SUPPORTING INFORMATION

From Bacteria to Cancer: A Benzothiazole-based DNA Gyrase B Inhibitor Redesigned for Hsp90 C-terminal Inhibition

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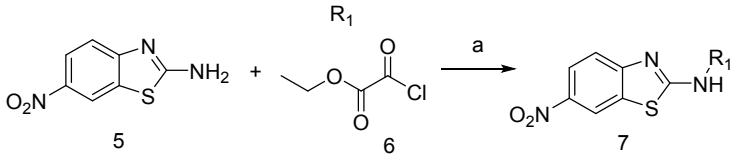
[1]These authors contributed equally to this work

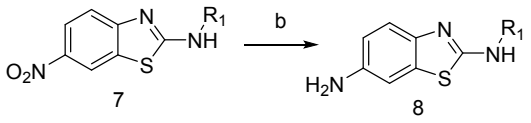
Table of Contents **Page No:**

8. Quantification of **Figure 3** **S12**

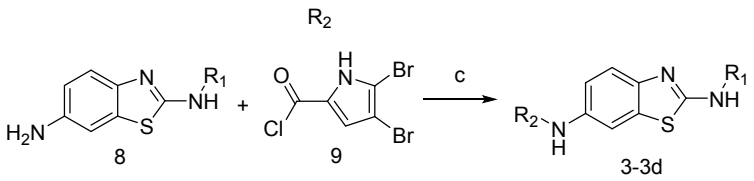
1. **General procedure for preparation of benzothiazole derivatives 3-3d**



(a) A 50 mL round bottom flask was charged with 6-nitrobenzo[d]thiazol-2-amine (**5)** (500 mg, 2.56 mmol) and pyridine (20 mL) at 0° C. Acid chloride **6** (3.13 mmol) was added dropwise to the stirred solution at 0°C, under an argon atmosphere. The reaction was warmed to rt and stirred for 12 h. The reaction was then concentrated, and the resulting residue dissolved in EtOAc (20 mL). The organic layer was washed with 6N HCl (1x20 mL) and followed by brine (1x20 mL). The combined organic layers were collected, dried with anhydrous Na_2SO_4, and concentrated. Hexane (10 mL) was added to the resulting residue, which was filtered and washed with hexanes (10 mL) once more to yield 6-nitrobenzo[d]thiazole intermediate (**7**) as a brown/yellow solid.



(b) 6-nitrobenzo[d]thiazole intermediate (**7**) (120 mg, 814 µmol) in THF (12 mL) and methanol (12 mL) was purged with argon for 5 min before palladium on carbon (3.4 mg, 34 µmol) was added and the argon evacuated. A hydrogen balloon was applied at rt for 12 h while stirring vigorously. The resulting slurry was filtered through a pad of celite and the eluent concentrated to give 6-aminobenzo[d]thiazole intermediate (**8**) as a yellow solid.

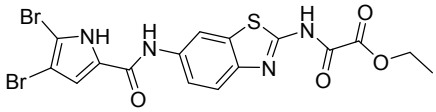


(c) 6-aminobenzo[d]thiazole intermediate (**8**) (100 mg, 0.512 mmol) and acid chloride **9** (0.259 mmol) were dissolved in DCM (10 mL) under an argon atmosphere. Triethylamine (34.6 mg, 0.049

mL, 0.346 mmol) was added, and the reaction stirred at rt for 12 h. Reaction was concentrated, excess ethanol added, and the solution refluxed for 3 h. The ethanol solution was evaporated, and the final compound purified via flash chromatography (SiO_2, 99:1, DCM:MeOH) to yield a light yellow solid.

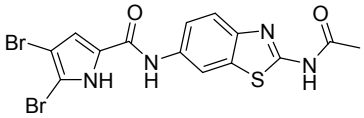
2. **HNMR data for compounds (3-3d)**

Compound 3



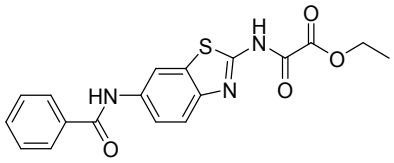
Ethyl 2-((6-(4,5-dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-2-yl)amino)-2-oxoacetate (3): Data and synthesis reported in references.[7]

Compound 3a



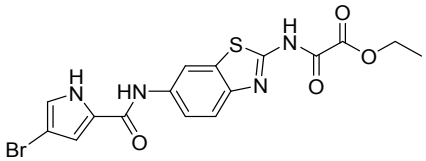
N-(2-acetamidobenzo[d]thiazol-6-yl)-4,5-dibromo-1H-pyrrole-2-carboxamide (3a):
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.94 (s, 1H), 12.31 (s, 1H), 10.00 (s, 1H), 8.36 (dd, *J* = 2.1, 0.6 Hz, 1H), 7.71 (dd, *J* = 8.7, 0.6 Hz, 1H), 7.66 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.25 (s, 1H), 2.20 (s, 3H). HRMS-ESI: calcd. for $C_{14}H_{10}Br_2N_4O_2S$, 456.8937 [M+1]+ ; found: 456.8964.

Compound 3b



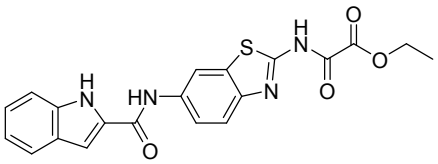
ethyl 2-((6-benzamidobenzo[d]thiazol-2-yl)amino)-2-oxoacetate (3b):
^{1}H NMR (400 MHz, DMSO-d_6) δ 13.19 (s, 1H), 10.48 (s, 1H), 8.53 (t, *J* = 1.4 Hz, 1H), 8.02 – 7.95 (m, 2H), 7.80 (d, *J* = 1.3 Hz, 2H), 7.62 – 7.53 (m, 3H), 4.33 (q, *J* = 7.1 Hz, 2H), 1.33 (t, *J* = 7.1 Hz, 3H). HRMS-ESI: calcd. for $C_{18}H_{15}N_3O_4S$, 370.0826 [M+1]+ ; found: 370.0856.

Compound 3c



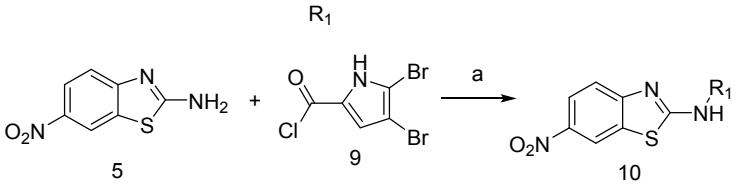
ethyl 2-((6-(4-bromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-2-yl)amino)-2-oxoacetate (3c):
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.09 (s, 1H), 10.01 (s, 1H), 8.43 (d, *J* = 2.0 Hz, 1H), 7.76 (d, *J* = 8.8 Hz, 1H), 7.72 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.19 (dd, *J* = 2.7, 1.6 Hz, 1H), 7.12 (dd, *J* = 3.1, 1.6 Hz, 1H), 4.32 (q, *J* = 7.1 Hz, 2H), 1.33 (t, *J* = 7.1 Hz, 3H). HRMS-ESI: calcd. for $C_{16}H_{13}BrN_4O_4S$, 436.9891 [M+1]+ ; found: 436.9914.

Compound 3d

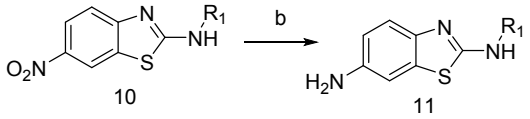


ethyl 2-((6-(1H-indole-2-carboxamido)benzo[d]thiazol-2-yl)amino)-2-oxoacetate (3d):
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (d, *J* = 2.2 Hz, 1H), 10.44 (s, 1H), 8.55 (t, *J* = 1.3 Hz, 1H), 7.86 – 7.77 (m, 2H), 7.73 – 7.66 (m, 1H), 7.52 – 7.44 (m, 2H), 7.24 (ddd, *J* = 8.2, 6.9, 1.1 Hz, 1H), 7.11 – 7.03 (m, 1H), 4.37 – 4.30 (m, 2H), 1.36 – 1.31 (m, 3H). HRMS-ESI: calcd. for $C_{14}H_{10}Br_2N_4O_2S$, 409.0936 [M+1]+ ; found: 409.0965.

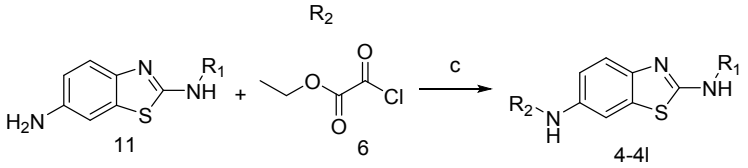
3. **General procedure for preparation of benzothiazole derivatives 4-4k**



(a) Acid chloride **9** (1 mmol) was dissolved in anhydrous dichloromethane (10 mL) at 0°C under an argon atmosphere. 6-nitrobenzo[d]thiazol-2-amine (**5**) (100 mg, 0.512 mmol) and triethylamine (0.346 mmol) were added dropwise, and the reaction warmed to rt and stirred for 12 h. The reaction was concentrated, and the resulting residue dissolved in EtOAc (10 mL). The organic layer was washed with 6N HCl (1x10 mL) and followed by brine (1x10 mL). The combined organic layers were collected, dried with anhydrous Na_2SO_4, and concentrated. Hexane (5 mL) was added to resulting residue, which was filtered and washed with hexane (10 mL) once more to yield 6-nitrobenzo[d]thiazole intermediate (**7**) as a brown/yellow solid.



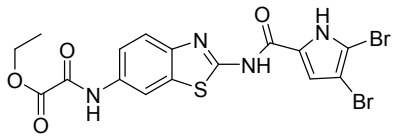
(b) 6-nitrobenzo[d]thiazole intermediate (**10**) (120 mg, 814 µmol) in THF (12 mL) and methanol (12 mL) was purged with argon for 5 min before palladium on carbon (3.4 mg, 34 µmol) was added and the argon evacuated. A hydrogen balloon was applied at rt for 12 h while stirring vigorously. The resulting slurry was filtered through a pad of celite and the eluent concentrated to give 6-aminobenzo[d]thiazole intermediate (**11**) as a yellow solid.



(c) 6-aminobenzo[d]thiazole intermediate (**11**) (58.5 mg, 0.173 mmol) was dissolved in pyridine (6 mL) and ethyl 2-chloro-2-oxoacetate (**6**) (35 mg, 0.029 mL, 0.259 mmol) was added to the solution dropwise at 0℃, under an argon atmosphere. The reaction was warmed to rt and stirred for 12 h. The reaction was dissolved in EtOAc (10 mL) and washed with 6N HCl (1x10 mL), followed by brine (1x10 mL). The combined organic layers were collected, dried with anhydrous Na2SO4, and concentrated. Final compound was purified via flash chromatography to yield a light yellow solid (SiO_2, 99:1, DCM:MeOH).

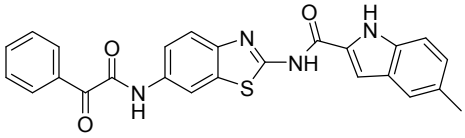
4. **Hnmr data for compounds (4-4k)**

Compound 4



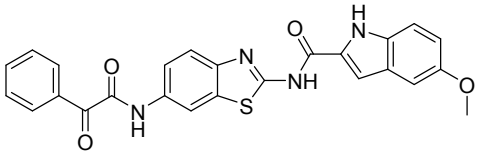
Ethyl 2-((2-(4,5-dibromo-1H-pyrrole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetate (4): Data and synthesis reported in references.[7]

Compound 4a



5-methyl-N-(6-(2-oxo-2-phenylacetamido)benzo[d]thiazol-2-yl)-1H-indole-2-carboxamide (4a):
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.90 (s, 1H), 11.87 (s, 1H), 11.16 (s, 1H), 8.49 (d, *J* = 2.1 Hz, 1H), 8.11 – 8.06 (m, 2H), 7.83 – 7.73 (m, 3H), 7.68 – 7.61 (m, 3H), 7.48 (dt, *J* = 1.7, 0.8 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.12 (dd, *J* = 8.5, 1.6 Hz, 1H), 2.39 (d, *J* = 1.0 Hz, 3H). HRMS-ESI: calcd. for $C_{25}H_{18}N_4O_3S$, 455.1151 [M+1]+ ; found: 455.1172.

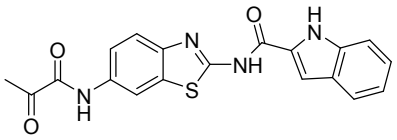
Compound 4b



5-methoxy-N-(6-(2-oxo-2-phenylacetamido)benzo[d]thiazol-2-yl)-1H-indole-2-carboxamide (4b):

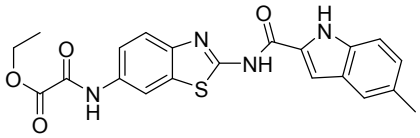
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.92 (s, 1H), 11.87 (s, 1H), 11.17 (s, 1H), 8.49 (d, *J* = 2.1 Hz, 1H), 8.11 – 8.07 (m, 2H), 7.82 – 7.73 (m, 3H), 7.67 – 7.61 (m, 3H), 7.39 (dt, *J* = 8.9, 0.7 Hz, 1H), 7.16 (d, *J* = 2.5 Hz, 1H), 6.94 (dd, *J* = 8.9, 2.5 Hz, 1H), 3.79 (s, 3H). HRMS-ESI: calcd. for $C_{25}H_{18}N_4O_4S$, 471.1095 [M+1]+ ; found: 471.1122.

Compound 4c



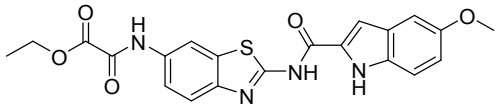
N-(6-(2-oxopropanamido)benzo[d]thiazol-2-yl)-1H-indole-2-carboxamide (4c):
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.95 (s, 1H), 12.00 (s, 1H), 10.64 (s, 1H), 8.51 (d, *J* = 2.1 Hz, 1H), 7.87 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.79 – 7.68 (m, 3H), 7.53 – 7.47 (m, 1H), 7.28 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 1H), 7.10 (ddd, *J* = 8.1, 6.9, 1.0 Hz, 1H), 2.47 (s, 3H). HRMS-ESI: calcd. for $C_{19}H_{14}N_4O_3S$, [M+1]+ ; found: .

Compound 4d



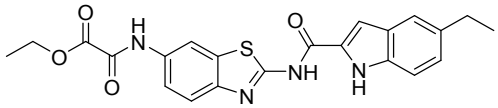
ethyl 2-((2-(5-methyl-1H-indole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetate (4d):
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.90 (s, 1H), 11.88 (s, 1H), 10.98 (s, 1H), 8.43 (t, *J* = 1.3 Hz, 1H), 7.78 (d, *J* = 1.6 Hz, 2H), 7.67 – 7.62 (m, 1H), 7.47 (dq, *J* = 1.7, 0.8 Hz, 1H), 7.41 – 7.35 (m, 1H), 7.12 (dd, *J* = 8.5, 1.7 Hz, 1H), 4.33 (q, *J* = 7.1 Hz, 2H), 2.39 (d, *J* = 1.0 Hz, 3H), 1.34 (t, *J* = 7.1 Hz, 3H). HRMS-ESI: calcd. for $C_{21}H_{18}N_4O_4S$, 423.1093 [M+1]+ ; found: 423.1122.

Lead compound 4e



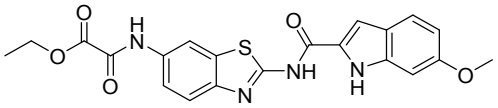
ethyl 2-((2-(5-methoxy-1H-indole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetate (4e): Data is shown in section 4 of SI.

Compound 4f



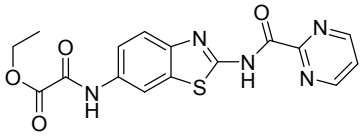
ethyl 2-((2-(5-ethyl-1H-indole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetate (4f):
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.91 (s, 1H), 11.89 – 11.84 (m, 1H), 10.98 (s, 1H), 8.43 (d, *J* = 1.4 Hz, 1H), 7.78 (d, *J* = 1.5 Hz, 2H), 7.70 – 7.65 (m, 1H), 7.55 – 7.46 (m, 1H), 7.40 (d, *J* = 8.5 Hz, 1H), 7.16 (dd, *J* = 8.5, 1.6 Hz, 1H), 4.34 (q, *J* = 7.1 Hz, 2H), 2.69 (q, *J* = 7.6 Hz, 2H), 1.34 (t, *J* = 7.1 Hz, 3H), 1.23 (t, *J* = 7.6 Hz, 3H). HRMS-ESI: calcd. for $C_{22}H_{20}N_4O_4S$, 437.1250 [M+1]+ ; found: 437.1278.

Compound 4g



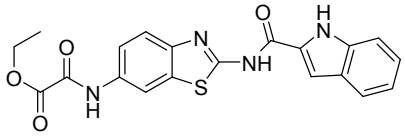
ethyl 2-((2-(6-methoxy-1H-indole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetate (4g):
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.82 (s, 1H), 11.81 (s, 1H), 10.96 (s, 1H), 8.41 (t, *J* = 1.3 Hz, 1H), 7.76 (d, *J* = 1.8 Hz, 2H), 7.69 – 7.65 (m, 1H), 7.57 (d, *J* = 8.8 Hz, 1H), 6.91 (d, *J* = 2.2 Hz, 1H), 6.75 (dd, *J* = 8.8, 2.3 Hz, 1H), 4.33 (q, *J* = 7.1 Hz, 2H), 3.79 (s, 3H), 1.33 (t, *J* = 7.1 Hz, 3H). HRMS-ESI: calcd. for $C_{21}H_{18}N_4O_5S$, 439.1043 [M+1]+ ; found: 439.1071.

Compound 4h



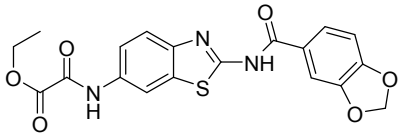
ethyl 2-oxo-2-((2-(pyrimidine-2-carboxamido)benzo[d]thiazol-6-yl)amino)acetate (4h):
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.67 (s, 1H), 11.00 (s, 1H), 9.09 (d, *J* = 4.9 Hz, 2H), 8.47 (dd, *J* = 1.8, 0.9 Hz, 1H), 7.84 – 7.77 (m, 3H), 4.33 (q, *J* = 7.1 Hz, 2H), 1.34 (t, *J* = 7.1 Hz, 3H). HRMS-ESI: calcd. for $C_{15}H_{13}N_5O_4S$, 372.0737 [M+1]+ ; found: 372.0761.

Compound 4i



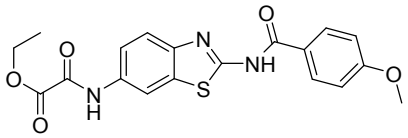
ethyl 2-((2-(1H-indole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetate (4i):
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.02 (s, 1H), 10.99 (s, 1H), 8.44 (s, 1H), 7.79 (s, 2H), 7.76 – 7.69 (m, 2H), 7.50 (d, *J* = 8.3 Hz, 1H), 7.29 (t, *J* = 7.7 Hz, 1H), 7.11 (t, *J* = 7.4 Hz, 1H), 4.34 (q, *J* = 7.1 Hz, 2H), 1.34 (t, *J* = 7.1 Hz, 3H). HRMS-ESI: calcd. for $C_{20}H_{16}N_4O_4S$, 409.0923 [M+1]+ ; found: 409.0965.

Compound 4j



ethyl 2-((2-(benzo[d][1,3]dioxole-5-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetate (4j):
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.73 (s, 1H), 10.97 (s, 1H), 8.42 (t, *J* = 1.4 Hz, 1H), 7.82 – 7.65 (m, 4H), 7.10 (d, *J* = 8.2 Hz, 1H), 6.17 (s, 2H), 4.33 (q, *J* = 7.1 Hz, 2H), 1.34 (t, *J* = 7.1 Hz, 3H). HRMS-ESI: calcd. for $C_{19}H_{15}N_3O_6S$, 414.0737 [M+1]+ ; found: 414.0754.

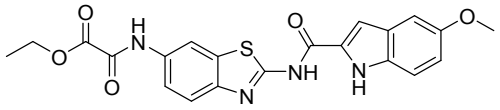
Compound 4k



ethyl 2-((2-(4-methoxybenzamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetate (4k):
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.72 (s, 1H), 10.95 (s, 1H), 8.41 (s, 1H), 8.15 (d, *J* = 8.7 Hz, 2H), 7.77 (d, *J* = 1.6 Hz, 2H), 7.11 (d, *J* = 8.8 Hz, 2H), 4.34 (q, *J* = 7.1 Hz, 2H), 3.87 (s, 3H), 1.34 (t, *J* = 7.1 Hz, 3H). HRMS-ESI: calcd. for $C_{19}H_{17}N_3O_5S$, 400.0941 [M+1]+ ; found: 400.0962.

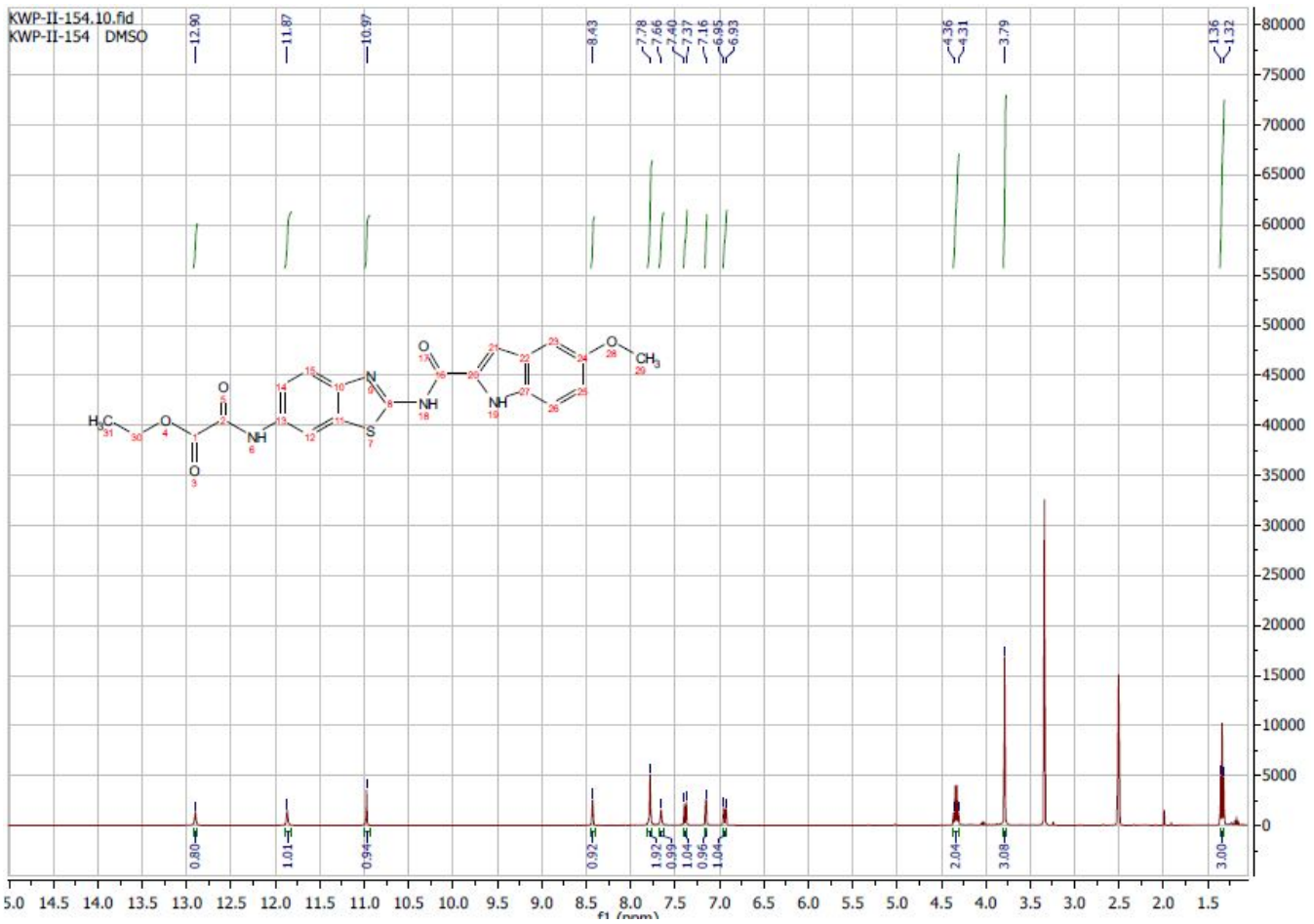
5. Lead Compound 4e Spectra/Data

Lead compound 4e

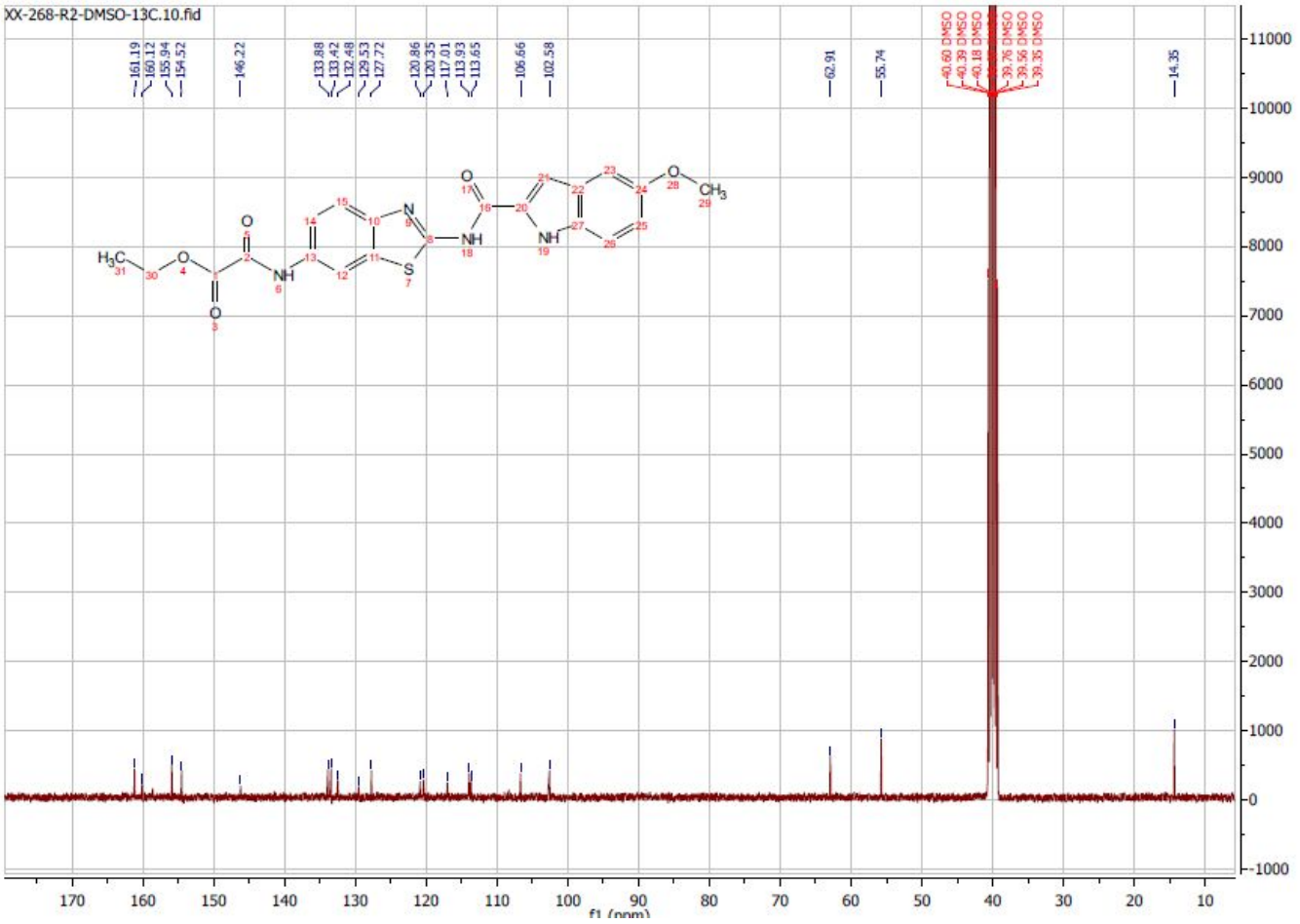


ethyl 2-((2-(5-methoxy-1H-indole-2-carboxamido)benzo[d]thiazol-6-yl)amino)-2-oxoacetate (4e):
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.90 (s, 1H), 11.87 (s, 1H), 10.97 (s, 1H), 8.43 (s, 1H), 7.78 (s, 2H), 7.66 (s, 1H), 7.38 (d, *J* = 8.9 Hz, 1H), 7.15 (d, *J* = 2.5 Hz, 1H), 6.94 (dd, *J* = 9.0, 2.5 Hz, 1H), 4.34 (q, *J* = 7.1 Hz, 2H), 3.79 (s, 3H), 1.34 (t, *J* = 7.1 Hz, 3H). 13C NMR (101 MHz, DMSO-d_6) δ 161.16, 160.09, 158.59, 155.94, 154.51, 146.22, 133.88, 133.42, 132.47, 129.53, 127.71, 120.84, 120.34, 117.01, 113.91, 113.63, 106.66, 102.57, 62.88, 55.72, 14.37. HRMS-ESI: calcd. for $C_{21}H_{18}N_4O_5S$, 439.1050 [M+1]+ ; found: 439.1071.

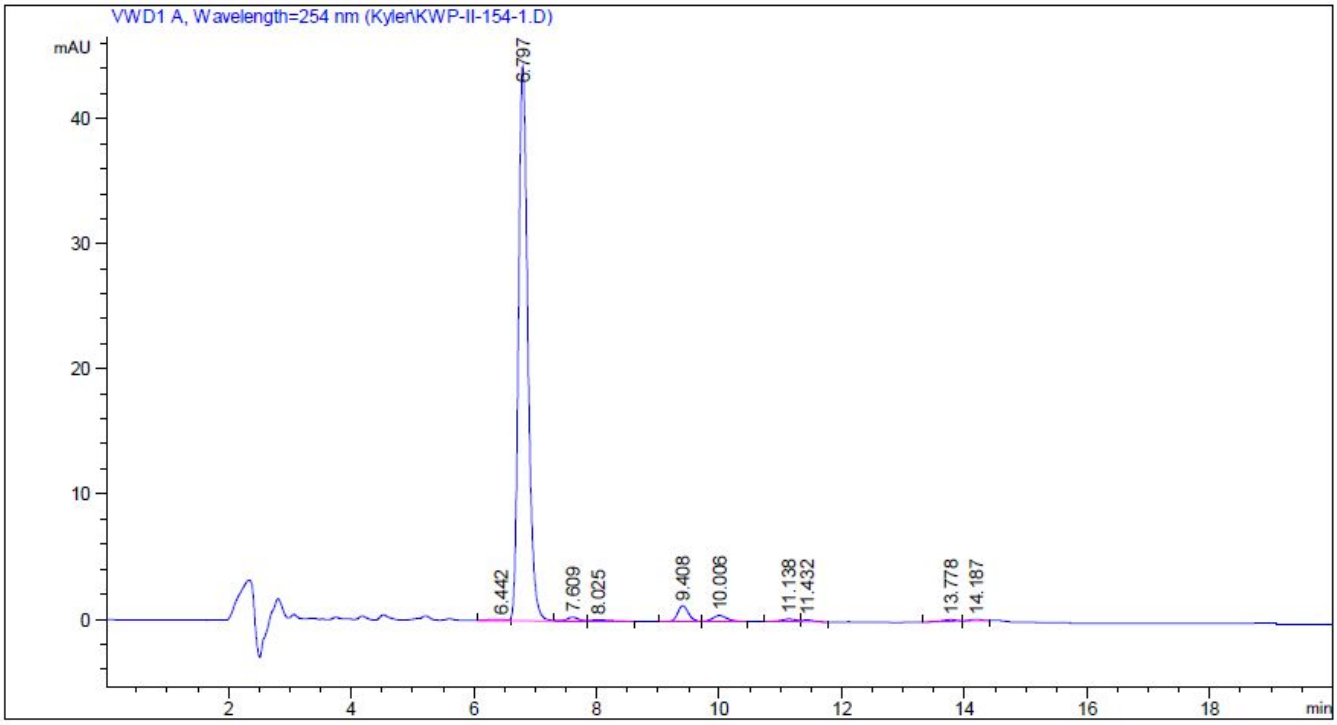
400 MHZ-^{1}H NMR spectrum for (4e) in DMSO-d_6



400 MHZ-^{13}C NMR spectrum for (4e) in DMSO-d_6


XX-268-R2-DMSO-13C.10.fid
161.19
160.12
155.94
154.52
146.22
133.88
133.42
132.48
129.53
127.72
120.86
120.35
117.01
113.93
113.65
106.66
102.58
62.91
55.74
40.60 DMSO
40.39 DMSO
40.18 DMSO
39.76 DMSO
39.56 DMSO
39.35 DMSO
14.35
f1 (ppm)

HPLC for (4e): >90%



Data File C:\Chem32\1\Data\Kyler\KWP-II-154-1.D
Sample Name: KWP-II-154-1

Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.442	BV E	0.2473	2.20585	1.19507e-1	0.4287
2	6.797	VV R	0.1642	471.15283	44.27013	91.5668
3	7.609	VV E	0.1954	4.20432	3.24085e-1	0.8171
4	8.025	VB E	0.2820	2.59261	1.29254e-1	0.5039
5	9.408	BV	0.1935	15.85783	1.27149	3.0819
6	10.006	VB	0.2612	8.28514	4.90136e-1	1.6102
7	11.138	BV	0.2521	3.79641	2.25830e-1	0.7378
8	11.432	VB	0.2007	1.99270	1.48314e-1	0.3873
9	13.778	BV	0.2470	2.51328	1.56776e-1	0.4884
10	14.187	VB	0.2299	1.94433	1.36743e-1	0.3779
Totals :				514.54530	47.27227	

*** End of Report ***

6. **Figure 1S.** Western blot of compound **4**

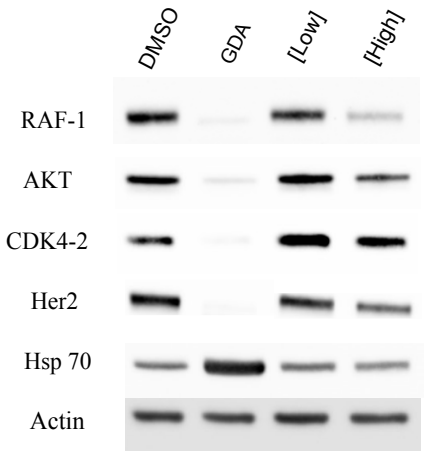


Figure 1S. Western blot analyses from SKBr3 lysates after administration of compound 4 for 24 hr. [4 Low]= 0.5 x EC_{50} [4 High]= 5 x EC_{50}. [GDA]= 500 nM

7. **HPLC Method**

The target compound purity analysis was accomplished utilizing the Agilent 1260 Infinity II HPLC system with an autosampler (Agilent, Santa Clara, CA), eluting a C-18 reverse-phase column (Agilent Eclipse plus C18, 3.5 µm, 4.6 mm × 100 mm) with a gradient mobile phase flow (1.0 mL/min; 0-100% Acetonitrile with 0.1% TFA-water with 0.1% TFA to 100-0% Acetonitrile with 0.1% TFA-water with 0.1% TFA; 20 min), and the samples were monitored under UV light at 254 nm. All target compounds were established to be ≥95% pure (major peak area/total combined area of peaks).

8. Quantification Data Figure 3

Compound 4e SKBR3 Actin 2.0						
Lane No.	Adj. Total Band Vol. (Int)	Total Band Vol. (Int)	Adj. Total Lane Vol. (Int)	Total Lane Vol. (Int)	Bkgd. Vol. (Int)	Norm. Factor
1	3806992	3806992	4602248	4602248	0	N/A
2	3560816	3560816	4442088	4442088	0	N/A
3	3972330	3972330	4825734	4825734	0	N/A
4	4341126	4341126	5206716	5206716	0	N/A
5	2876048	2876048	3869096	3869096	0	N/A
6	981176	981176	2170672	2170672	0	N/A