Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Synthesis and SAR of *o*-sulfonamido-arylhydrazide as LL-diaminopimelate aminotransferase (LL-DAP-AT) inhibitors

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Cloning, expression and purification of LL-DAP-AT

The DNA encoding LL-DAP-AT from *Arabidopsis thaliana* fused to a C-terminal histidine-tag (His_6) was obtained from BioBasic Inc. (Ontario, Canada) and the codon usage was optimized for expression in *E. coli*. The cloning, expression and purification of LL-DAP-AT with a C-terminal His_6 has been described previously.[1] The enzyme was stored at -80 °C in 200 mM NaCl, 20 mM Hepes-KOH (pH 7.6), 3 mM DTT.

General Chemistry Methods

All chemicals and solvents used in this study were purchased from Sigma-Aldrich, AB Chem, Inc. and Alfa Aesar. Infrared spectra were obtained using Nicolet Magna 750 FTIR Spectrometer and Nic-Plan FTIR Microscope. Agilent/Varian Inova 400 MHz, Agilent/Varian Mercury 400 MHz, and Agilent/Varian VNMRS 500 MHz two-channel spectrometers and Agilent/Varian Inova 500 MHz four-channel spectrometer were used to acquire ^{1}H and ^{13}C NMR spectra. Chloroform-D, CD_3OD and DMSO-d_6 were used as NMR solvents. Spin multiples are listed as s (singlet), d (double), t (triplet), q (quartet), m (multiplet), and br (broad) and coupling constant (*J*) values were estimated in hertz (Hz). Low-resolution mass spectra were obtained using Agilent Technologies 1100MSD (Single Quadrupole, positive and negative ion ESI, Santa Clara, CA, USA). High-resolution mass spectra were obtained using Applied BioSystems Mariner BioSpectrometry Workstation (orthogonal acceleration Time-of-Flight, positive and negative ion ESI and APCI, Foster City, CA, USA).

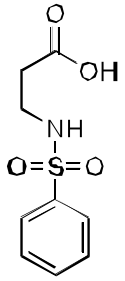
General Procedure for the Preparation of Analogues

Synthesis of sulfonamide - carboxylic acid intermediate:

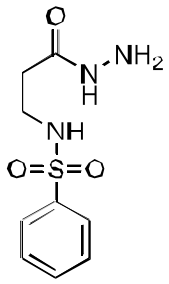
One equivalents of a proper amino acid and 2.5 equivalents of sodium carbonate were mixed with distilled water in a 0.4 M amino acid concentration. The mixture was heated in an oil-bath to ~60 °C. The proper sulfonyl chloride (1.25 equ.) was slowly added into the hot mixture during the course of 15 min. The mixture was then heated to 85 °C for a further 3 h. Norite (~20 mg) may applied if the mixture was in dark red or brown colour. The hot mixture was filtered through a pre-heated funnel. The hot filtrate was slowly poured with vigorous swirling into a 50 mL Erlenmeyer flask, which contained of 6 N hydrochloride acid (1 mL). After solid formation upon cooling, the solid was filtered and washed with of 1 N hydrochloride acid (2 mL). The product was used in next step without any further purification.

Synthesis of sulfonamide – hydrazide analogues:

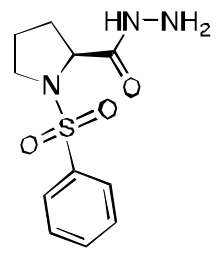
One equivalents of a proper sulfonamide – carboxylic acid intermediate and 1.2 equivalents of carbonyl diimidazole were dissolved in dry DMF (substrate concentration in 0.075 M). The mixture was stirred for 4 hours. Hydrazine monohydrate (2 equ.) was dissolved in dry DMF (0.15 M). The activated sulfonamide – carboxylic acid intermediate was added in the hydrazine solution slowly. The reaction was allowed to stir for 16 h at room temperature. The solvent was then removed in *vacuo*, and the residue was purified by column chromatograph to yield a proper sulfonamido–hydrazide analogue.



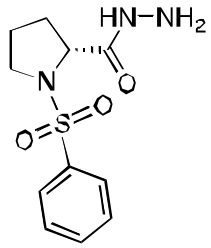
3-(Phenylsulfonamido)propanoic acid (**2a**): white solid; 72% yield; IR (Microscope): 3273, 3066, 3150 – 2830 (br), 1701, 1447, 1435, 1413, 1168 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 7.85 (m, 2H), 7.63 (m, 1H), 7.56 (m, 2H), 3.10 (t, *J* = 7.0 Hz, 2 H), 2.44 (t, *J* = 7.0 Hz, 2 H); ^{13}C NMR (125 MHz, CD_3OD): δ = 174.8, 141.8, 133.7, 130.3, 128.0, 39.9, 35.4; ESI-HRMS *m/z* calcd for $C_9H_{10}NO_4S$: 228.0336 $[M-H]^-$, found: 228.0333.



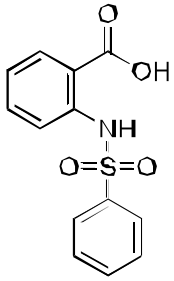
N-(3-Hydrazinyl-3-oxopropyl)benzenesulfonamide (**2**): white solid; 32% yield; IR (KBr pellet): 3417, 3302, 3246, 3194, 3096, 3057, 2914, 2859, 1643, 1534, 1445 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): δ = 7.85 (m, 2H), 7.57 (m, 3H), 3.11 (t, *J* = 6.8 Hz, 2H), 2.33 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ = 171.1, 140.3, 132.3, 128.8, 126.6, 39.0, 34.0; ESI-HRMS *m/z* calcd for $C_9H_{13}N_3NaO_3S$: 266.0568 $[M+Na]^+$, found: 266.0570.



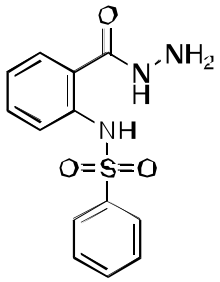
(*S*)-1-(Phenylsulfonyl)pyrrolidine-2-carbohydrazide (**3**): white solid; 25% yield over two steps; IR (KBr pellet): 3600 – 3120 (br), 3061, 2953, 2926, 2874, 1659, 1512, 1479, 1446 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): δ = 7.85 (m, 2H), 7.66 (m, 3H), 4.10 (dd, *J* = 4.0, 8.4 Hz, 1H), 3.55 (m, 1H), 3.23 (td, *J* = 7.2, 10.0 Hz, 1H), 1.84 (m, 3H), 1.56 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 173.0, 137.6, 134.2, 130.2, 128.5, 62.1, 50.3, 31.4, 25.1; ESI-HRMS *m/z* calcd for $C_{11}H_{16}N_3O_3S$: 270.0907 $[M+H]^+$, found: 270.0908; $[\alpha]_D^{23}$ = -130.23 (c 0.19, CH_3OH).



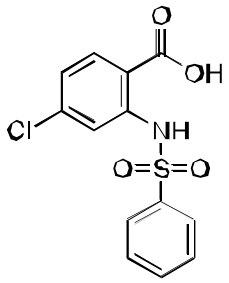
(*R*)-1-(Phenylsulfonyl)pyrrolidine-2-carbohydrazide (**4**): white solid; 28 % yield over two steps; IR (KBr pellet): 3600 – 3050 (br), 3061, 2976, 2921, 2874, 1660, 1515, 1480, 1446 cm^{-1}; 1H NMR (500 MHz, CD_3OD): δ = 7.88 (m, 2H), 7.68 (m, 1H), 7.60 (m, 2H), 4.11 (dd, *J* = 4.0, 9.0), 3.55 (ddd, *J* = 4.5, 7.0, 10.0 Hz, 1H), 3.23 (td, *J* = 7.0, 10.0 Hz, 1H), 1.84 (m, 3H), 1.54 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ = 173.4, 137.9, 134.6, 130.6, 128.9, 62.6, 50.7, 32.0, 25.5; ESI-HRMS *m/z* calcd for $C_{11}H_{15}N_3NaO_3S$: 292.0726 $[M+Na]^+$, found: 292.0726; $[\alpha]_D^{23}$ = 144.46 (c 0.165, CH_3OH).



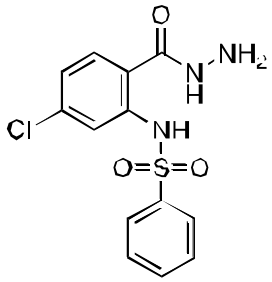
2-(Phenylsulfonamido)benzoic acid (**5a**): light brown solid; 68 % yield; IR (microscope): 3179, 3100, 3200 – 2850 (br), 2885, 1680, 1600, 1582, 1492, 1448, 1431 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.87 (m, 1H), 7.80 (m, 2H), 7.63 (m, 1H), 7.53 (m, 4H), 7.10 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 170.4, 140.5, 139.3, 135.2, 134.3, 132.2, 130.2, 127.5, 124.1, 119.2, 117.5; ESI-HRMS *m/z* calcd for $C_{13}H_{11}NO_4S$: 276.0336 $[M-H]^-$, found: 276.0336.



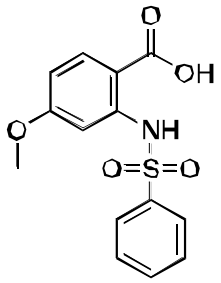
N-(2-(Hydrazinecarbonyl)phenyl)benzenesulfonamide (**5**): white solid; 76 % yield; IR (CH_2Cl_2 cast): 3327, 3400 – 2900 (br), 3064, 1631, 1598, 1518, 1494, 1447 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.78 (m, 2H), 7.65 (m, 1H), 7.51 (m, 1H), 7.39 (m, 4H), 7.07 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.2, 139.5, 138.6, 133.2, 129.3, 129.2, 127.4, 126.9, 124.3, 122.0, 120.5; ESI-HRMS *m/z* calcd for $C_{13}H_{13}N_3NaO_3S$: 314.0570 $[M+Na]^+$, found: 314.0571.



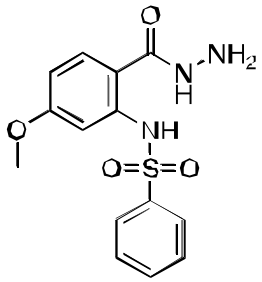
4-Chloro-2-(phenylsulfonamido)benzoic acid (**6a**): light brown solid; 85 % yield; IR (microscope): 3179, 3106, 3060, 3200 – 2700 (br),1672, 1597, 1566, 1488, 1449, 1433 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.88 (d, J = 8.5 Hz, 1H), 7.82 (m, 2H), 7.66 (m, 1H), 7.58 (m, 2H), 7.45 (d, J = 1.5 Hz, 1H), 7.16 (dd, J = 1.5, 8.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 168.9, 141.3, 138.6, 138.5, 133.7, 133.2, 129.6, 126.7, 123.1, 117.7, 116.0; ESI-HRMS *m/z* calcd for $C_{13}H_9ClNO_4S$: 309.9946 $[M-H]^-$, found: 309.9945.



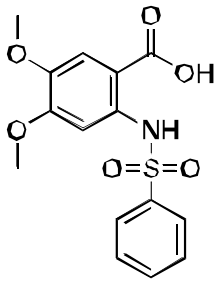
N-(5-Chloro-2-(hydrazinecarbonyl)phenyl)benzenesulfonamide (**6**): light yellow solid; 58 % yield; IR (CH_2Cl_2 cast): 3328, 3400 – 2900 (br), 3066, 1633, 1593, 1520, 1492, 1447 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 7.75 (m, 2H), 7.60 (m, 1H), 7.56 (m, 1H), 7.46 (m, 3H), 7.08 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ = 168.6, 140.8, 140.2, 139.1, 134.5, 130.3, 130.2, 128.3, 125.2, 122.3, 121.0; ESI-HRMS *m/z* calcd for $C_{13}H_{12}ClN_3NaO_3S$: 348.0180 $[M+Na]^+$, found: 348.0180.



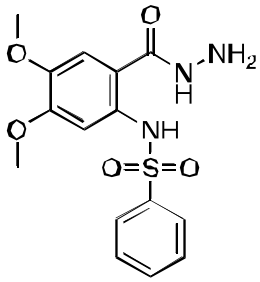
4-Methoxy-2-(phenylsulfonamido)benzoic acid (**7a**): light grey solid; 88 % yield; IR (microscope): 3170, 2975, 3200 – 2700 (br), 1637, 1615, 1570, 1510, 1441 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.83 (m, 3H), 7.65 (m, 1H), 7.57 (m, 2H), 6.97 (d, J = 2.5 Hz, 1H), 7.67 (dd, J = 2.5, 9.0 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 169.7, 163.6, 141.8, 138.4, 133.7, 133.5, 129.6, 126.8, 108.9, 108.6, 103.1, 55.6; ESI-HRMS m/z calcd for $C_{14}H_{12}NO_5S$: 306.0442 $[M-H]^-$, found: 306.0439.



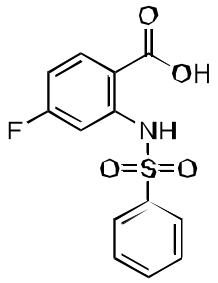
N-(2-(Hydrazinecarbonyl)-5-methoxyphenyl)benzenesulfonamide (**7**): white solid; 74 % yield; IR (CH_2Cl_2 cast): 3328, 3400 – 2900 (br), 3065, 3009, 2968, 2843, 1611, 1578, 1503, 1464, 1447 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 7.75 (m, 2H), 7.53 (m, 1H), 7.45 (m, 3H), 7.13 (d, J = 2.5 Hz, 1H), 6.60 (dd, J = 2.5, 9.0 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ = 169.7, 164.1, 141.6, 140.4, 134.3, 130.3, 130.2, 128.4, 114.0, 110.7, 107.2, 56.1; ESI-HRMS m/z calcd for $C_{14}H_{15}N_3NaO_4S$: 344.0675 $[M+Na]^+$, found: 344.0680.



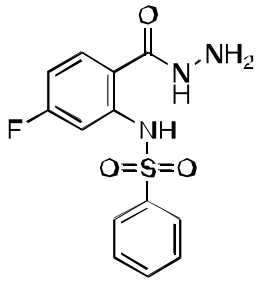
4,5-Dimethoxy-2-(phenylsulfonamido)benzoic acid (**8a**): grey solid; 70 % yield; IR (microscope): 3160, 3078, 3030, 2976, 2941, 2843, 3300 – 2700 (br), 1662, 1610, 1587, 1520, 1448, 1417 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.74 (m, 2H), 7.61 (m, 1H), 7.52 (m, 2H), 7.26 (s, 1H), 7.11 (s, 1H), 3.79 (s, 3H), 3.68 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.9, 153.8, 145.0, 138.8, 135.4, 134.0, 129.9, 127.3, 113.2, 109.2, 103.2, 56.2, 56.0; ESI-HRMS *m/z* calcd for $C_{15}H_{14}NO_6S$: 336.0547 $[M-H]^-$, found: 336.0549.



N-(2-(Hydrazinecarbonyl)-4,5-dimethoxyphenyl)benzenesulfonamide (**8**): white solid; 37 % yield; IR ($CHCl_3$ cast): 3335, 3400 – 3250 (br), 3022, 2917, 2849, 1606, 1515, 1465, 1448 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.73 (m, 2H), 7.52 (m, 1H), 7.41 (m, 2H), 7.25 (s, 1H), 6.77 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 168.8, 152.6, 145.8, 139.0, 133.1, 132.9, 128.8, 127.3, 112.9, 108.7, 106.5, 56.3, 56.2; ESI-HRMS *m/z* calcd for $C_{15}H_{17}N_3NaO_5S$: 374.0781 $[M+Na]^+$, found: 374.0781.

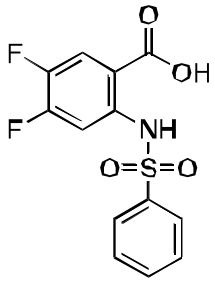


4-Fluoro-2-(phenylsulfonamido)benzoic acid (**9a**): light yellow solid; 76 % yield; IR (microscope): 3490, 3101, 3300 – 2750 (br), 1670, 1613, 1592, 1506, 1448, 1430 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.97 (dd, *J* = 6.5, 9.0 Hz, 1H), 7.86 (m, 2H), 7.67 (m, 1H), 7.58 (m, 2H), 7.24 (dd, *J* = 2.5, 11.0 Hz, 1H), 6.97 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.5, 165.4 (d, *J* = 250.4 Hz), 142.5 (d, *J* = 11.9 Hz), 138.7, 134.9 (d, *J* = 10.9 Hz), 134.3, 130.2, 127.3, 113.6 (d, *J* = 2.9 Hz), 111.0 (d, *J* = 21.6 Hz), 105.4 (d, *J* = 27.0 Hz); ^{19}F NMR (380 MHz, DMSO-d_6): δ = -102.2 (ddd, *J* = 7.2, 7.2, 12.1 Hz); ESI-HRMS *m/z* calcd for $C_{13}H_9FNO_4S$: 294.0242 $[M-H]^-$, found: 294.0245.

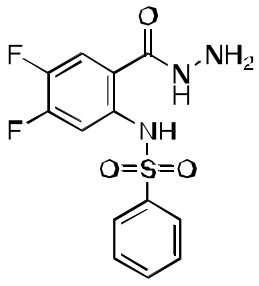


N-(5-Fluoro-2-(hydrazinecarbonyl)phenyl)benzenesulfonamide (**9**): light yellow solid; 58 % yield; IR (CH_2Cl_2 cast): 3334, 3400 – 3250 (br), 3093, 3027, 1631, 1596, 1502, 1448, 1423 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.85 (m, 2H), 7.56 (m, 1H), 7.46 (m, 2H), 7.41 (m, 2H), 6.75 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 168.5, 165.0 (d, *J* = 252.3 Hz), 141.2 (d, *J* = 11.8 Hz), 139.1, 133.3, 129.2, 128.7 (d, *J* = 10.3 Hz), 127.2, 115.2 (d, *J* = 3.0 Hz), 110.7 (d, *J* = 22.4 Hz), 107.8 (d, *J* = 26.4 Hz); ^{19}F NMR (380 MHz, $CDCCl_3$):

δ = -103.0 (ddd, *J* = 7.6, 7.6, 11.0 Hz); ESI-HRMS *m/z* calcd for $C_{13}H_{12}FN_3NaO_3S$: 332.0476 $[M+Na]^+$, found: 332.0475.

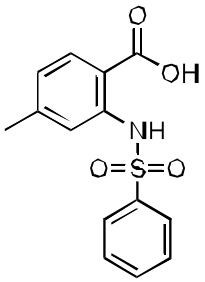


4,5-Difluoro-2-(phenylsulfonamido)benzoic acid (**10a**): light brown solid; 89 % yield; ; IR (microscope): 3161, 3087, 3400 – 2750 (br), 1686, 1604, 1522, 1481, 1449, 1401 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.84 (dd, *J* = 9.0, 11.0 Hz, 1H), 7.80 (m, 2H), 7.64 (m, 1H), 7.55 (m, 2H), 7.45 (dd, *J* = 7.0, 12.5 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 168.4 (d, *J* = 1.0 Hz), 153.0 (dd, *J* = 13.6, 252.0 Hz), 145.6 (dd, *J* = 12.9, 243.0 Hz), 138.6, 137.7 (dd, *J* = 2.5, 9.7 Hz), 134.3, 130.1, 127.3, 120.5 (dd, *J* = 2.1, 19.2 Hz), 115.1 (dd, *J* = 3.3, 4.7 Hz), 108.8 (d, *J* = 21.9 Hz); ^{19}F NMR (380 MHz, DMSO-d_6): δ = -127.4 (ddd, *J* = 9.9, 12.2, 23.2 Hz), -142.9 (ddd, *J* = 7.2, 11.0, 23.2 Hz); ESI-HRMS *m/z* calcd for $C_{13}H_8F_2NO_4S$: 312.0148 $[M-H]^-$, found: 312.0151.

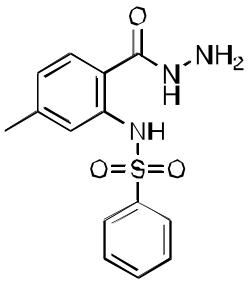


N-(4,5-Difluoro-2-(hydrazinecarbonyl)phenyl)benzenesulfonamide (**10**): light yellow solid; 48 % yield; IR (microscope): 3345, 3400 – 3250 (br), 3065, 1606, 1509,1448, 1392

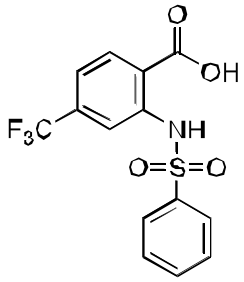
cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.80 (m, 2H), 7.58 (m, 2H), 7.47 (m, 2H), 7.22 (dd, *J* = 8.5, 10.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 167.4, 152.6 (dd, *J* = 13.1, 254.6 Hz), 146.4 (dd, *J* = 13.0, 247.3 Hz), 138.8, 135.9 (dd, *J* = 2.8, 9.3 Hz), 133.4, 129.2, 127.2, 116.4, 115.5 (d, *J* = 17.8 Hz), 111.5 (d, *J* = 21.5 Hz); ^{19}F NMR (380 MHz, $CDCl_3$): δ = -127.3 (ddd, *J* = 9.5, 11.8, 20.7 Hz), -140.7 (ddd, *J* = 7.6, 9.9, 22.8 Hz); ESI-HRMS *m/z* calcd for $C_{13}H_{11}F_2N_3NaO_3S$: 350.0382 $[M+Na]^+$, found: 350.0381.



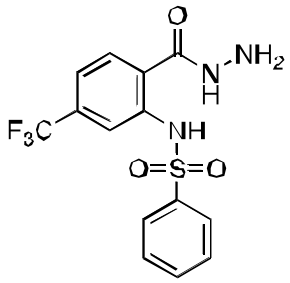
4-Methyl-2-(phenylsulfonamido)benzoic acid (**11a**): light grey solid; 93 % yield; IR ($CHCl_3$ cast): 3178, 3071, 3100 – 2750 (br), 2871, 1644, 1568, 1479, 1447 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.79 (m, 2H), 7.75 (d, *J* = 8.0 Hz, 1H), 7.62 (m, 1H), 7.54 (m, 2H), 7.33 (m, 1H), 6.92 (m, 1H), 2.28 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 170.2, 145.6, 140.3, 139.0, 134.0, 131.9, 130.0, 127.3, 124.7, 119.2, 114.4, 21.9; ESI-HRMS *m/z* calcd for $C_{14}H_{13}NNaO_4S$: 314.0457 $[M+Na]^+$, found: 314.0459.



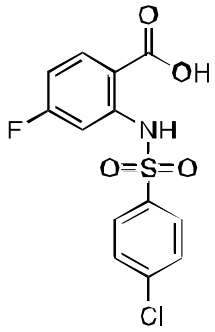
N-(2-(Hydrazinecarbonyl)-5-methylphenyl)benzenesulfonamide (**11**): light yellow solid; 55 % yield; IR ($CHCl_3$ cast): 3328, 3400 – 3250 (br), 3059, 3028, 2918, 1630, 1523, 1447 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ = 7.71 (d, *J* = 7.5 Hz, 2H), 7.54 (m, 1H), 7.43 (m, 3H), 7.37 (d, *J* = 8.0 Hz, 1H), 6.90 (d, *J* = 8.0 Hz, 1H), 2.30 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ = 169.6, 144.4, 140.6, 139.7, 134.1, 130.1, 128.7, 128.3, 125.9, 123.4, 120.1, 21.6; ESI-HRMS *m/z* calcd for $C_{14}H_{15}N_3NaO_3S$: 328.0726 $[M+Na]^+$, found: 328.0722.



2-(Phenylsulfonamido)-4-(trifluoromethyl)benzoic acid (**12a**): light orange solid; 94 % yield; IR ($CHCl_3$ cast): 3184, 3300 – 2750 (br), 3105, 3066, 1683, 1584, 1540, 1511, 1449, 1420 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 8.05 (d, *J* = 8.0 Hz, 1H), 7.79 (m, 2H), 7.67 (d, *J* = 1.5 Hz, 1H), 7.62 (m, 1H), 7.55 (m, 2H), 7.39 (dd, *J* = 1.5, 8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 168.8, 141.3, 139.3, 133.5 (d, *J* = 84.4 Hz), 133.5 (d, *J* = 32.0 Hz), 130.0, 127.7, 127.2, 125.0, 122.2 (d, *J* = 9.2), 119.6 (d, *J* = 3.1 Hz), 115.2 (m); ^{19}F NMR (380 MHz, DMSO-d_6): δ = -62.3; ESI-HRMS *m/z* calcd for $C_{14}H_9F_3NO_4S$: 344.0210 $[M-H]^-$, found: 344.0240.

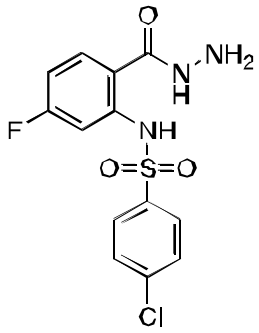


N-(2-(Hydrazinecarbonyl)-5-(trifluoromethyl)phenyl)benzenesulfonamide (**12**): light yellow solid; 36 % yield; IR ($CHCl_3$ cast): 3331, 3400 – 3250 (br), 3069, 1647, 1610, 1521, 1449, 1422 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ = 7.84 (d, *J* = 1.5 Hz, 1H), 7.74 (m, 2H), 7.64 (d, *J* = 8.5 Hz, 1H), 7.57 (m, 1H), 7.47 (m, 2H), 7.37 (dd, *J* = 1.5, 8.5, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ = 168.1, 139.9 (d, *J* = 23 Hz), 134.6 (q, *J* = 32.8 Hz), 134.6, 130.4, 129.9, 128.3, 126.4, 125.8, 123.6, 121.7 (q, *J* = 3.5 Hz), 119.4 (q, *J* = 3.8 Hz); ^{19}F NMR (380 MHz, CD_3OD): δ = -65.0; ESI-HRMS *m/z* calcd for $C_{14}H_{12}F_3N_3NaO_3S$: 382.0444 $[M+Na]^+$, found: 382.0440.

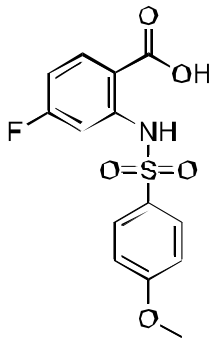


2-(4-Chlorophenylsulfonamido)-4-fluorobenzoic acid (**13a**): yellow solid; 37 % yield; IR ($CHCl_3$ cast): 3099, 3300 – 2750 (br), 1662, 1608, 1588, 1501, 1476, 1443, 1424 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.98 (dd, *J* = 6.5, 9.0 Hz, 1H), 7.87 (m, 2H), 7.66 (m, 2H), 7.23 (dd, *J* = 3.0, 11.0 Hz, 1H), 7.00 (ddd, *J* = 3.0, 8.5, 9.0 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 168.9, 164.9 (d, *J* = 250.8 Hz), 141.7 (d, *J* = 11.8 Hz), 138.7,

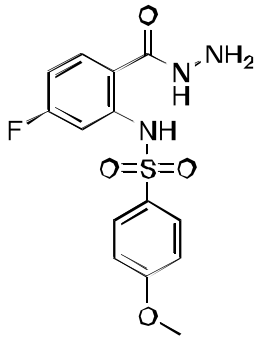
137.2, 134.5 (d, J = 11.0 Hz), 129.8 (d, J = 8.0 Hz), 128.8, 113.6 (d, J = 2.3 Hz), 110.7 (d, J = 21.3 Hz), 105.2 (d, J = 27.0 Hz); ^{19}F NMR (380 MHz, DMSO-d_6): δ = -102.2 (q, J = 8.7 Hz); ESI-HRMS *m/z* calcd for $C_{13}H_8ClFNO_4S$: 327.9852 $[M-H]^-$, found: 327.9849.



4-Chloro-*N*-(5-fluoro-2-(hydrazinecarbonyl)phenyl)benzenesulfonamide (**13**): light yellow solid; 40 % yield; IR ($CHCl_3$ cast): 3327, 3400 – 3250 (br), 3093, 2924, 1639, 1594, 1505, 1478, 1423 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 7.74 (m, 2H), 7.55 (dd, J = 6.0, 9.0 Hz, 1H), 7.49 (m, 2H), 7.35 (dd, J = 3.0, 11.0 Hz, 1H), 6.84 (ddd, J = 2.5, 8.0, 8.5 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ = 168.7, 165.9 (d, J = 250.3 Hz), 141.7 (d, J = 11.4 Hz), 140.8, 139.0, 131.2 (d, J = 10.3 Hz), 130.5 (d, J = 3.9 Hz), 130.0, 118.6 (d, J = 3.1 Hz), 112.1 (d, J = 21.9 Hz), 109.3 (d, J = 26.5 Hz); ^{19}F NMR (380 MHz, CD_3OD): δ = -107.2 (ddd, J = 7.6, 7.6, 9.9 Hz); ESI-HRMS *m/z* calcd for $C_{13}H_{11}ClFN_3NaO_3S$: 366.0086 $[M+Na]^+$, found: 366.0086.

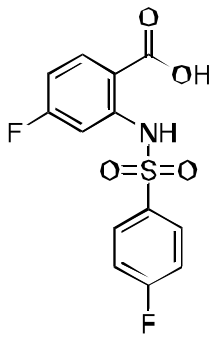


4-Fluoro-2-(4-methoxyphenylsulfonamido)benzoic acid (**14a**): yellow solid; 63 % yield; IR ($CHCl_3$ cast): 3099, 3300 – 2750 (br), 2954, 2923, 2852, 1690, 1672, 1612, 1595, 1500, 1462, 1429 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 7.97 (dd, J = 6.5, 9.0 Hz, 1H), 7.79 (m, 2H), 7.23 (dd, J = 2.5, 11.0 Hz, 1H), 7.09 (m, 2H), 6.96 (ddd, J = 2.5, 8.0, 9.0 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ = 169.0, 164.9 (d, J = 250.4 Hz), 163.1, 142.3 (d, J = 12.0 Hz), 134.4 (d, J = 11.1 Hz), 129.6, 129.2, 114.8, 112.9, 110.2 (d, J = 21.8 Hz), 104.6 (d, J = 27.0 Hz), 55.7; ^{19}F NMR (380 MHz, DMSO-d_6): δ = -102.2 (ddd, J = 7.6, 7.6, 11.0 Hz); ESI-HRMS *m/z* calcd for $C_{14}H_{11}FNO_5S$: 324.0347 $[M-H]^-$, found: 324.0346.

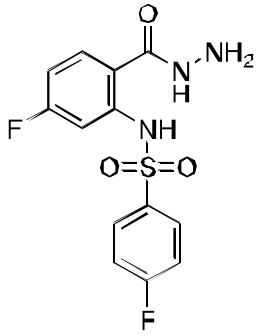


N-(5-Fluoro-2-(hydrazinecarbonyl)phenyl)-4-methoxybenzenesulfonamide (**14**): light yellow solid; 58 % yield; IR ($CHCl_3$ cast): 3330, 3400 – 3250 (br), 3099, 3022, 2973, 2946, 2843, 1633, 1596, 1499, 1463, 1440, 1422 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ =

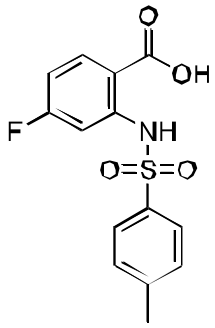
7.79 (m, 2H), 7.54 (dd, *J* = 6.0, 8.8 Hz, 1H), 7.33 (dd, *J* = 2.8, 10.8 Hz, 1H), 6.96 (m, 2H), 6.81 (ddd, *J* = 2.4, 8.0, 8.8 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 167.5, 164.5 (d, *J* = 249.0 Hz), 163.6, 140.7 (d, *J* = 11.5 Hz), 130.1, 129.7 (d, *J* = 10.1 Hz), 129.1, 116.9 (d, *J* = 3.1 Hz), 114.0, 110.3 (d, *J* = 22.3 Hz), 107.6 (d, *J* = 26.5 Hz); ^{19}F NMR (380 MHz, CD_3OD): δ = -107.6 (ddd, *J* = 7.6, 7.6, 11.4 Hz); ESI-HRMS *m/z* calcd for $C_{14}H_{14}FN_3NaO_4S$: 362.0581 $[M+Na]^+$, found: 362.0578.



4-Fluoro-2-(4-fluorophenylsulfonamido)benzoic acid (**15a**): yellow solid; 53 % yield; IR (microscope): 3169, 3106, 3071, 3300 – 2750 (br), 1653, 1611, 1592, 1508, 1498, 1445, 1422 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD): δ = 8.02 (dd, *J* = 6.6, 9.0 Hz, 1H), 7.89 (m, 2H), 7.39 (dd, *J* = 2.4, 10.8 Hz, 1H), 7.25 (m, 2H), 6.84 (ddd, *J* = 2.4, 8.4, 9.0 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ = 170.6, 167.2 (d, *J* = 251.8 Hz), 166.9 (d, *J* = 253.0 Hz), 144.0 (d, *J* = 12.0 Hz), 136.4 (d, *J* = 3.0 Hz), 135.6 (d, *J* = 10.8 Hz), 131.4 (d, *J* = 9.8 Hz), 117.5 (d, *J* = 23.0 Hz), 114.4 (d, *J* = 2.9 Hz), 111.6 (d, *J* = 22.3 Hz), 107.0 (d, *J* = 27.4 Hz); ^{19}F NMR (380 MHz, CD_3OD): δ = -103.9 (ddd, *J* = 7.2, 7.2, 11.2 Hz), -106.4 (ddd, *J* = 4.9, 8.7, 13.7 Hz); ESI-HRMS *m/z* calcd for $C_{13}H_8F_2NO_4S$: 312.0148 $[M-H]^-$, found: 312.0153.

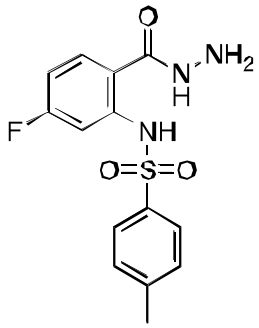


4-Fluoro-*N*-(5-fluoro-2-(hydrazinecarbonyl)phenyl)benzenesulfonamide (**15**): white solid; 43 % yield; IR ($CHCl_3$ cast): 3331, 3400 – 3250 (br), 3106, 1637, 1593, 1496, 1423 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ = 7.83 (m, 2H), 7.56 (dd, *J* = 8.0, 11.0 Hz, 1H), 7.36 (dd, *J* = 3.5, 13.5 Hz, 1H), 7.22 (m, 2H), 6.85 (ddd, *J* = 3.0, 10.0, 11.0 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ = 168.7, 166.8 (d, *J* = 252.5 Hz), 165.8 (d, *J* = 249.4 Hz), 141.8 (d, *J* = 11.4 Hz), 136.4 (d, *J* = 3.0 Hz), 131.3 (d, *J* = 9.8 Hz), 131.2 (d, *J* = 10.3 Hz), 118.7 (d, *J* = 2.6 Hz), 117.4 (d, *J* = 23.0 Hz), 112.1 (d, *J* = 22.0 Hz), 109.4 (d, *J* = 26.4 Hz); ^{19}F NMR (380 MHz, CD_3OD): δ = -106.7 (ddd, *J* = 4.9, 8.4, 13.3 Hz), -107.3 (ddd, *J* = 7.2, 7.2, 11.0 Hz); ESI-HRMS *m/z* calcd for $C_{13}H_{12}F_2N_3O_3S$: 328.0562 $[M+H]^+$, found: 328.0561.

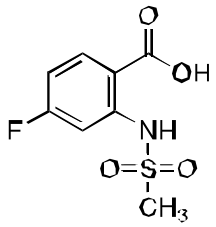


4-Fluoro-2-(4-methylphenylsulfonamido)benzoic acid (**16a**): yellow solid; 34 % yield; IR (microscope): 3192, 3103, 3300 – 2750 (br), 2925, 2864, 1681, 1611, 1593, 1504, 1436,

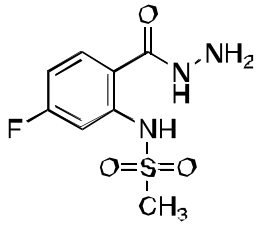
1397 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 8.00 (dd, *J* = 6.5, 9.0 Hz, 1H), 7.71 (d, *J* = 8.5 Hz, 2H), 7.37 (dd, *J* = 2.5, 11.0 Hz, 1H), 7.31 (d, *J* = 8.5 Hz, 2H), 6.80 (ddd, *J* = 2.5, 8.0, 9.0 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ = 170.6, 167.2 (d, *J* = 251.4 Hz), 146.0, 144.3 (d, *J* = 12.1 Hz), 137.3, 135.6 (d, *J* = 10.8 Hz), 130.9, 128.4, 114.0 (d, *J* = 2.8 Hz), 111.2 (d, *J* = 22.3 Hz), 106.7 (d, *J* = 27.4 Hz), 21.5; ^{19}F NMR (380 MHz, CD_3OD): δ = -104.2 (ddd, *J* = 7.2, 7.2, 12.2 Hz); ESI-HRMS *m/z* calcd for $C_{14}H_{11}FNO_4S$: 308.0398 $[M-H]^-$, found: 308.0404.



N-(5-Fluoro-2-(hydrazinecarbonyl)phenyl)-4-methylbenzenesulfonamide (**16**): yellow solid; 77 % yield; IR ($CHCl_3$ cast): 3329, 3400 – 3250 (br), 3094, 2926, 1636, 1596, 1503, 1423 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 7.64 (m, 2H), 7.54 (dd, *J* = 6.5, 9.0 Hz, 1H), 7.34 (dd, *J* = 2.5, 11.0 Hz, 1H), 7.28 (m, 2H), 6.81 (ddd, *J* = 2.5, 8.0, 9.0 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ = 168.8, 165.8 (d, *J* = 250.0 Hz), 145.8, 142.1 (d, *J* = 11.4 Hz), 137.3, 131.1 (d, *J* = 10.3 Hz), 130.8, 128.3, 118.2 (d, *J* = 2.9 Hz), 111.7 (d, *J* = 22.3 Hz), 109.0 (d, *J* = 26.5 Hz), 21.5; ^{19}F NMR (380 MHz, CD_3OD): δ = -107.4 (ddd, *J* = 7.2, 7.2, 9.9 Hz); ESI-HRMS *m/z* calcd for $C_{14}H_{15}FN_3O_3S$: 324.0813 $[M+H]^+$, found: 324.0813.

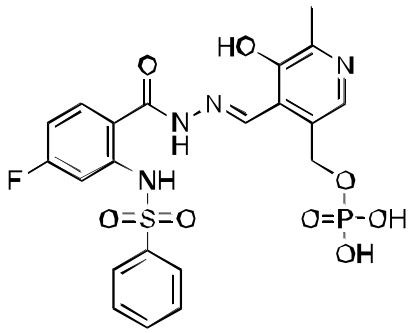


4-Fluoro-2-(methylsulfonamido)benzoic acid (**17a**): grey solid; 41 % yield; IR ($CHCl_3$ cast): 3220, 3109, 3300 – 2750 (br), 2931, 2851, 1660, 1623, 1588, 1506, 1430, 1416 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ = 8.16 (dd, *J* = 6.5, 9.0 Hz, 1H), 7.44 (dd, *J* = 2.5, 11.0 Hz, 1H), 6.89 (ddd, *J* = 2.4, 8.0, 8.8 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ = 170.6, 167.5 (d, *J* = 251.1 Hz), 144.7 (d, *J* = 12.0 Hz), 135.8 (d, *J* = 11.0 Hz), 113.5 (d, *J* = 2.5 Hz), 110.8 (d, *J* = 22.4 Hz), 105.8 (d, *J* = 27.8 Hz), 40.2; ^{19}F NMR (380 MHz, CD_3OD): δ = -103.9 (ddd, *J* = 7.6, 7.6, 11.4 Hz); ESI-HRMS *m/z* calcd for $C_8H_7FNO_4S$: 232.0085 $[M-H]^-$, found: 232.0085.



N-(5-Fluoro-2-(hydrazinecarbonyl)phenyl)methanesulfonamide (**17**): white solid; 88 % yield; IR ($CHCl_3$ cast): 3300, 3400 – 3250 (br), 3093, 3026, 2932, 1641, 1595, 1506, 1426 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ = 7.71 (dd, *J* = 6.0, 8.5 Hz, 1H), 7.41 (dd, *J* = 2.5, 11.0 Hz, 1H), 6.90 (ddd, *J* = 2.5, 8.0, 9.0 Hz, 1H), 3.06 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ = 169.1, 166.2 (d, *J* = 248.9 Hz), 142.5 (d, *J* = 11.3 Hz), 131.4 (d, *J* = 10.3 Hz), 117.5 (d, *J* = 3.1 Hz), 111.2 (d, *J* = 22.3 Hz), 107.6 (d, *J* = 27.1 Hz), 40.0; ^{19}F

NMR (380 MHz, CD_3OD): δ = -107.2 (ddd, *J* = 7.2, 7.2, 11.0 Hz); ESI-HRMS *m/z* calcd for $C_8H_{10}FN_3NaO_3S$: 270.0319 $[M+Na]^+$, found: 270.0319.



(*E*)-(4-((2-(4-Fluoro-2-(phenylsulfonamido)benzoyl)hydrazono)methyl)-5-hydroxy-6-methylpyridin-3-yl)methyl dihydrogen phosphate (**19**): Compound **9** (7.1 mg, 2.3×10^{-5} mol) and PLP (5.7 mg, 2.3×10^{-5} mol) were mixed in 1 mL of CD_3OD and 1 mL of D_2O. The yellow solution was analyzed by ^1H and ^{13}C NMR. The mixture was dried in *vacuo*, then IR and HRMS data of solid material were collected. Yellow solid; IR ($CHCl_3$ cast): 3500 – 2500 (br), 3062, 2918, 1651, 1605, 1511, 1480, 1447, 1427 cm^{-1}; ^1H NMR (500 MHz, D_2O): δ = 8.54 (s, 1H), 7.95 (s, 1H), 7.71 (m, 3H), 7.56 (m, 1H), 7.49 (m, 2H), 7.14 (m, 1H), 6.91 (m, 1H), 5.00 (d, *J* = 5 Hz, 2H), 2.47 (s, 3H); ^{13}C NMR (125 MHz, D_2O): δ = 166.8, 165.7, 164.8, 153.5 (d, *J* = 4.9 Hz), 149.1, 147.7, 142.2 (m), 139.6, 136.6 (m), 134.3, 132.5 (d, *J* = 10.8 Hz), 131.6 (d, *J* = 6.1 Hz), 130.4, 127.5 (d, *J* = 5.1 Hz), 123.4 (d, *J* = 2.8 Hz), 120.7 (d, *J* = 3.1 Hz), 112.4 (m), 62.9 (d, *J* = 3.0 Hz), 18.1; ^{19}F NMR (380 MHz, D_2O): δ = -105.0 (m); ESI-HRMS *m/z* calcd for $C_{21}H_{19}FN_4O_8PS$: 537.0651 $[M-H]^-$, found: 537.0652.

Determination of IC_{50}

Aliquot buffer solution: 420 mg of 2-oxoglutarate and 480 mg of racemic DAP (commercial) were dissolved in 280 mL of 100 mM HEPES-KOH, pH 7.6. 9 mL of this solution was used to dissolve 9 mg of 2-aminobenzaldehyde (OAB) and 850 μL of this stock was added into 8 assay cells (1mL). The final concentration of each component in the stock solution is: 2-OG (10 mM); OAB (8.3 mM); LL-DAP (assuming 25% of total DAP: 2.3 mM). Upon dilution to 1 mL in the assay cells, the final concentrations are: 2-OG (8.5 mM); OAB (7 mM); LL-DAP (2.0 mM).

Preparation of inhibitor working stock: 4μmol of inhibitor was dissolved in 1 mL of DMSO, which resulted 4 μmol/mL in concentration. The serial dilution gave 2 μmol/mL, 1 μmol/mL, 0.5 μmol/mL, 0.25 μmol/mL, 0.125 μmol/mL, 0.0625 μmol/mL, and 0.03125 μmol/mL solutions. In the final assay cell, the inhibitor concentrations were 100 μM, 50 μM, 25 μM, 12.5 μM, 6.25 μM, 3.125 μM and 1.5625 μM, respectively.

Addition of inhibitor: 50 μL of each concentration of inhibitor solutions was added into cell 2 to 8. And 50 μL of pure DMSO was added into cell 1 and 9, which are control and negative control. The Varian Cary 100 Bio UV-Visible spectrophotometer was used to measure the absorbance at 440 nm. Under the program of Enzyme Kinetics the absorbance for all the cells were then multi-zeroed.

Addition of enzyme: A 0.043 mg/mL solution of LL-DAP-AT in 100 mM HEPES-KOH pH 7.6 was prepared and 100 uL of this was added to each cell, except the negative

control (cell 9). The final concentration of the enzyme was 0.0043 mg/mL. The absorbance was recorded for 120 min with 20 sec/cycle. The initial rate of increase of the absorbance is proportional to the rate of reaction. By comparing the initial slope of each absorbance curve of the inhibitor assay cell to the control cell, the percentage inhibition was determined and the IC_{50} value was calculated based on this data.

Reference

1. N. Watanabe, M. M. Cherney, M. J. van Belkum, S. L. Marcus, M. D. Flegel, M. D. Clay, M. K. Deyholos, J. C. Vederas and M. N. James, *J Mol Biol*, 2007, **371**, 685.