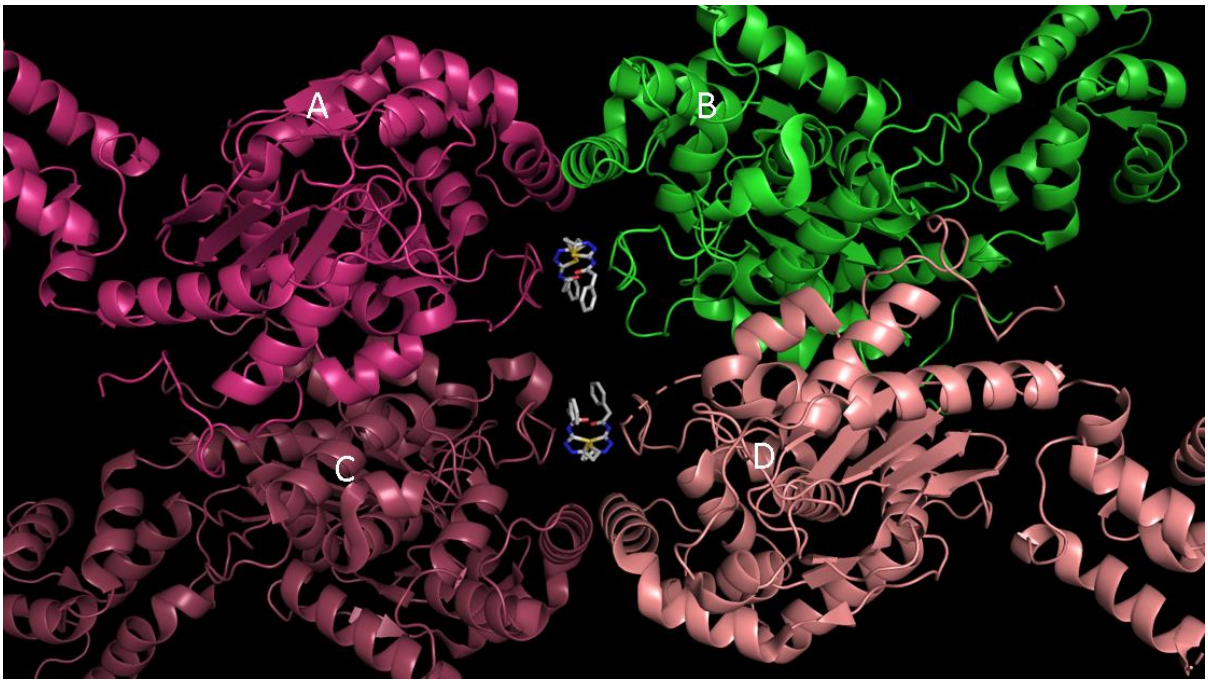
Supplementary data

GAC inhibitors with a 4-hydroxypiperidine spacer: Requirements for potency

Lee McDermott[a,b *], David Koes[c], Shabber Mohammed[a], Prema Iyer[a], Melissa Boby[a], Venkatakrishnan Balasubramanian[a,d], Mackenzie, Geedy[a], William Katt[e] and Richard Cerione[e,f,g]

[a]*University of Pittsburgh, Department of Pharmaceutical Sciences, Pittsburgh, PA 15260, USA*
[b]*University of Pittsburgh, Drug Discovery Institute, Pittsburgh, PA 15269, USA*
[c]*University of Pittsburgh, Department of Computational and Systems Biology, Pittsburgh, PA 15260, USA*
[d]*SASTRA Deemed University, Department of Chemical Engineering, Tamil Nadu, Tirumalaisamudram, 613401, India*
[e]*Cornell University, Department of Molecular Medicine, Ithaka, NY 14853, USA*
[f]*Cornell University, Cornell High Energy Synchrotron Source (CHESS), Ithaca, NY 14853, USA*
[g]*Cornell University, Department of Chemistry and Chemical Biology, Ithaca, New York 14853, United States*



Suppl. Figure 1. Binding of BPTES (in white) in the allosteric pocket formed between interacting GAC dimmers (A-B) and (C-D). PDB: 3UO9

Compounds UPGL00019, UPMP00020, 2, 3, 4a, **5a**, **6j**

Preparation and spectral data for UPGL00019, UPMP00020, **2**, **3**, **4a**, **5a**, **6j** have been previously disclosed.[1] In that disclosure UPGL00019 is noted as **14d**, UPGL00020 as **14i**, intermediate **2** as **11d**, intermediate **3** as **12d**, intermediate **4a** as **15d**, intermediate **5a** as **16d**, and compound **6j** as **14j**

∗ Corresponding author. E-mail: lam179@pitt.edu

Acylation of intermediates 3 and 5

General acylation procedure 1 (HATU coupling)
Desired intermediate 4-hydroxypiperidino-aminothiadiazole (1 eq) in dry DMF (approximately 0.05 mL/mg of limiting reagent) was treated with Et_3N (3 eq) and the carboxylic acid chosen as acylation partner (1.1 eq). To this mixture HATU was then added at room temperature. The reaction mixture was allowed to stir until consumption of the limiting reagent was judged complete by TLC. Followed concentration of the volatiles to a small volume or evaporation to dryness and then treatment with water to form a suspension of the product. Filtration of the solids, followed by wash with water (3X) and then drying afforded the desired compound. Further purification, if needed, was effected via silica gel column chromatography or trituration.

General acylation procedure 2 (Acyl chloride/acyl anhydride coupling)
Desired 4-hydoxypiperidino-aminothiadiazole (1 eq) in dry DMF (approximately 0.05 mL/mg of limiting reagent) was treated with Et_3N (3 eq) and a chosen acyl chloride or acyl anhydride partner (1.2 eq) at room temperature. The reaction mixture was stirred at room temperature until consumption of the limited reagent was judged complete by TLC. Followed concentration of the volatiles to small volume and then treatment with water to form a suspension of the crude product. Solids, were filtered washed with water (3X) and dried to afford the desired compound. Further purification, if needed, was effected via silica gel column chromatography or trituration.

General procedure 3, Synthesis of intermediates 5 (DMSO solvent)
Desired N-Boc protected 4-hydoxypiperydinyl thiadiazole **4** (1 eq.) was dissolved in 4N HCl in dioxane at room temperature and the mixture was stirred until TLC indicated consumption of the starting material. Followed evaporation of the volatiles to dryness and then treatment of the resulting residue with DMSO (approximately 0.05 mL/mg based on N-Boc protected piperidinyl thiadiazole weight). The mixture was then treated with 2-amino-5-bromothiadiazole (1 eq.) and K_2CO_3 (4 eq.) and stirred at 50-55 ^oC until TLC indicated consumption of 2-amino-5-bromothiazole. Reaction mixture was then treated with excess of water and the pH of the mixture was adjusted to 6 with dilute aqueous HCl. The suspension formed from this operation was filtered, and the solid obtained was washed with water and dried to afford the corresponding monoacyl bis thiadiazole derivative **5**. Further purification, if needed, was effected via silica gel column chromatography.

General procedure 4, Synthesis of intermediates 5 (EtOH solvent)
Desired N-Boc protected piperydinyl thiadiazole **4** (1 eq.) was dissolved in 4N HCl in dioxane at room temperature. The mixture was stirred until TLC indicated consumption of the starting material. Followed evaporation of the volatiles to dryness and then treatment of the resulting residue with EtOH. This mixture was treated with 2-amino-5-bromothiadiazole (1 eq.) and Et_3N (3 eq.) and then stirred at 80 ^oC (EtOH) until TLC indicated consumption of 2-amino-5-bromothiazole. Followed evaporation of the solvent to dryness and addition of water. The suspension formed from this operation was filtered and the solid obtained washed with water and dried to afford the corresponding monoacyl bis thiadiazole derivative **5**. Further purification, if needed, was effected via silica gel column chromatography.

***tert*-Butyl 4-((5-pivalamido-1,3,4-thiadiazol-2-yl)oxy)piperidine-1-carboxylate (4b)**
Compound was prepared from *tert*-butyl 4-((5-amino-1,3,4-thiadiazol-2-yl)oxy)piperidine-1-carboxylate (**3**)[1] and pivaloyl chloride according to general acylation procedure 2. The product was isolated as a tan solid after silica gel column chromatography with 0-5% MeOH in dichloromethane (223 mg, 69% yield). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 1.22 (s, 9H), 1.40 (s, 9H), 1.64 (dddd, J=17.2, 12.8, 8.8, 4.0 Hz, 2H), 1.98-2.06 (m, 2H), 3.19 (apparent broad t, J=9.6 Hz, 2H), 3.62-3.69 (m, 2H), 5.05-5.12 (m, 1H), 11.93 (s, 1H).

***tert*-Butyl 4-((5-(cyclobutanecarboxamido)-1,3,4-thiadiazol-2-yl)oxy)piperidine-1-carboxylate (4c)**
Compound was prepared from *tert*-butyl 4-((5-amino-1,3,4-thiadiazol-2-yl)oxy)piperidine-1-carboxylate (**3**)[1] and cyclobutanecarboxylic acid according to general acylation procedure 1. The compound was isolated as an off-white solid after silica gel column chromatography using 0-100% EtOAc in hexanes gradient (200 mg, 78% yield).
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9H), 1.84-1.90 (m, 2H), 1.95-2.09 (m overlapping, 4H), 2.26-2.35 (m, 2H), 2.36-2.47 (m, 2H), 3.31 (ddd, *J*=12.4, 8.0, 3.6 Hz, 2H), 3.41-3.49 (m, 1H), 3.71-3.79 (m, 2H), 5.12-5.19 (m, 1H).). ^{1}H NMR (600 MHz, DMSO-*d6*) δ 1.40 (s, 9H), 1.64 (ddd, *J*=17.4, 12.6, 4.2 Hz, 2H), 1.78-1.84 (m, 1H), 1.90-1.99 (m. 1H), 1.99-2.06 (m, 2H), 2.07-2.16 (m, 2H), 2.16-2.23 (m, 2H), 3.19 (apparent bs, 2H), 3.29-2.30 (m, 1H), 3.65 (m, 2H), 5.03-5.09 (m, 1H), 12.16 (s, 1H). ATR-IR (cm^{-1}) 3168, 3099, 2971, 2932, 2868, 2828, 2752, 1693, 1567, 1508, 1478, 1454, 1405, 1364, 1319, 1302, 1262, 1240, 1219, 1202, 1164, 1132, 1096, 1049, 1017, 946, 867. 837, 821, 785, 771, 741, 694, 669. LC-MS (ESI) *m/z* calculated for $C_{17}H_{26}N_4O_4S$: 382.17, observed [M+H]: 383.5

***tert*-butyl 4-((5-(2-cyclopropylacetamido)-1,3,4-thiadiazol-2-yl)oxy)piperidine-1-carboxylate (4d)**
Compound was prepared from *tert*-butyl 4-((5-amino-1,3,4-thiadiazol-2-yl)oxy)piperidine-1-carboxylate (**3**)[1] and 2-cyclopropylacetic acid according to general acylation procedure 1. Desired compound was isolated as an off-white/beige solid (60 mg, 81% yield)
^{1}H NMR (600 MHz, DMSO-*d6*) δ 0.30-0.34 (m, 2H), 0.63-0.67 (m, 2H), 1.17-1.24 (m, 1H), 1.50 (s, 9H), 1.86-1.91 (m, 2H), 2.07-2.14 (m, 2H), 2.54 (d, *J*=7.2 Hz, 2H), 3.34 (dddd, *J*=13.2, 12.0, 8.4, 3.6 Hz, 2H), 3.74-3.81 (m, 2H), 5.13-5,17 (m, 1H), 11.84 (s, 1H). LC-MS (ESI) *m/z* calculated for $C_{17}H_{26}N_4O_4S$: 382.17, observed [M+H]: 383.6.

N-(5-((1-(5-amino-1,3,4-thiadiazol-2-yl)piperidin-4-yl)oxy)-1,3,4-thiadiazol-2-yl)pivalamide (5b)
The compound was prepared from *tert*-butyl 4-((5-pivalamido-1,3,4-thiadiazol-2-yl)oxy)piperidine-1-carboxylate **(4b)** according to general procedure 4. The product was isolated as an off-white, beige solid after silica gel column purification with 0-10% MeOH in CH_2Cl_2 (93 mg, 47% yield).
^{1}H NMR (400 MHz, DMSO-*d6*) δ 1.22 (s, 9H), 1.77-1.87 (m, 2H), 2.05-2.15 (m, 2H), 3.19-3.3.26 (m, 2H), 3.48-3.56 (m, 2H), 5.08-5.15 (s, 1H), 6.48 (s, 2H), 11.96 (s, 1H). ATR-IR (cm^{-1}) 3424, 3392, 3226, 3117, 2969, 2957, 2933, 2828, 1661, 1625, 1603, 1548, 1490, 1460, 1399, 1370, 1333, 1307, 1296, 1270, 1260, 1250, 1183, 1163, 1149, 1120, 1044, 1012, 944, 925, 899, 821, 752, 690. LC-MS (ESI) *m/z* calculated for $C_{14}H_{21}N_7O_2S_2$: 383.12, observed [M-H]: 382.5.

N-(5-((1-(5-amino-1,3,4-thiadiazol-2-yl)piperidin-4-yl)oxy)-1,3,4-thiadiazol-2-yl) cyclobutanecarboxamide (5c)
Compound was prepared from *tert*-butyl 4-((5-(cyclobutanecarboxamido)-1,3,4-thiadiazol-2-yl)oxy)piperidine-1-carboxylate **(4c)** as described in general procedure 3. The product was isolated as an off-white/tan solid. (40 mg, 80% yield).
^{1}H NMR (600 MHz, DMSO-*d6*) δ 1.77-1.86 (m, 3H), 1.90-1.99 (m, 1H), 2.05-2.16 (m, 4H), 2.17-2.24 (m, 2H), 3.22 (ddd, *J*=12.6, 8.4, 3.6 Hz, 2H), 3.29-3.36 (m, 1H), 3.47-3.59 (m, 2H), 5.07-5.13 (m, 1H), 6.52 (s, 2H), 12.17 (s, 1H). ATR-IR (cm^{-1}) 3395, 3281, 3169, 2938, 2840, 2747, 1683, 1561, 1498, 1452, 1373, 1337, 1298, 1252, 1229, 1202, 1175, 1116, 1090, 1044, 1015, 947, 919, 895, 869, 831, 791, 757, 733, 690. LC-MS (ESI) *m/z* calculated for $C_{14}H_{19}N_7O_2S_2$: 381.10, observed [M+H]: 382.6

N-(5-((1-(5-amino-1,3,4-thiadiazol-2-yl)piperidin-4-yl)oxy)-1,3,4-thiadiazol-2-yl)-2-cyclopropylacetamide (5d)
The compound was prepared from *tert*-butyl 4-((5-(2-cyclopropylacetamido)-1,3,4-thiadiazol-2-yl)oxy)piperidine-1-carboxylate **(4d)** according to general procedure 3. It was isolated as an off-white beige solid (57 mg, 95% yield).
^{1}H NMR (600 MHz, DMSO-*d6*) δ 0.172 (ddd, *J*=9.6, 6.0, 4.8 Hz, 2H), 0.47 (dddd, *J*=10.2, 7.8, 6.0, 4.2 Hz, 2H), 0.98-1.07 (m, 1H), 1.75-1.86 (m, 2H), 2.10-2.15 (m, 2H), 2.30 (d, *J*=7.2 Hz, 2H), 3.22 (ddd, *J*=

12.6, 9.0, 3.6 Hz, 2H), 3.49-3.54 (m, 2H), 5.08-5.13 (m, 1H), 6.49 (s, 2H), 12.24 (s, 1H). ATR-IR (cm^{-1}) 3360, 3276, 3103, 2999, 2959, 2933, 2850, 2761, 2697, 1669, 1637, 1579, 1560, 1507, 1499, 1465, 1410, 1385, 1370, 1352, 1338, 1310, 1292, 1256, 1224, 1197, 1117, 1014, 968, 951, 938, 925, 910, 827, 810, 798, 769, 719, 688. LC-MS (ESI) *m/z* calculated for $C_{14}H_{19}N_7O_2S_2$: 381.10, observed [M-H]: 380.6.

N-(5-(4-((5-(2-phenylacetamido)-1,3,4-thiadiazol-2-yl)oxy)piperidin-1-yl)-1,3,4-thiadiazol-2-yl)propionamide (6a)

Compound was prepared from N-(5-((1-(5-amino-1,3,4-thiadiazol-2-yl)piperidin-4-yl)oxy)-1,3,4-thiadiazol-2-yl)-2-phenylacetamide **(5a)** and propionic anhydride according to general procedure 2. The product was isolated as an off-white/beige solid (30 mg, 88% yield).

^{1}H NMR (600 MHz, DMSO-*d6*) δ 1.06 (t, J=7.2 Hz, 3H), 1.85 (dddd, J=16.8, 12.0, 8.4, 3.6 Hz, 2H), 2.12-2.17 (m, 2H), 2.39 (q, J=7.8 Hz, 2H), 3.37 (ddd, J=12.6, 9.0, 3.6 Hz, 2 H), 3.64-3.69 (m, 2H), 3.76 (s, 2H), 5.12-5.16 (m, 1H), 7.24-7.34 (m, 5H), 11.99 (s, 1H), 12.58 (s, 1H). ATR-IR (cm^{-1}) 3268, 3171, 3089, 3043, 2905, 2832, 2758, 1688, 1571, 1496, 1453, 1418, 1384, 1366, 1352, 1315, 1297, 1269, 1253, 1232, 1208, 1192, 1143, 1116, 1015, 974, 948, 922, 907, 816, 796, 782, 756, 727, 698. LC-MS (ESI) *m/z* calculated for $C_{20}H_{23}N_7O_3S_2$: 473.13, observed [M-H]:472.5

N-(5-(4-((5-(2-phenylacetamido)-1,3,4-thiadiazol-2-yl)oxy)piperidin-1-yl)-1,3,4-thiadiazol-2-yl)isobutyramide (6b)

This compound was prepared from N-(5-((1-(5-amino-1,3,4-thiadiazol-2-yl)piperidin-4-yl)oxy)-1,3,4-thiadiazol-2-yl)-2-phenylacetamide **(5a)** and isobutyrylchloride according to general procedure 2. The product was isolated as a white/off-white solid after silica gel column purification with 0-5% MeOH in CH_2Cl_2 (90 mg, 58 % yield).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 1.09 (d, J=6.8 Hz, 6H), 1.79-1.89 (m, 2H), 2.11-2.18 (m, 2H), 2.69 (q, J=6.8 Hz, 1H); 3.34-3.341 (m, 2H); 3.64-3.71 (m, 2H); 3.76 (s, 2H), 5.12-5.18 (m, 1H), 7.25-7.37 (m, 5H), 11.98 (s, 1H), 12.56 (s, 1H). ATR-IR (cm^{-1}) 3172, 3091, 2969, 2903, 2849, 1686, 1565, 1501, 1452, 1385, 1375, 1348, 1299, 1253, 1205, 1159, 1136, 1095, 1053, 1020, 968, 943, 901, 872, 840, 817, 780, 756, 744, 724, 694, 642. LC-MS (ESI) *m/z* calculated for $C_{21}H_{25}N_7O_3S_2$: 487.15, observed [M-H]: 486.3.

N-(5-(4-((5-(2-phenylacetamido)-1,3,4-thiadiazol-2-yl)oxy)piperidin-1-yl)-1,3,4-thiadiazol-2-yl)cyclopropanecarboxamide (6c)

The compound was prepared via the acylation of N-(5-((1-(5-amino-1,3,4-thiadiazol-2-yl)piperidin-4-yl)oxy)-1,3,4-thiadiazol-2-yl)-2-phenylacetamide **(5a)** with cyclopropanecarboxylic acid according to general procedure 1. The product was isolated as a white/off-white solid after trituration with hot CH_2Cl_2 (72 mg, 63 % yield).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 0.85-0.92 (m, 4H), 1.80-1.92 (m, 3H), 2.1-2.16 (broad m, 2H), 3.38-3.41 (m, 2H), 3.64-3.39 (m, 2H), 3.76 (s, 2H), 5.10-5.17 (m, 1H), 7.24-7.36 (m, 5H), 12.30 (s, 1H), 12.56 (s, 1H). ATR-IR (cm^{-1}) 3175, 3096, 3047, 3007, 2945, 2924, 2883, 2845, 2757, 1687, 1575, 1499, 1457, 1405, 1391, 1349, 1337, 1311, 1261, 1243, 1221, 1199, 1182, 1159, 1144, 1109, 1089, 1062, 1047, 1017, 958, 920, 876, 837, 795, 784, 755, 734, 723, 696, 670. LC-MS (ESI) *m/z* calculated for $C_{21}H_{23}N_7O_3S_2$: 485.13, observed [M-H]: 484.2.

N-(5-(4-((5-(2-phenylacetamido)-1,3,4-thiadiazol-2-yl)oxy)piperidin-1-yl)-1,3,4-thiadiazol-2-yl)cyclobutanecarboxamide (6d)

Compound prepared from N-(5-((1-(5-amino-1,3,4-thiadiazol-2-yl)piperidin-4-yl)oxy)-1,3,4-thiadiazol-2-yl)-2-phenylacetamide **(5a)** and cyclobutanecarboxylic acid according to general procedure 1. The compound was isolated as a white solid after a suspension/trituration with hot CH_2Cl_2 (56 mg, 76% yield).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 1.75-1.98 (m overlapping, 4H), 2.10-2.24 (m, 6H), 3.31-3.41 (m, 2H), 3.64-3.70 (m, 2H), 3.76 (s, 2H), 5.10-5.17 (m, 1H), 7.24-7.36 (m, 5H), 11.88 (s, 1H), 12.57 (s, 1H). ATR-IR (cm^{-1}) 3172, 3094, 3044, 2974, 2934, 2899, 2840, 2752, 1697, 1685, 1562, 1496, 1450, 1374, 1314,

1297, 1263, 1252, 1201, 1129, 1077, 1052, 1020, 991, 968, 950, 919, 897, 870, 835, 815, 780, 757, 743, 722, 696, LC-MS (ESI) *m/z* calculated for $C_{22}H_{25}N_7O_3S_2$: 499.15, observed [M-H]: 498.2.

2-cyclopropyl-N-(5-(4-((5-(2-phenylacetamido)-1,3,4-thiadiazol-2-yl)oxy)piperidin-1-yl)-1,3,4-thiadiazol-2-yl)acetamide (6e)

This compound was prepared via the acylation of N-(5-((1-(5-amino-1,3,4-thiadiazol-2-yl)piperidin-4-yl)oxy)-1,3,4-thiadiazol-2-yl)-2-phenylacetamide **(5a)** with 2-cyclopropylacetic acid according to the general procedure 1. The product was obtained as an off-white/tan solid after trituration with hot CH_2Cl_2 (27 mg, 38%).

^{1}H NMR (300 MHz, DMSO-*d6*) δ 0.08-0.26 (m, 2H), 0.35-0.54 (m, 2H), 0.92-1.11 (m, 1H), 1.73-1.98 (m, 2H), 2.05-2.22 (m, 2H), 2.28 (d, *J*=6.0 Hz, 2H), 3.33-3.48 (m, 2H), 3.58-3.72 (m, 2H), 3.76 (s, 2H), 5.05-5.25 (m, 1H), 7.15-7.45 (m, 5H), 11.96 (s, 1H), 12.57 (s, 1H). ATR-IR (cm^{-1}) 3169, 3082, 2927, 2894, 2825, 2766, 1681, 1567, 1495, 1457,1434, 1379, 1330, 1312, 1268, 1250, 1212, 1191, 1143, 1024, 975, 955, 928, 898, 830, 800, 781, 753, 733, 722, 695, 652. LC-MS (ESI) *m/z* calculated for $C_{22}H_{25}N_7O_3S_2$: 499.15, observed [M-H]: 498.4

N-(5-((1-(5-(2-phenylacetamido)-1,3,4-thiadiazol-2-yl)piperidin-4-yl)oxy)-1,3,4-thiadiazol-2-yl)pivalamide (6f)

Compound was prepared from (N-(5-((1-(5-amino-1,3,4-thiadiazol-2-yl)piperidin-4-yl)oxy)-1,3,4-thiadiazol-2-yl)pivalamide) **(5b)** and phenylacetic acid according to general procedure 1. Isolated as an off white solid (40 mg, 44% yield).

^{1}H NMR (400 MHz, DMSO-*d6*) δ 1.22 (s, 9H), 1.84 (dddd, *J*=16.8, 12.4, 8.4, 4.0 Hz, 2H), 2.10-2.17 (m, 2H), 3.37 (ddd, *J*=12.8, 8.4, 3.6 Hz, 2H), 3.63-3.71 (m, 2H), 3.73 (s, 2H), 5.12-5.19 (m, 1H), 7.22-7.35 (m, 5H), 11.95 (s, 1H), 12.30 (s, 1H). ATR-IR (cm^{-1}) 3156, 3087, 3029, 2965, 2928, 2906, 2870, 1677, 1566, 1549, 1493, 1457, 1400, 1380, 1350, 1332, 1302, 1277, 1260, 1223, 1187, 1152, 1129, 1074, 1055, 1023, 970, 954, 925, 899, 855, 824, 806, 754, 716, 692, 646. LC-MS (ESI) *m/z* calculated for $C_{22}H_{27}N_7O_3S_2$: 501.16, observed [M-H]: 500.3.

N-(5-((1-(5-(2-cyclopropylacetamido)-1,3,4-thiadiazol-2-yl)piperidin-4-yl)oxy)-1,3,4-thiadiazol-2-yl)cyclobutanecarboxamide (6g)

The compound was prepared from N-(5-((1-(5-amino-1,3,4-thiadiazol-2-yl)piperidin-4-yl)oxy)-1,3,4-thiadiazol-2-yl) cyclobutanecarboxamide **(5c)** and 2-cyclopropylacetic acid according to general procedure 1. The product obtained after filtration was chromatographed with silica gel column and 0-15% MeOH in CH_2Cl_2 gradient. The chromatographed product was then dissolved in 10% MeOH in CH_2Cl_2. Followed filteration through a PTFE 0.2 μm filter and evaporation of the solvent to a solid that was suspended in CH_2Cl_2 and treated with excess of hexanes to afford the desired compound as an off-white/beige solid (37 mg, 76% yield).

^{1}H NMR (600 MHz, DMSO-*d6*) δ 0.17 (ddd, *J*=9.0, 5.4, 4.8 Hz, 2H), 0.46 (dddd, *J*=12.6, 8.4, 6.0, 4.8 Hz, 2H), 0.99-1.06 (m, 1H), 1.75-1.89 (m, 3H), 1.92-1.99 (m, 1H), 2.10-2.24 (m, 6H), 2.28 (d, *J*=6.6 Hz, 2H), 3.31-3.41 (m, 3H), 3.65-3.70 (m, 2H), 5.12-5.17 (m, 1H), 11.98 (s, 1H), 12.17 (s, 1H). ATR-IR (cm^{-1}) 3169, 3089, 2976, 2940, 2898, 2849, 2751, 1700, 1676, 1570, 1500, 1460, 1450, 1374, 1329, 1302, 1287, 1262, 1249, 1205, 1188, 1150, 1124, 1111, 1048, 1020, 972, 955, 930, 902, 890, 837, 811, 795, 728, 692. LC-MS (ESI) *m/z* calculated for $C_{19}H_{25}N7O_3S_2$: 463.15, observed [M-H]: 462.4

N-(5-((1-(5-isobutyramido-1,3,4-thiadiazol-2-yl)piperidin-4-yl)oxy)-1,3,4-thiadiazol-2-yl)cyclobutanecarboxamide (6h)

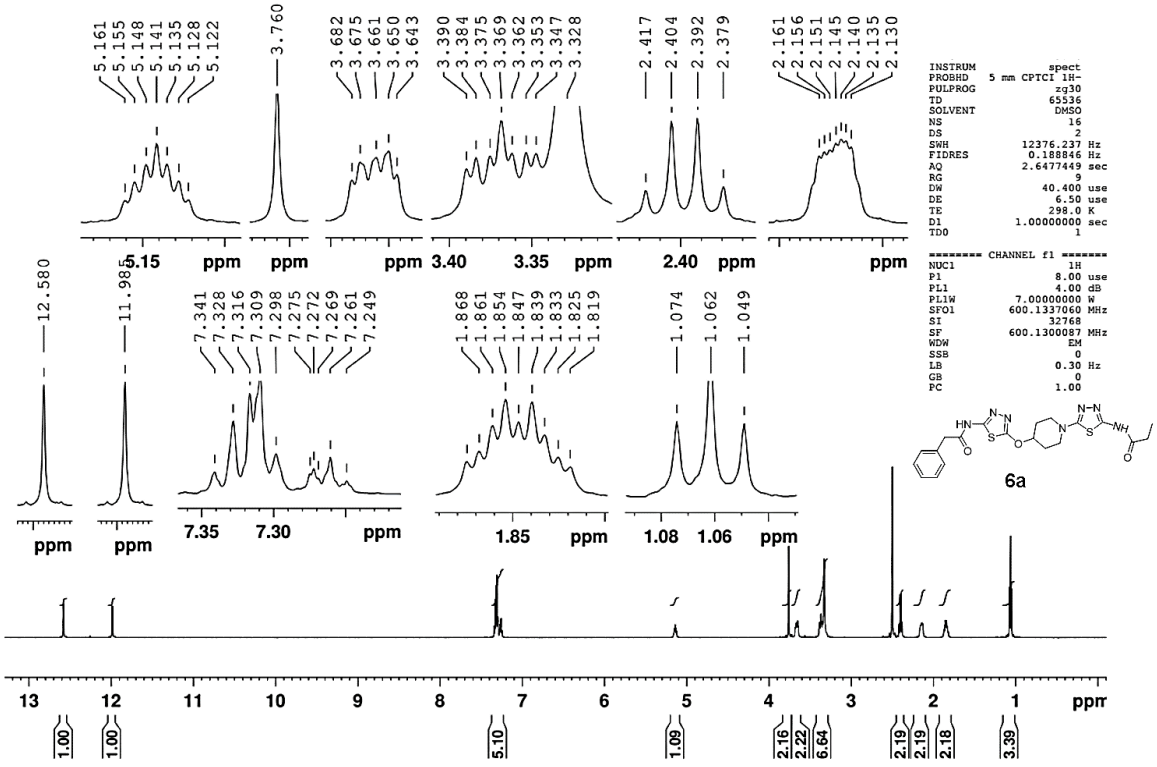
Compound was prepared from N-(5-((1-(5-amino-1,3,4-thiadiazol-2-yl)piperidin-4-yl)oxy)-1,3,4-thiadiazol-2-yl) cyclobutanecarboxamide **(5c)** and isobutyryl chloride according to the general procedure 2. The product was isolated as off-white solid after purification with silica gel column and 0-10% MeOH in EtOAc and then a suspension in CH_2Cl_2 and treatment with excess of hexanes (8 mg, 13% yield).

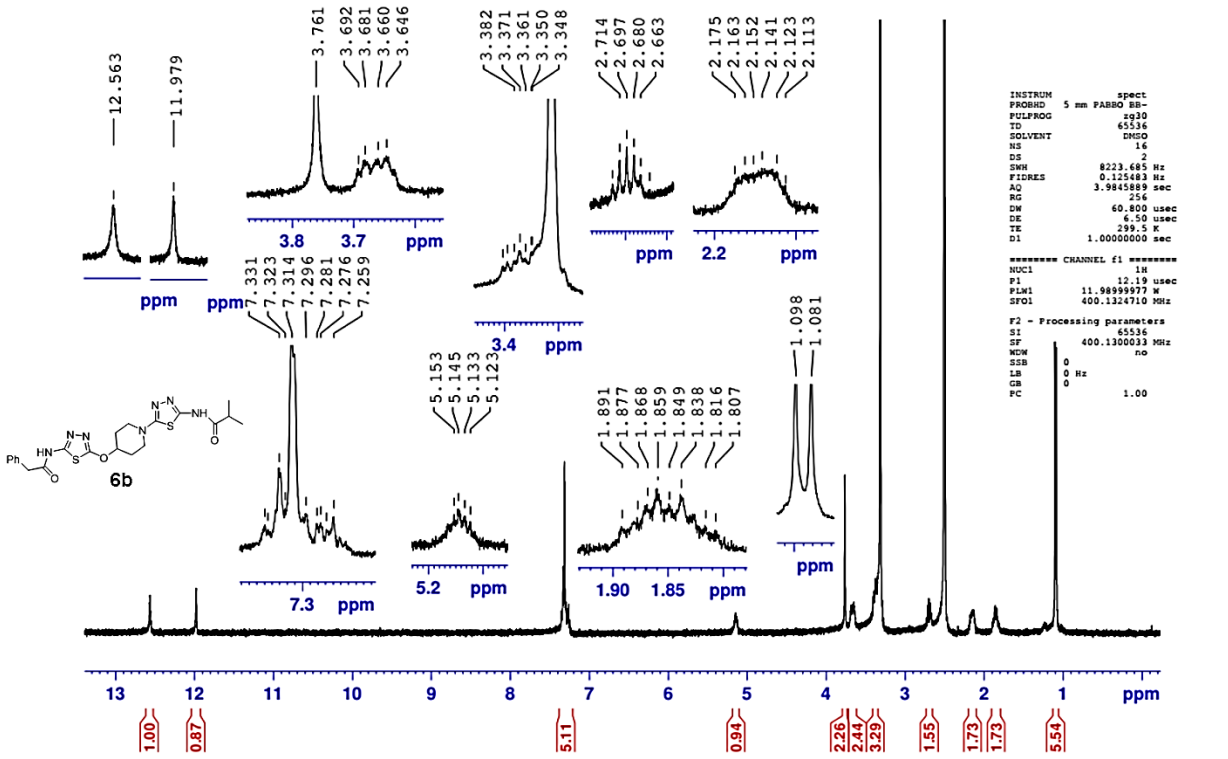
^{1}H NMR (600 MHz, DMSO-*d6*) δ 1.09 (d, *J*=7.2 Hz, 6H), 1.77-1.89 (m, 3H), 1.90-1.99 (m, 1H), 2.10-2.24 (m, 6H), 2.69 (p, *J*=6.6 Hz, 1H), 3.38 (ddd, *J*=12.6, 8.4, 2.6 Hz, 2H), 3.65-3.70 (m, 2H), 5.12-5.17 (m, 1H), 12.00 (s, 1H), 12.17 (s, 1H). ATR-IR (cm^{-1}) 3252, 3162, 3093, 2967, 2931, 2907, 2863, 2746, 1683, 1562, 1500, 1452, 1385, 1374, 1298, 1251, 1231, 1203, 1175, 1125, 1117, 1099, 1022, 955, 943, 904, 875, 837, 788, 747, 736, 696, 660. LC-MS (ESI) *m/z* calculated for $C_{18}H_{25}N_7O_3S_2$: 451.15, observed [M-H]: 450.5.

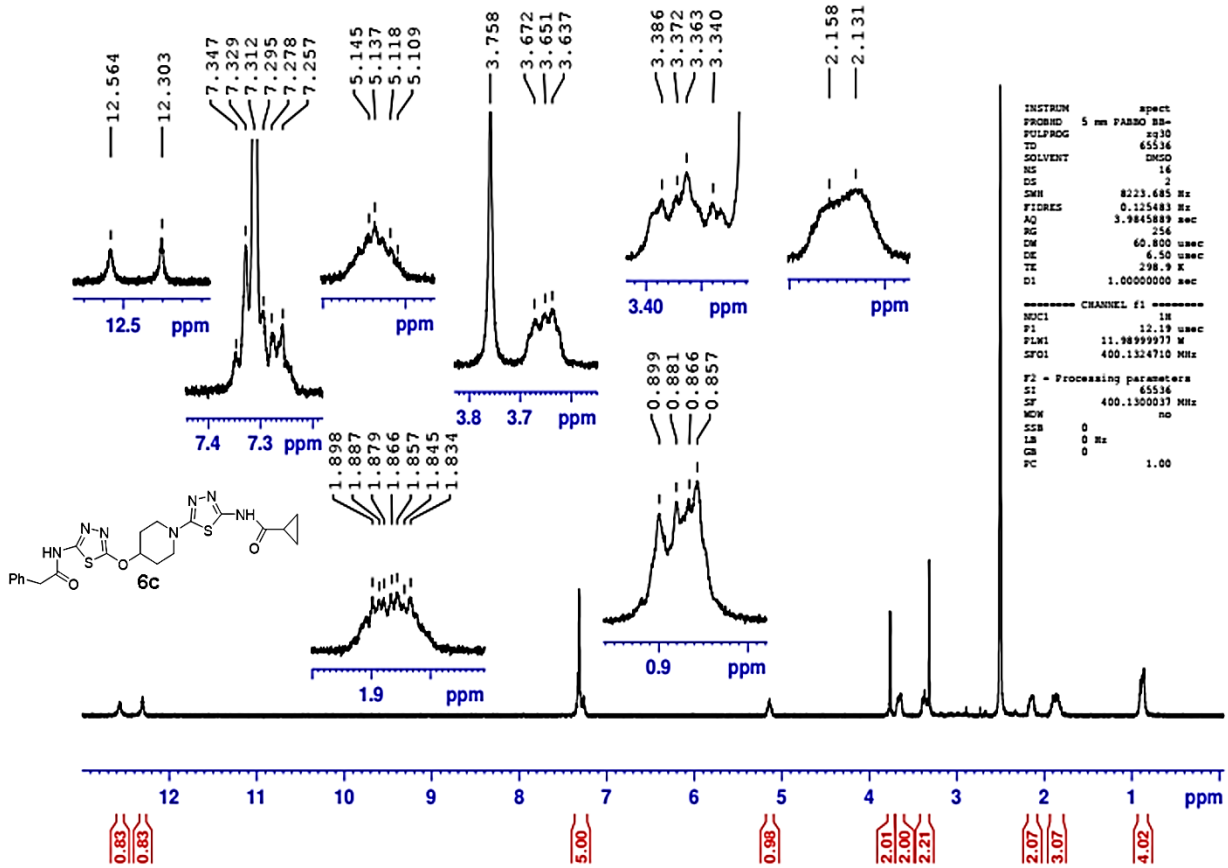
N-(5-(4-((5-(2-cyclopropylacetamido)-1,3,4-thiadiazol-2-yl)oxy)piperidin-1-yl)-1,3,4-thiadiazol-2-yl)isobutyramide (6i)

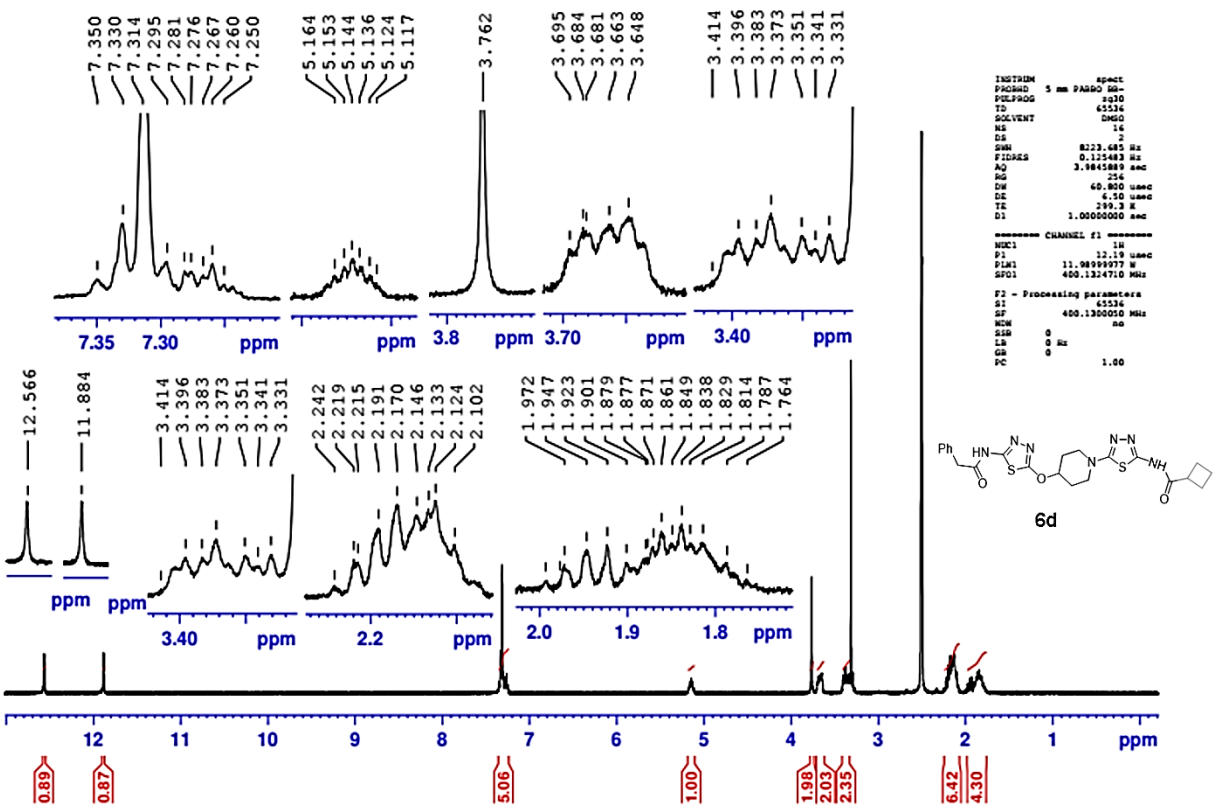
The compound was prepared from N-(5-((1-(5-amino-1,3,4-thiadiazol-2-yl)piperidin-4-yl)oxy)-1,3,4-thiadiazol-2-yl)-2-cyclopropylacetamide **(5d)** and isobutyryl chloride according to the general procedure 2. It was isolated as an off-white/beige solid after purification with silica gel column and 0-10% MeOH in EtOAc gradient followed by suspension in CH_2Cl_2 and treatment with excess of hexanes.
^{1}H NMR (300 MHz, DMSO-*d6*) 0.00 (ddd, *J*=9.3, 5.7, 4.5 Hz, 2H), 0.31 (dddd, *J*=10.2, 8.1, 6.0, 4.5 Hz, 2H), 0.81-0.9 (m, 1H), 0.91 (d, *J*=6.9 Hz, 6H), 1.65-1.74 (m, 2H), 1.96-2.05 (m, 2H), 2.14 (d, *J*=7.2 Hz, 2H), 2.53 (p, *J*=6.9 Hz, 1H), 3.12-3.25 (m, 2H), 3.47-3.56 (m, 2H), 4.95-5.20 (m, 1H), 11.82 (s, 1H), 12.07 (s, 1H). ATR-IR (cm^{-1}) 3274, 3176, 3104, 3049, 2976, 2933, 2912, 2875, 2846, 2760, 1689, 1576, 1503, 1454, 1414, 1386, 1370, 1341, 1294, 1273, 1250, 1231, 1202, 1187, 1162, 1132, 1202, 1187, 1162, 1132, 1114, 1097, 1018, 970, 952, 942, 908, 875, 833, 816, 799, 772, 725, 690. LC-MS (ESI) *m/z* calculated for $C_{18}H_{25}N_7O_3S_2$: 451.15, observed [M-H]: 450.6

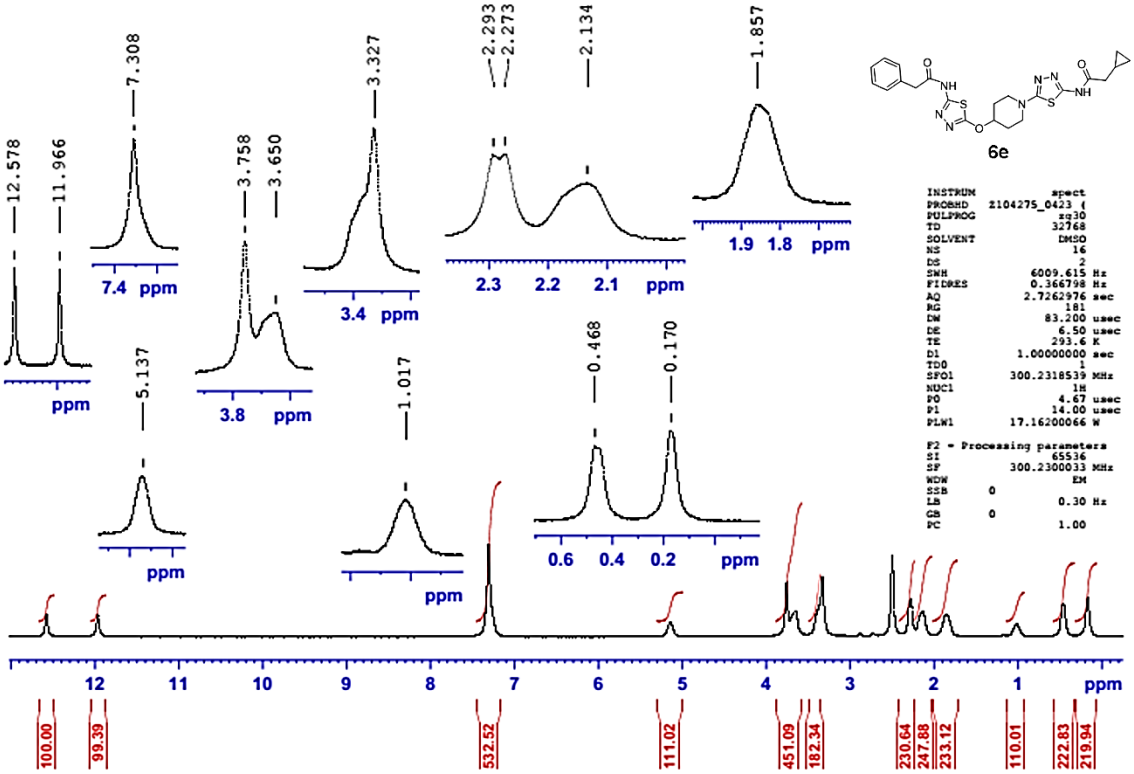
1H NMR spectra for compounds 6a-i

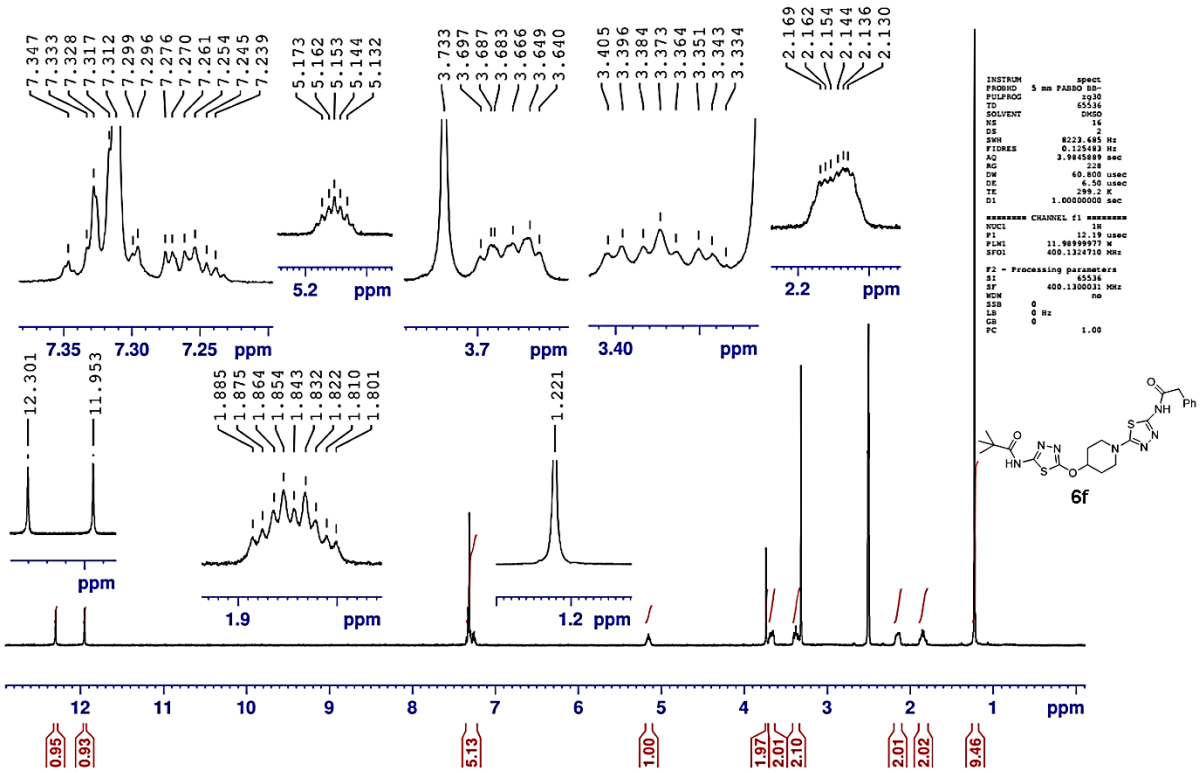


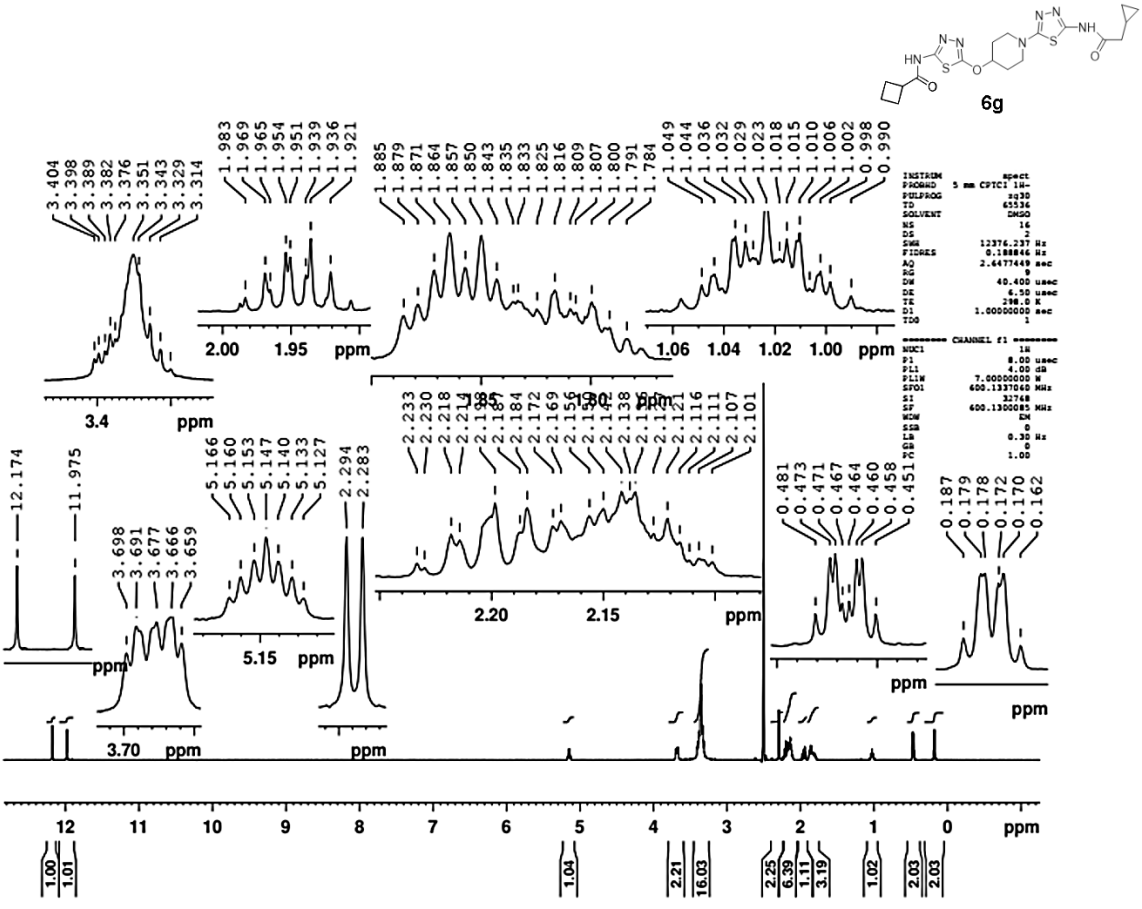

12.563
11.979
3.761
3.692
3.681
3.660
3.646
3.382
3.371
3.361
3.350
3.348
2.714
2.697
2.680
2.663
2.175
2.163
2.152
2.141
2.123
2.113
7.331
7.323
7.314
7.296
7.281
7.276
7.259
5.153
5.145
5.133
5.123
1.891
1.877
1.868
1.859
1.849
1.838
1.816
1.807
1.098
1.081
6b
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9845889 sec
RG 256
DW 60.800 usec
DE 6.50 usec
TE 299.5 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 12.19 usec
PLW1 11.98999977 W
SFO1 400.1324710 MHz
F2 - Processing parameters
SI 65536
SF 400.1300033 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00
1.00
0.87
5.11
0.94
2.26
2.44
3.29
1.55
1.73
1.73
5.54

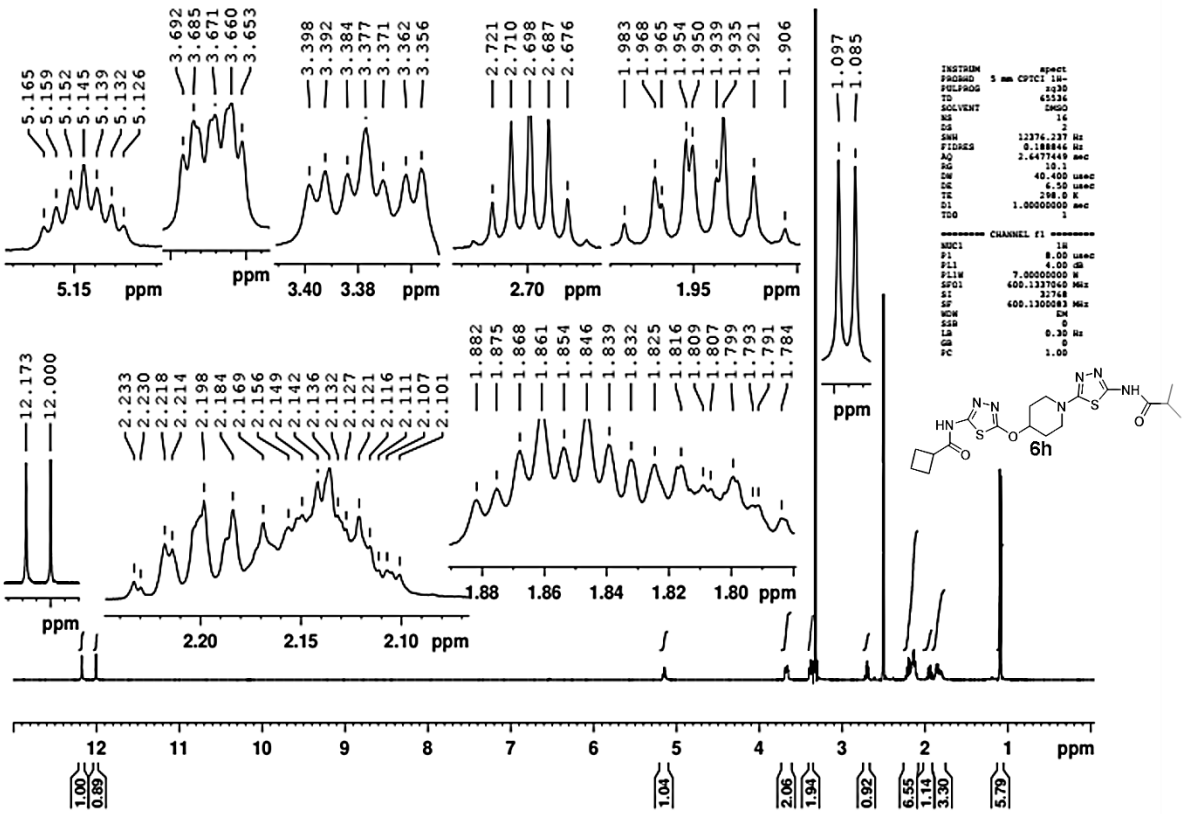

12.564
12.303
7.347
7.329
7.312
7.295
7.278
7.257
5.145
5.137
5.118
5.109
3.758
3.672
3.651
3.637
3.386
3.372
3.363
3.340
2.158
2.131
1.898
1.887
1.879
1.866
1.857
1.845
1.834
0.899
0.881
0.866
0.857
6c
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9845889 sec
RG 256
DW 60.800 usec
DE 6.50 usec
TE 298.9 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 12.19 usec
PLW1 11.98999977 W
SFO1 400.1324710 MHz
F2 - Processing parameters
SI 65536
SF 400.1300037 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00
0.83
0.83
5.00
0.98
2.01
2.00
2.21
2.07
3.07
4.02

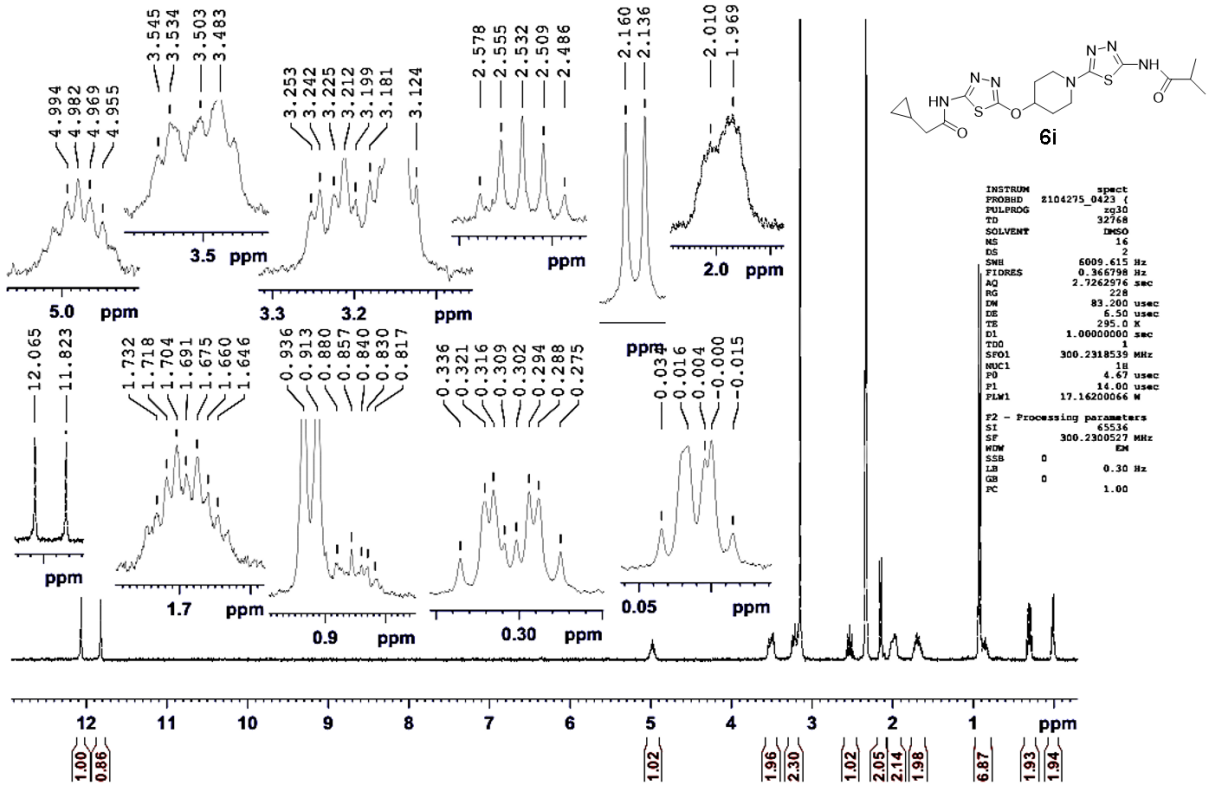

7.350
7.330
7.314
7.295
7.281
7.276
7.267
7.260
7.250
5.164
5.153
5.144
5.136
5.124
5.117
3.762
3.695
3.684
3.681
3.663
3.648
3.414
3.396
3.383
3.373
3.351
3.341
3.331
12.566
11.884
2.242
2.219
2.215
2.191
2.170
2.146
2.133
2.124
2.102
1.972
1.947
1.923
1.901
1.879
1.877
1.871
1.861
1.849
1.838
1.829
1.814
1.787
1.764
7.35 7.30 ppm
3.8 ppm
3.70 ppm
3.40 ppm
ppm
3.40 ppm
2.2 ppm
2.0 1.9 1.8 ppm
12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.89
0.87
5.06
1.00
1.98
2.03
2.35
6.42
4.30
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9845889 sec
RG 256
DW 60.800 usec
DE 6.50 usec
TE 299.3 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 12.19 usec
PLW1 11.98999977 W
SFO1 400.1324710 MHz
F2 - Processing parameters
SI 65536
SF 400.1300050 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00
Ph
HN
O
S
N
O
NH
O
6d


12.578
11.966
7.308
3.758
3.650
3.327
2.293
2.273
2.134
1.857
5.137
1.017
0.468
0.170
ppm
7.4 ppm
3.8 ppm
3.4 ppm
2.3 2.2 2.1 ppm
1.9 1.8 ppm
0.6 0.4 0.2 ppm
12 11 10 9 8 7 6 5 4 3 2 1 ppm
100.00
99.39
532.52
111.02
451.09
182.34
230.64
247.88
233.12
110.01
222.83
219.94
NH
S
N
O
NH
O
6e
INSTRUM spect
PROBHD 2104275_0423 (
PULPROG zg30
TD 32768
SOLVENT DMSO
NS 16
DS 2
SWH 6009.615 Hz
FIDRES 0.366798 Hz
AQ 2.7262976 sec
RG 181
DW 83.200 usec
DE 6.50 usec
TE 293.6 K
D1 1.00000000 sec
TD0 1
SFO1 300.2318539 MHz
NUC1 1H
P0 4.67 usec
P1 14.00 usec
PLW1 17.16200066 W
F2 - Processing parameters
SI 65536
SF 300.2300033 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00


7.347 7.333 7.328 7.317 7.312 7.299 7.296 7.276 7.270 7.261 7.254 7.245 7.239
5.173 5.162 5.153 5.144 5.132
3.733 3.697 3.687 3.683 3.666 3.649 3.640
3.405 3.396 3.384 3.373 3.364 3.351 3.343 3.334
2.169 2.162 2.154 2.144 2.136 2.130
12.301 11.953
1.885 1.875 1.864 1.854 1.843 1.832 1.822 1.810 1.801
1.221
6f
12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.95 0.93 5.13 1.00 1.97 2.01 2.10 2.01 2.02 9.46


6g
3.404 3.398 3.389 3.382 3.376 3.351 3.343 3.329 3.314
1.983 1.969 1.965 1.954 1.951 1.939 1.936 1.921
1.885 1.879 1.871 1.864 1.857 1.850 1.843 1.835 1.833 1.825 1.816 1.809 1.807 1.800 1.791 1.784
1.049 1.044 1.036 1.032 1.029 1.023 1.018 1.015 1.010 1.006 1.002 0.998 0.990
12.174 11.975
3.698 3.691 3.677 3.666 3.659
5.166 5.160 5.153 5.147 5.140 5.133 5.127
2.294 2.283
2.233 2.230 2.218 2.214 2.184 2.172 2.169 2.156 2.138 2.121 2.116 2.111 2.107 2.101
0.481 0.473 0.471 0.467 0.464 0.460 0.458 0.451
0.187 0.179 0.178 0.172 0.170 0.162
12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
1.00 1.01 1.04 2.21 16.03 2.25 6.39 1.11 3.19 1.02 2.03 2.03


6h


6i

References

1. McDermott, L. A.; Iyer, P.; Vernetti, L.; Rimer, S.; Sun, J.; Boby, M.; Yang, T.; Fioravanti, M.; O'Neill, J.; Wang, L.; Drakes, D.; Katt, W.; Huang, Q.; Cerione, R., Design and evaluation of novel glutaminase inhibitors. *Bioorg Med Chem* **2016,** *24* (8), 1819-39.