Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supplementary Material for:

Achieving Improved Permeability by Hydrogen Bond Donor Modulation in a Series of MGAT2 Inhibitors

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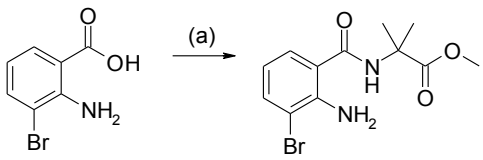
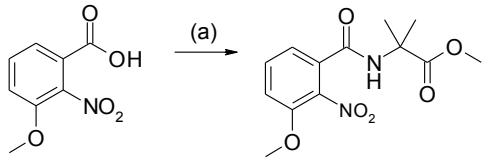
General experimental details: All solvents and chemicals used were reagent grade. Flash column chromatography was carried out using prepacked silica cartridges (from 4 g up to 330 g) from Redisep, Biotage, or Crawford and eluted using an Isco Companion system. Purity and characterization of compounds were established by a combination of liquid chromatography-mass spectroscopy (LC-MS) and NMR analytical techniques and was >95% for all compounds. ^{1}H NMR were recorded on a Bruker Avance DPX400 (400 MHz) and were determined in $CDCl_3$ or DMSO-d_6. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) (0.00 ppm) or solvent peaks as the internal reference and coupling constant (J) values are reported in Hertz (Hz). Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Merck precoated thin layer chromatography (TLC) plates (silica gel 60 F_{254}, 0.25 mm, art. 5715) were used for TLC analysis. Solutions were dried over anhydrous magnesium sulphate, and solvent was removed by rotary evaporation under reduced pressure.

Anthranilic acid coupling (Scheme 1, step a)



To a stirred solution of 2-amino-3-bromobenzoic acid (5.00 g, 23.14 mmol), methyl 2-amino-2-methylpropanoate hydrochloride (3.91 g, 25.46 mmol) and O-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (9.68 g, 25.46 mmol) in DMF (74.6 ml) under an atmosphere of nitrogen was added *N*-ethyldiisopropylamine (18.02 ml, 104.15 mmol) and the mixture was stirred at ambient temperature for 16 hours. The DMF was evaporated *in vacuo* to a residue which was taken up in ethyl acetate (200 mL), washed with brine, dried ($MgSO_4$) and evaporated *in vacuo* to a residue which was taken up in dichloromethane (75 mL) and filtered The filtrates were evaporated *in vacuo* to a residue which was chromatographed on silica with 30% ethyl acetate in heptane as eluant to give methyl 2-(2-amino-3-bromobenzamido)-2-methylpropanoate (7.21 g, 99 %). ^{1}H NMR (400 MHz, $CDCl_3$, 30 °C) δ 1.58 (6H, s), 3.70 (3H, s), 5.96 (2H, s), 6.44 - 6.50 (2H, m), 7.25 (1H, dd), 7.44 (1H, dd); m/z (ES+) [M+H]+ = 317.

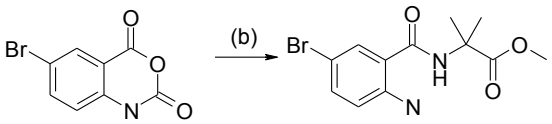
Nitrobenzoic acid coupling (Scheme 1, step a)



To a stirred solution of 3-methoxy-2-nitrobenzoic acid (1.00 g, 5.07 mmol), methyl 2-amino-

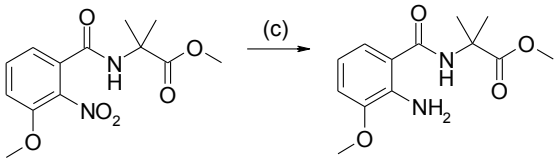
2-methylpropanoate hydrochloride (0.935 g, 6.09 mmol) and O-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (2.12 g, 5.58 mmol) in DMF (16.34 ml) under an atmosphere of nitrogen was added *N*-Ethyldiisopropylamine (3.95 ml, 22.83 mmol) and the mixture stirred at ambient temperature for 16 hours. The DMF was evaporated *in vacuo* to a residue which was taken up in ethyl acetate (300 mL), washed with brine, dried ($MgSO_4$) and evaporated *in vacuo* to a residue which was chromatographed on silica with ethyl acetate as eluant to give methyl 2-(3-methoxy-2-nitrobenzamido)-2-methylpropanoate (1.400 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$, 30 °C) δ 1.65 (6H, s), 3.80 (3H, s), 3.95 (3H, s), 6.65 (1H, s), 7.10 - 7.20 (2H, m), 7.45 (1H, t); m/z (ES-) (M-H)- = 295.

Ring opening of isatoic anhydride (Scheme 1, step b)



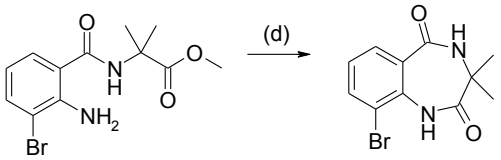
Methyl 2-amino-2-methylpropanoate hydrochloride (0.982 g, 6.40 mmol) was added in one portion to 6-bromo-1H-benzo[d][1,3]oxazine-2,4-dione (1.29 g, 5.33 mmol) in DMF (5 mL) followed by *N*-ethyldiisopropylamine (1.383 mL, 8.00 mmol) at 20 °C under nitrogen. The resulting suspension was stirred at 20 °C for 30 minutes then 40 °C for 2 hours then 60 °C for 2 hours. The reaction mixture was diluted with EtOAc (100 mL), and washed sequentially with 2M K_2CO_3 (20 mL), water (25 mL), and saturated brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product. The crude product was purified by flash silica chromatography, elution gradient 0 to 100% EtOAc in heptane. Pure fractions were evaporated to dryness to afford methyl 2-(2-amino-5-bromobenzamido)-2-methylpropanoate (0.920 g, 55%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$, 30 °C) δ 1.64 (6H, s), 3.77 (3H, s), 5.46 (2H, s), 6.47 (1H, s), 6.55 (1H, d), 7.27 (1H, dd), 7.44 (1H, d); m/z (ES+) (M+H)+ = 317.

Aniline formation (Scheme 1, step c)



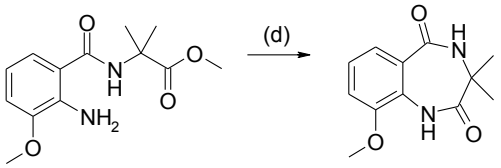
To a stirred solution of methyl 2-(3-methoxy-2-nitrobenzamido)-2-methylpropanoate (1.40 g, 4.73 mmol) in ethanol (95 ml) under an atmosphere of nitrogen was added palladium on carbon (0.503 g, 0.47 mmol). The mixture was stirred at ambient temperature under an atmosphere of hydrogen for 16 hours. The catalyst was filtered off, washed with ethanol (20 mL) and the combined filtrates evaporated *in vacuo* to a residue which was filtered through a silica column to remove a trace of colloidal palladium to give methyl 2-(2-amino-3-methoxybenzamido)-2-methylpropanoate (1.230 g, 98 %). ^{1}H NMR (400 MHz, $CDCl_3$, 30° C) δ 1.65 (6H, s), 3.75 (3H, s), 3.85 (3H, s), 5.75 (2H, s), 6.55 (1H, s), 6.60 (1H, dd), 6.80 (1H, d), 7.00 (1H, d); m/z (ES+) (M+H)+ = 267.

Pivalic acid ring closure (Scheme 1, step d)



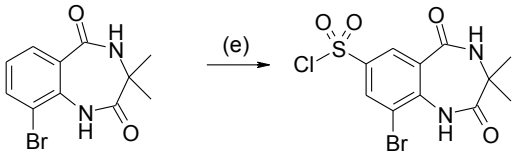
Methyl 2-(2-amino-3-bromobenzamido)-2-methylpropanoate (7.2 g, 22.85 mmol) in pivalic acid (45.7 ml) was split in to 3 x 20 mL vials and heated at 200 °C in a Biotage Initiator Microwave for 4 hours. The mixture was cooled to ambient temperature, the precipitated solid was off, washed with diethyl ether (2 x 50 mL) and crystallised from ethyl acetate to give 9-bromo-3,3-dimethyl-3,4-dihydro-1H-benzo[e][1,4]diazepine-2,5-dione (3.99 g, 62%). The crystallisation liquors were evaporated *in vacuo* to a residue which was chromatographed on basic alumina with 50% ethyl acetate in heptane as eluant to give a further crop of product (770 mg, 12%; total 74%). ^{1}H NMR (400 MHz, DMSO-d_6, 30° C) δ 1.19 (6H, s), 7.19 (1H, t), 7.75 (1H, dd), 7.88 (1H, dd), 8.59 (1H, s), 9.36 (1H, s); m/z (ES+) [M+H]+ = 285.

Acetic acid ring closure (Scheme 1, step d)



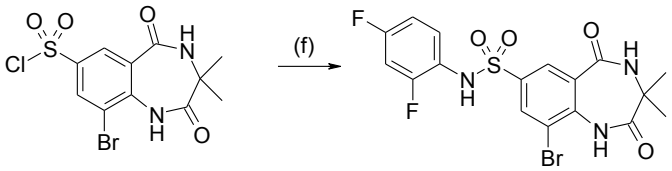
In a microwave vial, methyl 2-(2-amino-3-methoxybenzamido)-2-methylpropanoate (1.20 g, 4.51 mmol) in acetic acid (9.01 ml) was heated at 200 ° C in a Biotage Initiator Microwave for 30 minutes. The mixture was poured onto cold water (9 mL) and stirred for 5 minutes. The water and acetic acid were evaporated *in vacuo* to a residue which was chromatographed on silica with 2.5% methanol in dichloromethane as eluant to give a solid which was triturated with ethyl acetate (25 mL) to give 9-methoxy-3,3-dimethyl-3,4-dihydro-1H-benzo[e][1,4]diazepine-2,5-dione (0.442 g, 42%). ^{1}H NMR (400 MHz, DMSO-d_6, 30 ° C) δ 1.20 (6H, s), 3.85 (3H, s), 7.15 - 7.25 (2H, m), 8.35 (1H, s), 9.05 (1H, s); m/z (ES+) (M+H)+ = 235.

Sulphonyl chloride formation (Scheme 1, step e)



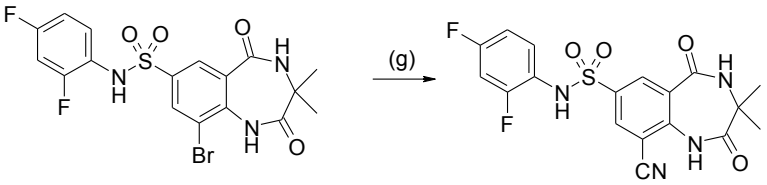
To 9-bromo-3,3-dimethyl-3,4-dihydro-1H-benzo[e][1,4]diazepine-2,5-dione (2.83 g, 10.00 mmol) at ambient temperature was added chlorosulfonic acid (8.15 ml, 123.6 mmol). When the addition was completed, the stirred mixture was heated at 65 ° C for 16 hours, cooled to ambient temperature, poured cautiously onto ice-cold water (135 mL) and stirred for 5 minutes. The precipitated solid was filtered off, washed with cold water (20 mL) and dried over P_2O_5 to give 9-bromo-3,3-dimethyl-2,5-dioxo-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepine-7-sulfonyl chloride (2.430 g, 64%). ^{1}H NMR (400 MHz, DMSO-d_6, 30 ° C) δ 1.20 (6H, s), 7.92 (1H, d), 7.95 (1H, d), 8.61 (1H, s), 9.40 (1H, s); m/z (ES-) [M-H]- = 381.

Sulphonamide formation (Scheme 1, step f)



To a stirred mixture of 9-bromo-3,3-dimethyl-2,5-dioxo-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepine-7-sulfonyl chloride (2.42 g, 2.85 mmol) and 2,4-difluoroaniline (0.316 ml, 3.14 mmol) in dichloromethane (10 ml) at ambient temperature was added pyridine (4.62 ml, 57.07 mmol) and the mixture stirred at ambient temperature for 16 hours. The dichloromethane and pyridine were evaporated *in vacuo* to a residue which was taken up in ethyl acetate (50 mL), washed with 1M citric acid (25 mL), brine, dried ($MgSO_4$) and evaporated *in vacuo* to a residue which was crystallised from methanol to give 9-bromo-*N*-(2,4-difluorophenyl)-3,3-dimethyl-2,5-dioxo-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepine-7-sulfonamide (0.630 g, 47%). The crystallisation liquors were evaporated *in vacuo* to a residue (1.4g) which was purified by preparative HPLC (Waters SunFire column, 5µ silica, 50 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 0.1% formic acid) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to give a further crop of product (190 mg, 14%; total 61%). ^{1}H NMR (400 MHz, DMSO-d_6, 30° C) δ 1.27 (6H, s), 7.09 - 7.17 (1H, m), 7.25 - 7.36 (2H, m), 8.00 (1H, d), 8.10 (1H, d), 8.91 (1H, s), 9.82 (1H, s), 10.36 (1H, s); m/z (ES-) [M-H]- = 472.

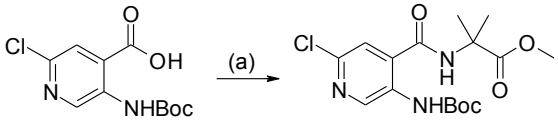
Nitrile formation (Scheme 1, step g)



15

9-Bromo-*N*-(2,4-difluorophenyl)-3,3-dimethyl-2,5-dioxo-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepine-7-sulfonamide (150 mg, 0.32 mmol), tris(dibenzylideneacetone)dipalladium(0) (7.2 mg, 7.9 µmol), dicyclohexyl(2',4',6'-triisopropylbiphenyl-2-yl)phosphine (15.1 mg, 0.03 mmol), zinc (2.1 mg, 0.03 mmol), and dicyanozinc (22.3 mg, 0.19 mmol) were placed in a microwave tube under nitrogen. Degassed DMA (2.3 mL) was added *via* syringe and the resulting mixture was heated under microwave irradiation at 120 °C for 2 hours. The reaction mixture was diluted with EtOAc (50 mL) and filtered. The filtrate was washed with saturated brine (50 mL) and water (25 mL), the organic layer was dried over $MgSO_4$, filtered and evaporated to afford crude product that was purified by flash silica chromatography, elution gradient 20 to 100% EtOAc in heptane. Pure fractions were evaporated to dryness to afford 9-cyano-*N*-(2,4-difluorophenyl)-3,3-dimethyl-2,5-dioxo-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepine-7-sulfonamide **15** (109 mg, 82%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, 30 °C) δ 1.24 (6H, s), 7.02 - 7.11 (1H, m), 7.20 - 7.32 (2H, m), 8.20 - 8.27 (2H, m), 8.95 (1H, s), 10.36 (1H, s), 10.83 (1H, s); m/z (ES+) [M+MeCN]+ = 462.

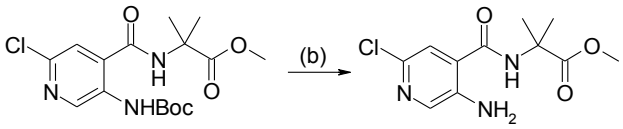
Methyl 2-(5-(tert-butoxycarbonylamino)-2-chloroisonicotinamido)-2-methylpropanoate (Scheme 2, step a)



36 **37**

O-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) (570 mg, 1.50 mmol) was added to a stirred solution of 5-((tert-butoxycarbonyl)amino)-2-chloroisonicotinic acid (409 mg, 1.50 mmol), methyl 2-amino-2-methylpropanoate hydrochloride (323 mg, 2.10 mmol) and *N*,*N*-diisopropylethylamine (1.310 mL, 7.50 mmol) in *N*,*N*-dimethylformamide (5 mL). The resulting mixture was stirred for 45 min at room temperature. The mixture was diluted with ethyl acetate (100 mL) and washed with saturated $NaHCO_3$ (25 mL), twice with water (2 x 25 mL) and brine (25 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The crude product was purified by flash silica chromatography, elution gradient 0 to 30% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford methyl 2-(5-((tert-butoxycarbonyl)amino)-2-chloroisonicotinamido)-2-methylpropanoate (503 mg, 90%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.48 (15H, d), 3.63 (3H, s), 7.77 (1H, s), 9.05 (1H, s), 9.22 (1H, s), 9.52 (1H, s); LRMS (ES+) m/z $(M+H)^+$ = 372.

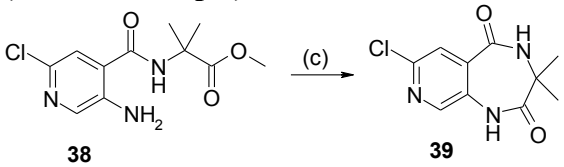
Methyl 2-(5-amino-2-chloroisonicotinamido)-2-methylpropanoate (Scheme 2, step b)



37 **38**

Trifluoroacetic acid (3 mL) was added to a stirred solution of methyl 2-(5-((tert-butoxycarbonyl)amino)-2-chloroisonicotinamido)-2-methylpropanoate (473 mg, 1.27 mmol) in dichloromethane (20 mL). The mixture was stirred for 3 hours and concentrated *in vacuo*. The crude product was purified by ion exchange chromatography, using an SCX column. The desired product was eluted from the column using 7M NH_3/methanol and pure fractions were evaporated to dryness to afford methyl 2-(5-amino-2-chloroisonicotinamido)-2-methylpropanoate (347 mg, 100%) as a oil which solidified on standing. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.46 (6H, s), 3.60 (3H, s), 6.42 (2H, s), 7.57 (1H, s), 7.94 (1H, s), 8.76 (1H, s); LRMS (ES+) m/z $(M+H)^+$ = 272.

7-Chloro-3,3-dimethyl-3,4-dihydro-1*H*-pyrido[*3,4-e*][*1,4*]diazepine-2,5-dione (Scheme 2, step c)

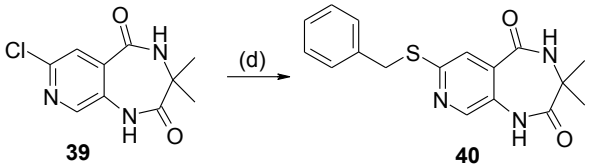


38 **39**

Methyl 2-(5-amino-2-chloroisonicotinamido)-2-methylpropanoate (112.5 mg, 0.41 mmol) in pivalic acid (2 mL, 17.72 mmol) was sealed into a microwave tube. The reaction was heated to 200 °C for 3 hours in the microwave reactor and cooled to room temperature. The mixture was concentrated *in vacuo*. The crude product was purified by flash silica chromatography, elution gradient 0 to 10% methanol in dichloromethane. Pure fractions were evaporated to dryness and the solid residue was washed with a small amount of dichloromethane to afford 7-chloro-3,3-dimethyl-3,4-dihydro-1*H*-pyrido[*3,4-e*][*1,4*]diazepine-2,5-dione (35.0 mg, 35%)

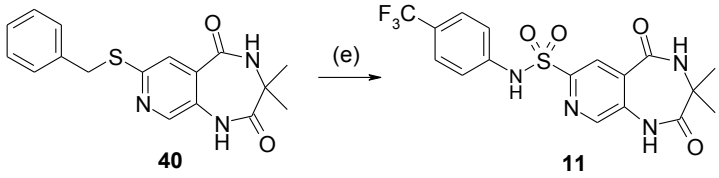
as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.27 (6H, s), 7.71 (1H, s), 8.31 (1H, m), 8.83 (1H, s), 10.78 (1H, s); LRMS (ES+) m/z $(M+H)^+$ = 240.

7-(Benzylthio)-3,3-dimethyl-3,4-dihydro-1*H*-pyrido[*3,4-e*][*1,4*]diazepine-2,5-dione (Scheme 2, step d)



Potassium 2-methylpropan-2-olate (775 mg, 6.90 mmol) was added to a stirred solution of phenylmethanethiol (2.5 mL, 21.30 mmol) and 7-chloro-3,3-dimethyl-3,4-dihydro-1*H*-pyrido[*3,4-e*][*1,4*]diazepine-2,5-dione (275.8 mg, 1.15 mmol) in DMF (4 mL). The reaction mixture was stirred at 80 °C for 3 hours. After cooling to room temperature, solvent was removed under reduced pressure. The crude product was then purified by flash column chromatography eluting with 10% MeOH in DCM to provide 7-(benzylthio)-3,3-dimethyl-3,4-dihydro-1*H*-pyrido[*3,4-e*][*1,4*]diazepine-2,5-dione (353 mg, 94%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.23 (s, 6H), 4.40 (s, 2H), 7.23 (m, 2H), 7.29 (m, 2H), 7.38 (d, *J* = 8 Hz, 2H), 7.53 (1H, s), 8.37 (1H, s), 8.70 (1H, s), 10.59 (1H, s); LRMS (ES+) m/z $(M+H)^+$ = 328.

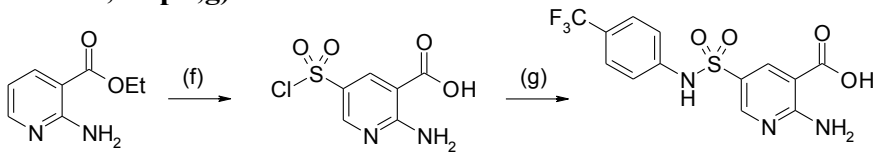
3,3-Dimethyl-2,5-dioxo-*N*-(4-(trifluoromethyl)phenyl)-2,3,4,5-tetrahydro-1*H*-pyrido[*3,4-e*][*1,4*]diazepine-7-sulfonamide
(Scheme 2, step e)



1-Chloropyrrolidine-2,5-dione (547 mg, 4.10 mmol) was added to a stirred solution of 7-(benzylthio)-3,3-dimethyl-3,4-dihydro-1*H*-pyrido[*3,4-e*][*1,4*]diazepine-2,5-dione (353 mg, 1.08 mmol) in acetic acid (5 mL) and water (0.1 mL). The reaction mixture was stirred for 10 min, before being filtered and the solid was washed with dichloromethane (100 ml). The filtrate was dried ($MgSO_4$), filtered and concentrated under reduced pressure to provide crude 3,3-dimethyl-2,5-dioxo-2,3,4,5-tetrahydro-1*H*-pyrido[*3,4-e*][*1,4*]diazepine-7-sulfonyl chloride (327 mg, 100%). LRMS (ES-) m/z (M-H)- = 302.
4 -(Trifluoromethyl)aniline (0.203 mL, 1.61 mmol) was added to a mixture of pyridine (9 mL) and 4 x 4A molecular sieves followed by 3,3-dimethyl-2,5-dioxo-2,3,4,5-tetrahydro-1*H*-pyrido[*3,4-e*][*1,4*]diazepine-7-sulfonyl chloride (327 mg, 1.08 mmol). The resulting mixture was stirred at ambient temperature for 3 h. The solid was filtered off and washed through with dichloromethane. The filtrate was concentrated under reduced pressure. The crude product were then purified by preparative HPLC (Waters SunFire column, 5µ silica, 19 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 0.1% formic acid) and MeCN as eluents, followed by HPLC (Waters XBridge Prep C18 OBD column, 5µ silica, 19 mm diameter, 100 mm length), using decreasingly polar mixtures of water (containing 1% NH_3) and MeCN as eluents. Fractions containing the desired compound were evaporated to dryness to afford 3,3-dimethyl-2,5-dioxo-*N*-(4-(trifluoromethyl)phenyl)-2,3,4,5-tetrahydro-1*H*-pyrido[*3,4-e*][*1,4*]diazepine-7-sulfonamide (102 mg, 22%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.22 (s, 6H), 7.33 (d, *J* = 8.5 Hz, 2H), 7.60 (d, *J* =

8.6 Hz, 2H), 8.24 (s, 1H), 8.51 (s, 1H), 8.91 (s, 1H), 11.06 (d, *J* = 9.7 Hz, 2H); LRMS (ES-) m/z $(M-H)^-$ = 427.

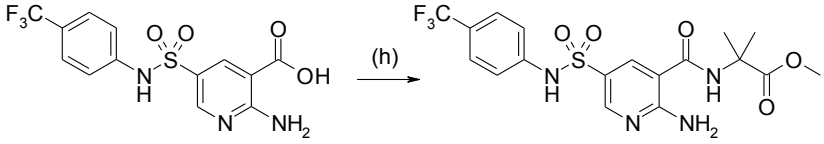
2-Amino-5-(*N*-(4-(trifluoromethyl)phenyl)sulfamoyl)nicotinic acid
(Scheme 2, step f,g)



41 **42** **43**

Chlorosulfonic acid (1.0 mL, 15.77 mmol) was added to methyl 2-aminonicotinate (200 mg, 1.31 mmol) over a period of 5 minutes under nitrogen. The resulting mixture was stirred at 65 °C for 18 hours. It was cooled to room temperature and poured cautiously onto crushed ice (around 5 g) and partitioned immediately with CH_2Cl_2 (2 x 75 mL). Combined organics were dried (sodium sulphate) and concentrated *in vacuo* to a yellow solid (118 mg).
Pyridine (4.16 mL, 51.38 mmol) was added to methyl 2-amino-5-(chlorosulfonyl)nicotinate (115 mg, 0.46 mmol) and 4-aminobenzotrifluoride (86 µl, 0.69 mmol) under nitrogen. The resulting solution was stirred at 20 °C for 3 days. The pyridine was removed *in vacuo* to leave a yellow residue (358 mg). The crude product was purified by flash silica chromatography, elution gradient 0 to 25% methanol in dichloromethane. Pure fractions were evaporated to dryness to afford methyl 2-amino-5-(*N*-(4-(trifluoromethyl)phenyl)sulfamoyl)nicotinate (18.0 mg, 4% over 2 steps) as a yellow solid; LRMS (ES+) m/z (M+H)+ = 376 and 2-amino-5-(*N*-(4-(trifluoromethyl)phenyl)sulfamoyl)nicotinic acid (44.0 mg, 9% over 2 steps) as an off white solid. LRMS (ES-) m/z (M-H)- = 360.

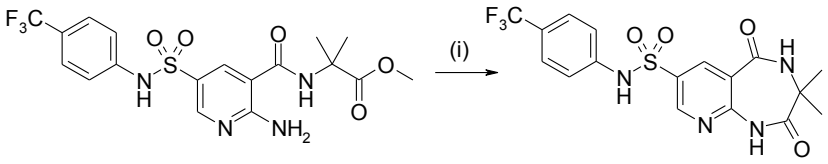
Methyl 2-(2-amino-5-(*N*-(4-(trifluoromethyl)phenyl)sulfamoyl)nicotinamido)-2-methylpropanoate
(Scheme 2, step h)



43 **44**

O-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HATU) (173 mg, 0.45 mmol) was added to 2-amino-5-(*N*-(4-(trifluoromethyl)phenyl)sulfamoyl)nicotinic acid (164 mg, 0.45 mmol), methyl 2-amino-2-methylpropanoate hydrochloride (69.7 mg, 0.45 mmol) and *N*,*N*-diisopropylethylamine (0.317 mL, 1.82 mmol) in DMF (10 mL). The resulting solution was stirred at 20 °C for 18 hours. The solvent was removed *in vacuo* to leave a brown oil (997 mg). It was taken up in a mixture of methanol and DCM, concentrated *in vacuo* and adsorbed onto silica. The crude product was purified by flash silica chromatography, elution gradient 50 to 100% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford methyl 2-(2-amino-5-(*N*-(4-(trifluoromethyl)phenyl)sulfamoyl)nicotinamido)-2-methylpropanoate (60.0 mg, 29%) as a light brown gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (s, 6H), 3.78 (s, 3H), 6.74 (s, 1H), 6.92 (s, 2H), 7.17 (s, 1H), 7.23 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 2H), 8.00 (d, *J* = 2.3 Hz, 1H), 8.49 (d, *J* = 2.3 Hz, 1H); LRMS (ES+) m/z (M+H)+ = 461.

3,3-Dimethyl-2,5-dioxo-*N*-(4-(trifluoromethyl)phenyl)-2,3,4,5-tetrahydro-1*H*-pyrido[*2,3-e*][*1,4*]diazepine-7-sulfonamide
(Scheme 2, step i)



44 **12**

Pivalic acid (333 mg, 3.26 mmol) was added to methyl 2-(2-amino-5-(*N*-(4-(trifluoromethyl)phenyl)sulfamoyl)nicotinamido)-2-methylpropanoate (60 mg, 0.13 mmol) and warmed to around 40 °C. The resulting solution was transferred to a microwave vessel which was then sealed and was heated within the microwave reactor at 200 °C for 4 hours. The sealed microwave vessel was heated thermally at 200 °C for 24 hours. It was allowed to cool to room temperature and the pivalic acid removed *in vacuo*. The residue was azeotroped once with toluene. The crude product was purified by flash silica chromatography, elution gradient 20 to 90% ethyl acetate in heptane. Pure fractions were evaporated to dryness to afford 3,3-dimethyl-2,5-dioxo-*N*-(4-(trifluoromethyl)phenyl)-2,3,4,5-tetrahydro-1*H*-pyrido[*2,3-e*][*1,4*]diazepine-7-sulfonamide (31.0 mg, 56%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, 100 °C) δ 1.29 (s, 6H), 7.36 (d, J = 8.1 Hz, 2H), 7.63 (d, J = 8.2 Hz, 2H), 8.41 (s, 1H), 8.55 (s, 1H), 8.83 (s, 1H), 10.83 (s, 1H); LRMS (ES-) m/z (M-H)- = 427.

Procedures for determination of physicochemical properties:
$logD_{7.4}$, plasma-protein binding and solubility measurements were made as described in; Buttar, D.; Colclough, N.; Gerhardt, S.; MacFaul, P. A.; Phillips, S. D.; Plowright, A.; Whittamore, P.; Tam, K.; Maskos, K.; Steinbacher, S.; Steuber, H. A. Combined spectroscopic and crystallographic approach to probing drug–human serum albumin interactions. *Bioorg. Med. Chem.* **2010**, *18,* 7486-7496.

$logD_{7.4}$:
$LogD_{7.4}$ measurements were made using a shake-flask method where the extent of partitioning between pH 7.4 buffer and octanol was measured. Compounds were dissolved in a known volume buffer, and following the addition of a known amount of octanol, the solutions were shaken for 30 min. Following centrifugation, analysis of the aqueous layer was performed by LC–UV to quantify the amount of compound in solution and then compared to analysis of the compound in solution before the addition of octanol to calculate the partitioning coefficient, $D_{7.4}$.

Solubility:
Assessments of aqueous solubility were made after an incubation of 24 h in pH 7.4 phosphate buffer. After centrifugation, analysis of the supernatant liquid was performed by LC–UV to quantify the amount of compound in solution.

Protein binding strength *via* equilibrium dialysis:
Dialysis membranes (Spectra/Por 2, 12–14 kDa molecular weight cut-off, 47 mm diameter, Spectrum Laboratories) were prepared for use by washing with distiled water and subsequent soaking in phosphate buffer (pH 7.4). Membranes were then blotted dry and placed between two 1 mL Teflon dialysis half-cells (Braun ScienceTec, Les Ulis, France). Each half-cell was filled individually with 1 mL of protein solution containing the compound of interest, while the corresponding half-cell was filled with 1 mL of isotonic phosphate buffer. Dialysis units were immersed in a 37 °C temperature-controlled water bath and rotated at 30 rpm for 18–19 h using a Dianorm apparatus (Braun ScienceTec). After this period, samples from both the half-cell containing buffer (protein free) and the half-cell containing protein were submitted for HPLC analysis using an Agilent 1100 series HPLC with a 110 binary pump and a UV

diode ray detector. Acquisition and integration were carried out using Chemstation software (Agilent Technologies) version A.06.03 with relevant customised macro software. Integration of the subsequent chromatograms, are used to calculate the concentration of drug in the protein containing solution (Dp) and in the protein-free solutions (Df), which are then used to derive the binding constant for the test compound (K_1) assuming a 1:1 binding model as shown in Eq. 1 where the compound can only bind to a single site on the protein molecule. This is expressed mathematically in Eq. 2 where D and Df are the total and free drug concentrations, respectively, and Pr is the total protein concentration.

$$\mathrm{D} + \mathrm{P} \overset{K_1}{\rightleftharpoons} \mathrm{DP} \qquad \text{Eq. 1}$$

$$D = (D_\mathrm{f} + D_\mathrm{p}) = \frac{K_1 \cdot D_\mathrm{f} \cdot \mathrm{Pr}}{1 + K_1 \cdot D_\mathrm{f}} + D_\mathrm{f} \qquad \text{Eq. 2}$$

Pharmacokinetic data: All experiments were performed in compliance with the relevant laws and institutional guidelines by the AstraZeneca *in vivo* group.