArH), 7.37 (dd, J = 7.5 Hz, 1H, ArH), 7.49-7.57 (m, 2H, ArH), 7.60 (d, J = 8.7 Hz, 1H, ArH), 7.77 (d, J = 8.3 Hz, 1H, ArH), 7.81-8.15 (m, 2H, ArH), 8.54 (brs, 1H, ArH), 9.96 (brs, 0.7H, NH), 10.17 (brs, 1H, NH), 10.81 (brs, 1H, NH), 11.19-11.96 (m, 1.7H, OH and NH). ¹³C NMR (100 MHz, DMSO-d₆): δC (ppm) 110.71 (Ar), 118.75 (Ar), 124.03 (Ar), 125.59 (Ar), 125.94 (Ar), 126.83 (Ar), 128.58 (Ar), 128.91 (Ar), 130.30 (Ar), 130.92 (Ar), 136.13 (Ar), 139.40 (Ar), 154.37 (Ar-OH), 167.81 (C=O), 180.84 (C=S). HRMS (ESI⁺): m/z calcd for C₁₈H₁₄Cl₂N₃O₂S (M+H)⁺ 406.01783, found 406.01713.

1-(4-Oxoquinazolin-3(4H)-yl)-3-phenylthiourea (52). This compound was synthesized according to the general procedure described above using phenyl isothiocyanate (0.42 g, 3.1 mmol) and 3-aminoquinazoline-4(3H)one (0.5 g, 3.1 mmol) in ethanol (40 mL) at reflux. After 4 h of reaction, pure product was collected as white crystals after evaporation of the solvent under reduced pressure, recrystallization from ethanol and then a column chromatography (EtOAc/EP 8:2) (0.088 g, 10 %). Rf 0.19 (PE/EtOAc 5:5). Mp: 165.2-167.1°C. ¹H NMR (400 MHz, DMSO-d₆): δH (ppm) 7.22 (dd, J = 7.4 Hz, 1H, ArH), 7.39 (dd, J = 7.6 Hz, 2H, ArH), 7.43-7.57 (m, 2H, ArH), 7.61 (ddd, J = 7.2 Hz, J = 1.0 Hz, 1H, ArH), 7.75 (d, J = 8.0 Hz, 1H, ArH), 7.90 (ddd, J = 7.5 Hz, J = 1.5 Hz, 1H, ArH), 8.21 (dd, J = 8.0 Hz, J = 1.2 Hz, 1H, ArH), 8.32 (brs, 1H, CH), 10.00-11.00 (m, 2H, NH). ¹³C NMR (100 MHz, DMSO-d₆): δC (ppm)
122.58 (Ar), 124.82 (Ar), 125.57 (Ar), 126.42 (Ar), 127.33 (Ar), 127.48 (Ar), 128.62 (Ar), 134.80 (Ar), 138.48 (Ar), 147.33 (CH), 149.48 (Ar), 158.89 (C=O), 182.47 (C=S). HRMS (ESI\(^+\)): \(m/z\) calcd for \(\text{C}_{15}\text{H}_{13}\text{N}_4\text{O}_S\) (M+H\(^+\)) 297.08046, found 297.08016.

PDB Coordinates for Computational Models.

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| ATOM H | 2148 | HD11 LEU A 142| 3.185 -1.089  14.782 1.00  0.00 |
| ATOM H | 2149 | HD12 LEU A 142| 3.185  0.689  14.774 1.00  0.00 |
| ATOM H | 2150 | HD13 LEU A 142| 3.755 -0.193  16.208 1.00  0.00 |
| ATOM H | 2151 | HD21 LEU A 142| -0.438  0.052  15.297 1.00  0.00 |
| ATOM H | 2152 | HD22 LEU A 142| 0.762  0.820  14.233 1.00  0.00 |
| ATOM H | 2153 | HD23 LEU A 142| 0.557 -0.947  14.213 1.00  0.00 |
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HETATM11080  H41  POB A 321  7.553  -9.985  -1.482  1.00  5.53
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HETATM11082  H43  POB A 321  9.074  -10.881  -1.712  1.00  5.53
HETATM11083  H1'  POB A 321  12.501  -9.889  2.446  1.00  5.75
HETATM11084  H1'1 POB A 321  13.856  -7.700  3.530  1.00  5.46
HETATM11085  H1'2 POB A 321  14.262  -9.298  3.571  1.00  5.46
HETATM11086  H1'3 POB A 321  12.864  -8.783  4.279  1.00  5.46
HETATM11087  H2'1 POB A 321  13.951  -7.582  0.992  1.00  5.41
HETATM11088  H2'2 POB A 321  13.230  -8.990  0.176  1.00  5.41
HETATM11089  H2'3 POB A 321  14.639  -9.205  1.241  1.00  5.41

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REFERENCES


