Dual Glycolate Oxidase/Lactate Dehydrogenase A Inhibitors for Primary Hyperoxaluria

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Supporting Information

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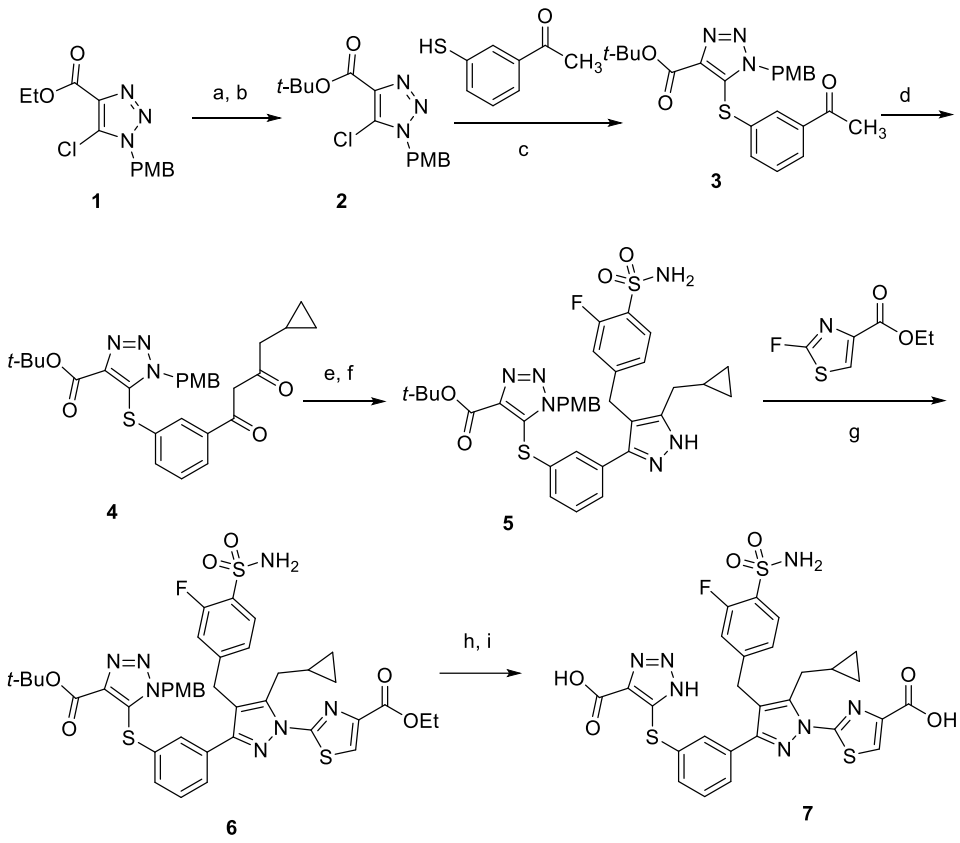
General Information:

The starting materials used for the synthesis were prepared according to known literature procedures or obtained from commercial sources, such as, but not limited to, Sigma-Aldrich, Fluka, Acros Organics, Alfa Aesar, VWR Scientific, and the like. Nuclear Magnetic Resonance (NMR) analysis was conducted using a 400 MHz spectrometer with an appropriate deuterated solvent. Chemical shift (δ) is expressed in units of parts per million (ppm). LCMS analysis was conducted using a Shimadzu LCMS-2020 with a Ascentis Express C18 2.7 µM, 3.0 × 50 mm column, eluting with 95:5 to 0:100 H_2O:MeCN + 0.05% trifluoroacetic acid at a flow rate of 1.5 mL/min over 3.0 minutes. The QDA MS detector was set up to scan under both positive and negative mode ions ranging from 100-1200 Daltons. General methods for the preparation of compounds can be modified by the use of appropriate reagents and conditions for the introduction of the various moieties found in the structures as provided herein.

Preparation of 2-(3-(3-((4-carboxy-1*H*-1,2,3-triazol-5-yl)thio)phenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1*H*-pyrazol-1-yl)thiazole-4-carboxylic acid (Compound 7)



Step a: Preparation of 5-chloro-1-(4-methoxybenzyl)-1*H*-1,2,3-triazole-4-carboxylic acid

Into a 1 L round-bottom flask was placed ethyl 5-chloro-1-(4-methoxybenzyl)-1*H*-1,2,3-triazole-4-carboxylate (20.0 g, 67.6 mmol, 1.0 equiv), THF (200 mL), solid LiOH (16.2 g, 676 mmol, 10 equiv) and water (400 mL). The resulting solution was stirred at 25 °C for 4 h. The resulting mixture was concentrated under reduced pressure and the residue was acidified to pH=2 with 1 M aqueous HCl solution. The precipitated solids were collected by filtration and washed with water (2 × 10 mL). The title compound, 5-chloro-1-(4-methoxybenzyl)-1*H*-1,2,3-triazole-4-carboxylic acid, was obtained as a white solid (12 g, 66% yield).

Step b: Preparation of *tert*-butyl 5-chloro-1-(4-methoxybenzyl)-1*H*-1,2,3-triazole-4-carboxylate

Into a 500 mL round-bottom flask equipped with a magnetic stir bar and under a nitrogen atmosphere was placed 5-chloro-1-[(4-methoxyphenyl)methyl]-1,2,3-triazole-4-carboxylic acid (10.0 g, 37.3 mmol, 1.0 equiv), Boc_2O (16.3 g, 74.7 mmol, 2.0 equiv), DMAP (0.90 g, 7.5 mmol, 0.2 equiv), $EtN(iPr)_2$ (15.3 mL, 112 mmol, 3.0 equiv) and CH_2Cl_2 (200 mL). The resulting solution was stirred at 25 °C for 3 h. The reaction was then quenched by the addition of 100 mL of water. The resulting solution was poured into a separatory funnel and extracted with ethyl acetate (3 × 200 mL). The combined organic layers were concentrated, and the residue was applied onto a silica gel column. The column was eluted with ethyl acetate/petroleum ether (1:10). The fractions containing *tert*-butyl 5-chloro-1-(4-methoxybenzyl)-1*H*-1,2,3-triazole-4-carboxylate were isolated, concentrated and dried under vacuum to afford a yellow solid (10 g, 83% yield).

Step c: Preparation of *tert*-butyl 5-(3-acetylphenyl)sulfanyl-3-[(4-methoxyphenyl)methyl]triazole-4-carboxylate

Into a 100 mL round bottom flask was added *tert*-butyl 5-chloro-1-[(4-methoxyphenyl)methyl]triazole-4-carboxylate (1.1 equiv, 2.3 g, 7.2 mmol), 1-(3-sulfanylphenyl)ethanone (1.0 equiv, 1.0 g, 6.6 mmol), potassium carbonate (2.5 equiv, 2.3 g, 16 mmol) and DMF (22 mL). The mixture was stirred at 130 °C for 18 hours. The reaction was filtered and loaded directly on a 25 g normal phase pre-cartridge. Purification by column chromatography through silica gel [330 g gold Teledyne silica gel cartridge] on a Teledyne ISCO Rf system, eluting with 95:5 to 50:50 hexanes:EtOAc as a gradient over 35 minutes, collecting all peaks. The desired product containing fractions were concentrated and dried under vacuum to afford a clear oil (2.5 g, 87% yield).

Step d: Preparation of *tert*-butyl 5-[3-(4-cyclopropyl-3-oxo-butanoyl)phenyl]sulfanyl-1-[(4-methoxyphenyl)methyl]triazole-4-carboxylate

To a mixture of *tert*-butyl 5-(3-acetylphenyl)sulfanyl-3-[(4-methoxyphenyl)methyl]triazole-4-carboxylate (1.0 equiv, 2.0 g, 4.5 mmol), 1-(benzotriazol-1-yl)-2-cyclopropyl-ethanone (1.5 equiv, 1.4 g, 6.8 mmol) and magnesium bromide ethyl etherate (2.5 equiv, 2.9 g, 11 mmol) in CH_2Cl_2 (25 mL), cooled over an ice bath was added $EtN(iPr)_2$ (4.6 g, 36 mmol, 6.3 mL) dropwise over 10 minutes using an addition funnel. After the addition was complete, the ice bath was removed and the reaction was stirred at 23 °C for 18 hours. An additional amount of CH_2Cl_2 (19 mL) was added to make stirring more effective. After this time, the reaction mixture was acidified with 1M aqueous HCl (48 mL) and stirred for 5 minutes. The layers were partitioned and the aqueous was back extracted with CH_2Cl_2 (30 mL). The combined organic extracts were washed with water (20 mL), dried over $MgSO_4$, filtered and then concentrated under vacuum to an oil. This material was applied to a silica gel pre-cartridge and then purified by column chromatography using a 120g silica Teledyne ISCO cartridge eluting with a 0:100 to 25:75 EtOAc:hexanes as a gradient. The fractions from the first peak were combined and concentrated under vacuum, resulting in the title product as a light yellow oil (1.2 g, 51% yield).

Step e: Preparation of *tert*-butyl 5-[3-[4-cyclopropyl-2-[(3-fluoro-4-sulfamoyl-phenyl)methyl]-3-oxo-butanoyl]phenyl]sulfanyl-1-[(4-methoxyphenyl)methyl]triazole-4-carboxylate

Into a 200 mL round-bottom flask equipped with a magnetic stir bar and under N_2 was added *tert*-butyl 5-[3-(4-cyclopropyl-3-oxo-butanoyl)phenyl]sulfanyl-1-[(4-methoxyphenyl)methyl]triazole-4-carboxylate (1.0 equiv, 4.3 g, 8.3 mmol) and DMSO (25 mL). The solution was treated with cesium carbonate (1.2 equiv, 3.3 g, 9.9 mmol) and stirred for 10 minutes. 4-(Bromomethyl)-2-fluorobenzenesulfonamide (1.2 equiv, 2.7 g, 9.9 mmol) was then added. The mixture was stirred at room temperature for 2 h. LCMS analysis after this time reveals product formation. The reaction mixture was quenched with 1M aqueous HCl solution (50 mL) and water (100 mL). The mixture was poured into a 250 mL separatory funnel and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was loaded onto a 20 g silica gel pre-cartridge and dried. Purification by column chromatography through silica gel on the ISCO Rf [120 g GOLD], eluting with 95:5 to 10:90 hexanes:EtOAc as a gradient over 30 minutes, collect all peaks. The product containing fractions were combined, concentrated and further dried under vacuum, yielding the title product as a clear oil (1.3 g, 78% yield).

Step f: Preparation of *tert*-butyl 5-[3-[5-(cyclopropylmethyl)-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]-1*H*-pyrazol-3-yl]phenyl]sulfanyl-1-[(4-methoxyphenyl)methyl]triazole-4-carboxylate

Into a 100 mL round-bottom flask equipped with a magnetic stir bar and under N_2 was added *tert*-butyl 5-[3-[4-cyclopropyl-2-[(3-fluoro-4-sulfamoyl-phenyl)methyl]-3-oxo-butanoyl]phenyl]sulfanyl-1-[(4-methoxyphenyl)methyl]triazole-4-carboxylate (1.0 equiv, 1.3 g, 1.8 mmol), hydrazine-monohydrate (2.0 equiv, 180 μL, 3.7 mmol) and ethanol (6.1 mL). The solution was heated to 70 °C in an oil bath for 2 h. LCMS after this time reveals product formation. The reaction mixture was cooled to room temperature and concentrated under reduced

pressure. The residue was further dried via co-evaporation with EtOH to drive off water and excess hydrazine. The resulting crude oil was dried under vacuum for 18 h overnight to afford the title product as a yellow foam (1.2 g, 93% yield), which was used in next step without further purification.

Step g: Preparation of ethyl 2-[3-[3-[5-*tert*-butoxycarbonyl-3-[(4-methoxyphenyl)methyl]triazol-4-yl]sulfanylphenyl]-5-(cyclopropylmethyl)-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]pyrazol-1-yl]thiazole-4-carboxylate

Into a 20 mL vial equipped with a magnetic stir bar and under N_2 was added *tert*-butyl 5-[3-[5-(cyclopropylmethyl)-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]-1*H*-pyrazol-3-yl]phenyl]sulfanyl-1-[(4-methoxyphenyl)methyl]triazole-4-carboxylate (1.0 equiv, 400 mg, 0.6 mmol), ethyl 2-fluorothiazole-4-carboxylate (1.2 equiv, 120 mg, 0.70 mmol), sodium *tert*-pentoxide (2.5 equiv, 160 mg, 1.4 mmol) and DMF (2.3 mL). The mixture was stirred at room temperature for 1 hour. LCMS analysis indicated formation of desired product. The reaction was quenched by formic acid (0.1 mL), loaded directly onto a 20 g silica gel pre-cartridge and dried under vacuum. Purification by column chromatography through silica gel [80 g gold Teledyne silica gel cartridge] on a Teledyne ISCO Rf system, eluting with 100:0 to 0:100 hexanes:EtOAc as a gradient over 45 minutes, collecting all peaks. The desired product containing fractions were concentrated and dried under vacuum to afford a clear oil (104 mg, 21% yield).

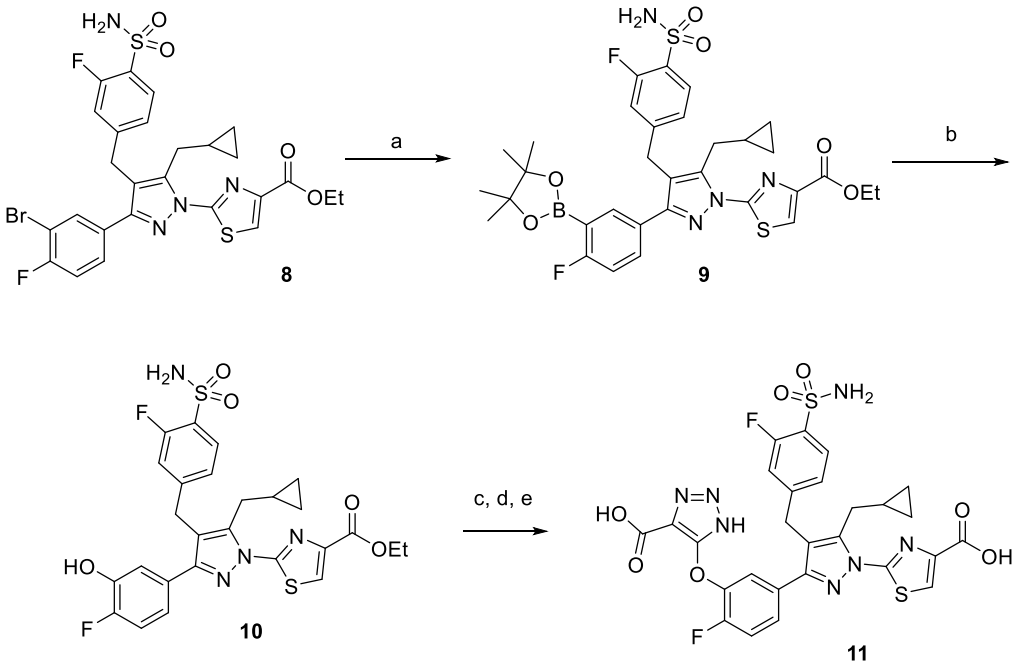
Step h: Preparation of 2-(5-(cyclopropylmethyl)-3-(3-((4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-5-yl)thio)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1*H*-pyrazol-1-yl)thiazole-4-carboxylic acid

Into a 4 mL reaction vial was added ethyl 2-[3-[3-[5-*tert*-butoxycarbonyl-3-[(4-methoxyphenyl)methyl]triazol-4-yl]sulfanylphenyl]-5-(cyclopropylmethyl)-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]pyrazol-1-yl]thiazole-4-carboxylate (1.0 equiv, 100 mg, 0.1 mmol), triethylsilane (14 equiv, 250 μL, 1.6 mmol) and CH_2Cl_2 (250 μL). The mixture was stirred at 23 °C for 5 min. Trifluoroacetic acid (57 equiv, 500 μL, 7 mmol) was added slowly. The mixture was stirred at 23 °C and monitored by LCMS. Upon completion of reaction, the reaction was concentrated. The resulting crude mixture was dissolved in methanol and injected onto a preparative HPLC (ACCQPrep HP125 UV). Purification by a reverse phase prep HPLC column chromatography (C18 column dimensions: 20 mm × 150 mm, 5 μm), eluting with 90:10 to 0:100 water:MeCN + 0.1% formic acid as a gradient over 35 minutes, collecting all peaks. The desired product containing fractions were concentrated and lyophilized overnight to yield the title product as a white powder (15 mg, 18% yield).

Step i: Preparation of 2-(3-(3-((4-carboxy-1*H*-1,2,3-triazol-5-yl)thio)phenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1*H*-pyrazol-1-yl)thiazole-4-carboxylic acid

In a 4 mL reaction vial was added 2-(5-(cyclopropylmethyl)-3-(3-((4-(ethoxycarbonyl)-1*H*-1,2,3-triazol-5-yl)thio)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1*H*-pyrazol-1-yl)thiazole-4-carboxylic acid (1.0 equiv, 15 mg) and 1 M aqueous LiOH/THF/MeOH (10 equiv, 660 μL, v/v/v, 1/1/1) and stirred at 23°C for 18 hours. LCMS analysis indicated completion of hydrolysis. The reaction was quenched dropwise with concentration formic acid until the pH was ~2. The mixture was concentrated and injected directly to prep HPLC (ACCQPrep HP125 UV). Purification by a reverse phase preparative HPLC column chromatography (C18 column dimensions: 20 mm × 150 mm, 5 μm), eluting with 90:10 to 0:100 water:MeCN + 0.1% formic acid as a gradient over 25 minutes, collecting all peaks. The desired product containing fractions were concentrated and lyophilized overnight to yield the title product as a white powder (8.2 mg, 45% yield). ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.29 (s, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.62 (s, 1H), 7.56 (s, 2H), 7.45 (t, *J* = 7.0 Hz, 1H), 7.39 (s, 1H), 7.09 (d, *J* = 11.2 Hz, 1H), 6.99 (d, *J* = 8.2 Hz, 1H), 4.11 (s, 2H), 2.41 (d, *J* = 6.9 Hz, 2H), 0.92 – 0.88 (m, 1H), 0.41 – 0.30 (m, 2H), 0.19 – 0.09 (m, 2H). LC-MS (ESI) *m/z* 656 $(M+H)^+$; [$(M+H)^+$ calculated for $C_{27}H_{22}FN_7O_6S_3$, 655.69].

Preparation of 2-(3-(3-((4-carboxy-1*H*-1,2,3-triazol-5-yl)oxy)-4-fluorophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1*H*-pyrazol-1-yl)thiazole-4-carboxylic acid (Compound 11)



Step a: Preparation of ethyl 2-(5-(cyclopropylmethyl)-3-(4-fluoro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1*H*-pyrazol-1-yl)thiazole-4-carboxylate

To a degassed solution of ethyl 2-[3-(3-bromo-4-fluoro-phenyl)-5-(cyclopropylmethyl)-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]pyrazol-1-yl]thiazole-4-carboxylate (1.0 equiv, 700 mg, 1.1 mmol, synthesized by following published procedures (WO 2016/109559)), bis(pinacolato)diboron (2.0 equiv, 560 mg, 2.2 mmol) and potassium acetate (3.0 equiv, 320 mg, 3.3 mmol) in 1,4-dioxane (3.1 mL) was added [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (0.1 equiv, 90 mg, 0.1 mmol). The reaction was heated to 100 °C overnight. This mixture was diluted with EtOAc (5 mL), filtered and concentrated under vacuum. This residue was purified by column chromatography through silica gel [40 g gold Teledyne silica gel cartridge] on a Teledyne ISCO Rf system, eluting with 100:0 to 0:100 hexanes:EtOAc as a gradient over 25 minutes, collecting all peaks. The fractions from the major peak were combined, concentrated under vacuum, and further dried under high vacuum overnight, yielding the title product as a while solid (342 mg, 46% yield).

Step b: Preparation of ethyl 2-(5-(cyclopropylmethyl)-3-(4-fluoro-3-hydroxyphenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1*H*-pyrazol-1-yl)thiazole-4-carboxylate

Into a round bottom flask was added ethyl 2-[5-(cyclopropylmethyl)-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]-3-[4-fluoro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pyrazol-1-yl]thiazole-4-carboxylate (1.0 equiv, 340 mg, 0.5 mmol), [5-[5-(cyclopropylmethyl)-1-(4-ethoxycarbonylthiazol-2-yl)-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]pyrazol-3-yl]-2-fluoro-phenyl]boronic acid (1.0 equiv, 300 mg, 0.5 mmol), urea hydrogen peroxide adduct (4.0 equiv, 190 mg, 2.0 mmol) and methanol (1.7 mL). The mixture was stirred at 23 °C and monitored by LCMS. After stirring for 2 hours, LCMS analysis indicated completion of reaction by LCMS. The reaction was quenched by adding 10% aqueous sodium thiosulfate. The mixture was loaded directly on reverse phase C18 pre-cartridge and dried. Purification by a reverse phase column chromatography through silica gel [80 g gold

C18 Teledyne silica gel cartridge] on a Teledyne ISCO Rf system, eluting with 95:5 to 0:100 water:CH_3CN + 0.1% formic acid as a gradient over 30 minutes, collecting all peaks. The product-containing fractions were combined, concentrated under vacuum and further dried under high vacuum overnight, yielding the title product as a while solid (237 mg, 82% yield).

Step c: Preparation of ethyl 2-[3-[3-[5-*tert*-butoxycarbonyl-3-[(4-methoxyphenyl)methyl]triazol-4-yl]oxy-4-fluoro-phenyl]-5-(cyclopropylmethyl)-4-[(3-fluoro-4-sulfamoylphenyl)methyl]pyrazol-1-yl]thiazole-4-carboxylate

Into a 4 mL reaction vial was added *tert*-butyl 5-chloro-1-[(4-methoxyphenyl)methyl]triazole-4-carboxylate (0.8 equiv, 0.14 g, 0.42 mmol), ethyl 2-[5-(cyclopropylmethyl)-3-(4-fluoro-3-hydroxy-phenyl)-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]pyrazol-1-yl]thiazole-4-carboxylate (1.0 equiv, 0.3 g, 0.5 mmol), potassium carbonate (3.0 equiv, 220 mg, 1.6 mmol) and DMF (2.6 mL). The mixture was stirred at 110 °C for 2 hours. LCMS analysis indicated product formation. The reaction was filtered and loaded directly onto a 20 g silica gel pre-cartridge and dried under vacuum. The mixture was purified by column chromatography through silica gel [40 g gold Teledyne silica gel cartridge] on a Teledyne ISCO Rf system, eluting with 95:5 to 0:100 hexanes:EtOAc as a gradient over 25 minutes, collecting all peaks. The desired product containing fractions were concentrated and dried under vacuum to afford a clear oil (88 mg, 19% yield).

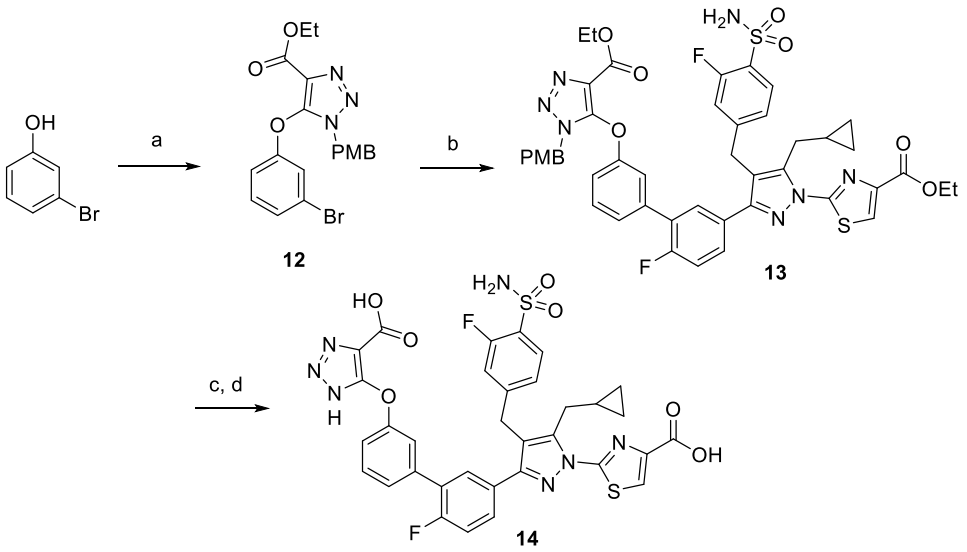
Step d: Preparation of 4-[5-[5-(cyclopropylmethyl)-1-(4-ethoxycarbonylthiazol-2-yl)-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]pyrazol-3-yl]-2-fluoro-phenoxy]-*1H*-triazole-5- carboxylic acid

Into a 4 mL reaction vial was added ethyl 2-[3-[3-[5-*tert*-butoxycarbonyl-3-[(4-methoxyphenyl)methyl]triazol-4-yl]oxy-4-fluoro-phenyl]-5-(cyclopropylmethyl)-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]pyrazol-1-yl]thiazole-4-carboxylate (1.0 equiv, 88 mg, 0.10 mmol), triethylsilane (12 equiv, 0.20 mL, 1.3 mmol) and CH_2Cl_2 (0.2 mL). The mixture was stirred at 23 °C for 5 min. Trifluoroacetic acid (52 equiv, 0.40 mL, 5.3 mmol) was added dropwise. The mixture was stirred at 40 °C and monitored by LCMS. After stirring at 40 °C for 4 hours, LCMS analysis indicated completion of reaction. The reaction was concentrated to remove most of trifluoroacetic acid. The resulting crude oil was injected directly onto a preparative HPLC (ACCQPrep HP125 UV). Purification by a reverse phase prep HPLC column chromatography (C18 column dimensions: 20 mm × 150 mm, 5 µm), eluting with 90:10 to 0:100 water:MeCN + 0.1% formic acid as a gradient over 35 minutes, collecting all peaks. The desired product containing fractions were concentrated and lyophilized overnight to yield the title product as a white powder (26 mg, 37% yield).

Step e: Preparation of 2-(3-(3-((4-carboxy-1*H*-1,2,3-triazol-5-yl)oxy)-4-fluorophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1*H*-pyrazol-1-yl)thiazole-4-carboxylic acid

Into a 4 mL reaction vial was added 4-[5-[5-(cyclopropylmethyl)-1-(4-ethoxycarbonylthiazol-2-yl)-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]pyrazol-3-yl]-2-fluoro-phenoxy]-*1H*-triazole-5-carboxylic acid (1.0 equiv, 26 mg, 0.038 mmol), 1 M aqueous LiOH (6.0 equiv, 0.23 mL, 0.23 mmol), THF (0.2 mL) and methanol (0.2 mL). The mixture was stirred at 23 °C for 18 hours. LCMS analysis indicated completion of hydrolysis. The reaction was quenched with formic acid until the pH was~2. The mixture was concentrated and injected directly to prep HPLC (ACCQPrep HP125 UV). Purification by a reverse phase prep HPLC column (C18 column dimensions: 20 mm × 150 mm, 5 µm), eluting with 90:10 to 0:100 water:MeCN + 0.1% formic acid as a gradient over 25 minutes, collecting all peaks. The desired product containing fractions were concentrated and lyophilized overnight to yield the title product as a white powder (13 mg, 52% yield). ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.30 (s, 1H), 7.64 (t, *J* = 7.9 Hz, 2H), 7.61 (*br* s, 1H), 7.51 – 7.42 (m, 1H), 7.40 (s, 1H), 7.39 – 7.32 (m, 1H), 7.09 (d, *J* = 11.3 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 4.11 (s, 2H), 3.14 (d, *J* = 6.9 Hz, 2H), 1.18 – 1.04 (m, 1H), 0.40 – 0.30 (m, 2H), 0.25 – 0.15 (m, 2H). LC-MS (ESI) *m/z* 658 $(M+H)^+$; [$(M+H)^+$ calculated for $C_{27}H_{21}F_2N_7O_7S_2$, 657.62].

Preparation of 2-[3-[3-[3-[(5-carboxy-1*H*-triazol-4-yl)oxy]phenyl]-4-fluoro-phenyl]-5-(cyclopropylmethyl)-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]pyrazol-1-yl]thiazole-4-carboxylic acid (Compound 14)



Step a: Preparation of ethyl 5-(3-bromophenoxy)-1-[(4-methoxyphenyl)methyl]triazole-4-carboxylate

Into a 200 mL round bottom flask was added ethyl 5-chloro-1-[(4-methoxyphenyl)methyl]triazole-4-carboxylate (1.0 equiv, 1.0 g, 3.4 mmol), *m*-bromophenol (1.0 equiv, 590 mg, 3.4 mmol), potassium carbonate (3.0 equiv, 1.4 g, 10 mmol) and DMF (11 mL). The mixture was stirred at 130 °C for 2 hours. LCMS analysis indicated reaction was not completed. The mixture was stirred for additional 7 hours at 130 °C. The mixture was partitioned in a 1 L separatory funnel with 450 mL water and 150 mL of EtOAc. The aqueous layer was back extracted with EtOAc (2 x 150 mL). The combined organic layers were concentrated and loaded directly on 20 g silica gel pre-cartridge. The mixture was purified by column chromatography through silica gel [80 g gold Teledyne silica gel cartridge] on a Teledyne ISCO Rf system, eluting with 100:0 to 30:70 hexanes:EtOAc as a gradient over 25 minutes, collecting all peaks. The desired product containing fractions were concentrated and dried under vacuum to afford a clear oil which crystallized slowly upon standing (1.4 g, 93% yield).

Step b: Preparation of ethyl 2-[5-(cyclopropylmethyl)-3-[3-[3-[5-ethoxycarbonyl-3-[(4-methoxyphenyl)methyl]triazol-4-yl]oxyphenyl]-4-fluoro-phenyl]-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]pyrazol-1-yl]thiazole-4-carboxylate

Into a 20 mL reaction vial was added [5-[5-(cyclopropylmethyl)-1-(4-ethoxycarbonylthiazol-2-yl)-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]pyrazol-3-yl]-2-fluoro-phenyl]boronic acid (1.0 equiv, 100 mg, 0.2 mmol), 2 M aqueous potassium phosphate solution (3.0 equiv, 0.25 mL, 0.50 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (0.05 equiv, 7 mg, 0.008 mmol), ethyl 5-(3-bromophenoxy)-1-[(4-methoxyphenyl)methyl]triazole-4-carboxylate (1.0 equiv, 72 mg, 0.17 mmol) and 1,4-dioxane (1.6 mL). The mixture was purged with nitrogen for 10 min, then heated at 130 °C for 30 min. The reaction mixture was loaded onto a 20 g silica gel pre-cartridge and dried under vacuum. The mixture was purified by column chromatography through silica gel [24g gold Teledyne silica gel cartridge] on a Teledyne ISCO Rf system, eluting with 95:5 to 0:100 hexanes:EtOAc as a gradient over 20 minutes, collecting all peaks. The desired

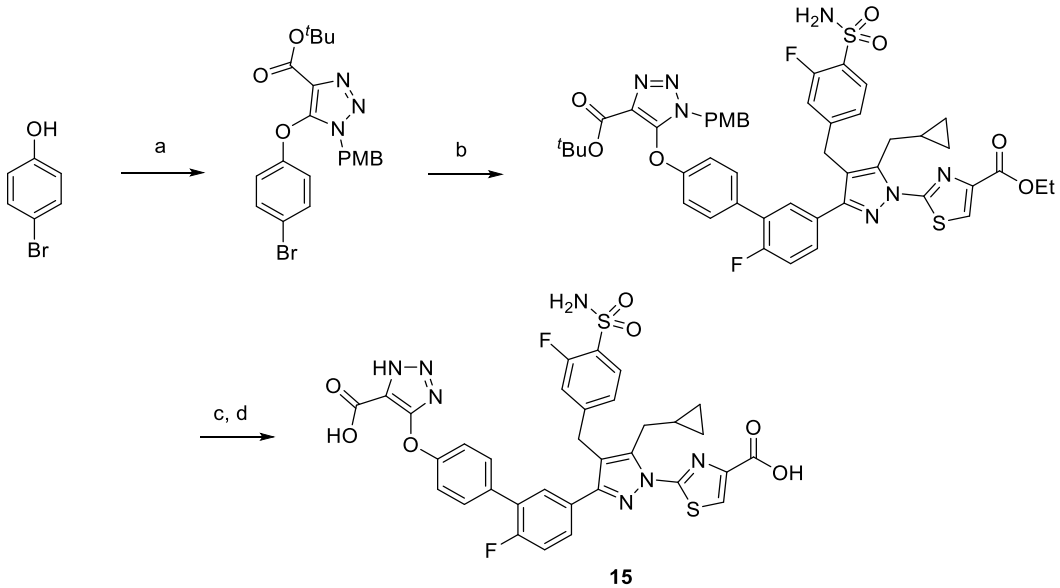
product containing fractions were concentrated and dried under vacuum to afford a clear oil which crystallized slowly upon standing (91 mg, 60% yield).

Step c and d: Preparation of 2-[3-[3-[3-[(5-carboxy-1*H*-triazol-4-yl)oxy]phenyl]-4-fluoro-phenyl]-5-(cyclopropylmethyl)-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]pyrazol-1-yl]thiazole-4-carboxylic acid

Into a 10 mL reaction vial was added ethyl 2-[5-(cyclopropylmethyl)-3-[3-[3-[5-ethoxycarbonyl-3-[(4-methoxyphenyl)methyl]triazol-4-yl]oxyphenyl]-4-fluoro-phenyl]-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]pyrazol-1-yl]thiazole-4-carboxylate (1.0 equiv, 91 mg, 0.10 mmol), CH_2Cl_2 (0.15 mL) and triethylsilane (3.1 equiv, 0.050 mL, 0.31 mmol). The mixture was stirred for 2 min at 23 °C. Trifluoroacetic acid (53 equiv, 0.60 mL, 5.3 mmol) was added dropwise. The reaction was heated at 50 °C and monitored by LCMS. After stirring at 50 °C for 4 hours, LCMS analysis indicated completion of reaction. The mixture was concentrated and injected directly onto a preparative HPLC column (ACCQPrep HP125 UV). Purification by a reverse phase preparative HPLC column chromatography (C18 column dimensions: 20 mm × 150 mm, 5 µm), eluting with 90:10 to 0:100 water:MeCN + 0.1% formic acid as a gradient over 35 minutes, collecting all peaks. The desired product containing fractions were concentrated and lyophilized overnight to yield the di-ester product as a white powder (47 mg, 59% yield).

The di-ester product was treated with 1 M aqueous LiOH/THF/MeOH (v/v/v, 1/1/1, 0.6 mL) and stirred at 23 °C for 18 hours. LCMS analysis indicated completion of hydrolysis. The reaction was quenched with concentrated formic acid until the pH was~2. The mixture was concentrated and injected directly onto a preparative HPLC column (ACCQPrep HP125 UV). Purification by a reverse phase preparative HPLC column chromatography (C18 column dimensions: 20 mm × 150 mm, 5 µm), eluting with 90:10 to 0:100 water:MeCN + 0.1% formic acid as a gradient over 25 minutes, collecting all peaks. The desired product containing fractions were concentrated and lyophilized overnight to yield the title product as a white powder (15 mg, 15% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 8.18 (s, 1H), 7.73 (t, *J* = 7.7 Hz, 1H), 7.64 – 7.57 (m, 2H), 7.43 (t, *J* = 8.0 Hz, 1H), 7.27 – 7.02 (m, 6H), 4.19 (s, 2H), 3.26 (d, *J* = 6.8 Hz, 3H), 1.18 – 1.07 (m, 1H), 0.42 – 0.34 (m, 2H), 0.26 – 0.21 (m, 2H). LC-MS (ESI) *m/z* 734 $(M+H)^+$; [$(M+H)^+$ calculated for $C_{33}H_{25}F_2N_7O_7S_2$, 733.72].

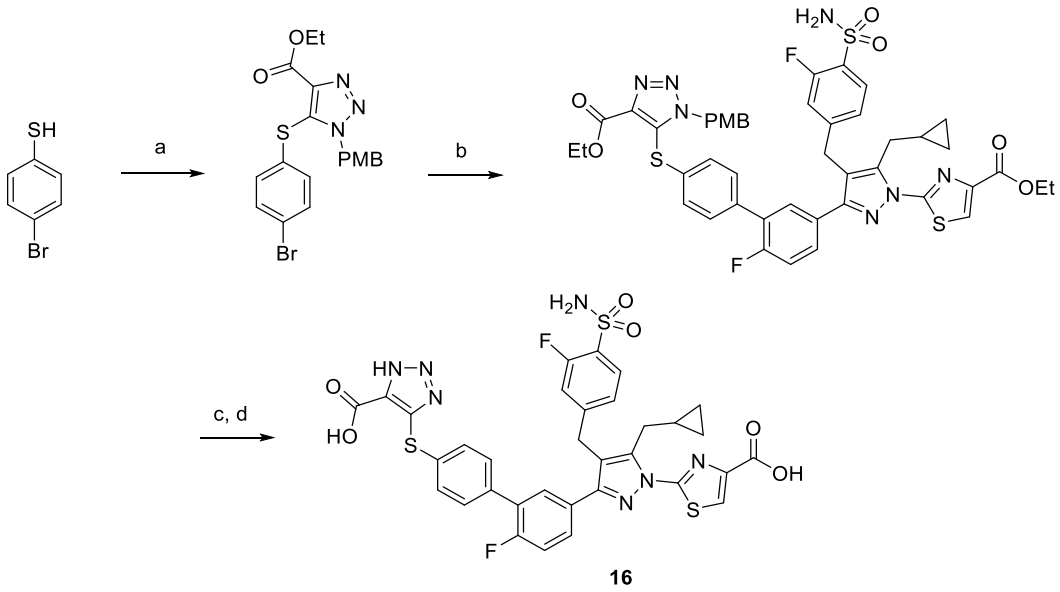
Preparation of 2-(3-(4'-((5-carboxy-1*H*-1,2,3-triazol-4-yl)oxy)-6-fluoro-[1,1'-biphenyl]-3-yl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1*H*-pyrazol-1-yl)thiazole-4-carboxylic acid (Compound 15)



15

Compound 15 was prepared in the same manner as described in above synthesis of **Compound 14**, starting from *para*-bromophenol. ^{1}H NMR (400 MHz, d_6-DMSO) δ 13.19 (*br* s, 1H), 8.31 (s, 1H), 7.68 (t, *J* = 7.9 Hz, 1H), 7.62 (d, *J* = 2.6 Hz, 1H), 7.59 (*br* s, 3H), 7.46 – 7.40 (m, 1H), 7.41 – 7.32 (m, 2H), 7.22 – 7.12 (m, 3H), 7.08 (d, *J* = 8.1 Hz, 1H), 4.19 (s, 2H), 3.18 (d, *J* = 6.9 Hz, 2H), 1.22 – 1.11 (m, 1H), 0.38 – 0.32 (m, 2H), 0.27 – 0.19 (m, 2H). LC-MS (ESI) *m/z* 734 $(M+H)^+$; [$(M+H)^+$ calculated for $C_{33}H_{25}F_2N_7O_7S_2$, 733.72].

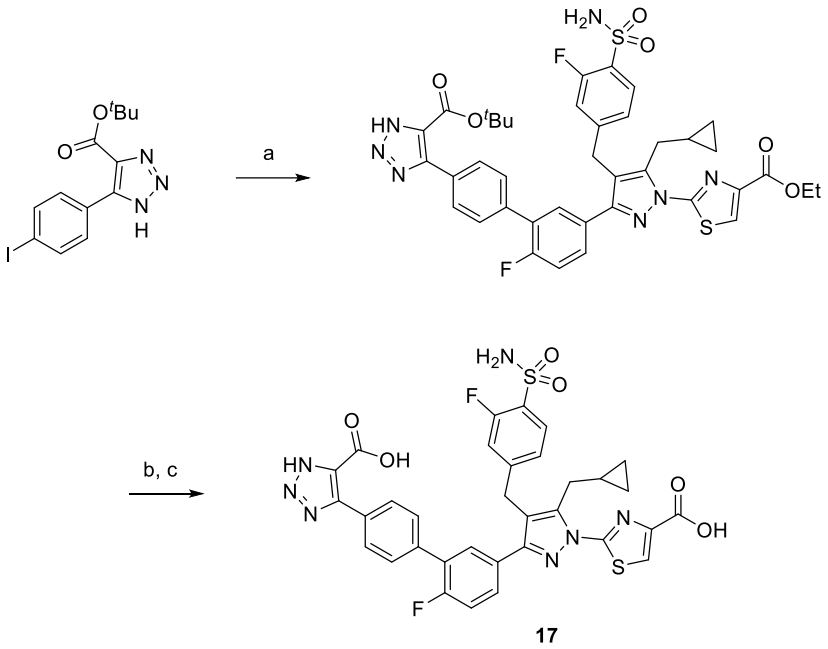
Preparation of 2-(3-(4'-((5-carboxy-1*H*-1,2,3-triazol-4-yl)thio)-6-fluoro-[1,1'-biphenyl]-3-yl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1*H*-pyrazol-1-yl)thiazole-4-carboxylic acid (Compound 16)



16

Compound 16 was prepared in the same manner as described in above synthesis of **Compound 14**, starting from *para*-bromothiophenol. ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.31 (s, 1H), 7.67 (t, *J* = 8.0 Hz, 2H), 7.65 – 7.59 (m, 3H), 7.55 – 7.47 (m, 2H), 7.46 – 7.35 (m, 3H), 7.18 (d, *J* = 11.2 Hz, 1H), 7.07 (d, *J* = 8.2 Hz, 1H), 4.19 (s, 2H), 3.18 (d, *J* = 6.9 Hz, 2H), 1.21 – 1.09 (m, 1H), 0.38 – 0.31 (m, 2H), 0.26 – 0.20 (m, 2H). LC-MS (ESI) *m/z* 749 $(M+H)^+$; [$(M+H)^+$ calculated for $C_{33}H_{25}F_2N_7O_6S_3$, 749.78].

Preparation of 2-(3-(4'-(5-carboxy-1*H*-1,2,3-triazol-4-yl)-6-fluoro-[1,1'-biphenyl]-3-yl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1*H*-pyrazol-1-yl)thiazole-4-carboxylic acid (Compound 17)



Step a: Preparation of ethyl 2-(3-(4'-(5-(*tert*-butoxycarbonyl)-1*H*-1,2,3-triazol-4-yl)-6-fluoro-[1,1'-biphenyl]-3-yl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1*H*-pyrazol-1-yl)thiazole-4-carboxylate

Into a 20 mL reaction vial was added ethyl 2-[5-(cyclopropylmethyl)-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]-3-[4-fluoro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]pyrazol-1-yl]thiazole-4-carboxylate (1.0 equiv, 200 mg, 0.3 mmol), 2 M aqueous potassium phosphate solution (3.0 equiv, 0.44 mL, 0.88 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (0.1 equiv, 24 mg, 0.03 mmol), *tert*-butyl 4-(4-iodophenyl)-1*H*-triazole-5-carboxylate (1.0 equiv, 110 mg, 0.29 mmol) and 1,4-dioxane (1.7 mL). The mixture was purged with nitrogen for 10 min, then heated at 100 °C for 30 min. LCMS analysis indicated completion of reaction. The mixture was concentrated under reduced pressure, loaded onto a 20 g silica gel pre-cartridge and dried under vacuum. The mixture was purified by column chromatography through silica gel [40 g gold Teledyne silica gel cartridge] on a Teledyne ISCO Rf system, eluting with 95:5 to 0:100 hexanes:EtOAc as a gradient over 25 minutes, collecting all peaks. The desired product containing fractions were concentrated and dried under vacuum to afford a clear oil which crystallized slowly upon standing (104 mg, 44% yield).

Step b and c: Preparation of 2-(3-(4'-(5-carboxy-1*H*-1,2,3-triazol-4-yl)-6-fluoro-[1,1'-biphenyl]-3-yl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1*H*-pyrazol-1-yl)thiazole-4-carboxylic acid

Into a reaction vial was added ethyl 2-[3-[3-[4-(5-tert-butoxycarbonyl-1*H*-triazol-4-yl)phenyl]-4-fluoro-phenyl]-5-(cyclopropylmethyl)-4-[(3-fluoro-4-sulfamoyl-phenyl)methyl]pyrazol-1-yl]thiazole-4-carboxylate (1.0 equiv, 104 mg, 0.13 mmol), dichloromethane (0.4 mL) and triethylsilane (9.6 equiv, 0.20 mL, 1.3 mmol). The mixture was stirred for 2 min at 23 °C. Trifluoroacetic acid (41 equiv, 0.60 mL, 5.3 mmol) was added dropwise. The reaction was heated at 50 °C and monitored by LCMS. After stirring at 50 °C for 4 hours, LCMS analysis indicated completion of reaction. The mixture was concentrated and injected directly onto a preparative HPLC column (ACCQPrep HP125 UV). Purification by reverse phase preparative HPLC column chromatography (C18 column dimensions: 20 mm × 150 mm, 5 µm), eluting with 90:10 to 0:100 water:MeCN + 0.1% formic acid as a gradient over 35 minutes, collecting all peaks. The desired product containing fractions were concentrated and lyophilized overnight to yield 4-(5'-(5-(cyclopropylmethyl)-1-(4-(ethoxycarbonyl)thiazol-2-yl)-4-(3-fluoro-4-sulfamoylbenzyl)-1*H*-pyrazol-3-yl)-2'-fluoro-[1,1'-biphenyl]-4-yl)-1*H*-1,2,3-triazole-5-carboxylic acid as a white powder (54 mg, 55% yield).

The ester product was treated with 1 M aqueous LiOH/THF/MeOH (1.5 mL, v/v/v, 1/1/1) and stirred at 23 °C for 18 hours. LCMS analysis indicated completion of hydrolysis. The reaction was quenched with concentration formic acid until the pH was acidic. The mixture was concentrated and injected directly onto a preparative HPLC column (ACCQPrep HP125). Purificaiton by reverse phase preparative HPLC column chromatography (C18 column dimensions: 20 mm × 150 mm, 5 µm), eluting with 90:10 to 0:100 water:MeCN + 0.1% formic acid as a gradient over 25 minutes, collecting all peaks. The desired product containing fractions were concentrated and lyophilized overnight to yield the title product as a white powder (35 mg, 37% yield). ^{1}H NMR (400 MHz, d_6-DMSO) δ 13.30 – 13.00 (m, 1H), 8.29 (s, 1H), 8.05 – 7.80 (m, 2H), 7.68 – 7.55 (m, 4H), 7.55 – 7.45 (m, 2H), 7.39 (t, *J* = 9.6 Hz, 1H), 7.17 (d, *J* = 11.1 Hz, 1H), 7.07 (d, *J* = 8.1 Hz, 1H), 4.18 (s, 2H), 3.16 (d, *J* = 7.0 Hz, 2H), 1.20 – 1.05 (m, 1H), 0.37 – 0.29 (m, 2H), 0.24 – 0.17 (m, 2H). LC-MS (ESI) *m/z* 718 $(M+H)^+$; [$(M+H)^+$ calculated for $C_{33}H_{25}F_2N_7O_6S_2$, 717.72].

Human GO Production and Purification:

The method was previously described by Murray *et al*[1]. Briefly, C41(DE3) *Escherichia coli* cells containing pET28a-HAOX1 plasmid were grown in LB Miller + 50 ug/ml kanamycin at 37° C to an OD_{600} of 0.8. The culture temperature was decreased to 16 °C, and protein expression was induced overnight by adding 0.5 mM IPTG. Cells were harvested by centrifugation and stored at -80° C until further use. Cells were lysed in the presence of 0.05 mg/ml FMN in buffer A [20 mM HEPES pH 7.9, 500 mM KCl, 5 mM imidazole, 10% glycerol, 0.1% Triton X-100, 0.1 µg/ml benzamidine, 0.1 µg/ml PMSF, 1 mM $MgCl_2$, and 0.005% DNAse I], using an Avestin Emulsiflex C3 homogenizer at 15,000-20,000 psi (3 passes). The lysate was cleared by centrifugation at 39,000 x g for 45 min. The His-tagged protein was isolated from a cobalt NTA column (HisPur Cobalt Superflow Agarose; Thermo 25229), using a 5-250 mM imidazole gradient in buffer A without Triton X-100 and glycerol. The pooled fractions were dialyzed overnight against 20 mM HEPES pH 7.5, 100 mM NaCl, 10% glycerol and 0.1 mM EDTA. Biotinylated thrombin (0.1 U/mg) was added to the dialysis bag to cleave the His-tag. Cleavage was confirmed via mass spectrometry. The salt content of the buffer was increased to 500 mM NaCl to facilitate protein concentration. Excess FMN was added to the protein for 1 h prior to concentration and separation over a Superdex 200 column with 20 mM HEPES pH7.5, 500 mM NaCl, 10% glycerol. Pooled fractions were concentrated with 10,000 MWCO centrifugal concentrator (Sartorius Vivaspin 20, CS2002). Aliquots of protein were flash frozen with liquid nitrogen and stored at -80 °C.

Human LDHA Production and Purification:

BL21 gold (DE3) *E. coli* cells containing the LDHA-pET28b plasmid were grown at 37 °C to an OD_{600} of 0.8. The expression protocol, buffers used, lysis and affinity column conditions were the same as for GO above. LDHA containing fractions were pooled and dialyzed into 20 mM HEPES pH 7.5, 100 mM NaCl, and 0.2 mM EDTA. For this protein the His-tag was not removed. The dialyzed protein was filtered (0.2 µm), concentrated, and passed over a Superdex 200 size exclusion column and stored as indicated above.

Crystallization and Structure Determination:

Briefly, crystals of GO and LDHA were obtained by co-crystallization with compound **15** (5-fold molar excess). Crystals were cryo protected and flash frozen directly in the 100 K nitrogen cryo stream. Data were collected on a rotating anode home X-ray diffractometer (Rigaku Micromax 007 X-ray source with VariMax-HF Confocal Optic System coupled to Pilatus3M detector) operated at 40 kV and 20 mA. The data were integrated and scaled with HKL-3000. Both the structures were solved by molecular replacement using GO (PDB code 2RDT) and LDHA (PDB code 5W8I) as search models.[1,2] Models were prepared by trimming off all the non-protein molecules and active site residues. Models were manually rebuilt in COOT and refined with REFMAC.[3,4] Model validation was performed with PROCHECK and MOLPROBITY.[5,6] Details of crystallization conditions, structure solution and refinement statistics are summarized in **Table S1**. Figures were prepared with PyMOL (Shrödinger).

Table S1. Data Collection and Refinement Statistics

Dataset	**GO-compound 15**	**LDHA-compound 15**
Data Collection		
X-ray source	MicroMax-007	MicroMax-007
Wavelength (Å)	1.5418	1.5418
Resolution range (Å)	50.00-2.07 (2.11-2.07)	50.00-2.50 (2.54-2.50)
Space group	*I*4	*I*121
Unit cell (Å, °)	96.9 96.9 79.5 90 90 90	83.9 131.0 215.5 90 99.3 90
Total reflections	134115	364240
Unique reflections	22486 (1133)	78979 (3992)
Multiplicity	6.0 (2.0)	4.6 (2.9)
Completeness (%)	99.9 (99.4)	100 (99.9)
Mean I/σI	14.6 (3.2)	13.5 (3.0)
Wilson B-factor (Å^2)	17.9	31.9
R-merge	0.095 (0.273)	0.104 (0.407)
R-meas	0.103 (0.364)	0.117 (0.497)
R-pim	0.040 (0.239)	0.053 (0.280)
CC$^{1/2}$ (highest resolution shell)	0.797	0.870
CC* (highest resolution shell)	0.942	0.965
Refinement		
Reflections used in refinement	21419 (1534)	74825 (4086)
Reflections used for R-free	1065 (85)	3910 (213)
R-work	0.1259	0.2093
R-free	0.1660	0.2576
Number of non-hydrogen atoms	2972	11585
macromolecules	2651	10187
solvent	202	539
ligands	119	859
RMS(bonds)	0.011	0.008
RMS(angles)	1.754	1.461
Ramachandran favored (%)	93.2	92.5
Ramachandran allowed (%)	5.4	7.4
Generously allowed (%)	1.4	
Clashscore	4.5	2.79
Average B-factor (Å^2)		
macromolecules	21.4	39.47
solvent	29.3	40.24
Cryo	20% Ethylene Glycol in mother liquor	20% Ethylene Glycol in mother liquor
Crystallization conditions	0.1 M Tris-HCl pH 8.5 25% (w/v) PEG 1000	0.2 M Lithium Sulfate 0.1 M Sodium Cacodylate: HCl pH 6.5 30% (v/v) PEG 400
PDB code	7M2O	7M2N

LDHA Biochemical Assay:

Briefly, compounds were serially diluted (3-fold in 100% DMSO) for a 10-concentration dose response. A solution of 50 µL of diluted recombinant human LDHA (WFSM – Lowther Lab) or mouse LDHA (Creative Biomart) in assay buffer (50 mM Tris pH 7.5 and 100 mM NaCl) was preincubated with 1 µL compound in black, clear bottom plates (Greiner Bio-One) at room temperature for 10 min. The reaction was initiated with the addition of 50 µL of diluted sodium pyruvate (Sigma Aldrich) and NADH (Sigma Aldrich). The plate was shaken briefly and immediately transferred to an absorbance plate reader. The oxidation of NADH was tracked at 340 nm for 15 min. The final assay conditions had 0.035 µg/mL hLDHA or 0.012 µL/mL mLDHA, 50 µM sodium pyruvate, 50 µM NADH, and 1% DMSO.

GO Biochemical Assay:

Briefly, compounds were serially diluted (3-fold in 100% DMSO) for a 10-concentration dose response. A solution of 50 µL of diluted recombinant human GO (WFSM – Lowther Lab) or mouse GO (Creative Biomart) in assay buffer (25 mM HEPES pH 8.0) was preincubated with 1 µL compound in black, opaque plates (Greiner Bio-One) at room temperature for 10 min. The reaction was initiated with the addition of 50 µL of a diluted substrate mixture of glycolate (Sigma Aldrich), horseradish peroxidase (Sigma Aldrich), and Amplex Red (Invitrogen). The plate was shaken briefly and immediately transferred to a fluorescent plate reader. Fluorescence was obtained from the reaction of H_2O_2 and Amplex Red and tracked for 10 min (Ex: 544 nm, Em: 590 nm). The final assay conditions had 0.04 µg/mL hGO or 0.4 µg/mL mGO, 60 µM glycolate, 0.01 U/mL HRP, 50 µM Amplex Red, and 1% DMSO.

Mouse Primary Hepatocyte Assay

A mouse model with hepatic knockdown of the *Agxt* gene was developed. The model was generated through systemic administration of 0.4 mg/kg siRNA to CD1 male mice (8 – 12 weeks of age, Charles River Labs). The *Agxt* siRNA was encapsulated in a lipid nanoparticle (XL-10 (KL-52) LNP as described in WO2016/205410) and its sequence was: 5'-AcAAcuGGAGGGAcAucGudTsdT-3' (modified sense strand sequence, N: RNA residues; dN: DNA residues; n: 2'-O-methyl residues; s: phosphorothioate residues) and 5'-ACGAUGUCCCUCcAGUUGUdTsdT-3' (modified antisense strand sequence, see annotation above for residue modifications). Administration of the *Agxt* siRNA was done intravenously on day 0 and day 7 to maintain >90% knockdown of hepatic *Agxt* expression throughout the experimental study. The *Agxt*-KD model presented robust elevation of the urinary oxalate excretion within 7 days post-administration to a similar extent as *Agxt*-null mice.[7]

Hepatocytes from naïve CD1 or *Agxt* knockdown mice were freshly isolated on the day of the assay. In brief, mouse portal vein was cannulated and perfused at a flow of 2 mL/min and the inferior vena cava was cut for drainage. The liver was perfused in HBSS containing 10 mM HEPES at pH 7.4 for 4 min at 10 mL/min followed by perfusion in the digestion media (HBSS, 10 mM HEPES, pH 7.4, 1 mM $CaCl_2$ and 50 U/mL collagenase (Fisher #NC9919937)) for 10 min at 10 mL/min. The liver membrane was then disrupted in the hepatocyte wash media (Fisher #17704024) on ice, and the dissociated hepatocytes were washed twice in the hepatocyte wash media. Cells were then resuspended in 30 mL hepatocyte wash media and 20 mL of Percoll solution was added followed by centrifugation at 120 x g for 4 min. Mouse hepatocytes were resuspended in suspension media (Krebs-Henseleit Buffer, 5 mM HEPES, pH 7.4) and incubated at 37 °C for up to 2 h with shaking at 900 rpm in 5% CO_2 before use. To assess the potency of compounds in blocking the conversion of pyruvate to lactate in naïve mouse hepatocytes or the conversion of glycolate to oxalate in *Agxt* knockdown mouse primary hepatocytes, compounds were serially diluted in assay buffer (Krebs-Heinseleit Buffer, 1 M HEPES, pH 7.4, 0.2% DMSO) containing either 2 mM pyruvate or 10 mM glycolate. Equal volumes of diluted compounds were incubated with naïve mouse hepatocytes (50 µL, 100,000 cells/well) or *Agxt* knockdown hepatocytes (100 µL, 200,000 cells/well) in Krebs-Heinseleit Buffer, 1 M HEPES, pH 7.4 at 37 °C in 5% CO_2 with shaking at 900 rpm. To measure the levels of pyruvate to lactate conversion

in naïve mouse hepatocytes, after 30 min compound incubation the cells were quenched with 100 µM ^{13}C-lactate in acetonitrile. The cells were then centrifuged for 10 min at 3700 rpm and 100 µL of supernatant was transferred to a new plate and mixed with 100 µL H_2O before being injected into LC-MS for pyruvate and lactate quantification. Chromatographic separation was performed using an Agilent Zorbax XDB-18 4.6 x 50 mm column, with 0.1% acetic acid in water (mobile phase A) and acetonitrile (mobile phase B) run at 1 mL/min as per the following gradient: 5% B for 1 min, 5-95% B over 0.5 min, 95% B for 1 min, 95-5% B over 0.1 min, and 5% B for 1.50 min. The samples were analyzed using Sciex 4000 mass spectrometer operated in multiple reaction monitoring (MRM) mode under negative ionization. The MRM transitions were m/z 89 to m/z 43 for lactate and m/z 90 to m/z 44 for ^{13}C-lacate. To measure the conversion of glycolate to oxalate in *Agxt* knockdown mouse hepatocytes, after 2 h compound incubation the cells were centrifuged at 2 min at 3200 x g, and 100 µL of supernatant was transferred to a new plate and mixed with 10 µL of 1 N aqueous HCl solution to bring the pH < 2.0. Acidification both limits potential non-enzymatic breakdown of ascorbic acid to oxalate and loss of oxalate through crystallization of calcium oxalate. The amount of oxalate in each sample was then quantified by ion chromatography coupled with negative electrospray mass spectrometry at UAB, as previously described.[8]

Mouse PK/PD experiment of Compound 7 and 14:

Male C57BL/6 mice were purchased from Charles River Laboratories. The animals were 8 weeks old with body weights 20-23 g on the dosing date. The animals were housed in a 12-h light/12-h dark cycle environment, having access to standard rodent chow and water ad libitum. **Compound 7** and **14** were formulated as a solution at a dose volume of 10 mg/mL in 0.5% methylcellulose containing 2 equivalents of sodium hydroxide. Both compounds were orally administered to three mice per group at a dose of 100 mg/kg. At 6 h, animals were anesthetized using isoflurane, blood samples were collected into pre-cooled tubes containing K_3-EDTA from each animal via cardiac puncture. These blood samples were maintained on ice and centrifuged within an hour at 6000 x g for 10 min at 4 °C to obtain plasma. The plasma samples were collected into polypropylene tubes, quickly frozen on dry ice and stored at -80 °C until analysis. Following blood collection, animals were euthanized by cervical dislocation under deep isoflurane anesthesia, and a piece of liver (~100-200 mg) was collected, snap frozen in liquid nitrogen, and then stored at -80 °C until analysis.

PK Analysis

To quantify **Compound 7** and **14** in mouse livers by LC-MS/MS, mouse liver tissues were homogenized in 4× (w:v) of acetonitrile/H_2O (1:1) using a bead ruptor. Liver homogenates (25 µL) were subjected to protein precipitation with 150 µL of acetonitrile containing 0.2 µM of **CHK-12289** (in-house GO inhibitor, MW = 289.23) as the internal standard (IS). The samples were then centrifuged at 4,300 × g for 20 min at 4 °C, and 100 µL of the supernatants were transferred to a deep well 96 well plate containing equal volume of LC-MS grade H_2O. The plate was mixed for 5 min at 23 °C, and 5 µL of the sample was injected into UPLC. Chromatographic separation was achieved using Agilent Zorbax SB-C8 RRHD 2.1 × 50 mm column, with 0.1% acetic acid in water (mobile phase A) and 0.1% acetic acid in acetonitrile (mobile phase B) run at 0.4 mL/min as per the following gradient: 5% B for 0.5 min, 5-90% B over 0.5 min, 95% B for 1 min, 95-5% B over 0.1 min, and 5% B for 1.40 min. Total run time was 3.5 min. The samples were analyzed using Sciex QTRAP 5500 linear ion trap mass spectrometer operated in multiple reaction monitoring (MRM) mode under negative ionization. The MRM transitions were m/z 653.86 to m/z 610.0 for **Compound 7**, m/z 731.93 to m/z 577.10 for **Compound 14**, and m/z 287.88 to m/z 215.9 for the IS.

PD Analysis

To quantify glycolate levels in mouse plasma by LC-MS/MS, plasma samples (25 µL) were subjected to protein precipitation with 150 µL of acetonitrile containing 100 µM of $^{13}C_2$-glycolate as the internal standard (IS), followed by centrifugation at 4,300 x g for 20 min at 4 °C. Supernatants (120 µL) were transferred to a deep well 96 well plate containing 50 µL of LC-MS grade H_2O. The plate was mixed for 5 min at 23 °C , and 5 µL of the sample was injected into UPLC. Chromatographic separation was achieved using Atlantis HILIC Silica 3 µm, 2.1 × 50 mm column, with mobile phase A (10 mM ammonium acetate in water) and mobile phase B (10 mM ammonium acetate in 90% acetonitrile and 10% water), run at 0.75 mL/min as per the following gradient 100% B for 0.5 min, 100-30% B over 1 min, 30% B for 0.5 min, 30-100% over 0.1 min, and then 100% B for 3.4 min. Total run time was 5.5 min. The samples were analyzed using Sciex QTRAP 5500 linear ion trap mass spectrometer operated in multiple reaction monitoring (MRM) mode under negative ionization. The MRM transitions were m/z 74.877 to m/z 47.1 for glycolate and m/z 76.863 to m/z 48.1 for $^{13}C_2$-glycolate (IS).

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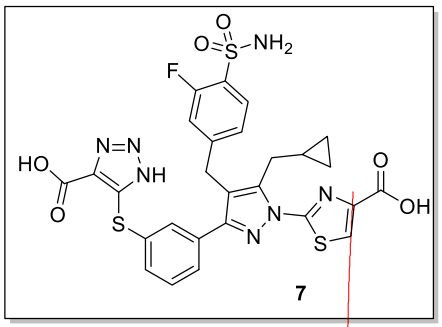
JD0211-063-1HNMR

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PROCNO                   1

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PULPROG              zg30
TD                  65536
SOLVENT              DMSO
NS                     16
DS                      2
SWH              8250.825 Hz
FIDRES           0.125898 Hz
AQ               3.9715316 sec
RG                  1149.4
DW                 60.600 usec
DE                 86.57 usec
TE                 292.6 K
D1            1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                   1H
P1                  9.00 usec
PL1                 0.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                  32768
SF           400.1300112 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
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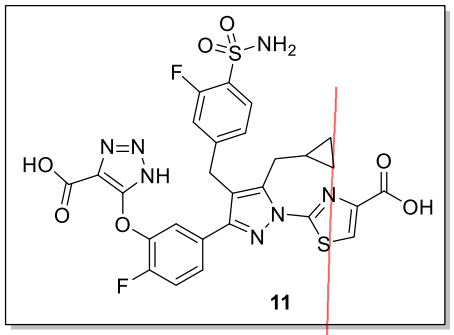
JD0211-046-1HNMR

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PROCNO                   1

F2 - Acquisition Parameters
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PULPROG              zg30
TD                  65536
SOLVENT              DMSO
NS                     16
DS                      2
SWH              8250.825 Hz
FIDRES           0.125898 Hz
AQ              3.9715316 sec
RG                  574.7
DW                 60.600 usec
DE                 86.57 usec
TE                 293.8 K
D1            1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                   1H
P1                  9.00 usec
PL1                 0.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                  32768
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SSB                     0
LB                   0.30 Hz
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PC                   1.00
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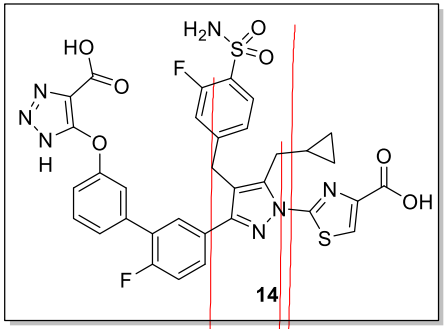
JD0211-075-1HNMR

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Current Data Parameters
NAME      JD0211-075-1HNMR
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
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Time                 15.01
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PROBHD    5 mm Multinucl
PULPROG              zg30
TD                  65536
SOLVENT              MeOD
NS                      4
DS                      2
SWH              8250.825 Hz
FIDRES           0.125898 Hz
AQ               3.9715316 sec
RG                  1149.4
DW                 60.600 usec
DE                 86.57 usec
TE                 294.5 K
D1            1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                   1H
P1                  9.00 usec
PL1                 0.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                  32768
SF           400.1300112 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
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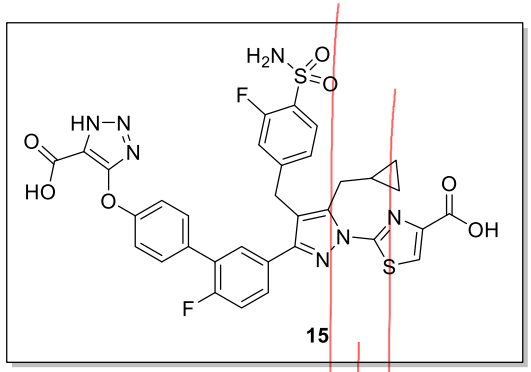
JD0211-087-1HNMR



DMSO

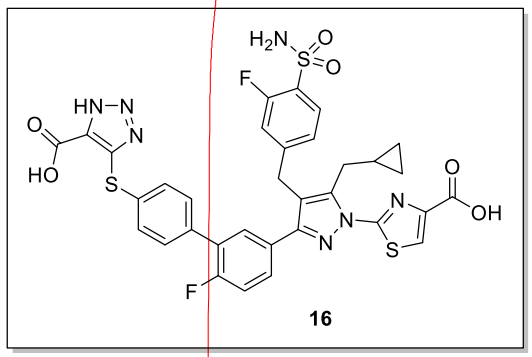
water

```
Current Data Parameters
NAME      JD0211-087-1HNMR
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20190814
Time                 16.28
INSTRUM              spect
PROBHD    5 mm Multinucl
PULPROG               zg30
TD                   65536
SOLVENT               DMSO
NS                       4
DS                       2
SWH               8250.825 Hz
FIDRES            0.125898 Hz
AQ               3.9715316 sec
RG                   574.7
DW                  60.600 usec
DE                  86.57 usec
TE                  297.0 K
D1             1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    1H
P1                   9.00 usec
PL1                  0.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                   32768
SF           400.1300015 MHz
WDW                     EM
SSB                      0
LB                   0.30 Hz
GB                       0
PC                   1.00
```

9 8 7 6 5 4 3 2 1 0 ppm

0.97 1.63 3.35 2.30 0.85 3.08 0.87 2.23 2.96 1.02 1.27 2.06 2.14

JD0211-083-1HNMR



DMSO

water

Current Data Parameters
NAME JD0211-083-1HNMR
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
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Time 10.13
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PROBHD 5 mm Multinucl
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 8250.825 Hz
FIDRES 0.125898 Hz
AQ 3.9715316 sec
RG 1149.4
DW 60.600 usec
DE 86.57 usec
TE 293.8 K
D1 1.00000000 sec
TD0 1

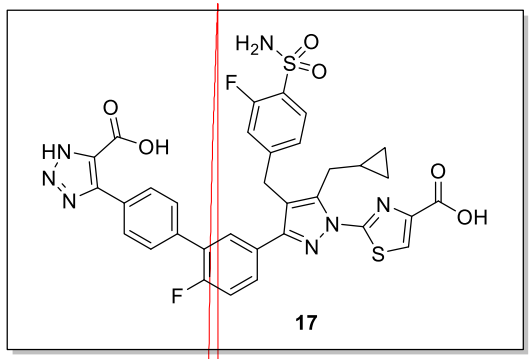
======== CHANNEL f1 ========
NUC1 1H
P1 9.00 usec
PL1 0.00 dB
SFO1 400.1324710 MHz

F2 - Processing parameters
SI 32768
SF 400.1300013 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

8 7 6 5 4 3 2 1 0 ppm

0.94 4.61 1.87 2.83 1.06 1.00 2.04 3.41 1.33 2.05 2.10

JD0211-085-1HNMR




```
Current Data Parameters
NAME      JD0211-085-1HNMR
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20190808
Time                 10.23
INSTRUM              spect
PROBHD    5 mm Multinucl
PULPROG               zg30
TD                   65536
SOLVENT               DMSO
NS                      16
DS                       2
SWH               8250.825 Hz
FIDRES           0.125898 Hz
AQ               3.9715316 sec
RG                  1625.5
DW                  60.600 usec
DE                  86.57 usec
TE                  293.9 K
D1             1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    1H
P1                   9.00 usec
PL1                  0.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                   32768
SF           400.1300112 MHz
WDW                     EM
SSB                      0
LB                   0.30 Hz
GB                       0
PC                    1.00
```