

Supporting Information for:

A Basis for the Reduced Chemical Library Inhibition of Firefly Luciferase
Obtained from Directed Evolution

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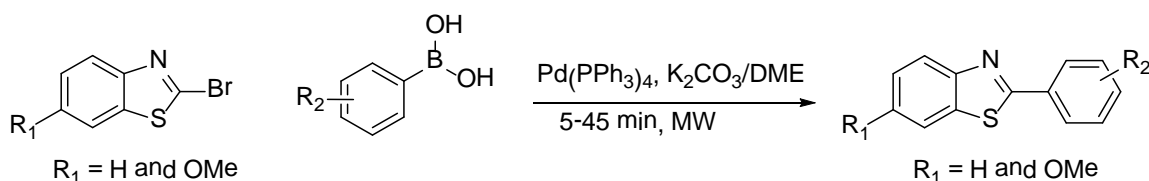
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General Methods:

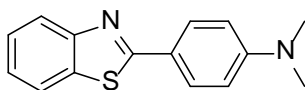
All commercially available reagents and solvents (anhydrous and non-anhydrous) were purchased from Aldrich (Milwaukee, WI), Acros (Pittsburgh, PA), Sigma (St. Louis, MO), Strem (Newburyport, MA), and Fisher Scientific (Fair Lawn, NJ) and used as obtained. All microwave reactions were performed utilizing a Biotage Initiator 1.2 microwave system and Biotage 2.0–5.0 mL vessel with a crimped top. Purification was accomplished via chromatographic methods using forced flow mobile phase of the indicated solvent system on Biotage KP-Sil pre-packed cartridges and using the Biotage SP-1 automated chromatography system. ^1H - and ^{13}C NMR spectra were recorded on a Varian Inova 400 MHz spectrometer. Chemical shifts are reported in ppm with the solvent resonance as the internal standard (CDCl_3 7.26 ppm, for ^1H). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants, and number of protons. Low resolution mass spectra (electrospray ionization) were acquired on an Agilent Technologies 6130 quadrupole spectrometer coupled to an Agilent Technologies 1200 series HPLC. High resolution mass spectral data was collected using and Agilent 6210 time-of-flight mass spectrometer.

The General Procedure for Suzuki Coupling:

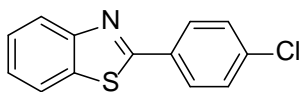


To a mixture of 2-bromobenzo[d]thiazole or 2-bromo-6-methoxybenzo[d]thiazole (0.25 mmol) and selected arylboronic acids/boronates (0.325 mmol) in 2 mL DME in a microwave tube (2-5 mL capacity) was added Pd(PPh₃)₄ (5 mol %, 0.012 mmol) and a 2.0 M aqueous solution of K₂CO₃ (0.25 mmol). The mixture was irradiated with MW for 5-45 minutes at 150 °C, cooled, diluted with 25 mL ethyl acetate and filtered through celite. The solvent was evaporated and the crude material was purified on a Biotage® silica gel column. Gradient elution with ethyl acetate in hexanes (proportions changed based upon the R_f value of the products) gave the coupled products as solids. Samples were analyzed for purity on an Agilent 1200 series LC/MS equipped with a Zorbax™ Eclipse XDB-C18 reverse phase (5 micron, 4.6 x 150 mm) column having a flow rate of 1.1 mL/min. A gradient of 5% to 100% acetonitrile over 8 minutes was used during analytical analysis. Purity of final compounds was determined to be >90%, using a 5 µL injection with quantitation by AUC at 220 and 254 nM.

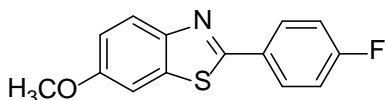
Characterization data:



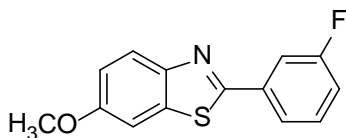
4-(Benzo[d]thiazol-2-yl)-N,N-dimethylaniline (7a): ¹H-NMR (CDCl₃) δ 3.01 (s, 6H, CH₃), 6.74 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.27-7.46 (m, 2H, Ar-H), 7.83 (d, *J* = 8.4 Hz, 1H, Ar-H) and 7.94-7.98 (m, 3H, Ar-H). HRMS (ESI) calcd for C₁₅H₁₅N₂S [M+H]⁺ 255.0956, found 255.0958.



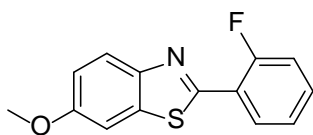
2-(4-Chlorophenyl)benzo[d]thiazole (7b): $^1\text{H-NMR}$ (CDCl_3) δ 7.38-7.62 (m, 3H, Ar-H), 7.69 (d, $J = 8.8$ Hz, 2H, Ar-H), 7.90-8.05 (m, 2H, Ar-H) and 8.17 (d, $J = 8.8$ Hz, 1H, Ar-H); HRMS (ESI) calcd for $\text{C}_{13}\text{H}_9\text{ClNS}$ $[\text{M}+\text{H}]^+$ 246.0144, found 246.0140.



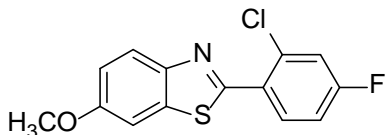
2-(4-Fluorophenyl)-6-methoxybenzo[d]thiazole (7c): $^1\text{H-NMR}$ (CDCl_3) δ 3.90 (s, 3H, OCH_3), 7.08-7.11 (m, 1H, Ar-H), 7.15-7.19 (m, 2H, Ar-H), 7.35 (d, $J = 2.8$ Hz, 1H, Ar-H), 7.93 (d, $J = 9.2$ Hz, 1H, Ar-H) and 8.02-8.05 (m, 2H, Ar-H); HRMS (ESI) calcd for $\text{C}_{14}\text{H}_{11}\text{FNOS}$ $[\text{M}+\text{H}]^+$ 260.0545, found 260.0544.



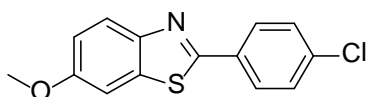
2-(3-Fluorophenyl)-6-methoxybenzo[d]thiazole (7d): $^1\text{H-NMR}$ (CDCl_3) δ 3.90 (s, 3H, OCH_3), 7.11 (dd, $J = 8.8$ Hz and 2.4 Hz, 1H, Ar-H), 7.14-7.18 (m, 1H, Ar-H), 7.36 (d, $J = 2.8$ Hz, 1H, Ar-H), 7.41-7.47 (m, 1H, Ar-H), 7.76-7.81 (m, 2H, Ar-H) and 7.95 (d, $J = 9.2$ Hz, 1H, Ar-H); HRMS (ESI) calcd for $\text{C}_{14}\text{H}_{11}\text{FNOS}$ $[\text{M}+\text{H}]^+$ 260.0545, found 260.0546.



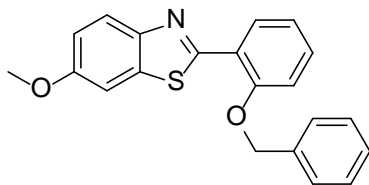
2-(2-Fluorophenyl)-6-methoxybenzo[d]thiazole (7e): $^1\text{H-NMR}$ (CDCl_3) δ 3.91 (s, 3H, OCH_3), 7.11-7.14 (m, 1H, Ar-H), 7.22-7.31 (m, 2H, Ar-H), 7.38 (d, $J = 2.8$ Hz, 1H, Ar-H), 7.41-7.44 (m, 1H, Ar-H), 7.99 (d, $J = 8.4$ Hz, 1H, Ar-H) and 8.34-8.38 (m, 1H, Ar-H); HRMS (ESI) calcd for $\text{C}_{14}\text{H}_{11}\text{FNOS}$ $[\text{M}+\text{H}]^+$ 260.0545, found 260.0543.



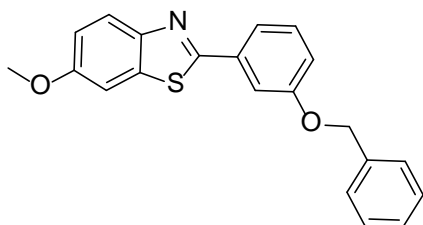
2-(2-Chloro-4-fluorophenyl)-6-methoxybenzo[d]thiazole (7f): $^1\text{H-NMR}$ (CDCl_3) δ 3.91 (s, 3H, OCH_3), 7.11-7.15 (m, 2H, Ar-H), 7.27-7.29 (m, 1H, Ar-H), 7.38 (d, $J = 2.4$ Hz, 1H, Ar-H), 7.99 (d, $J = 8.4$ Hz, 1H, Ar-H) and 8.21 ((dd, $J = 8.8$ Hz and 2.0 Hz, 1H, Ar-H); HRMS (ESI) calcd for $\text{C}_{14}\text{H}_{10}\text{ClFNOS}$ $[\text{M}+\text{H}]^+$ 294.0156, found 294.0157.



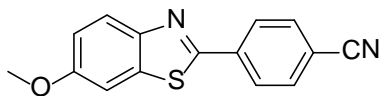
2-(4-Chlorophenyl)-6-methoxybenzo[d]thiazole (7g): $^1\text{H-NMR}$ (CDCl_3) δ 3.90 (s, 3H, OCH_3), 7.10 (dd, $J = 8.8$ Hz and 2.8 Hz, 1H, Ar-H), 7.35 (d, $J = 2.4$ Hz, 1H, Ar-H), 7.45 (d, $J = 8.8$ Hz, 2H, Ar-H) and 7.93-7.98 (m, 3H, Ar-H); HRMS (ESI) calcd for $\text{C}_{14}\text{H}_{11}\text{ClNOS}$ $[\text{M}+\text{H}]^+$ 276.0250, found 276.0252.



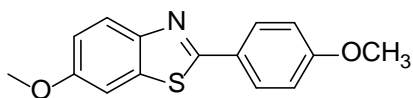
2-(2-(Benzyloxy)phenyl)-6-methoxybenzo[d]thiazole (7h): $^1\text{H-NMR}$ (CDCl_3) δ 3.88 (s, 3H, OCH_3), 5.33 (s, 2H, OCH_2), 7.07-7.14 (m, 3H, Ar-H), 7.32 (d, $J = 2.4$ Hz, 1H, Ar-H), 7.37-7.44 (m, 4H, Ar-H), , 7.53-7.55 (m, 2H, Ar-H), 7.96 (d, $J = 9.2$ Hz, 1H, Ar-H) and 8.48-8.51 (m, 1H, Ar-H); HRMS (ESI) calcd for $\text{C}_{21}\text{H}_{18}\text{NO}_2\text{S}$ $[\text{M}+\text{H}]^+$ 348.1058, found 348.1059.



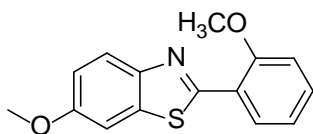
2-(3-(Benzyloxy)phenyl)-6-methoxybenzo[d]thiazole (7i): $^1\text{H-NMR}$ (CDCl_3) δ 3.88 (s, 3H, OCH_3), 5.33 (s, 2H, OCH_2), 7.07-7.14 (m, 3H, Ar-H), 7.32 (d, $J = 2.4$ Hz, 1H, Ar-H), 7.37-7.44 (m, 4H, Ar-H), 7.54 (d, $J = 7.2$ Hz, 2H, Ar-H), 7.96 (d, $J = 9.2$ Hz, 1H, Ar-H) and 8.48-8.51 (m, 1H, Ar-H) ; HRMS (ESI) calcd for $\text{C}_{21}\text{H}_{18}\text{NO}_2\text{S}$ $[\text{M}+\text{H}]^+$ 348.1058, found 348.1054.



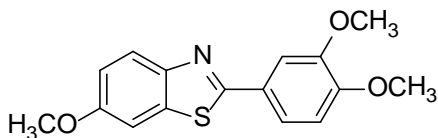
4-(6-Methoxybenzo[d]thiazol-2-yl)benzonitrile (7j): $^1\text{H-NMR}$ (CDCl_3) δ 3.91 (s, 3H, OCH_3), 7.12-7.15 (m, 1H, Ar-H), 7.36 (d, $J = 2$ Hz 1H, Ar-H), 7.75 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.97 (d, $J = 8.8$ Hz, 1H, Ar-H) and 8.13 (d, $J = 8.4$ Hz, 2H, Ar-H); HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{11}\text{N}_2\text{OS}$ $[\text{M}+\text{H}]^+$ 267.0592, found 267.0591.



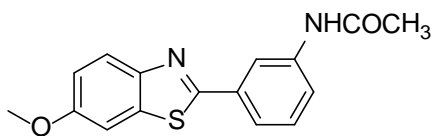
6-Methoxy-2-(4-methoxyphenyl)benzo[d]thiazole (7k): $^1\text{H-NMR}$ (CDCl_3) δ 3.87 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3) 6.98 (d, $J = 8.8$ Hz, 2H, Ar-H), 7.06 (dd, $J = 8.8$ Hz and 2.4 Hz, 1H, Ar-H), 7.33 (d, $J = 2.8$ Hz, 1H, Ar-H), 7.90 (d, $J = 8.8$ Hz, 1H, Ar-H) and 7.97 (d, $J = 8.8$ Hz, 2H, Ar-H); HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{14}\text{NO}_2\text{S}$ $[\text{M}+\text{H}]^+$ 272.0745, found 272.0742.



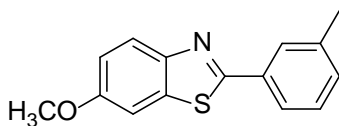
6-Methoxy-2-(2-methoxyphenyl)benzo[d]thiazole (7l): $^1\text{H-NMR}$ (CDCl_3) δ 3.90 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 7.05-7.14 (m, 3H, Ar-H), 7.37 (d, $J = 2.4$ Hz, 1H, Ar-H), 7.41-7.46 (m, 1H, Ar-H), 7.97 (d, $J = 8.8$ Hz, 1H, Ar-H) and 8.46 (dd, $J = 8.0$ Hz and 1.6 Hz, 1H, Ar-H); HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{14}\text{NO}_2\text{S}$ $[\text{M}+\text{H}]^+$ 272.0745, found 272.0745.



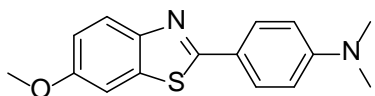
2-(3,4-Dimethoxyphenyl)-6-methoxybenzo[d]thiazole (7m): $^1\text{H-NMR}$ (CDCl_3) δ 3.89 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 6.93 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.07 (dd, $J = 8.8$ Hz and 2.4 Hz, 1H, Ar-H), 7.34 (d, $J = 2.4$ Hz, 1H, Ar-H), 7.54 (dd, $J = 8.4$ Hz and 2.0 Hz, 1H, Ar-H), 7.66 (d, $J = 2.4$ Hz, 1H, Ar-H) and 7.91 (d, $J = 9.2$ Hz, 1H, Ar-H) ; HRMS (ESI) calcd for $\text{C}_{16}\text{H}_{16}\text{NO}_3\text{S}$ $[\text{M}+\text{H}]^+$ 302.0851, found 302.0851.



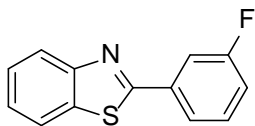
***N*-(3-(6-Methoxybenzo[d]thiazol-2-yl)phenyl)acetamide (7n):** $^1\text{H-NMR}$ (CDCl_3) δ 2.22 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 7.06 (dd, $J = 9.2$ Hz and 2.4 Hz, 1H, Ar-H), 7.36 (d, $J = 2.4$ Hz, 1H, Ar-H), 7.41-7.45 (m, 1H, Ar-H), 7.75-7.80 (m, 2H, Ar-H), 7.93 (d, $J = 8.8$ Hz, 1H, Ar-H) and 8.07 (s, 1H, Ar-H); HRMS (ESI) calcd for $\text{C}_{16}\text{H}_{15}\text{N}_2\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$ 299.0861, found 299.0859.



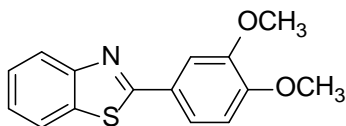
6-Methoxy-2-*m*-tolylbenzo[d]thiazole (7o): $^1\text{H-NMR}$ (CDCl_3) δ 2.45 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 7.74-7.10 (m, 1H, Ar-H), 7.27-7.29 (m, 1H, Ar-H), 7.34-7.38 (m, 2H, Ar-H), 7.80-7.89 (m, 2H, Ar-H) and 7.94 (d, $J = 8.8$ Hz, 1H, Ar-H); HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{14}\text{NOS}$ $[\text{M}+\text{H}]^+$ 256.0796, found 256.0797.



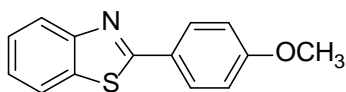
4-(6-Methoxybenzo[d]thiazol-2-yl)-*N,N*-dimethylaniline (7p): $^1\text{H-NMR}$ (CDCl_3) δ 3.01 (s, 6H, CH_3), 3.87 (s, 3H, OCH_3), 6.73 (d, $J = 9.2$ Hz, 2H, Ar-H), 7.02 (dd, $J = 8.8$ Hz and 2.4 Hz, 1H, Ar-H), 7.31 (d, $J = 2.8$ Hz, 1H, Ar-H), 7.85 (d, $J = 9.2$ Hz, 1H, Ar-H) and 7.90 (d, $J = 9.2$ Hz, 2H, Ar-H); HRMS (ESI) calcd for $\text{C}_{16}\text{H}_{17}\text{N}_2\text{OS}$ $[\text{M}+\text{H}]^+$ 285.1062, found 285.1059.



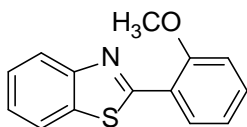
2-(3-Fluorophenyl)benzo[d]thiazole (7q): $^1\text{H-NMR}$ (CDCl_3) δ 7.17-7.23 (m, 1H, Ar-H), 7.40-7.54 (m, 3H, Ar-H), 7.81-7.87 (m, 2H, Ar-H), 7.91-7.94 (m, 1H, Ar-H) and 8.08-8.11 (m, 1H, Ar-H); HRMS (ESI) calcd for $\text{C}_{13}\text{H}_9\text{FNS}$ $[\text{M}+\text{H}]^+$ 230.0440, found 230.0445.



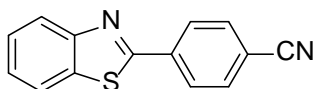
2-(3,4-Dimethoxyphenyl)benzo[d]thiazole (7r): $^1\text{H-NMR}$ (CDCl_3) δ 3.96 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 6.95 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.34-7.38 (m, 1H, Ar-H), 7.46-7.50 (m, 1H, Ar-H), 7.61 (dd, $J = 8.4$ Hz and 2 Hz, 1H, Ar-H), 7.71 (d, $J = 2$ Hz, 1H, Ar-H), 7.87-7.89 (m, 1H, Ar-H) and 8.03-8.05 (m, 1H, Ar-H); HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{14}\text{NO}_2\text{S}$ $[\text{M}+\text{H}]^+$ 271.0745, found 271.0744.



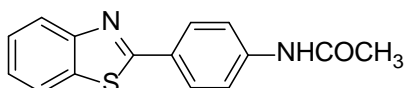
2-(4-Methoxyphenyl)benzo[d]thiazole (7s): $^1\text{H-NMR}$ (CDCl_3) δ 3.88 (s, 3H, OCH_3), 6.99 (d, $J = 8.8$ Hz, 2H, Ar-H), 7.32-7.36 (m, 1H, Ar-H), 7.44-7.48 (m, 1H, Ar-H), 7.86-7.88 (m, 1H, Ar-H) and 8.01-8.04 (m, 3H, Ar-H); HRMS (ESI) calcd for $\text{C}_{14}\text{H}_{12}\text{NOS}$ $[\text{M}+\text{H}]^+$ 242.0640, found 242.0643.



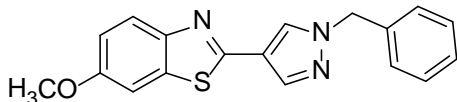
2-(2-Methoxyphenyl)benzo[d]thiazole (7t): $^1\text{H-NMR}$ (CDCl_3) δ 4.01 (s, 3H, OCH_3), 7.07-7.25 (m, 2H, Ar-H), 7.36-7.40 (m, 1H, Ar-H), 7.45-7.52 (m, 2H, Ar-H), 7.92-7.94 (m, 1H, Ar-H), 8.08-8.11 (m, 1H, Ar-H) and 8.51-8.53 (m, 1H, Ar-H); HRMS (ESI) calcd for $\text{C}_{14}\text{H}_{12}\text{NOS}$ $[\text{M}+\text{H}]^+$ 242.0640, found 242.0637.



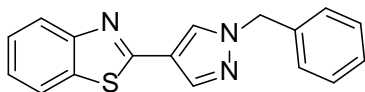
4-(Benzo[d]thiazol-2-yl)benzonitrile (7u): $^1\text{H-NMR}$ (CDCl_3) δ 7.43-7.48 (m, 1H, Ar-H), 7.53-7.57 (m, 1H, Ar-H), 7.79 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.95 (d, $J = 8.0$ Hz, 1H, Ar-H), 8.11 (d, $J = 8.4$ Hz, 1H, Ar-H) and 8.20 (d, $J = 8.4$ Hz, 2H, Ar-H); HRMS (ESI) calcd for $\text{C}_{14}\text{H}_9\text{N}_2\text{S}$ $[\text{M}+\text{H}]^+$ 237.0486, found 237.0485.



N-(4-(benzo[d]thiazol-2-yl)phenyl)acetamide (7v): $^1\text{H-NMR}$ (CDCl_3) δ 2.22 (s, 3H, CH_3) 7.35-7.39 (m, 1H, Ar-H), 7.46-7.50 (m, 1H, Ar-H), 7.64-7.69 (m, 2H, Ar-H), 7.88-7.90 (m, 1H, Ar-H) and 8.03-8.06 (m, 3H, Ar-H); HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{13}\text{N}_2\text{OS}$ $[\text{M}+\text{H}]^+$ 269.0749, found 269.0750.



2-(1-Benzyl-1H-pyrazol-4-yl)-6-methoxybenzo[d]thiazole (7w): $^1\text{H-NMR}$ (CDCl_3) δ 3.87 (s, 3H, OCH_3), 5.35 (s, 2H, CH_2), 7.03-7.06 (m, 1H, Ar-H), 7.28-7.39 (m, 6H, Ar-H), 7.83 (d, $J = 8.8$ Hz, 1H, Ar-H), 7.95 (s, 1H, Het-H) and 8.02 (s, 1H, Het-H); HRMS (ESI) calcd for $\text{C}_{18}\text{H}_{16}\text{N}_3\text{OS}$ $[\text{M}+\text{H}]^+$ 322.1014, found 322.1011.



2-(1-Benzyl-1H-pyrazol-4-yl)benzo[d]thiazole (7x): $^1\text{H-NMR}$ (CDCl_3) δ 5.37 (s, 2H, CH_2), 7.31-7.47 (m, 7H, Ar-H), 7.83-7.85 (m, 1H, Ar-H), 7.95 (d, $J = 9.0$ Hz, 1H, Ar-H), 8.00 (s, 1H, Het-H) and 8.07 (s, 1H, Het-H); HRMS (ESI) calcd for $\text{C}_{17}\text{H}_{14}\text{N}_3\text{S}$ $[\text{M}+\text{H}]^+$ 292.0908, found 292.0907.