Supporting infomation

Discovery of AZD2716: a novel, potent secreted phospholipase A_2 ($sPLA_2$) inhibitor for the treatment of coronary artery disease

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Discovery Sciences

Sections of [d]Structure & Biophysics, [e]Reagents and Assay Development and [f]Screening Sciences and Sample Management.

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General synthesis information

All solvent and reagents used were reagent grade. Purity and characterization of compounds were established by a combination of liquid chromatography–mass spectroscopy (LC-MS), and NMR analytical techniques. High resolution LC-MS was detected on a Waters LCTp ToF MS using electrospray ionization (ESI-MS). The MS inlet consisted of a Waters Acquity UPLC system, and the separation was performed on a Waters C18 XBridge at 45-50 °C. The separation was done with a 2-95% ACN gradient over 3 min at pH 10 (40 mM NH_3 and 5 mM H_2CO_3). A measure of related impurities was assessed at 210 nM. ^{1}H NMR were recorded on a Bruker Avance DPX400 (400 MHz), AV500 (500 MHz) or AV600 (600 MHz) and were determined in $CHCl_3$-*d*, DMSO-d_6 and MeOH-d_4 with trimethylsilane (TMS) (0.00 ppm) or solvent peaks as the internal reference. Chemical shifts are reported in ppm relative to solvent signal at 7.26 ppm ($CDCl_3$), 2.50 ppm (DMSO) and 3.30 ppm (MeOD) and

coupling constant (*J*) values are reported in Hertz (Hz). Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad peak. The final compounds were purified by RP-HPLC on a Waters Fraction Lynx system equipped with a ZQ MS detector. The columns used were Waters Xbridge C18 OBD 5µ (pH 10, gradient 5-95 % ACN + 0.2 % NH_3) or Waters SunFire C18 OBD 5µ (pH 3, gradient 5-95 % ACN + 0.1 M Formic acid).

Synthesis and characterization of compound 2-9.

5'-Benzyl-2'-carbamoylbiphenyl-3-carboxylic acid (2)

4-benzylbenzonitrile

To a flask was added 4-bromobenzonitrile (4.6 g, 25.27 mmol) and tetrakis(triphenylphosphine)palladium(0) (1.168 g, 1.01 mmol) followed by tetrahydrofuran (30 mL). The solution was put under nitrogen atmosphere and then was added benzylzinc(II) bromide (0.5 M in THF) (65.7 mL, 32.85 mmol). The solution was heated at 60 °C for 2 h. The solution was cooled to RT and concentrated by evaporation. The residue was diluted with EtOAc and then washed with brine. After drying using a phase separator and evaporation the residue was purified by automated flash chromatography on a Biotage® KP-SIL 100g column. A gradient from 0% to 30% of EtOAc in heptane over 7V was used as mobile phase. The product was collected using the wavelength 254 nm to yield 4-benzylbenzonitrile (88 %, 4.3 g) as an oil that solidified upon standing. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90 (s, 2H), 7.02 (d, 2H), 7.07 – 7.25 (m, 5H), 7.42 (m, 2H).

5-benzyl-2-cyanophenylboronic acid

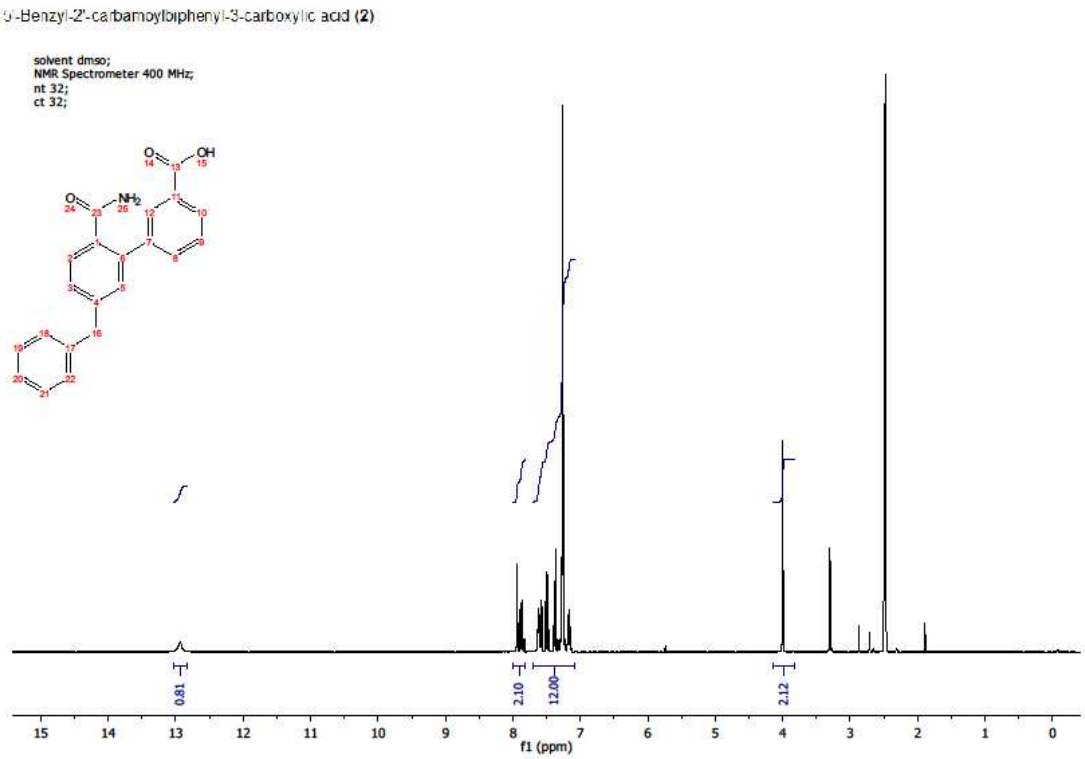
To a dried 500 ml three-necked flask put under nitrogen atmosphere was added dry tetrahydrofuran (180 mL) followed by 2,2,6,6-tetramethylpiperidine (22.2 mL, 131.53 mmol). The solution was cooled to -50 °C and then was added nBuLi (2.5 M in hexanes) (51.9 mL, 129.68 mmol) (the temperature reached -40 °C). After cooling to -78 °C was added triisopropyl borate (53.4 mL, 231.57 mmol) over 5 minutes. The temperature was reached ca -70 °C. After cooling the mixture to -78 °C was added 4-benzylbenzonitrile (17.9 g, 92.63 mmol) in tetrahydrofuran (20 mL) over 8 min. The temperature was kept at ca -70 °C. Upon addition a red/brown solution was obtained. After 15 min stirring was added water (40 ml) to the reaction mixture, still under nitrogen atmosphere and at -75 °C., The mixture turned yellow and was transfered to 1 L round bottom flask and concentrated by evaporation. To the residue was added EtOAc (100 ml) and water (100 ml). The mixture was transferred to a separatory funnel and shaken/separated. The aqueous phase was neutralized by addition of CO2(s) and then extracted twice with EtOAc. The organic phases were combined and then extracted (x5) with NaOH (40 ml, 1.5 M). The pH of the combined aqueous layer was adjusted to pH 4 by addition of HCl (10%) and the aqueous phase was extracted with EtOAc (60 x 3).). The organic phase was dried using a phase separator and evaporated (co-evaporated with ACN) to yield 5-benzyl-2-cyanophenylboronic acid (39%, 8.6 g); m/z (MH^-) 236. NMR very complex, please refer to attached spectra.

Ethyl 5'-benzyl-2'-cyanobiphenyl-3-carboxylate

5-benzyl-2-cyanophenylboronic acid (400 mg, 1.69 mmol), ethyl 3-iodobenzoate (470 mg, 1.70 mmol), $PdCl_2$(dppf) (100 mg, 0.14 mmol) and Cs_2CO_3 (891 mg, 2.73 mmol) were added to an empty vial. The reagents were flushed with N_2 gas during ca 5 min. DMF was then added and the mixture was stirred 1 h at 60 °C. Most of the DMF was evaporated and the residue was extracted with CH_2Cl_2/water. The organic layer was separated and evaporated. The residue was purified by chromatography on a silica column. The product was eluted with CH_2Cl_2/acetone (90:10) to give 515 mg product mixture that was used in the next step without further handling.

*5'-Benzyl-2'-carbamoylbiphenyl-3-carboxylic acid (**2**)*

Crude ethyl 5'-benzyl-2'-cyanobiphenyl-3-carboxylate(500 mg, ~1.4 mmol), NaOH (500 mg, 12.5 mmol) were added to n-propanol (5 ml). The mixture was stirred for 2 h at 90 °C. 1 ml acetic acid was added and the mixture was evaporated. The residue was extracted with CH_2Cl_2/water. The water phase was made acidic by adding HCl. The organic layer was separated and evaporated. The compound was purified by preparative HPLC on a Kromasil C8 column (10 µm 250x50 ID mm) using a gradient of 20-65% acetonitrile in H_2O/ACN/FA 95/5/0.2 buffer over 15 minutes with a flow of 100 mL/min. The compounds were detected by UV at 254 nm to 5'-Benzyl-2'-carbamoylbiphenyl-3-carboxylic acid (**2**)
(37%, 178 mg, purity LCMS 96%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.80-7.92 (m, 2H), 7.15-7.61 (m, 3.98 (s, 2H). HRMS Calcd for $[C_{21}H_{18}NO_3]$+: 332.1286; found: 332.1294. LCMS purity



2-(5'-Benzyl-2'-carbamoylbiphenyl-3-yl)acetic acid (3)

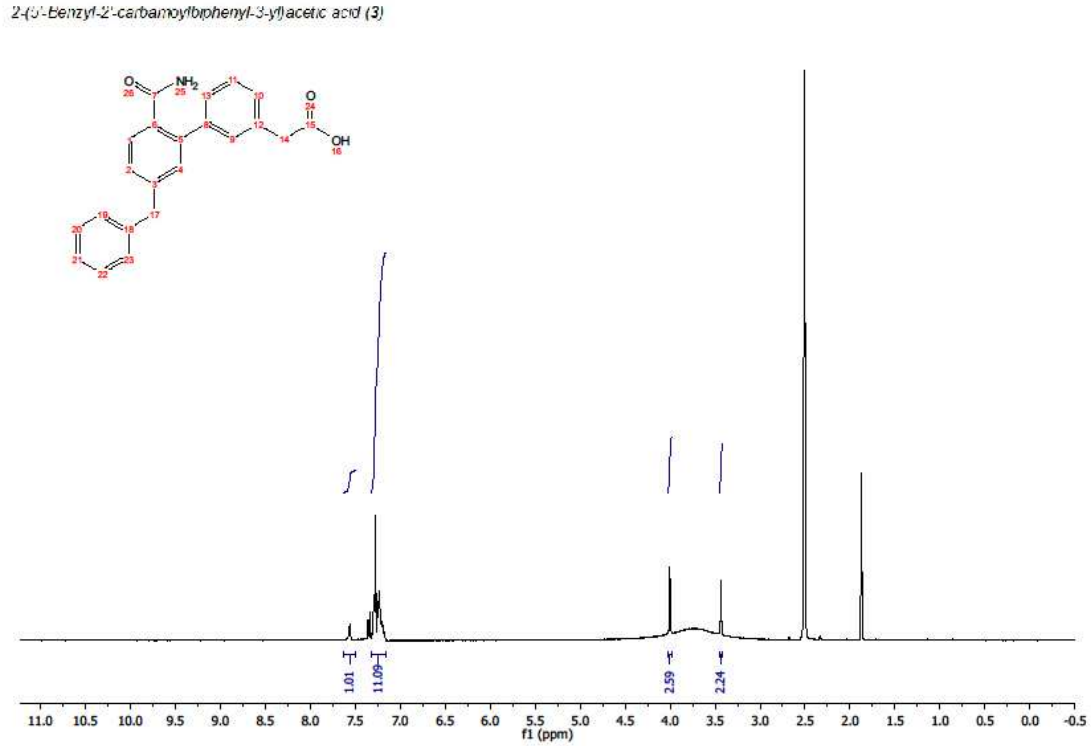
Methyl 2-(5'-benzyl-2'-cyanobiphenyl-3-yl)acetate

5-benzyl-2-cyanophenylboronic acid (see preparation of compound **2**) (0.27 g, 1.12 mmol), methyl 2-(3-bromophenyl)acetate (0.25, 1.12 mmol), Cs_2CO_3 (0.731 g, 2.24 mmol) and $PdCl_2$(dppf) (0.082 g, 0.11 mmol) were flushed with nitrogen and then dissolved in DMF (2

mL). The mixture was heated at 100 °C for 2 h. 2/3 of the DMF was evaporated and ethylacetate and water was added. The organic layer was separated, dried with a phase separator and evaporated. The residue was purified with flash chromatography on silica using CH_2Cl_2 as eluent. The pure fractions were evaporated to give methyl 2-(5'-benzyl-2'-cyanobiphenyl-3-yl)acetate (31%, 119 mg). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88 (m, 1H) 7.33 – 7.50 (m, 6H), 7.27 – 7.32 (m, 4H), 7.25 (m, 1H), 4.05 (s, 2H), 3.66 (s, 2H), 3.22 (s, 3H).

2-(5'-Benzyl-2'-carbamoylbiphenyl-3-yl)acetic acid (***3***)

Methyl 2-(5'-benzyl-2'-cyanobiphenyl-3-yl)acetate (0.119 g, 0.35 mmol) and sodium hydroxide (0.026 mL, 1.39 mmol) were solved in n-propanol (1 mL) and the solution was heated at 90 °C 3 h and then kept at 75 °C over night. Acetic acid (0.080 mL, 1.39 mmol) was added and the solvents were evaporated. The compound was purified by preparative HPLC on a Kromasil C8 column (10 µm 250x50 ID mm) using a gradient of 20-65% acetonitrile in H_2O/ACN/FA 95/5/0.2 buffer over 15 minutes with a flow of 100 mL/min. The compounds were detected by UV at 254 nm to yield 2-(5'-Benzyl-2'-carbamoylbiphenyl-3-yl)acetic acid (3) (66%, 78 mg, purity LCMS 97%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.58 (m, 1H), 7.22-7.63 (m, 11H), 4.0 (s, 2H), 3.43 (s, 2H). HRMS Calcd for $[C_{22}H_{20}NO_3]$+: 346.1443; found: 346.1448.



***3-(5'-Benzyl-2'-carbamoylbiphenyl-3-yl)propanoic acid* 4**

4-Benzyl-2-chlorobenzonitrile

To a flask was added 4-bromo-2-chlorobenzonitrile (1 g, 4.62 mmol) and $Pd(PPh_3)_4$ (0.214 g, 0.18 mmol) followed by THF (20 mL). The solution was put under nitrogen atmosphere and

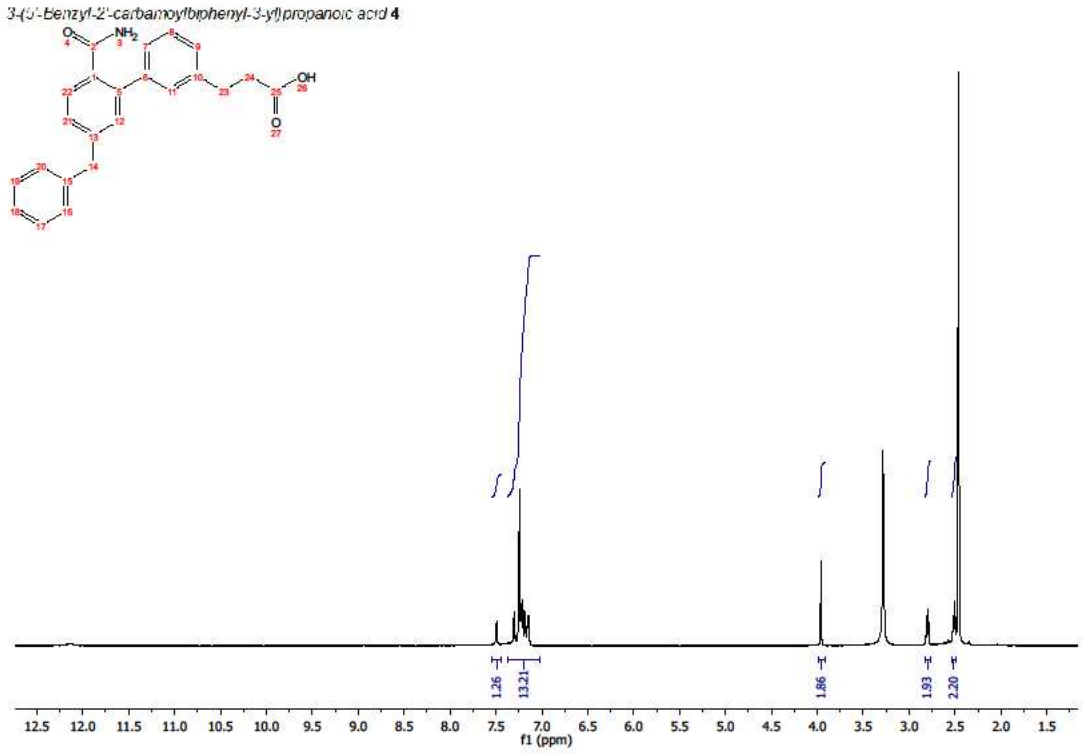
then was added benzylzinc(II) bromide (0.5 M in THF) (18.48 mL, 9.24 mmol). The solution was heated at 60 °C for 1h. LCMS and TLC showed complete conversion of starting material. The solution was cooled to RT and diluted with EtOAc and then washed with brine. After drying using a phase separator and evaporation was the residue by automated flash chromatography on a Biotage® KP-SIL 100g column. A gradient from 0% to 40% of EtOAc in heptane over 7 CV was used as mobile phase. The product was collected using the wavelength 254 nm to yield 4-benzyl-2-chlorobenzonitrile (77%, 0.81 g). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56 (m, 1H) 7.31 (m, 3H), 7.26-7.20 (m, 1H), 7.10 – 7.21 (m, 3H), 3.99 (s, 2H).

Methyl 3-(5'-benzyl-2'-cyanobiphenyl-3-yl)propanoate

To a vial was added 4-benzyl-2-chlorobenzonitrile (0.547 g, 2.40 mmol), 3-(3-methoxy-3-oxopropyl)phenylboronic acid (0.55 g, 2.64 mmol), $C_{S2}CO_3$ (2.349 g, 7.21 mmol), $Pd(PPh_3)4$ (0.194 g, 0.17 mmol) and DMF (15 mL). The mixture was put under nitrogen atmosphere and heated at 90 °C for 20 h. The mixture was allowed to reach RT and then diluted with EtOAc and washed with water and brine. The organic phase was dried using a phase separator and evaporated. The residue was purified by automated flash chromatography on a Biotage® KP-SIL 100g column. A gradient from 0% to 40% of EtOAc in heptane over 8 CV was used as mobile phase. The product was collected using the wavelength 254 nm to yield methyl 3-(5'-benzyl-2'-cyanobiphenyl-3-yl)propanoate (54%, 0.46 g). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.65 (m, 1H) 7.33 – 7.41 (m, 3H), 7.27 – 7.32 (m, 3H), 7.25 (m, 1H), 7.22 (m, 2H), 7.17 (m, 2H), 4.05 (s, 2H), 3.66 (s, 3H), 3.00 (m, 2H), 2.67 (m, 2H).

*3-(5'-Benzyl-2'-carbamoylbiphenyl-3-yl)propanoic acid (***4**)

To a microwawe vial was added methyl 3-(5'-benzyl-2'-cyanobiphenyl-3-yl)propanoate (0.4 g, 1.13 mmol), KOH (0.631 g, 11.25 mmol), MeOH (10 mL) and water (2.0 mL). The solution was stirred at 150 °C for 30 min. To the reaction solution was added acetic acid until pH 5. The reaction solution was evaporated and the residue uptaken in DMSO/water. The compound was purified by preparative HPLC on a Kromasil C8 column (10 µm 250x50 ID mm) using a gradient of 20-65% acetonitrile in H_2O/ACN/FA 95/5/0.2 buffer over 15 minutes with a flow of 100 mL/min. The compounds were detected by UV at 254 nm to yield 3-(5'-benzyl-2'-carbamoylbiphenyl-3-yl)propanoic acid (67%, 272 mg, purity LCMS 99%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.49 (m, 1H) 7.30 (m, 1H), 7.10 – 7.29 (m, 12H), 3.96 (s, 2H), 2.80 (m, 2H), 2.51 (m, 3H). HRMS Calcd for $[C_{23}H_{22}NO_3]$+: 360.1599; found: 360.1585.



4-(5'-benzyl-2'-carbamoylbiphenyl-3-yl)butanoic acid (5)

Methyl 4-(3-bromophenyl)butanoate

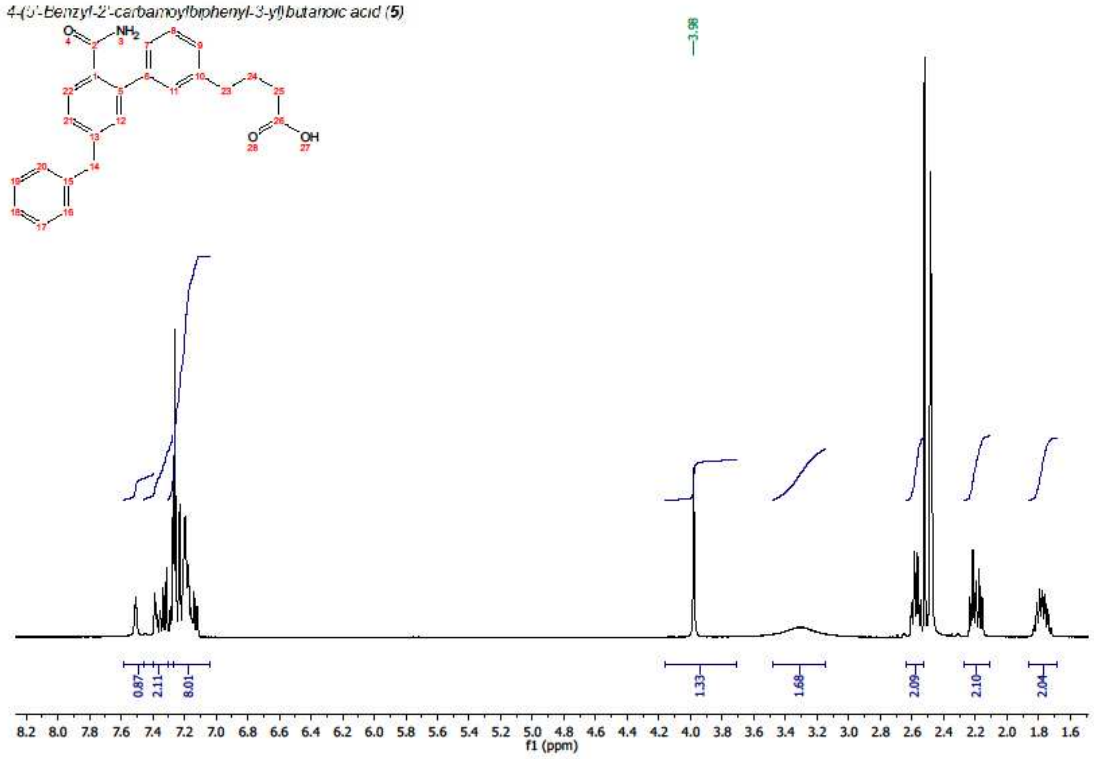
Thionyl chloride (0.075 mL, 1.03 mmol) was added dropwise to methanol (5 mL) (exothermic) and then 4-(3-bromophenyl)butanoic acid (0.5 g, 2.06 mmol) was added and the reaction stirred at rt for 3 h. The solvent was evaporated and EtOAc was added. The org phase was washed with $NaHCO_3$ (sat aq), separated with a phase separator and evaporated to give crude methyl 4-(3-bromophenyl)butanoate (92%, 0.485 g) as a pale yellow liquid that was used in the next step without further handling. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 (m, 2H) 7.15-728 (m, 2H), 3.56 (s, 3H), 3.33 (s, 2H), 2.59 (m, 2H), 2.30 (m, 2H), 1.84 (m, 2H).

Methyl 4-(5'-benzyl-2'-cyanobiphenyl-3-yl)butanoate

5-benzyl-2-cyanophenylboronic acid (see preparation of compound **2**) (0.150 g, 0.63 mmol),Cs_2CO_3 (0.412 g, 1.27 mmol), methyl 4-(3-bromophenyl)butanoate (0.163 g, 0.63 mmol) and $PdCl_2$(dppf) (0.046 g, 0.06 mmol) were suspended in DMF (2 mL) and the mixture was heated at 100 °C for 3 h. 2/3 of the DMF was evaporated and ethylacetate and water was added. The organic layer was separated, dried with a phase separator and evaporated. The residue was purified with flash chromatography on silica using CH_2Cl_2 as eluent. The pure fractions was evaporated to give methyl 4-(5'-benzyl-2'-cyanobiphenyl-3-yl)butanoate (53%, 124 mg) as the desired product. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82 (d, 1H), 7.29-7-5 (m, 11 H), 4.13 (s, 1H), 3.30 (s, 3H), 2.68 (m, 2H), 2.34 (m, 2H), 1.90 (m, 2H).

*4-(5'-Benzyl-2'-carbamoylbiphenyl-3-yl)butanoic acid (**5**)*

Methyl 4-(5'-benzyl-2'-cyanobiphenyl-3-yl)butanoate (0.124 g, 0.34 mmol) and sodium hydroxide (0.044 mL, 2.35 mmol) were added to n-propanol (1 mL) and the mixture was heated at 90 °C over night. The mixture was neutralized with acetic acid (0.134 mL, 2.35 mmol). The solvent was evaporated and the compound was purified by preparative HPLC on a Kromasil C8 column (10 µm 250x50 ID mm) using a gradient of 20-65% acetonitrile in H_2O/ACN/FA 95/5/0.2 buffer over 15 minutes with a flow of 100 mL/min. The compounds were detected by UV at 254 nm to yield 4-*(5'-benzyl-2'-carbamoylbiphenyl-3-yl)butanoic acid (5)* (50%, 60 mg, purity LCMS 97%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.51(s, 1H) 7.40-7.30 (m, 2H), 7.10 – 7.29 (m, 9H), 3.98 (s,2H), 2.56 (dd Jzz=7,7 Hz, 15.5 Hz, 2H), 2.19 (m, 2H), 1.77 (m, 2H). HRMS Calcd for [$C_{24}H_{23}NO_3$]+: 374.1756; found: 374.1753.



2-(5'-Benzyl-2'-carbamoylbiphenyl-3-yloxy)acetic acid (6)

Tert-butyl 2-(3-bromophenoxy)acetate

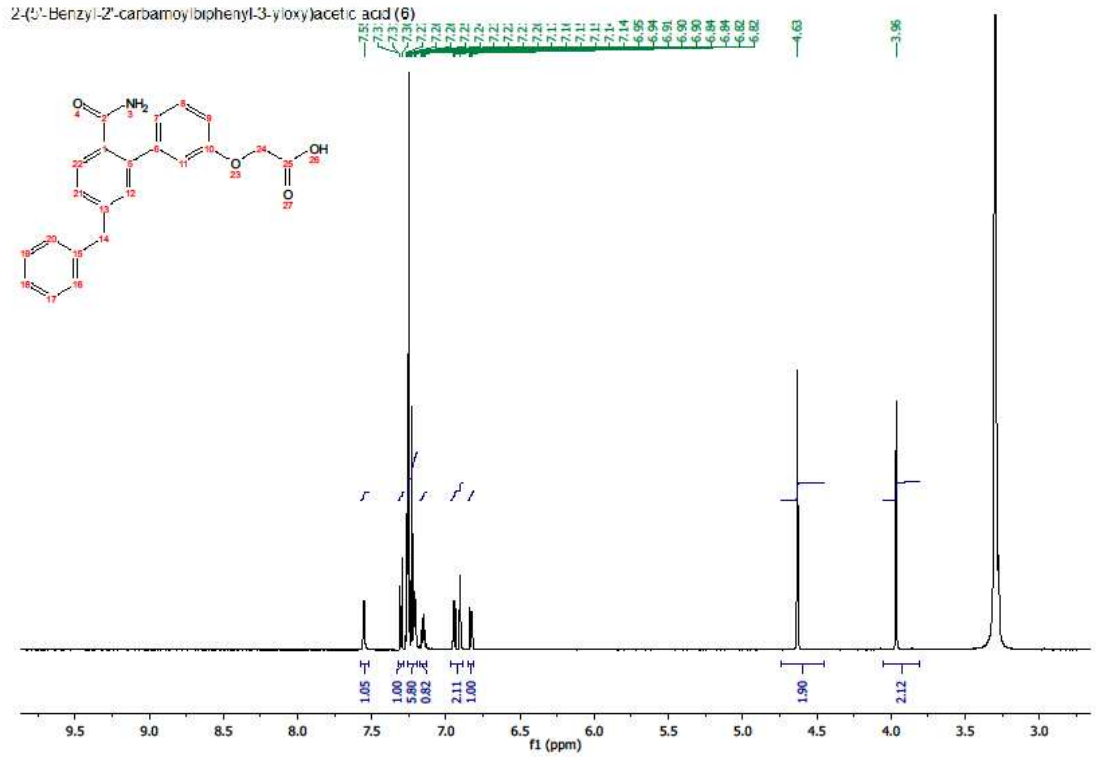
Tert-butyl 2-bromoacetate (0.594 mL, 3.68 mmol), 3-bromophenol (0.7 g, 4.05 mmol) and $C_{S2}CO_3$ (1.798 g, 5.52 mmol) were stirred in acetone (15 mL) at reflux for 1h. The reaction suspension was cooled to RT and then diluted with EtOAc and washed with water, dried using a phase separator and evaporated to yield tert-butyl 2-(3-bromophenoxy)acetate (93%, 0.98 g) as an oil. ^{1}H NMR (400 MHz, CDCl3) δ 7.15 (m, 2H), 7.03 (s, 1H), 6.60)m, 1H), 4.48 (s, 2H) 1.48 (s, 9H).

Tert-butyl 2-(5'-benzyl-2'-cyanobiphenyl-3-yloxy)acetate

To a vial was added 5-benzyl-2-cyanophenylboronic acid see preparation of compound **2**) (0.3 g, 1.27 mmol), tert-butyl 2-(3-bromophenoxy)acetate (0.280 g, 0.97 mmol), $C_{S2}CO_3$ (0.952 g, 2.92 mmol), $Pd(Ph_3P)_4$ (0.056 g, 0.05 mmol) and DMF (8 mL). The mixture was put under nitrogen atmosphere and heated at 90 ºC for 4 h when LCMS (showing M+ 344) showed almost complete conversion of starting material. The reaction suspension was diluted with EtOAc and then washed with water. The aqueous phase was then extracted once with EtOAc. The combined organic phases was then washed with water and brine, dried using a phase separator and then evaporated. The compound was purified by preparative HPLC on a Kromasil C8 column (10 µm 250x50 ID mm) using a gradient of 20-65% acetonitrile in H_2O/ACN/FA 95/5/0.2 buffer over 15 minutes with a flow of 100 mL/min. The compounds were detected by UV at 254 nm to yield tert-butyl 2-(5'-benzyl-2'-cyanobiphenyl-3-yloxy)acetate (70%, 272 mg). ^{1}H NMR (400 MHz, CDCl3) δ 7.64 (d, J=7.5 Hz, 1H), 7.36 (m, 1H), 7.30 (m, 3H), 7.22 (m, 1H), 7.16 (m, 4H), 7.02 (m, 1H), 6.94 (dd, J=8.4, 2.50 Hz, 1 H), 4.55 (s, 2H), 4.03 (s, 2H), 1.46 (s, 9H).

2-(5'-Benzyl-2'-carbamoylbiphenyl-3-yloxy)acetic acid (**6**)

Tert-butyl 2-(5'-benzyl-2'-cyanobiphenyl-3-yloxy)acetate (0.272 g, 0.68 mmol) and KOH (0.382 g, 6.81 mmol) were dissolved in MeOH (4 mL) and water (0.800 mL) in microwave vial. The mixture was heated in a microwawe at 130 ºC for 20 min. The pH was adjusted to ca 5 by addition of acetic acid. The volatiles were removed and the residue dissolved in DMSO/water and some drops of methanol. The compound was purified by preparative HPLC on a Kromasil C8 column (10 µm 250x50 ID mm) using a gradient of 20-65% acetonitrile in H_2O/ACN/FA 95/5/0.2 buffer over 15 minutes with a flow of 100 mL/min. The compounds were detected by UV at 254 nm to yield 2-(5'-benzyl-2'-carbamoylbiphenyl-3-yloxy)acetic acid (6) (67%, 165 mg, purity LCMS 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.56(br s, 1H) 7.32-7.28 (m, 1H), 7.26 – 7.15 (m, 6H), 7.15 (m, 1H), 6.91 (m, 2 H), 6.84 (dd J=8.3 Hz, 2.6 Hz, 1H), 4.63 (s, 2H), 3.94 (s, 2H). HRMS Calcd for $[C_{22}H_{20}NO_4]$+: 362.1392; found: 362.1413.



*Methyl 3-(5'-benzyl-2'-cyanobiphenyl-3-yl)-2-methylpropanoate (**7**, (*S*)-**7**and (*R*)-**7**)*

Dimethyl 2-(3-iodobenzyl)-2-methylmalonate

To a solution of 1-(bromomethyl)-3-iodobenzene (6.7 g, 22.56 mmol) and dimethyl 2-methylmalonate (3.15 mL, 23.69 mmol) in DMF (10 mL) was added Cs_2CO_3 (8.82 g, 27.08 mmol). The suspension was then stirred at 70 °C for 2 h. The reaction suspension was allowed to cool to RT. The volatiles were evaporated. The residue was partitionated between EtOAc (15 ml) and water (15 ml). The aqueous phase was then extracted once with EtOAc. The organic phases were combined and washed with brine and then dried using a phase separator. Evaporation of the solvents (co-evaporation with ACN) gave dimethyl 2-(3-iodobenzyl)-2-methylmalonate (96%, 7.86 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 – 7.30 (m, 2H), 6.88 (m, 2H), 3.64 – 3.53 (s, 6H), 3.01 (s, 2H), 1.23 (s, 3H).

Methyl 3-(3-iodophenyl)-2-methylpropanoate

Dimethyl 2-(3-iodobenzyl)-2-methylmalonate (8.2 g, 22.64 mmol) and NaOH (3.62 g, 90.57 mmol) were heated in water (15 mL) and MeOH (5 mL) at 80 °C for 2 h. The solution was allowed to cool to RT and then evaporation to dryness. To the mixture was added 10 ml water and the aqueous phase was washed twice with diethyl ether. The pH of the aqueous phase was made acidic by addition of HCl (10%). The aqueous phase was extracted with EtOAc.

The organic phase was dried using a phase separator and then evaporated to yield 2-(3-iodobenzyl)-2-methylmalonic acid (86%, 6.49 g); m/z (MH^-) 333.

A suspension of 2-(3-iodobenzyl)-2-methylmalonic acid (5.2 g, 15.56 mmol) in acetic acid (5 ml, 87.34 mmol) was heated, under nitrogen atmosphere, at 120 °C in round bottom flask equipped with a cooler for 10 h. The solution was allowed to cool to RT and the volatiles were evporated and the residue dissolved in EtOAc. The organic phase was washed with HCl (5%) and brine. The organic phase was dried using a phase separator and evaporated to yield 3-(3-iodophenyl)-2-methylpropanoic acid (95%, 4.2 g); m/z (MH^-) 289.

To 3-(3-iodophenyl)-2-methylpropanoic acid (4 g, 13.79 mmol) was added MeOH (3 mL) and HCl (1.25 M in MeOH) (2.206 mL, 2.76 mmol). The obtained solution was put under nitrogen atmosphere and then heated to 60 °C for 2.5 h. The solution was allowed to cool to RT and then evaporated. The residue was uptaken in EtOAc and washed with $NaHCO_3$ (10%) and brine. The organic phase was then dried using a phase separator and evaporated to yield methyl 3-(3-iodophenyl)-2-methylpropanoate (75% over three steps, 3.9 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.17 (d, 3H), 2.62 (m, 1H), 2.67 – 2.81 (m, 1H), 2.98 (m, 1H), 3.66 (s, 3H), 6.98 – 7.09 (m, 1H), 7.12 (m, 1H), 7.56 (m, 2H).

Methyl 2-methyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate

To a round bottom flask was added methyl 3-(3-iodophenyl)-2-methylpropanoate (3.04 g, 10.00 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (3.05 g, 12.00 mmol), potassium acetate (1.962 g, 19.99 mmol) and $Pd(dbpf)Cl_2$ (0.097 g, 0.15 mmol). The mixture was put under nitrogen atmosphere and dioxane (25 mL) was added. The mixture was put nder nitrogen atmosphere and heated in an oil bath at 90 °C. After 6 h heating was the reaction mixture allowed to cool to RT. The suspension was filtrated through a silica plug and the solvent then evaporated. The black residue was suspended in EtOAc and washed with NH_4Cl (sat). The aqueous phase was then extracted with EtOAc. The combined organic phase was then washed with water and then brine and then dried using a phase separator and evaporated. The residue was purified by automated flash chromatography on a Biotage® KP-SIL 100g column. A gradient from 0% to 25% of EtOAc in heptane over 10 CV was used as mobile phase. The product was collected using the wavelength 232/254 nm to yield methyl 2-methyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate (49%). ^{1}H NMR (400 MHz, $CDCl_3$, 21°C) δ 7.76 – 7.60 (m, 2H), 7.34 – 7.25 (m, 2H), 3.67 (s, 3H), 3.07 (m, 1H), 2.84 – 2.72 (m, 1H), 2.67 (m, 1H), 1.37 (s, 12H), 1.16 (d, 3H).

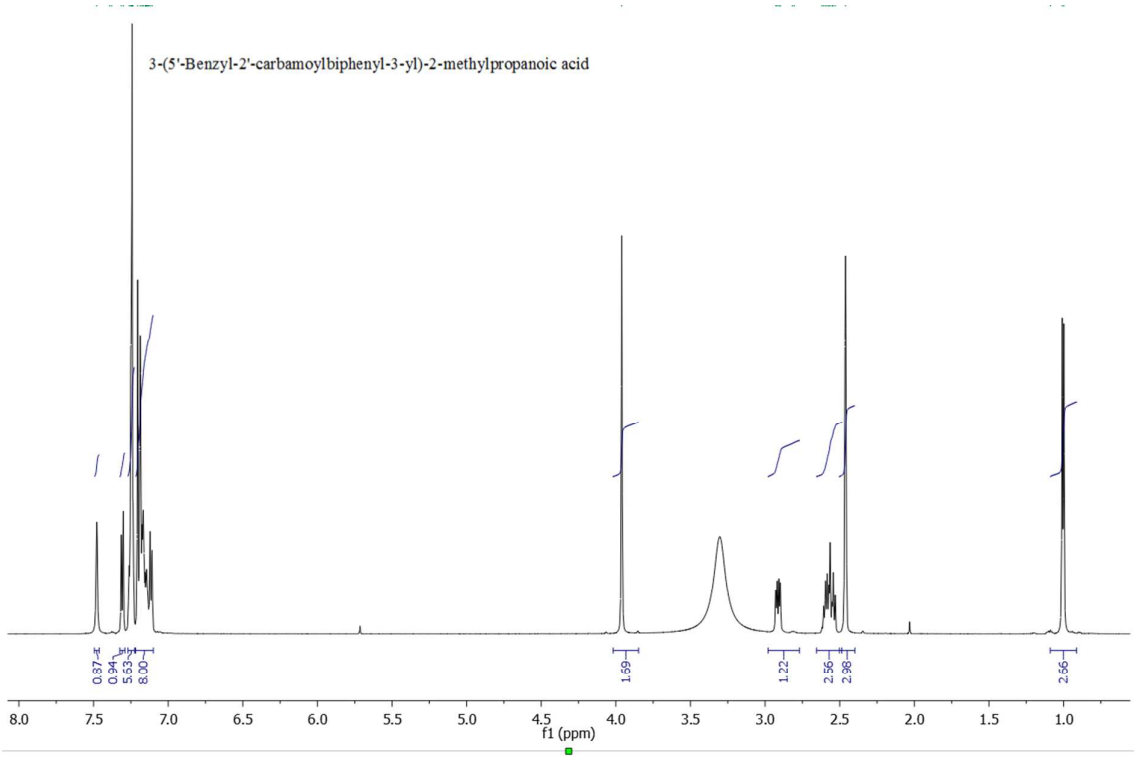
Methyl 3-(5'-benzyl-2'-cyanobiphenyl-3-yl)-2-methylpropanoate

To a round bottom flask was added 4-benzyl-2-chlorobenzonitrile (see preparation of compound **4**) (0.416 g, 1.83 mmol), methyl 2-methyl-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate (0.612 g, 2.01 mmol), Cs_2CO_3 (1.192 g, 3.66 mmol) and 1,1'-bis(di-tert-nutylphosphino)ferrocene palladium dichloride (10 mol%). The mixture was put under nitrogen atmosphere and then was added DMF (8 mL). The suspension was degassed and refilled with nitrogen (x3). The suspension was heated at 90 °C for 75 min. The reaction suspension which had turned brown was allowed to cool to RT and then diluted with EtOAc and washed with NH_4Cl (sat). The aqueous phase was then extracted with EtOAc (x2). The combined organic phase was washed with brine and then dried using a phase separator. After evaporation was the product purified by automated flash chromatography on a Biotage® KP-SIL 100 g column. A gradient from 0% to 40% of EtOAc in heptane over 10

CV was used as mobile phase. The product was collected using the wavelength 254 nm. Product fractions were collected and evaporated to yield methyl 3-(5'-benzyl-2'-cyanobiphenyl-3-yl)-2-methylpropanoate as an oil (89%, 0.60 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (m, 1H) 7.31 (m, 2H), 7.24 (m, 4H), 7.15 (m, 5H), 3.94 – 4.08 (m, 2H), 3.57 (s, 3H), 3.04 (m, 1H), 2.50 – 2.90 (m, 2H), 1.12 (d, 3H).

3-(5'-Benzyl-2'-carbamoylbiphenyl-3-yl)-2-methylpropanoic acid (**7**)

Methyl 3-(5'-benzyl-2'-cyanobiphenyl-3-yl)-2-methylpropanoate (0.443 g, 1.20 mmol) and KOH (0.673 g, 11.99 mmol) were dissolved in MeOH (8 mL) and water (1.600 mL) in a 5 mL microwave vial. The vial was capped and heated at 132 °C for 30 min and then at 142 °C for 20 min in a single node microwave reactor. Acetic acid (1 ml) was added and the solvents evaporated. The residue was dissolved in DMSO/MeOH/Water and the compound was purified by preparative HPLC on a Kromasil C8 column (10 µm 250x50 ID mm) using a gradient of 20-60% acetonitrile in H_2O/ACN/FA 95/5/0.2 buffer over 22 minutes with a flow of 100 mL/min. The compounds were detected by UV at 250 nm to yield 3-(2'-carbamoyl-5'-(4-chlorophenoxy)biphenyl-3-yl)-2-methylpropanoic acid (56%, 250 mg, purity LCMS 99%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.48 (s, 1H) 7.31 (m, 1H), 7.25 (m, 5H), 7.14 – 7.23 (m, 6H), 7.12 (m, 1H), 3.97 (s, 2H), 2.92 (dd, J=6.1, 13.0 Hz, 1H), 2.52 – 2.64 (m, 2H), 1.01 (d, J=6.9 Hz 3H). HRMS Calcd for $[C_{24}H_{24}NO_3]$+: 374.1756; found: 374.1750.



The racemic product was separated by chiral HPLC using a Chiralcel OJ 5 µm 20x250 mm column with heptane/EtOH/formic acid ((10:90:0.1; 15 ml/min, 40 °C, 260 nm) as mobile phase to yield (*S*)-**7** and (*R*)-**7** with identical ^{1}HNMR and HRMS data as racemic **7**. (*R*)-**7**: tR=5.8 min $[\alpha]_D^{20}$ 15.4 (*c* 0.5, ACN), 99.7 %ee. (*S*)-**7**: tR=9.2 min. 99.0 % ee.
Absolut configuration was determined by X-ray crystallography. Please see: Karlsson, S.; Sörensen, H.; Andersen, S. M.; Cruz, A.; Ryberg, P. An enantioselective hydrogenation of an

akenoic acid as a key step in the sysnthesis of AZD2716. *Org. Proc. Res. Dev.* **2016**, 20*(2)*, 262-269).

2-((5'-Benzyl-2'-carbamoylbiphenyl-3-yl)methyl)butanoic acid (8)

Diethyl 2-(3-bromobenzyl)-2-ethylmalonate

To a round bottom flask containing 1-bromo-3-(bromomethyl)benzene (2.18 g, 8.72 mmol) was added diethyl 2-ethylmalonate (1.66 g, 8.82 mmol) dissolved in DMF (10 mL). Cesium carbonate (3.17 g, 9.73 mmol) was added and the suspension was stirred at 60 ºC for 6 h. The reaction suspension was allowed to cool to RT and water (20 ml) was added. The mixture was extracted with EtOAc (3x 20ml). The organic phase was washed with brine and dried using a phase separator. The solvent was removed under reduced pressure to give diethyl 2-(3-bromobenzyl)-2-ethylmalonate as a yellow oil (3.13 g). H-NMR showed the desired product with traces of starting malonate and DMF. The compound was used in the next step without further purification. ^{1}H NMR (400 MHz, CDCl3) 7.35 (br d, J=7.7 Hz, 1H), 7.25 (m, 1H), 7.12 (t, J=7.7Hz, 1H), 7.03 (br d, J=8.7 Hz, 1 H), 4.18 (dq, J=2.8, 7.0 Hz, 4 H), 0 3.18 (s, 2H), 1.84 (q, J=7.2 Hz, 2H), 1.24 (t, 7.0 Hz, 6H), 0.92 (t, J=7.2 Hz, 3H).

Methyl 2-(3-bromobenzyl)butanoate

To a round bottom flask containing diethyl 2-(3-bromobenzyl)-2-ethylmalonate (2.49 g, 6.97 mmol) was added sodium hydroxide (1.12 g, 27.9 mmol), water (18 mL) and MeOH (5 mL). The suspension was divided into two microwave vials which were capped and heated in a microwave at 130 ºC for 1 h. The reaction mixtures were acidified to pH ca 2 with HCl (conc) while cooled in ice bath. The aqueous phase was extracted with dichloromethane (4 x). The solvent was removed under reduced pressure to give 1.91 g a yellow-white solid. The crude was dissolved in acetic acid (10 ml) and heated to reflux keeping the oil batch at 140 ºC for 15h. The acetic acid was removed under reduced pressure to yield 1.3 g oil. The crude acid was dissolved in hydrochloric acid in MeOH (10 mL, 1.3) and MeOH (10 mL) was added. The solution was refluxed for 2.5 h and the solution was allowed to cool and then concentrated under reduced pressure. The residue was dissolved in DCM and the organic layer was washed twice with $NaHCO_3$ (sat) and dried over $MgSO_4$. The solvent was evaporated to give crude methyl 2-(3-bromobenzyl)butanoate (53%,1.01 g) as a yellow oil which was used in the next step without further purification. ^{1}H NMR (400 MHz CDCl3) 7.32 (m, 2H), 7.17-7.07 (m, 2H), 3.62 (s, 3H), 2.90 (dd, J=8.8, 14.2 Hz, 1H), 2.91 (dd, J=6.8, 14.2 Hz, 1H), 2.58 (m, 1 H), 1.61 (m, 2H), 0.91 (t, J= 7.5 Hz, 3H).

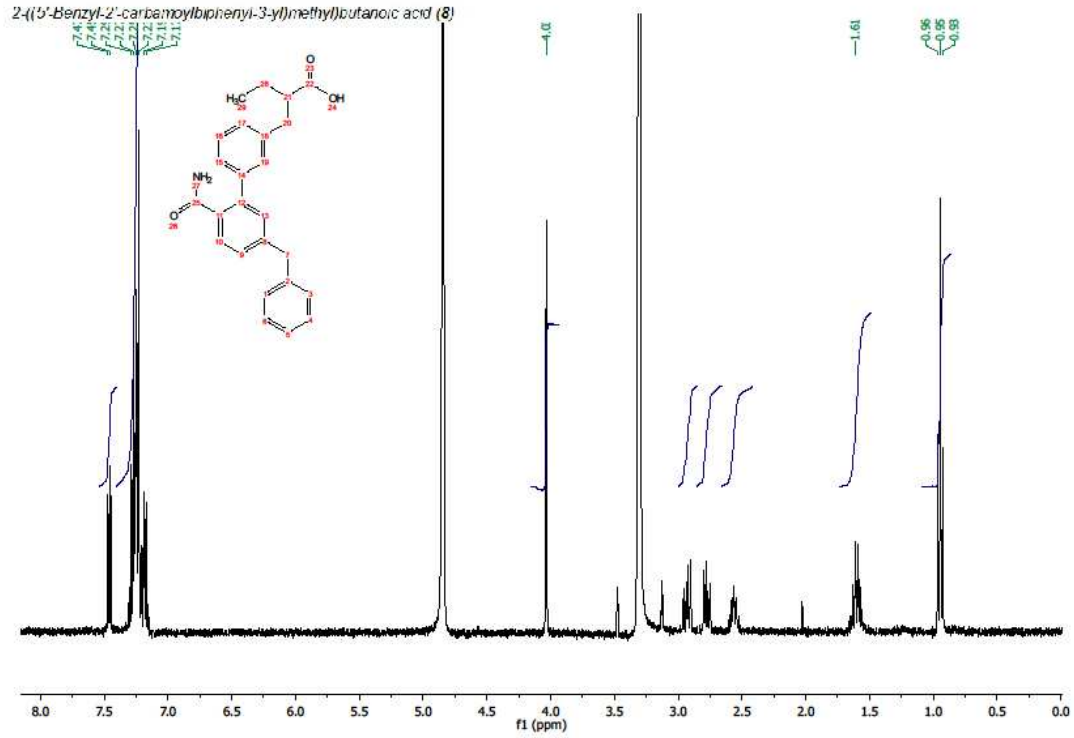
Methyl 2-((5'-benzyl-2'-cyanobiphenyl-3-yl)methyl)butanoate

To a vial containing methyl 2-(3-bromobenzyl)butanoate (154.5 mg, 0.57 mmol were added 5-benzyl-2-cyanophenylboronic acid (se preparation of **2**) (171.5 mg, 0.72 mmol), Pd-106 (44.5 mg, 0.06 mmol) and cesium carbonate (375 mg, 1.15 mmol). The vial was put under an inert atmosphere and DMF (4mL) was added. The solution was stirred at 90 ºC for 2 h and was then allowed to stand at rt overnight. The reaction mixture was diluted with EtOAc, washed with NH_4Cl (sat) followed by brine. The org phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure to give 292 mg of black-brown oil. The crude was purified with flash silica chromatography.A gradient from 0 to 65% EtOAc in heptane was used as a mobile phase. The pure fractions were collected and the solvent was removed under reduced pressure to give *methyl 2-((5'-benzyl-2'-cyanobiphenyl-3-yl)methyl)butanoate*

as a colourless oil (66%, 144 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J= 7.9 Hz, 1H), 7.38-7.27 (m, 11H), 4.07 (s, 2H), 3.61 (s, 3H), 3.03 (dd, J= 7.9, 13.5 Hz, 1H), 2.82 (dd, J=7.1, 13.5 Hz, 1H), 2.67 (m, 1H), 1.66 (m, 2H), +.93 (t, J=7.5 Hz, 3 H).

*2-((5'-Benzyl-2'-carbamoylbiphenyl-3-yl)methyl)butanoic acid (**8**)*

In a microwave vial methyl 2-((5'-benzyl-2'-cyanobiphenyl-3-yl)methyl)butanoate (138 mg, 0.36 mmol) was dissolved in MeOH (2.5 mL). Potassium hydroxide (202 mg, 3.60 mmol) was addded. By addition of water (0.4 mL) a suspension was observed. The vial was capped and the suspension was heated at 140 ºC for 1h in a microwawe. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc and washed with water. AcOH was added to the EtOAc/water mixture. The org phase was washed with brine and dried over a phase separator. The solvent was removed to give 157 mg of a crude colourless oil. The compound was purified by preparative HPLC on a Kromasil C8 column (10 µm 250x20 ID mm) using a gradient of 10-80% acetonitrile in H_2O/ACN/FA 95/5/0.2 buffer, over 20 minutes with a flow of 19 mL/min. The compounds were detected by UV at 254 nm. The pure fractions were collected and lyophilised to give 2-((5'-benzyl-2'-carbamoylbiphenyl-3-yl)methyl)butanoic acid (68%, 95 mg, purity LCMS 98%) as a white solid. ^{1}H NMR (400 MHz, MeOD) δ 7.45 (m, 1H), 7.33-7.14 (m, 11 H), 4.05 (s, 3H), 2.93 (dd, J=8.9, 13.4 Hz, 1H), 2.77 (dd, J=6.3, 13.4 Hz, 1H), 2.57 (m, 1H), 1.60 (m, 2H), 0.94 (t, J=7.05, 3H). HRMS Calcd for $[C_{25}H_{26}NO_3]$+: 388.1913; ; found: 388.1911.



1-((5'-benzyl-2'-carbamoylbiphenyl-3-yl)methyl)cyclopropanecarboxylic acid (9)

1-(3-Bromobenzyl)cyclopropanecarbonitrile

To a solution of diisopropylamine (0.570 mL, 4.00 mmol) in tetrahydrofuran (10 mL), put under nitrogen atmosphere, and cooled to -30 °C was added nBuLi (1.60 mL, 4.00 mmol). The solution was then cooled to -78 °C and cyclopropanecarbonitrile (0.30 mL, 4.00 mmol) in tetrahydrofuran (5.00 mL) was added. The solution was stirred at -78 ºC for 45 min and then was added 1-bromo-3-(bromomethyl)benzene (1.2 g, 4.80 mmol) in tetrahydrofuran (5.00 mL). The solution was stirred for 1 h and then allowed to reach RT over 10 min and then stirred for 16 h. The volatiles were then evaporated and the residue diluted with EtOAc and washed with water and brine. The organic phase was dried using a phase separator and evaporated. The residue was purified by automated flash chromatography on a Biotage® KP-SIL 100g column. A gradient from 0% to 50% of EtOAc in heptane over 10 CV was used as mobile phase. The product was collected using the wavelength 254 nm (low abs) to yield 1-(3-bromobenzyl)cyclopropanecarbonitrile (47%, 0.45 g) as an oil. ^{1}H NMR (400 MHz, CDCl3) δ 7.30 (m, 2H), 7.17 (m, 2H), 1.19 (m, 2H), 0.83 (m, 2H).

1-(3-Bromobenzyl)cyclopropanecarboxylic acid

1-(3-bromobenzyl)cyclopropanecarbonitrile (0.45 g, 1.88 mmol) and HCl (conc.) (3.87 ml, 47.1 mmol) were added to a 5 mL microwave vial. The vial was capped and heated at 125 °C for 90 min in a single node microwave reactor. The aqueous phase was extracted with EtOAc (x2). The organic phase was then extraxted with NaOH (1 M x2). The aqueous phase was then washed with EtOAc and then made acidic (pH<1) by addition of HCl. The emultion was then extracted with EtOAc (x2) and the organic phase washed with brine and dried using a phase separator and evaporated to yield crude 1-(3-bromobenzyl)cyclopropanecarboxylic acid (43%, 0.21g) as oil which was used in the next reaction step without further purification. LCMS M-: 254.

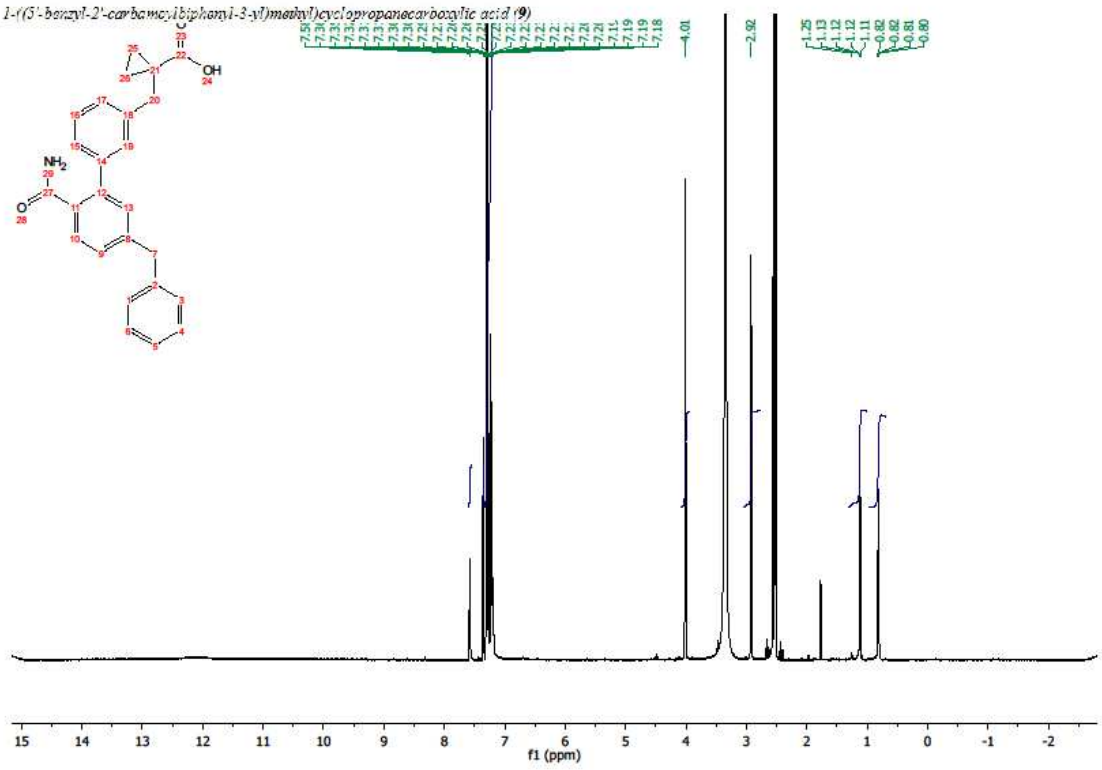
1-((5'-Benzyl-2'-cyanobiphenyl-3-yl)methyl)cyclopropanecarboxylic acid

To a vial was added 5-benzyl-2-cyanophenylboronic acid (0.086 g, 0.36 mmol), 1-(3-bromobenzyl)cyclopropanecarboxylic acid (0.077 g, 0.30 mmol), Cs_2CO_3 (0.25 g, 0.77 mmol) and Pd-118 (0.019 g, 0.03 mmol). The mixture was put under nitrogen atmosphere and DMF (2 mL) was added. The suspension was heated at 90 °C for 1 h. The reaction suspension was allowed to cool to RT and then diluted with EtOAc and washed with HCl (10% aq). The aqueous phase was extracted once with EtOAc and the organic phase was evaporated. The residue was taken to the next step without further handling. LCMS M^-:366.

*1-((5'-benzyl-2'-carbamoylbiphenyl-3-yl)methyl)cyclopropanecarboxylic acid (**9**)*

KOH (0.134 g, 2.39 mmol) and 1-((5'-benzyl-2'-cyanobiphenyl-3-yl)methyl)cyclopropanecarboxylic acid (0.088 g, 0.24 mmol) were dissolved in n-propanol (2 mL) and water (0.200 mL). The solution was heated at 95 °C for 8 h. The reaction solution was allowed to cool to RT and acetic acid (0.137 mL, 2.39 mmol) was added. The solvents were evaporated. The residue was dissolved in MeOH/Water/DMSO and purifed using Fractionlynx III, (Sunfire Prep C18 5µm OBD 19x150 mm column), with 5 to 95% acetonitrile in 0.2% acetic acid at pH 3 Product not pure enough and therefore purified again**.** The obtained product was dissolved in DMSO and purified using Fractionlynx I, (Xbridge Prep C18 5µm OBD 19x150 mm column), with 5 to 95% acetonitrile in 0.2% ammonia at pH 10 to yield 1-((5'-benzyl-2'-carbamoylbiphenyl-3-yl)methyl)cyclopropanecarboxylic acid as a white solid after freeze drying (19%, 17 mg, purity LCMS 98%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.58 (s, 1H), 7.36 (d, J-8.8 Hz, 1 H), 7.32 -714 (m, 10 H), 4.02 (s, 2H), 2.92 (s,

2H), 1.12 (m, 2H), 0.81 (m, 2H). HRMS Calcd for $[C_{25}H_{24}NO_3]$+: 386.1756; found: 386.1768.



Crystallization and structure determination

sPLA2-X protein production, crystallization and structure determination

sPLA2-X protein expression and crystallization have been described (Chen et al). In brief, human recombinant sPLA2-X was produced in BL21(DE3) Gold *E. coli* strain as inclusion bodies. The protein was refolded for 4-5 days and enzymatic activity was checked checked every 12 h using a spectrofluorimetric assay until a plateau was reached. This was followed by purification using a Phenyl Sepharose HP column and the protein was dialyzed against 20 mM Tris/HCl pH 8, 5 mM $CaCl_2$ to yield the final product.

Crystals of sPLA2-X was obtained with the hanging drop vapor diffusion method by mixing equal amounts of sPLA2-X (10mg/ml) with ~40% PEG 400, 0.1 M Bis-Tris pH 6.0 at 20 ºC. To obtain the complex structure with compound **1** crystals were soaked with 5 mM compound in 45% PEG 400, 5 mM $CaCl_2$ and 0.1 M Bis-Tris pH 7.4. The crystals were flash-frozen in liquid nitrogen and data collected on a Rigaku rotating anode.

The data was processed with programs from the CCP4 suite (Winn, M.D. et al. *Acta. Cryst.* **2011**, D67, 235-242). The structure was solved by molecular replacement with MOLREP, using an in house sPLA2-X crystal structure as starting model. The structure was refined by alternating cycles of manual rebuilding in Coot (Emsley, P. et al. *Acta. Cryst.* ***2004,*** D**60**, 2126-2132) and automated refinement with Refmac5 (Murshudov, G.N. et al. *Acta. Cryst*. **1997**, D53, 240-255). The final model contained two protein molecules with one calcium ion

and one copy of compound **1** bound in each active site. Details on data collection and refinement are available in Table S1.

sPLA2-IIa protein production, crystallization and structure determination

Expression and purification of ^{15}N labeled human sPLA2-IIa double mutant (N1A, T76S):

An overnight culture of vector pET24a-sPLA2 transformed BL21(DE3) cells, in supplemented minimal medium (M9 + Martek Celtone, glucose, $MgSO_4$, $CaCl_2$, Kanamycin) was added to a 20 L fermentor (Braun) with the same medium supplemented with antifoam. Harvest was done 4 h post-induction by centrifugation and the cell paste was stored at -80 ˚C. The cell pellet was thawed and resuspended in buffer (50 mM Tris/HCl, pH 8.3, 10 mM EDTA, 1 % Trition-X100) and disintegrated by Turrax and High-Pressure homogenizer followed by centrifugation. The cell pellet was washed with water and dissolved in solubilisation buffer (6 M Guanidine/HCl, 120 mM Borate, pH 8.5). After stirring for 2 hours the slurry was centrifugated and the supernatant was diluted 1:6 in refolding buffer (12 mM $CaCl_2$, 1.2 mM glutathione reduced (GSH), 1.2 mM glutathione oxidized (GSSG), pH 8.5). After 3 days in room temperature, the sample was concentrated with a Cross flow device (PALL) and then dialysed against 40 mM NaAc, pH 4 for 3 days in room temperature. The dialysate was purified by cation exchange chromatography with batch adsorption on Source 30 S resin (GE), eluted with a linear gradient over 30 column volumes. Fractions were pooled based on activity assay data. A buffer exchange step was done Superdex G-25 column (20 mM Hepes, pH 7.5), followed by a second cation exchange chromatography step on a Mono S column. Purified sPLA2 was eluted with a 20 column volume linear gradient with 20 mM Hepes, pH 7.5, 1.5 M KCl. Fractions were again pooled based on activity assay data. A final buffer exchange step was done on Sephadex G-25 (10 mM NaAc, pH 5.9, 50 mM NaCl, 50 mM $CaCl_2$).

Crystallization and structure determination:

The ^{15}N labeled sPLA2-IIa (N1A, T76S) was buffer exchanged and concentrated to 23.5 mg/ml in 1M NaCl, 10mM Tris pH 7.5, 10mM $CaCl_2$ and was crystallized in 1+1 µL drops in an EasyXtal Tool plate (Qiagen). Crystals grew after streak seeding in a grid of 3.0-3.5M sodium formate and 100mM HEPES pH 7.5 at 20 °C. All crystallization reagents were from Hampton Research. The crystals were soaked for more than 72 hours in a drop containing 10 mM of compound 4 dissolved in deuterated DMSO. Data was collected on an FR-E+ generator (Rigaku) using a Rigaku A200 detector. Details on data collection are available in Table S1. The protein crystallized in space group $P2_12_12_1$ with two molecules per asymmetric unit. The data was processed with Mosflm (Leslie, A. et al 2007)) and Scala (Winn, M. D. *et al*. 2011, Evans, P.R. 2006). The structure was solved by molecular replacement using rigid body refinement in the program Refmac5 (Murshudov, G. N. et al 1997)) using an in house sPLA2-IIa structure as starting model. Manual refinement was perfomed using the program Coot (Emsley, P. & Cowtan, K. 2004) with subsequent cycles of Refmac5 and Autobuster (Bricogne, G. et al 2010) refinement. Waters were added in Coot. Compound **4** could be unambiguously placed into the difference electron density in the active site of each subunit during the last cycles of refinement. Details on refinement are summarized in Table S1. The final model contained two copies of sPLA2-IIa (residues 1-123) with one inhibitor molecule and two calcium ions bound in each subunit.

Coordinates and structure factors

The coordinates and structure factors have been deposited in the Protein Data Bank with the accession codes 5g3m ($sPLA_2$-X:1) and 5g3n ($sPLA_2$-IIa).

sPLA2 assays

Human plasma sPLA2 inhibition assay

1.2 µl compound in DMSO, 0-10 µM final assay concentration, was preincubated for 10 minutes at 37 ^oC with 28.8 µl human heparin plasma pool diluted 1:8 in assay buffer 10mM TRIS-HCl pH 9.0, 10 mM $CaCl_2$ containing 0.1% FFA free BSA, Sigma (USA) in a 384 black well plates, Greiner (UK). 20 µl HPM substate: 1-Hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycero-3-phosphomethanol, Na-salt, FluoProbes, FP-31900A, Interchim (France) were added to each well, final assay concentration 6 µM. The assay where performed using ORCA Robotic system, Beckman Coulter (USA). Kinetics was monitored at room temperature up to 90 minutes using Molecular Devices Paradigm (USA) by measuring emitted light at 370 nm and exciting at 340 nm, inhibition of sPLA2 enzyme leads to a decreased fluorescence and 50 minute time point was used to calculate dose response IC50.

Human heparin plasma pool collected from healthy volunteers at AstraZeneca R&D after Ethical Committee approval and signed informed consent from donors.

sPLA2 inhibition assay using phosphatidylcholine as substrate

sPLA2 activity, i.e. the formation of free fatty acids, was determined with a NEFA C kit (Wako Chemicals GmbH), according to the following procedure. A substrate solution was prepared by dissolving 50 mg of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids 850457P) in 1ml 4% NonidetP40 (USB) and 2% deoxycholic acid (Sigma D6750)). 19ml of 0.12M Tris-Hcl pH 8.0 containing 12mM $CaCl_2$, and 0.1mM EDTA and BSA (Sigma A8806) to a final concentration of 0.1% was added and the solution was thorough vortexed. Before use the substrate solution was incubated at 37 °C for 30 min.

Recombinant sPLA2-IIa, -5 and -10 (in-house produced) was diluted to a concentration of 0.69 µg/ml (final assay conc. 28 nM) for type IIa and -X, and 0.34 µg/ml (final assay conc. 14nM) for type V in 50mM Tris-HCl pH 8.0 with 5 mM $CaCl_2$ and BSA to a final concentration of 0.1%.

To identify compounds that act as antagonists to the different sPLA2 subtypes the following protocol was applied. 0.6 µl compound in DMSO, in a 10 point 1/3 serial dilution/compound +14.5 µl of the respective enzyme solution was added to a 384-well plate (Greiner 781101) and the plates were incubated at 37 °C for 20 min. After the incubation 10 µl of the pre-incubated substrate solution was added, followed by a further incubation of 60 min at 37 °C. 60 µl of NEFA R1 from the NEFA kit was added to each well of the plates and incubated for 10 min at RT. Finally 30 µl of NEFA R2 was added and incubated for another 5 min at RT, and after that the absorbance was measured at 546 nM.

The average of the control wells included in the plate was used as Min (fully activated target) and Max (fully inhibited target). Curve fitting was performed using a Four Parameter Logistic equation in a non-linear regression model and estimation of IC_{50} values was made for test compounds.

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Table S1. Data collection and refinement statistics

	sPLA2-IIa:compound 4	sPLA2-X:compound 1
Data collection		
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions	*a*=52.1 Å, *b*=66.5 Å, *c*=73.8 Å	*a*=27.5 Å, *b*=85.1 Å, *c*=103.3 Å
Resolution (Å)	24.6 – 1.8 (1.85-1.8)[1]	103.3 – 1.8 (1.94-1.84)
R_{merge}	0.106 (0.516)	0.102 (0.655)
$\langle I / \sigma I \rangle$	6.3 (1.9)	12.1 (2.5)
Completeness (%)	96.8 (95.8)	96.3 (76.9)
Redundancy	2.5 (2.5)	5.0 (4.0)
Refinement		
Measured / unique refl.	59464 / 23401	105840 / 21292
R_{work} / R_{free}	0.195/ 0.231	0.199/0.232
No. atoms		
Protein	1947	1920
Water	227	101
Ligand	54	32
Ca^{2+}	4	2
Average *B*-factors		
Protein ($Å^2$)	24.9	19.6
Water ($Å^2$)	34.3	28.4
Ligand ($Å^2$)	19.3	19.9
Ca^{2+} ($Å^2$)	32.8	15.9
Ramachandran outliers (%)	0.9 (generously allowed)	0
R.m.s deviations		
Bond lengths (Å)	0.011	0.007
Bond angles (°)	1.1	1.3

[1] Values in parentheses refer to highest-resolution shell.

Table 3 details

Table 3[a]. Profile of compound 4.

Solubility (pH=7.4) (μM)[a]	98	
P_{app} (10^{-6} cm/s)[b]	40.1	
HEP Cl_{int} (μL/min/10^{-6} cells)[c]	5.2	
hERG, $Na_v1.5$, IKs, $K_v4.3$, $Ca_v3.2$, $Ca_v1.2$ IC_{50} (μM)[d]	>33.3	
"Worst" CYP450 IC_{50} (μM)[e]	>20	
OATP1B1 IC_{50} (μM)[f]	2.2	
PK[g]	Rat	Dog
Dose i.v./p.o. (μmol/kg)	2/4	1/2
CL (mL/min/kg)	1	0.3
V_{ss} (L/kg)	0.22	0.26
F (%)	81	82

[a]DMSO/HBSS solubility measured at pH=7.4 Compounds, dissolved in DMSO and stored in 96-well plates, are transferred and diluted with buffer into new plates. The plates are shaken for 24 hours, then filtered, and the filtered solutions are analysed to give an estimate of the solubility. As standards for the concentration estimations, samples with the same degree of dilution are prepared, but using organic solvent (ethanol, acetonitril, etc). The diluted samples, and the standards are analysed with LC-UV/MS.

[b]Permeability measured in Caco-2 cells in the A to B direction, pH=6.5. Please see for more details: Over B, McCarren P, Artursson P, Foley M, Giordanetto F, Grönberg G, Hilgendorf C, Lee MD,4th, Matsson P, Muncipinto G, Pellisson M, Perry MW, Svensson R, Duvall JR, Kihlberg J. Impact of stereospecific intramolecular hydrogen bonding on cell permeability and physicochemical properties. J Med Chem 57(6):2746-2754 (2014).

[c]Intrinsic clearance of test compounds after incubation with human hepatocytes. Hepatic Clint assessment of compounds: Incubation is done at 1 μM substrate concentration and 1 million cells/ml. The incubation media used is William's E supplemented with 25 mM HEPES and 2 mM L-glutamine and set to pH 7.4. The assay is run in 96-well plates where each plate represents a single time point and the time points used are normally 2, 15, 30, 45 and 60 min. The incubation is made at 37°C (5% CO_2 and >80% humidity). The incubation mixture is quenched with three volumes of stop solution. The assay data is run through Activity Base and the protocol is designed to be flexible and more than one specie can be combined on the same plate. The blank samples are handled on a separate plate.

[d]Patch clamp assay using IONWORKS™ technology in CHO cells expressing the relevant human ion channel.

[e]Inhibition of metabolic degradation of the corresponding substrate by human recombinant cytochrome P450s (3A4, 2C9, 2C19, 2D6, 1A2, 2C8) at 37 ºC. The percent inhibition is determined at five different concentrations and reported as IC_{50}.

[f]Inhibition of pivastatin uptake to HEK293 cells transfected with human OATP1B1. [g]Pharmacokinetic parameters calculated from noncompartmental analysis concentrations in fasted Sprague Dawley rats and Beagle dogs (i.v./p.o. N=2).

Abbrevations (se also Table 3 definitions)

Bpin: Bis(pinacolato)diboron

DMF: Dimethyl formamide

F_u: Fraction unbound, calculated as 100-human protein binding(%)

HEK293: Human Embryonic Kidney 293 cells

HMG-CoA: 3-Hydroxy-3-methylglutaryl-coenzyme A

HTS: High Throughput Screen

LE: Ligand efficiency, calculated as -RTln($sPLA_2$-IIa IC_{50}) / Heavy Atom Count

LLE: Ligand Lipophilicity efficiency, calculated as pIC_{50} ($sPLA_2$-IIa) – logD.

nBuLi: normal butyl lithium

OATP1B1: organic anion-transporting polypeptide 1B1

$P_{app.}$ Permeability measured in Caco-2 cells in the A to B direction, pH=6.5.PK: Pharmacokinetic

PD: Pharmacodynamic

$PdCl_2$(dppf): 1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane

$PdCl_2$(dbpf): 1,1'-BIS(DI-TERT-BUTYLPHOSPHINO)FERROCENE PALLADIUM DICHLORIDE

$sPLA_2$: Secreted phospholipase A_2

THF: Tetrahydrofuran