SUPPORTING INFORMATION

Novel carboxamide-based allosteric MEK inhibitors: discovery and optimization efforts toward XL518 (GDC-0973)

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Chemistry Experimental Section

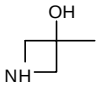
All reagents and solvents were purchased commercially and used without further purification unless otherwise indicated. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian Mercury Plus 400 MHz instrument. Chemical shifts are reported in parts per million (ppm) relative to an internal standard of tetramethylsilane in $CDCl_3$, CD_3OD or d_6-DMSO. Melting points were determined on a Barnstead Electrothermal melting point apparatus model 9100 and are uncorrected. Elemental analyses were conducted by Robertson Microlit Laboratories, Madison NJ. Positive ion FAB high resolution mass spectrometry was conducted by Analytical Instrument Group, Inc., Raleigh NC. The following purity and identity criteria were employed. For Compound (**1**) purity criteria included: i) Purity of 99% or greater with no single impurity present in excess of 0.5% as determined by analytical HPLC at a detector wavelength of 254 nm. ii) Elemental analysis to within 0.4% for C, H, N, F. Identity criteria for (**1**) included: i) Proton and Carbon NMR spectra consistent with assigned structure. ii) Low resolution (EI) MS and high resolution FAB MS consistent with assigned molecular formula. iii) Single molecule crystal structure consistent with assigned absolute stereochemistry. The optical purity of (**1**) was determined for the BOC-protected derivative 1,1-dimethylethyl (2S)-2-[1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)-3-hydroxyazetidin-3-yl]piperidine-1-carboxylate using chiral analytical HPLC and was consistently determined to be >98% enantiomeric excess. For compounds (**8**), (**12**), (**13**) and (**18**) purity criteria included >95% purity as determined by analytical HPLC at a detector wavelength of 254 nm and identity criteria included: i) Proton NMR spectra consistent with assigned structure. ii) Low resolution (EI) MS and high resolution FAB MS consistent with assigned molecular formula. For compounds (**3**), (**6**), (**7**), (**9-11**), (**15-17**) and (**19**) purity criteria included >95% purity as determined by analytical HPLC at a detector wavelength of 254 nm and identity criteria included: i) Proton NMR spectra consistent with assigned structure. ii) Low resolution (EI) MS consistent with assigned molecular formula.

Analytical HPLC was performed using a Shimadzu LC-10AT VP system equipped with a Shimadzu SPD-M10A VP diode array detector. Low resolution reverse phase analytical HPLC mass spectrometry was conducted on either a Waters Alliance HT 2795 Separations system equipped with a 2996 photodiode array detector and Micromass ZMD ESI mass detector or an Agilent 1100 series LC/MSD system. The Shimadzu analytical system is equipped with a YMC-Pack Pro 150x4.6 mm, 5µ C18 reverse phase

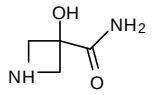
column and employs a two component mobile phase as follows: Component A (0.05% trifluoroacetic acid in water), Component B (0.1% trifluoroacetic acid in acetonitrile). The analytical method employed to establish purity criteria uses a 5-95% B gradient over 25 minutes at a 1.5 mL/min. flow rate and total run time of 27 minutes or a 5-95% B gradient over 18 minutes at a 1.5 mL/min. flow rate and total run time of 20 minutes. The Waters/Micromass system is equipped with a Phenomenex 30x2.0 mm, Synergi MAX-RP 4μ C18 reverse phase column and operates using a two component mobile phase as follows: Component A (0.05% trifluoroacetic acid in water), Component B (0.1% trifluoroacetic acid in acetonitrile). The analytical method employed uses a 5-95% B gradient over 6.5 minutes at a 1.5 mL/min. flow rate and total run time of 7 minutes. The Agilent system is equipped with an Agilent Eclipse XDB-C18 30x4.6 mm, 3.5μ C18 reverse phase column and operates using a two component mobile phase as follows: Component A (25 mM ammonium acetate in water), Component B (acetonitrile). The analytical method employed uses a 20-90% B gradient over 2 minutes at a 2 mL/min**.** flow rate and total run time of 3 minutes.

The chiral analytical HPLC methods employed in the determination of optical purity operate using a two component mobile phase component A (water), Component B (acetonitrile) as follows: Method 1 employs a Chiralcel OD-RH 150x4.6 mm cellulose polymer coated silica column using a 45-55% B gradient over 50 minutes at a 1 mL/minute flow rate and total run time of 50 minutes. Method 2 employs a Chiralcel OD-RH 250x10 mm cellulose polymer coated silica column using a 40-75% B gradient over 80 minutes at a 2 mL/minute flow rate and total run time of 85 minutes.

Synthesis of azetidine precursors:



3-methylazetidin-3-ol may be purchased commercially or prepared according to the literature method.[1]



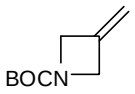
3-Hydroxyazetidine-3-carboxamide

1-(Diphenylmethyl)azetidin-3-ol hydrochloride (2.75 g, 9.98 mmol), 3Å molecular sieves and 4-methylmorpholine (1.1 mL, 10.0 mmol) were suspended in dichloromethane (20 mL) and cooled in an ice-water bath. 4-Methylmorpholine *N*-oxide (2.93 g, 25.0 mmol) and tetrapropylammonium perruthenate (140 mg, 0.399 mmol) were added and the mixture was stirred at ambient for 24 h. The mixture was filtered through a plug of silica using 5% triethylamine in ethyl acetate as eluent. The filtrate was concentrated *in vacuo* and the residue was partitioned between ethyl acetate and saturated sodium bicarbonate solution. The organic portion was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. Column chromatography (silica gel, 8:1 hexanes:ethyl acetate) gave 1-(diphenylmethyl)azetidin-3-one (871 mg, 3.68 mmol, 37% yield): ^{1}H NMR (400 MHz, $CDCl_3$): 7.50-7.46 (m, 4H), 7.33-7.27 (m, 4H), 7.27-7.19 (m, 2H), 4.59 (s, 1H), 4.01 (s, 4H)**;** MS (EI) for $C_{16}H_{15}NO$: 238 (MH^+).

1-(Diphenylmethyl)azetidin-3-one (600 mg, 2.53 mmol), was dissolved in dichloromethane (1 mL) and treated with triethylamine (0.5 mL, 3.59 mmol) and trimethylsilylcyanide (0.8 mL, 6.01 mmol) at ambient for 2 h and then the mixture was concentrated *in vacuo* to afford 1-(diphenylmethyl)-3-[(trimethylsilyl)oxy]azetidine-3-carbonitrile (774 mg, 2.30 mmol, 91% yield) as a yellow solid. 1-(diphenylmethyl)-3-[(trimethylsilyl)oxy]azetidine-3-carbonitrile as obtained (250 mg, 0.744 mmol) was dissolved in dichloromethane (2 mL) and cooled in an ice-ater bath then concentrated sulfuric acid (0.2 mL) was added drop-wise. The mixture was stirred at ambient for 2 h and then was cooled in an ice-water bath and 25% ammonium hydroxide solution was carefully added drop-wise to pH 10-11. The mixture was extracted twice with dichloromethane. The combined organic portion was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo* to afford a residue which was triturated with hexanes/ether to afford 1-(diphenylmethyl)-3-hydroxyazetidine-3-carboxamide (160 mg, 0.567 mmol, 76% yield) as an off-white solid: ^{1}H NMR (400

MHz, $CDCl_3$): 7.92 (br s, 1H), 7.39-7.34 (m, 4H), 7.33-7.27 (m, 4H), 7.27-7.19 (m, 2H), 5.61 (br s, 1H), 4.45 (s, 1H), 4.34 (s, 1H), 3.50 (dd, 2H), 3.20 (dd, 2H)**;** MS (EI) for $C_{17}H_{18}N_2O_2$: 283 (MH^+).

1-(Diphenylmethyl)-3-hydroxyazetidine-3-carboxamide as obtained was hydrogenated using 20 wt% palladium hydroxide on carbon in methanol at 40 psi using a Parr apparatus according to the literature method to afford 3-hydroxyazetidine-3-carboxamide as the hydrochloride salt.[2] MS (EI) for $C_4H_8N_2O_2$: 117 (MH^+).



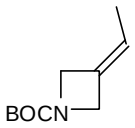
1,1-dimethylethyl 3-methylideneazetidine-1-carboxylate (**21**)

A mixture of 3-azetidinol hydrochloride (10 g, 91 mmol), di-*tert*-butyl dicarbonate (18.8 g, 86.3 mmol) and sodium bicarbonate (15.3 g, 182 mmol) in dioxane:water (400 mL, 1:1) was stirred at room temperature for 15 hours. The organic portion was removed *in vacuo* and the aqueous portion was extracted with ethyl acetate three times. The combined organic portion was washed with 5% aqueous HCl, water, brine, dried over sodium sulfate, filtered and concentrated *in-vacuo* to afford 12.8 g, 74 mmol (81%) of 1,1-dimethylethyl 3-hydroxyazetidine-1-carboxylate as a colorless oil that was used without further purification. ^{1}H NMR (400 MHz, DMSO): 5.62 (d, 1H), 4.40-4.33 (m, 1H), 4.02-3.95 (m, 2H), 3.62-3.54 (m, 2H), 1.37 (s, 9H). GCMS for $C_8H_{15}NO_3$: 173

A solution of oxalyl chloride (545 µl, 6.36 mmol) in dichloromethane (25 mL) was cooled to -78°C. While maintaining an internal temperature of -78°C, the drop-wise addition of DMSO (903 µl, 12.7 mmol) followed by 1,1-dimethylethyl 3-hydroxyazetidine-1-carboxylate (1 g, 5.78 mmol in 30 mL of dichloromethane) and finally triethylamine (3.25 mL, 23.1 mmol in DCM 20 mL) was performed. The mixture was allowed to warm to room temperature and was stirred for 15 hours. The reaction mixture was diluted with water and partitioned. The organic portion was washed twice with water then the combined aqueous portion was extracted once with dichloromethane.

The combined organic portion was washed with brine, dried over sodium sulfate, filtered and concentrated to afford a yellow oil which was purified by silica gel column chromatography (30% ethyl acetate in hexanes) to afford 1,1-dimethylethyl 3-oxoazetidine-1-carboxylate (893 mg, 90%) of as a colorless oil which solidified upon standing. ^{1}H NMR (400 MHz, DMSO): 4.67 (s, 4H), 1.42 (s, 9H). GCMS for $C_8H_{13}NO_3$: 171

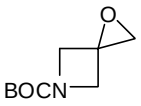
A mixture of potassium *tert*-butoxide (15.5 g, 137 mmol) and methyltriphenylphosphine bromide (49 g, 137 mmol) in diethyl ether (300 mL) was stirred at room temperature for 1 hour, followed by the addition of 1,1-dimethylethyl 3-oxoazetidine-1-carboxylate (10 g, 58 mmol in 100 mL diethyl ether). The mixture was stirred at 35°C for 2 hours and then allowed to cool to room temperature. The mixture was filtered through a pad of celite and the filtrate was partitioned with water. The organic solution was washed twice with water, brine, dried over sodium sulfate then filtered and concentrated to give an orange oil which was purified by silca gel column chromatography (10% ethyl acetate in hexanes) to afford 1,1-dimethylethyl 3-methylideneazetidine-1-carboxylate (9.80 g, 100%) of as a colorless oil. ^{1}H NMR (400 MHz, d_6-DMSO): 5.05-4.85 (m, 2H), 4.95-4.63 (m, 4H), 1.45 (s, 9H). GCMS for $C_9H_{15}NO_2$: 169.



1,1-dimethylethyl 3-ethylideneazetidine-1-carboxylate (**22**)

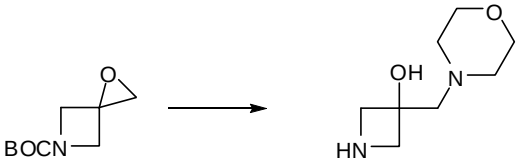
Potassium *tert*-butoxide (1.672 g, 14.9 mmol) and ethyltriphenylphosphonium bromide (5.538 g, 14.9 mmol) were stirred in ether (30 mL) at amibient for 1 h. 1,1-Dimethylethyl 3-oxoazetidine-1-carboxylate (954 mg, 6.0 mmol) was added and the mixture was warmed to 35 C for 4.5 h. The mixture was filtered through celite and the filtrate was washed with water, brine, dried over anhydrous sodium sulfate, filtered and concentrated. Silica gel column chromatography (20% ethyl ether in hexanes) gave 1,1-

dimethylethyl 3-ethylideneazetidine-1-carboxylate (506 mg, 49%). ^{1}H NMR (400 MHz, $CDCl_3$): 5.37-5.28 (m, 1H), 4.47-4.39 (m, 4H), 1.56-1.51 (m, 3H), 1.45 (s, 9H).



1,1-dimethylethyl 1-oxa-5-azaspiro[2.3]hexane-5-carboxylate (**23**)

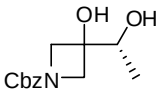
To a solution of (**21**) (2.96 g, 17.5 mmol) in chloroform (180 mL) was added 3-chloroperoxybenzoic acid (77%, 13.9 g, 62.0 mmol), and the resulting mixture was stirred at room temperature for 2 days. The reaction mixture was quenched with a 1:1 mixture of 10% aqueous sodium thiosulfate and saturated sodium bicarbonate solutions (150 mL). The organic portion was separated, dried over sodium sulfate, filtered and concentrated to give an oily residue which was then purified by silica gel column chromatography (15-50% ethyl acetate in hexanes) to give 1,1-dimethylethyl 1-oxa-5-azaspiro[2.3]hexane-5-carboxylate (1.65g, 51%). GCMS for $C_9H_{15}NO_3$: 185.



General procedure for amination of epoxide (**23**):

1,1-Dimethylethyl 1-oxa-5-azaspiro[2.3]hexane-5-carboxylate (51 mg, 0.28 mmol.) was taken into THF (1 mL) followed by addition of morpholine (123 μL, 1.4 mmol.) and the mixture was stirred for one hour at room temperature. The solution was then concentrated and the residue partitioned with ethyl acetate and water. The organic layer was washed once with water then brine and the organic layer dried over anhydrous sodium sulfate. Filtration and concentration gave a colorless oil that was purified by silica gel flash chromatography using ethyl acetate then 10% methanol in dichloromethane as eluent. The combined pure fractions were concentrated and the

residue treated with neat TFA (1 mL) for 5 minutes then concentrated. The residue was taken into methanol (2 mL) and basified to pH > 10 by addition of Biorad AG-1X hydroxide form resin. Filtration and concentration afforded 3-(morpholin-4-ylmethyl)azetidin-3-ol (11.6 mg, 24% yield) as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD): 3.69-3.66 (m, 4H), 3.55 (d, 2H), 3.49 (d, 2H), 2.66 (s, 2H), 2.57-2.55 (m, 4H).

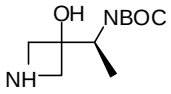


Phenylmethyl 3-hydroxy-3-[(1*R*)-1-hydroxyethyl]azetidine-1-carboxylate (**25**)

AD-mix-β (7 g) was dissolved in *tert*-butanol (25 mL) and water (25 mL) and methanesulfonamide (519 mg, 5.46 mmol) was added. The mixture was cooled in an ice-water bath and 1,1-dimethylethyl 3-ethylideneazetidine-1-carboxylate (**22**) (1 g, 5.46 mmol) was added. The mixture was stirred with cooling in an ice-water bath for 8 h and then at ambient for 15 h. The mixture was again cooled in an ice-water bath and treated with sodium sulfite (7 g) then the mixture was stirred at ambient for 4 days. The mixture was partitioned between ethyl acetate and 2N sodium hydroxide solution. The aqueous portion was extracted with ethyl acetate (3x). The combined organic portion was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. Silica gel column chromatography (70-80% ethyl acetate in hexanes) gave 1,1-dimethylethyl 3-hydroxy-3-[(1*R*)-1-hydroxyethyl]azetidine-1-carboxylate (1.035 g, 4.77 mmol, 87% yield): ^{1}H NMR (400 MHz, $CDCl_3$): 4.00-3.77 (m, 5H), 2.79 (br, 1H), 2.00 (br, 1H), 1.44 (s, 9H), 1.25 (d, 3H).

1,1-Dimethylethyl 3-hydroxy-3-[(1*R*)-1-hydroxyethyl]azetidine-1-carboxylate (600 mg, 2.76 mmol) was dissolved in methanol (5 mL) and treated with 4N hydrogen chloride in dioxane (1 mL) at reflux for 15 minutes then cooled to RT and concentrated. The residue was dissolved in dioxane:water (1:1; 10 mL). Sodium bicarbonate (465 mg, 5.54 mmol) was added and the mixture was cooled in an ice-water bath. Benzyl chloroformate (0.4 mL, 3.0 mmol) was added drop-wise and the mixture was stirred at ambient for 15 h. The mixture was concentrated to remove dioxane and the residual

aqueous portion was extracted with ethyl acetate (3x). The combined organic portion was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. Silica gel column chromatography (80% ethyl acetate in hexanes) gave phenylmethyl 3-hydroxy-3-[(1*R*)-1-hydroxyethyl]azetidine-1-carboxylate (650 mg, 94%): ^{1}H NMR (400 MHz, $CDCl_3$): 7.37-7.28 (m, 5H), 5.10 (s, 2H), 4.05-3.85 (m, 5H), 2.67 (br s, 1H), 1.83 (br d, 1H), 1.24 (d, 3H); MS (EI) for $C_{13}H_{17}NO_4$: 252 (MH^+).



1,1-dimethylethyl [(1*S*)-1-(3-hydroxyazetidin-3-yl)ethyl]carbamate (**26**)

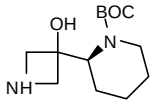
Phenylmethyl 3-hydroxy-3-[(1*R*)-1-hydroxyethyl]azetidine-1-carboxylate (**25**) (643 mg, 2.56 mmol) was dissolved in dichloromethane (15 mL) and pyridine (0.45 mL, 5.58 mmol) and cooled in an ice-water bath. Thionyl chloride (0.25 mL, 3.46 mmol) was added drop-wise and then the mixture was heated at reflux for 0.5 h. The mixture was cooled to ambient and thionyl chloride (0.1 mL, 1.38 mmol) was added drop-wise and the mixture was heated at reflux for a further 0.5 h then was concentrated. The residue was partitioned between ethyl ether and water and the aqueous portion was extracted once with ethyl ether. The combined organic portion was washed with 0.5M hydrochloric acid, brine, dried over anhydrous sodium sulfate, filtered and was concentrated to afford a colorless oil which was dissolved in acetonitrile (10 mL) and cooled in an ice-water bath. Ruthenium (III) chloride hydrate (5 mg) was added followed by sodium periodate (691 mg, 3.23 mmol) and water (10 mL). The mixture was stirred at ambient for 2 h and then was extracted with ethyl ether (3x). The combined organic portion was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. Silica gel column chromatography (100% ethyl ether) gave phenylmethyl (8*R*)-8-methyl-5,7-dioxa-6-thia-2-azaspiro[3.4]octane-2-carboxylate 6,6-dioxide (670 mg, 84%): ^{1}H NMR (400 MHz, $CDCl_3$): 7.41-7.32 (m, 5H), 5.12 (s, 2H), 5.05 (q, 1H), 4.49 (dd, 1H), 4.35 (qd, 2H), 4.11 (dd, 1H), 1.66 (d, 3H); GCMS for $C_{13}H_{15}NO_6S$: 313 (M^+).

Phenylmethyl (8*R*)-8-methyl-5,7-dioxa-6-thia-2-azaspiro[3.4]octane-2-carboxylate 6,6-dioxide (614 mg, 1.96 mmol), was dissolved in *N*,*N*-dimethylformamide (5 mL) and was treated with sodium azide (255 mg, 3.92 mmol) at 60 C for 1.5 h. Ethyl ether (10 mL) and 20% aqueous sulfuric acid (10 mL) were added and the mixture was stirred at ambient for 22 h. The aqueous portion was extracted with ethyl ether (3x). The combined organic portion was washed with water, brine, dried over anhydrous sodium sulfate, filtered and was concentrated. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate 1:1) to provide phenylmethyl 3-[(1*S*)-1-azidoethyl]-3-hydroxyazetidine-1-carboxylate as a colorless oil (412 mg, 77%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.30 (m, 5H), 5.10 (s, 2H), 4.02 (d, 1H), 3.94 (d, 1H), 3.89 (d, 1H), 3.87 (q, 1H), 2.36 (s, 1H), 1.34 (d, 3H).

To a solution of phenylmethyl 3-[(1*S*)-1-azidoethyl]-3-hydroxyazetidine-1-carboxylate (412 mg, 1.5 mmol) in THF (5 mL) and water (5 mL) was added triphenylphosphine (433 mg, 1.65 mmol). The mixture was heated to 70 ^{o}C for 1 h then cooled to RT. Water and ethyl acetate were added and the layers were partitioned. The aqueous phase was extracted three times with ethyl acetate and the combined organic extracts were dried over magnesium sulfate, filtered, and concentrated. The residue was dissolved in THF (5 mL), followed by addition of di-*tert*-butyl dicarbonate (502 mg, 2.3 mmol). The solution was stirred at rt for 2 h then concentrated. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate 1:1) to provide phenylmethyl 3-[(1*S*)-1-({[(1,1-dimethylethyl)oxy]carbonyl}amino)ethyl]-3-hydroxyazetidine-1-carboxylate as colorless oil (429.2 mg, 82% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36-7.29 (m, 5H), 5.10 (m, 2H), 4.77 (br s, 1H), 4.04 (d, 1H), 3.92 (d, 1H), 3.89-3.77 (m, 3H), 1.44 (s, 9H), 1.20 (d, 3H).

To a solution of phenylmethyl 3-[(1*S*)-1-({[(1,1-dimethylethyl)oxy]carbonyl}amino)ethyl]-3-hydroxyazetidine-1-carboxylate (429.2 mg, 1.2 mmol) in methanol (10 mL) was suspended 10% palladium on carbon (wet, 250 mg), and the mixture was hydrogenated (1 atm) for 1 h. The catalyst was removed by filtration and the filtrate was concentrated to provide 1,1-dimethylethyl [(1*S*)-1-(3-hydroxyazetidin-3-yl)ethyl]carbamate as a colorless oil (263 mg, 1.2 mmol, quantitative yield). 1,1-Dimethylethyl [(1*R*)-1-(3-hydroxyazetidin-3-yl)ethyl]carbamate (**27**) was

prepared from (**22**) in the identical manner by use of AD-mix-α in the dihydroxylation sequence to give (**24**) then proceeding as above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.87 (m, 1H), 3.92 (m, 1H), 3.78 (d, 1H), 3.66 (d, 1H), 3.48 (d, 2H), 1.44 (s, 9H), 1.15 (d, 3H); MS (EI) for $C_{10}H_{20}N_2O_3$: 161 (MH^+-*t*-butyl).



1,1-dimethylethyl (2S)-2-(3-hydroxyazetidin-3-yl)piperidine-1-carboxylate (**30**)

To a solution of 1,1-dimethylethyl 2-(3-hydroxy-1-{[(phenylmethyl)oxy]carbonyl}azetidin-3-yl)piperidine-1-carboxylate (**28**) (368 mg, 0.94 mmol.) in dichloromethane (5 mL) was added DMAP (115 mg, 0.94 mmol.) and the resulting solution was cooled to 0^{o}C. (R)-(-)-α-Methoxy-α-trifluoromethylphenylacetyl chloride (105 μL, 0.56 mmol.) was added to the solution by syringe and the mixture was allowed to warm to room temperature then stirred an additional 12 hours. The solution was then partitioned with saturated aqueous sodium bicarbonate and the organic phase dried over anhydrous magnesium sulfate then filtered and concentrated to an oily residue. Silica gel flash chromatography using hexanes:ethyl acetate 3:1 as eluent afforded the less polar 1,1-dimethylethyl (2R)-2-(1-{[(phenylmethyl)oxy]carbonyl}-3-{[(2R)-3,3,3-trifluoro-2-(methyloxy)-2-phenylpropanoyl]oxy}azetidin-3-yl)piperidine-1-carboxylate (27.5 mg, 5% yield), the more polar 1,1-dimethylethyl (2S)-2-(1-{[(phenylmethyl)oxy]carbonyl}-3-{[(2R)-3,3,3-trifluoro-2-(methyloxy)-2-phenylpropanoyl]oxy}azetidin-3-yl)piperidine-1-carboxylate (105 mg, 19% yield) and starting material (253 mg, 69% recovery). The starting material thus recovered was taken into dichloromethane (3 mL) followed by addition of DMAP (115 mg, 0.94 mmol.) and (R)-(-)-α-methoxy-α-trifluoromethylphenylacetyl chloride (105 μL, 0.56 mmol.) and the mixture was allowed to stir at room temperature over 12 hours. Proceeding as before afforded combined 1,1-dimethylethyl (2R)-2-(1-{[(phenylmethyl)oxy]carbonyl}-3-{[(2R)-3,3,3-trifluoro-2-(methyloxy)-2-phenylpropanoyl]oxy}azetidin-3-yl)piperidine-1-carboxylate (46.6 mg, 8% yield), the more polar 1,1-dimethylethyl (2S)-2-(1-

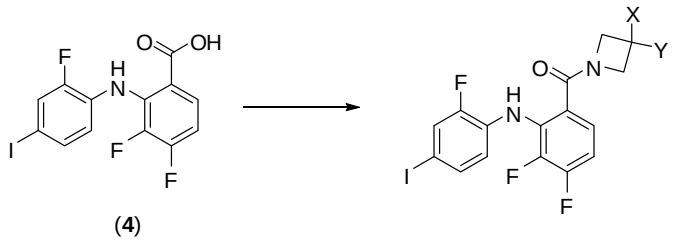
{[(phenylmethyl)oxy]carbonyl}-3-{[(2R)-3,3,3-trifluoro-2-(methyloxy)-2-phenylpropanoyl]oxy}azetidin-3-yl)piperidine-1-carboxylate (228 mg, 41% yield) and starting material (100.8 mg, 27% recovery). The starting material thus recovered was taken into tetrahydrofuran:dichloromethane (1:1, 2 mL) followed by addition of DMAP (47 mg, 0.39 mmol.) and (R)-(-)-α-methoxy-α-trifluoromethylphenylacetyl chloride (80 μL, 0.43 mmol.) and the mixture was heated to 60 ^{o}C over 12 hours. Proceeding as before afforded combined less polar 1,1-dimethylethyl (2R)-2-(1-{[(phenylmethyl)oxy]carbonyl}-3-{[(2R)-3,3,3-trifluoro-2-(methyloxy)-2-phenylpropanoyl]oxy}azetidin-3-yl)piperidine-1-carboxylate (144 mg, 26 % yield). The chiral ester derivatives thus obtained were again subject to silica gel flash chromatography using hexanes:ethyl acetate 3:1 as eluent to give the pure less polar 1,1-dimethylethyl (2R)-2-(1-{[(phenylmethyl)oxy]carbonyl}-3-{[(2R)-3,3,3-trifluoro-2-(methyloxy)-2-phenylpropanoyl]oxy}azetidin-3-yl)piperidine-1-carboxylate (122.8 mg, 22% yield) and the more polar 1,1-dimethylethyl (2S)-2-(1-{[(phenylmethyl)oxy]carbonyl}-3-{[(2R)-3,3,3-trifluoro-2-(methyloxy)-2-phenylpropanoyl]oxy}azetidin-3-yl)piperidine-1-carboxylate (177.6 mg, 32% yield) both as colorless amorphous residues.

1,1-Dimethylethyl (2S)-2-(1-{[(phenylmethyl)oxy]carbonyl}-3-{[(2R)-3,3,3-trifluoro-2-(methyloxy)-2-phenylpropanoyl]oxy}azetidin-3-yl)piperidine-1-carboxylate (177.6 mg) was taken into methanol (4 mL) followed by addition of 1M aqueous sodium hydroxide (1 mL) and the resulting solution was stirred for one hour at room temperature. The solution was then partitioned with ethyl acetate and 1M aqueous hydrochloric acid. The organic layer was washed with brine, dried over anhydrous magnesium sulfate then filtered and concentrated. The residue was purified by silica gel flash chromatography using hexanes:ethyl acetate 2:1 to give 1,1-dimethylethyl (2S)-2-(3-hydroxy-1-{[(phenylmethyl)oxy]carbonyl}azetidin-3-yl)piperidine-1-carboxylate (**29**) (87.4 mg, 75% yield). Analogously, 1,1-dimethylethyl (2R)-2-(3-hydroxy-1-{[(phenylmethyl)oxy]carbonyl}azetidin-3-yl)piperidine-1-carboxylate (60.8 mg, 81%) was obtained as a colorless amorphous solid.

1,1-Dimethylethyl (2R)-2-(3-hydroxy-1-{[(phenylmethyl)oxy]carbonyl}azetidin-3-yl)piperidine-1-carboxylate (60.8 mg, 0.16 mmol) and 1,1-dimethylethyl (2S)-2-(3-

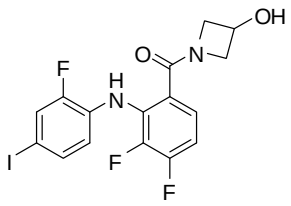
hydroxy-1-{[(phenylmethyl)oxy]carbonyl}azetidin-3-yl)piperidine-1-carboxylate (**29**) (87.4 mg, 0.23 mmol) were separately combined with 10% Pd/C (30 mg) and the mixtures taken into methanol (2 mL). Each was subject to hydrogenation at ambient pressure for one hour then filtered through a celite pad and the filtrate concentrated then dried *in vacuo* to afford 1,1-dimethylethyl (2S)-2-(3-hydroxyazetidin-3-yl)piperidine-1-carboxylate (**30**) and 1,1-dimethylethyl (2R)-2-(3-hydroxyazetidin-3-yl)piperidine-1-carboxylate as colorless solids. MS (EI) for $C_{13}H_{24}N_2O_3$: 201 (MH^+-*t*-butyl).

Synthesis of MEK inhibitors:



(**4**)

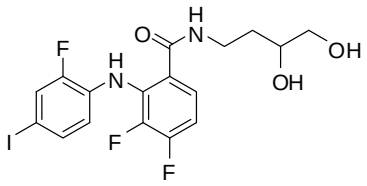
Method A: A general procedure is given below for the direct amide coupling of 3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino] benzoic acid (**4**) with the corresponding amine or azetidine under standard conditions was employed for the synthesis of compounds (**3**), (**6**), (**7**), (**9**), (**11**). 3,4-Difluoro-2-[(2-fluoro-4-iodophenyl)amino] benzoic acid was obtained according to the method described in the literature.[3]



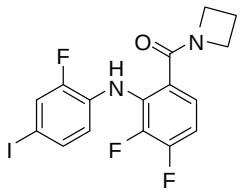
1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)azetidin-3-ol (**7**)

3,4-Difluoro-2-[(2-fluoro-4-iodophenyl)amino]benzoic acid (2.1 g, 5.3 mmol) was taken into DMF (10 mL) followed by addition of PyBOP (2.6 g, 5.3 mmol) and the mixture was allowed to stir at room temperature over 15 minutes. Azetidin-3-ol hydrochloride (870 mg, 8.0 mmol) and DIPEA (1.85 mL, 11.2 mmol) were then added and the mixture was allowed to stir an additional hour at room temperature. The mixture

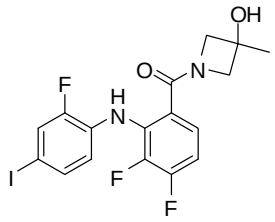
was then partitioned with ethyl acetate and 0.5 M aqueous sodium hydroxide solution. The organic layer was then washed with water (3x) then brine and dried over anhydrous sodium sulfate. Filtration and concentration followed by silica gel flash chromatography using ethyl acetate: hexanes (5:1) eluent afforded 1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)azetidin-3-ol (2.09 g, 87% yield) as a colorless amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): 8.47 (s, 1H), 7.39 (dd, 1H), 7.32 (d, 1H), 7.13-7.09 (m, 1H), 6.84-6.78 (m, 1H), 6.63-6.57 (m, 1H), 4.74-4.67 (m, 1H), 4.43-4.39 (m, 2H), 4.20-3.96 (br d, 2H), 2.50 (d, 1H). MS (EI) for $C_{16}H_{12}F_3N_2O_2$: 449 (MH^+).



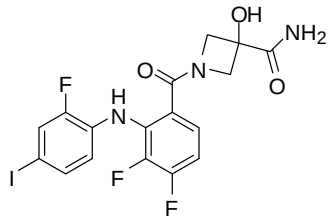
N-(3,4-Dihydroxybutyl)-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]benzamide (**3**): ^{1}H NMR (400 MHz, d_6-DMSO): 9.35 (s, 1H), 8.71 (tr, 1H), 7.60 (d, 1H), 7.55 (tr, 1H), 7.39 (d, 1H), 7.20 (dd, 1H), 6.70 (m, 1H), 3.51-3.45 (m, 1H), 3.39-3.21 (m, 4H), 1.74-1.67 (m, 1H), 1.48-1.39 (m, 1H); MS (EI) for $C_{17}H_{16}F_3IN_2O_3$: 481 (MH^+).



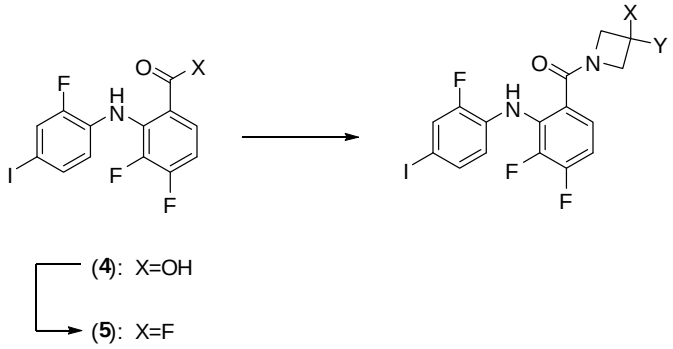
6-(Azetidin-1-ylcarbonyl)-2,3-difluoro-N-(2-fluoro-4-iodophenyl)aniline (**6**): ^{1}H NMR (400 MHz, $CDCl_3$): 8.57 (s, 1H), 7.41-7.38 (dd, 1H), 7.34-7.31 (dt, 1H), 7.13-7.09 (m, 1H), 6.83-6.77 (m, 1H), 6.64-6.58 (m, 1H), 4.27 (b, 2H), 4.18 (b, 2H), 2.38-2.30 (p, 2H); MS (EI) for $C_{16}H_{12}F_3IN_3O$: 433 (MH^+).



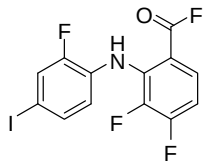
1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)-3-methylazetidin-3-ol (**9**): ^{1}H NMR (400 MHz, $CDCl_3$): 8.31 (br s, 1H), 7.40 (d, 1H), 7.33 (d, 1H), 7.15-7.11 (m, 1H), 6.85-6.78 (m, 1H), 6.65-6.59 (m, 1H), 4.24-4.04 (m, 4H), 1.55 (s, 3H); MS (EI) for $C_{17}H_{14}F_3IN_2O_2$: 463 (MH^+).



1-({3,4-Difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)-3-hydroxyazetidine-3-carboxamide (**11**): ^{1}H NMR (400 MHz, d_6-DMSO): 8.63 (br s, 1H), 7.58 (dd, 1H), 7.42-7.36 (m, 3H), 7.34-7.28 (m, 1H), 7.22-7.12 (m, 1H), 6.76-6.68 (m, 2H), 4.39 (d, 1H), 4.19 (d, 1H), 4.00 (d, 1H), 3.83 (d, 1H); MS (EI) for $C_{17}H_{13}F_3IN_3O_3$: 492 (MH^+).

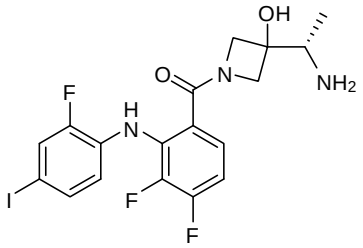


Method B: A procedure is given below for the preparation of benzoyl fluoride (**5**) and a general method for reaction with the corresponding azetidine to afford compounds (**1**), (**8**), (**12-19**).



3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino] benzoyl fluoride (**5**):

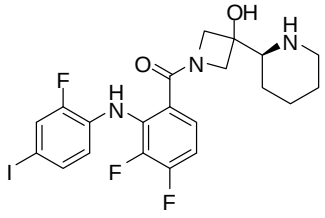
To a stirred mixture of 3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino] benzoic acid (12 g, 30.5 mmol) in dichloromethane (70 ml) at 0°C was added pyridine (2.5 ml, 30.8 mmol) followed by drop-wise addition of cyanuric fluoride (2.8 ml, 33.6 mmol). The reaction mixture was stirred at 0°C for 10 min. and then warmed to room temperature and stirred for 2 hours. The reaction mixture was diluted with water and extracted with dichloromethane (1x 100 ml). The aqueous layer was extracted once with dichloromethane (50 ml). The combined organic layers were washed with saturated aqueous sodium bicarbonate solution, brine, dried over anhydrous sodium sulfate then filtered and concentrated to give a brown solid. The residue was purified by silica gel flash chromatography (plug, 25% ethyl acetate in hexanes) to afford 3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino] benzoyl fluoride as a beige solid (11.8 g, 97% yield). ^{1}H NMR (400MHz, CD_3OD): 8.41 (s, 1H), 7.80-7.81 (m, 1H), 7.52 (dd, 1H), 7.43-7.47 (m, 1H), 6.96-7.03 (m, 1H), 6.85-6.92 (m, 1H).



3-[(1*S*)-1-aminoethyl]-1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)azetidin-3-ol (**18**):

To a solution of 1,1-dimethylethyl [(1*S*)-1-(3-hydroxyazetidin-3-yl)ethyl]carbamate (**26**) (263 mg, 1.2 mmol) in THF (8 mL) was added diisopropyethylamine (258 μL, 1.6 mmol) followed by 3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]benzoyl fluoride (**5**) (474 mg, 1.2 mmol). The mixture was stirred overnight at rt. After dilution with ethyl acetate, the solution was washed with 10% aqueous citric followed by saturated sodium bicarbonate. The organic extracts were dried over magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel flash chromatography (hexanes:ethyl acetate 1:1) to provide 1,1-dimethylethyl {(1*S*)-1-[1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)-3-hydroxyazetidin-3-yl]ethyl}carbamate as a white foam (564 mg, 0.95 mmol, 79% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (br s, 1H), 7.39 (dd, 1H), 7.32 (d, 1H), 7.12 (m, 1H), 6.81 (m, 1H), 6.61 (m, 1H), 4.73 (br s, 1H), 4.23 (d, 1H), 4.16-3.90 (br m, 3H), 3.77 (m, 1H), 1.43 (s, 9H), 1.18 (d, 3H); MS (EI) for $C_{23}H_{25}F_3IN_3O_4$: 590 (M-H).

A solution of 1,1-dimethylethyl {(1*S*)-1-[1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)-3-hydroxyazetidin-3-yl]ethyl}carbamate (564 mg, 0.95 mmol) in methanol (5 mL) was treated with hydrogen chloride in dioxane (4 N, 1.0 mL, 4.0 mmol). The mixture was heated to 55 ^{o}C for 2 h and was then cooled to rt. The resulting solution was concentrated and the residue purified by preparative reverse phase HPLC to provide 3-[(1*S*)-1-aminoethyl]-1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)azetidin-3-ol (**18**) as a solid (9.3 mg). ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.56 (s, 1H), 7.84 (br s, 3H), 7.60 (d, 1H), 7.39 (d, 1H), 7.35 (m, 1H), 7.21 (m, 1H), 6.69 (br s, 1H), 6.66 (br s, 1H), 4.31-3.95 (m, 3H), 3.80 (m, 1H), 3.49 (m, 1H), 1.11 (d, 3H); MS (EI) for $C_{18}H_{17}F_3IN_3O_2$: 492 (MH^+). High resolution positive ion FAB MS for $C_{18}H_{17}F_3IN_3O_2$: Calculated m/z 492.03954, Found m/z 492.03955 (MH^+).

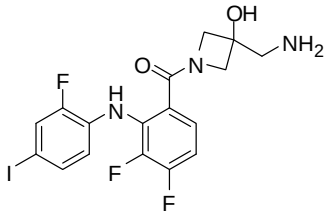


1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)-3-[(2S)-piperidin-2-yl]azetidin-3-ol (**1**): ^{1}H NMR (400 MHz, d_4-methanol): 7.49-7.46 (dd, 1H), 7.37-7.35(dt, 1H), 7.35-7.30 (m, 1H), 7.10-7.04 (m, 1H), 6.64-6.59 (m, 1H), 4.39-4.32 (dd, 1H), 4.21-4.18 (dd, 1H), 4.13-4.07 (m, 1H), 3.97-3.88 (dd, 1H), 3.57-3.32 (m, 1H), 3.02-2.96 (dd,1H), 1.90-1.50 (m, 7H); MS (EI) for $C_{21}H_{21}F_3IN_3O_2$: 532 (MH^+). ^{13}C NMR (400 MHz, $CDCl_3$): 169.38, 154.58, 154.20, 154.09, 152.11, 151.69, 151.56, 145.07, 142.59, 133.16 (d), 132.52 (br), 130.94 (d), 124.58, 124.37 (m), 119.85, 109.52 (t), 81.91 (d), 71.86, 65.21, 62.62, 60.31, 58.16, 46.82, 26.16 (d), 25.25 (m), 24.17. High resolution positive ion FAB MS for $C_{21}H_{21}F_3IN_3O_2$: Calculated m/z 532.071291, Found m/z 532.07129 (MH^+).

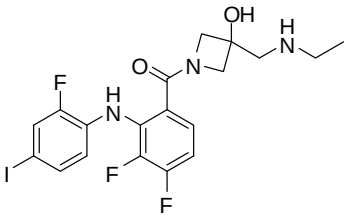
Mp: 171-172 °C for free base

Elemental Analysis for $C_{21}H_{21}F_3IN_3O_2$:

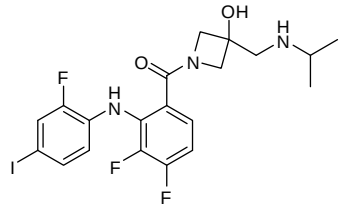
%C	calc.: 47.47	found: 47.44
%H	calc.: 3.98	found: 3.85
%N	calc.: 7.91	found: 7.70
%F	calc.: 10.73	found: 10.56



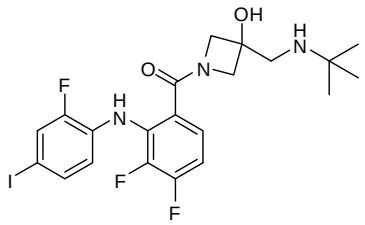
3-(aminomethyl)-1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)azetidin-3-ol (**8**): ^{1}H NMR (400 MHz, d_4-methanol): 7.46 (dd, 1H), 7.35 (d, 1H), 7.30 (tr, 1H), 7.04 (dd, 1H), 6.62 (ddd, 1H), 4.14 (dd AB, 2H), 3.98 (dd AB, 2H), 3.07 (s, 2H); MS (EI) for $C_{17}H_{15}F_3IN_3O_2$: 478 (MH^+). High resolution positive ion FAB MS for $C_{17}H_{15}F_3IN_3O_2$: Calculated m/z 478.02416, Found m/z 478.02417 (MH^+).



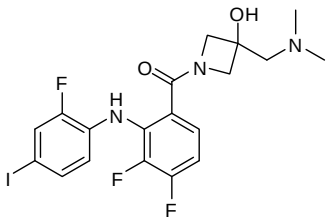
1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)-3-[(ethylamino)methyl]azetidin-3-ol (**12**): ^{1}H NMR (400 MHz, d_6-DMSO): 8.57 (s, 2H), 7.58 (dd, 1H), 7.37 (d, 1H), 7.32 (tr, 1H), 7.18 (dd, 1H), 6.68 (ddd, 1H), 3.97 (dd AB, 2H), 3.79 (dd AB, 2H), 2.64 (s, 2H), 2.52 (q, 2H), 0.96 (t, 3H). MS (EI) for $C_{19}H_{19}F_3IN_3O_2$: 506 (MH^+). High resolution positive ion FAB MS for $C_{19}H_{19}F_3IN_3O_2$: Calculated m/z 506.05538, Found m/z 506.05539 (MH^+).



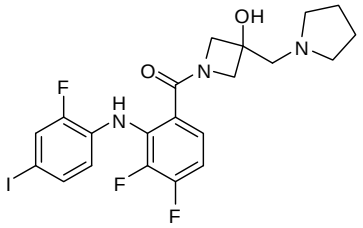
1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)-3-{[(1-methylethyl)amino]methyl}azetidin-3-ol (**13**): ^{1}H NMR (400 MHz, d_4-methanol): 7.47 (dd, 1H), 7.36 (d, 1H), 7.32 (tr, 1H), 7.06 (dd, 1H), 6.63 (ddd, 1H), 4.22 (dd AB, 2H), 4.05 (dd AB, 2H), 3.37 (m, 1H), 3.30 (s, 2H, buried), 1.33 (d, 6H). MS (EI) for $C_{20}H_{21}F_3IN_3O_2$: 520 (MH^+). High resolution positive ion FAB MS for $C_{20}H_{21}F_3IN_3O_2$: Calculated m/z 520.07081, Found m/z 520.07080 (MH^+).



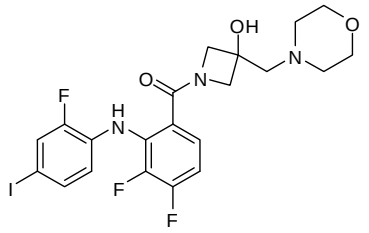
1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)-3-{[(1,1-dimethylethyl)amino]methyl}azetidin-3-ol (**14**): ^{1}H NMR (400 MHz, d_4-methanol): 7.44 (dd, 1H), 7.34 (d, 1H), 7.28 (ddd, 1H), 7.05 (dd, 1H), 6.61 (ddd, 1H), 4.05 (dd AB, 2H), 3.89 (dd AB, 2H), 2.73 (s, 2H), 1.08 (s, 9H). MS (EI) for $C_{21}H_{23}F_3IN_3O_4$: 534 (MH^+).



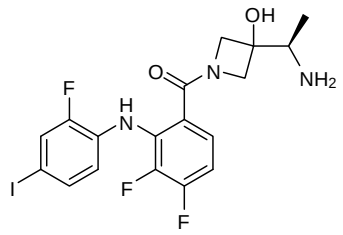
1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)-3-[(dimethylamino)methyl]azetidin-3-ol (**15**): ^{1}H NMR (400 MHz, d_6-DMSO): 8.56 (s, 1H), 7.59-7.56 (dd, 1H), 7.38-7.36 (dt, 1H), 7.34-7.33 (m, 1H), 7.21-7.14 (m, 1H), 6.71-6.65 (m, 1H), 5.55 (b, 1H), 4.07-4.05 (d, 1H), 3.89-3.84 (t, 2H), 3.74-3.719 (d, 1H), 2.46 (m, 2H), 2.19 (br s, 6H); MS (EI) for $C_{19}H_{19}F_3IN_3O_2$: 506 (MH^+).



1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)-3-(pyrrolidin-1-ylmethyl)azetidin-3-ol (**16**): ^{1}H NMR (400 MHz, d_4-methanol): 7.47 (dd, 1H), 7.37 (d, 1H), 7.33 (tr, 1H), 7.05 (dd, 1H), 6.63 (ddd, 1H), 4.29 (dd AB, 2H), 4.10 (dd AB, 2H), 3.65-3.48 (br s, 2H), 3.54 (s, 2H), 3.27-3.04 (br s, 2H), 2.07 (br s, 4H). MS (EI) for $C_{21}H_{21}F_3IN_3O_2$: 532 (MH^+).



1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)-3-(morpholin-4-ylmethyl)azetidin-3-ol (**17**): ^{1}H NMR (400 MHz, CD_3OD): 7.48 (d, 1H), 7.36 (d, 1H), 7.33-7.29 (m, 1H), 7.08-7.02 (m, 1H), 6.65-6.60 (m, 1H), 4.39 (br d, 1H), 4.24-4.18 (br, 2H), 4.08-3.96 (br m, 3H), 3.80 (br s, 2H), 3.51 (d, 2H), 3.40 (br s, 2H), 3.24 (br s, 2H). MS (EI) for $C_{21}H_{21}F_3IN_3O_3$: 548 (MH^+).



3-[(1R)-1-aminoethyl]-1-({3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]phenyl}carbonyl)azetidin-3-ol (**19**): ^{1}H NMR (400 MHz, d_6-DMSO): 8.56 (s, 1H), 7.84 (br s, 1H), 7.40-7.31 (m, 2H), 7.24-7.17 (m, 1H), 6.72-6.64 (m, 2H), 4.31-3.94 (m, 3H), 3.84-3.76 (m, 1H), 3.52-3.45 (m, 1H), 1.14-1.06 (m, 3H); MS (EI) for $C_{18}H_{17}F_3IN_3O_2$: 492 (MH^+).

References:

1) Palmer, Andreas M.; Chiesa, Vittoria; Schmid, Anja; Muench, Gabriela; Grobbel, Burkhard; Zimmermann, Peter J.; Brehm, Christof; Buhr, Wilm; Simon, Wolfgang-Alexander; Kromer, Wolfgang; Postius, Stefan; Volz, Jurgen; Hess, Dietmar. Tetrahydrochromenoimidazoles as Potassium-Competitive Acid Blockers (P-CABs): Structure-Activity Relationship of Their Antisecretory Properties and Their Affinity toward the hERG Channel. *J. Med. Chem.* **2010**, *53(9)*, 645-3674.

2) Nisato, Dino; Frigerio, Marco Synthesis of 3-aminoazetidine. *J. Het. Chem.* **1985**, *22(4)*, 961-3.
3) Barrett Stephen D; Bridges Alexander J; Dudley David T; Saltiel Alan R; Fergus James H; Flamme Cathlin M; Delaney Amy M; Kaufman Michael; LePage Sophie; Leopold Wilbur R; Przybranowski Sally A; Sebolt-Leopold Judith; Van Becelaere Keri; Doherty Annette M; Kennedy Robert M; Marston Dan; Howard W Allen Jr; Smith Yvonne; Warmus Joseph S; Tecle Haile The discovery of the benzhydroxamate MEK inhibitors CI-1040 and PD-0325901. *Bioorg. Med. Chem. Lett.* **2008**, *18(24)*, 6501-4.

Biochemical Assay Protocol: For a biochemical measurement of MEK1 inhibitory activity compounds were studied in a triple coupled cRaf-MEK1-ERK2 assay using AlphaScreen technology (Perkin Elmer). The compound prepared as a 10 μM DMSO stock solution, is serial diluted into an assay buffer composed of 20 mM Tris (pH 7.5), 10 mM magnesium chloride, 0.03% CHAPS and 1 mM DTT. Subsequently, 10 μL of substrate mixture is added composed of unactive MEK1 (3 nM), ATP (50 μM), unactive ERK2 (4 nM), biotinylated MBP peptide (b-FFKNIVTPRTPPPSQGK, 1 μM) and antiphospho MBP peptide (0.5 nM). The mixture is then gently shaken for 30 minutes at room temperature followed by addition of active cRaf (5 μL at 0.5 nM) to initiate reaction. The mixture is then shaken for 100 minutes at room temperature then quenched by addition of 10 μL of a mixture of 5μg/mL streptavidin donor beads and 5μg/mL protein A acceptor beads in detection buffer (75 mM Hepes pH = 7.5, 300 mM sodium chloride, 120 mM EDTA, 0.3% BSA and 0.03% Tween), followed by incubation overnight and signal detection on an AlphaQuest plate reader (Perkin Elmer). IC_{50} values reported are the average of a minimum of two independent determinations.

Kinase Selectivity: XL518 (**1**) is a highly specific inhibitor of MEK1/2. In vitro profiling of 103 protein kinases showed no cross reactivity and returned IC_{50} values > 10,000 nM.

Kinase	IC_{50} (nM)
MEK1[a]	0.9
MEK2[b]	199
Abl, Akt1, Akt2, ALK, AMPK, ASK1, Aurora-A, B-Raf, B-RafV600E, Btk, CamK2σ, CaMK1, CDK2/cyclinE, CDK6/cyclinD3. Chk1, Chk2, CK1, CK2, CLK3, c-Raf, DAPK1, DYRK2, EGFR, EMK, EphA2, EphB4, ErbB2, ERK1, ERK2, FAK, Fes, FGFR1, FGFR3, Flt-1, Flt-3, Flt-4, Fms, GRK2, GRK5, GSK3β, Hck, HIPK1, PKA, IGF-1R, IKKα, IRAK1, IRK, JAK2, JNK1, KDR, Kit, Lck, LOK, Lyn, MAP4K3, MAPKAP2, MARK1, Met, MINK, MKK4, MKK6, MKK7β, MLK1, MSK1, MST1, NEK3, MEK7, NLK, p38α, p70S6K, PAK2, PAK4, PAK6, PASK, PDGFRβ, PDGFRα, PDK1, PI3Kα, PI3Kβ, PI3Kγ, PIM1, PIM2, PIM3, PKCβII, PKCα, PKCγ, PDK2, Plk3, ROCK-1, RON, Rsk1, SGK, SYK, SRC, STK24, Tie-2, TrkB, TSSK1, WNK2, ZAP-70	> 10,000[c]

[a]Value determined using the coupled enzymatic cascade c-Raf/MEK1unactive/ERK2unactive at a saturating concentration of ATP (50 μM).
[b]Value determined using the coupled enzymatic cascade c-MEK2active/ERK2unactive at a saturating concentration of ATP (50 μM).
[c]Highest concentration tested. ATP concentration selected were proximal to the K_m values for the respective enzyme.

Cellular Assay Protocol: Cellular activity was determined using MDA-MB-231T cells in an endogenous ERK phosphorylation ELISA assay. MDA-MB-231T (ATCC) cells were plated at a density of 20000 cells/well onto 96-well microtiter plates (Costar), in DMEM (Cellgro) containing 10% FBS (heat-inactivated, Cellgro), and 1% Pen/Strep (Cellgro). The cells were incubated at 37°C , 5% CO_2 for 24 hr. Serum starvation was performed by replacing the medium with serum-free DMEM or MEM for an additional 24 hr. Serial dilutions of test compound in fresh serum-free medium in a final concentration of 0.3% DMSO (vehicle) were added to the cells and incubated for 1 hr. Negative controls were in serum-free medium + 0.3% DMSO only. After treatment, the cells were fixed with 4% formaldehyde, followed by quenching of endogenous peroxidases with 0.6% hydrogen peroxide. Plates were then blocked (10% FBS, Cellgro) and incubated with mouse monoclonal anti-phospho-p44/42 MAPK, E10 (1:2000, Cell

Signaling), followed by secondary antibody (HRP-conguated, goat anti-mouse IgE, 1:3000 (Jackson ImmunoResearch Laboratories, Inc). Washing of the plates was performed with PBS-T (0.1% Triton X-100) in between all incubation steps. A luminol-based substrate solution was then added and plates were read using the Victor Wallac machine. IC50 values were determined based on total ERK phosphorylation with compound treatment versus total ERK phosphorylation with 0.3% DMSO treatment alone.

In Vivo Studies:

Experimental Animals: Female CD rats (Sprauge Dawley) approximately 9-10 weeks of age and weighing approximately 201-225 grams were purchased from Charles River Laboratories. For pharmacokinetic studies the animals were fasted overnight prior to compound dosing and returned to food four hours post compound administration. Female nude mice (NCr) 5-8 weeks of age and weighing approximately 20 grams were purchased from Taconic. Prior to initiation of a study, the animals were allowed to acclimate for 48 hours. During studies the animals were provided food and water ad libitum and all animals were housed in a room conditioned at 70-75^{o}C and 60% relative humidity. A twelve hour light and dark cycle was maintained with automatic timers.

Rat Pharmacokinetics: All compounds were dosed at 5 mg/kg by both oral and IV administration. Experimental groups were comprised of three animals for each compound and dosing route. Blood was collected (100 μL) in heparinized tubes via a jugular catheter at timepoints 0.25, 0.5, 1, 2, 4, 8, 24 hours. The plasma obtained was stored at -80^{o}C and a volume of 50 μL was used for analytics. The concentration of compound was determined by HPLC/MS/MS analysis using a PE Sciex API-4000 MS/MS integrated with a Shimadzu LC-10AD, Leap HTS-PAL autosampler using Analyst 1.3 system software. An Eclipse XDB-C18, 50x4.6 mm Agilent column was employed for sample separation. The mobile phase was composed of 0.1% formic acid buffered aqueous acetonitrile. Samples for analysis were prepared by combination of the plasma aliquot (50 μL) with a 100μL volume of internal standard solution. Following vortex mixing and centrifugation, the supernatant was transferred to a 96-well microtiter

plate and a 20 μL sample was evaluated for compound concentration. A non-compartmental model was applied to calculate pharmacokinetic parameters for all routes of administration using WinNonlin 4.1 software.

Pharmacodynamic Studies: MDA-MB-231T human breast adenocarcinoma cells were cultured in vitro in DMEM (Mediatach) supplemented with 10% FBS (Hyclone), Penicillin-Streptomycin, and non-essential amino acids at 37^{o}C in a humidified, 5% CO_2 atmosphere. On day zero, cells were harvested by trypsinization, and 1x10^{6} cells in 0.1 mL ice-cold Hank's balanced salt solution were implanted subcutaneously into the animal mammary fat pad. A transponder was implanted in each mouse for identification and all animals were monitored daily for clinical symptoms and survival. For 2, 8 and 24 h dose-response/duration of action studies, whole blood was sampled at determined times post dose by cardiac puncture. The plasma concentrations of parent compound and the metabolite (**4**) were analyzed. After CO_2 euthanasia, tumors and brains from the animals corresponding to the 2, 8 and 24 h post single dose time point were harvested, minced and resuspended in ice-cold lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% sodium deoxycholate, 1 mM EDTA, 50 mM NaF, 1 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 2 mM phenylmethylsulfonyl fluoride, 10 mg/ml aprotinin, 5 mg/ml leupeptin and 5 mg/ml pepstatin A). Tumors and brains were homogenized using a Polytron PT 10/35-homogenizer (Brinkmann); a fraction of the crude lysate was saved to be analyzed by LC/MS/MS for tumor and brain parent compound and metabolite (**4**) concentrations, while the rest was centrifuged at 12,000 rpm for 14 minutes at 4°C. Supernatants (clarified lysates) were separated and stored at –80°C until assayed for individual target modulation. Protein concentrations in the tumor and brain lysates were measured by BCA method (Pierce). Lysates (25 μg) were mixed with 4x NuPage LDS buffer and reducing reagent. Samples were then heated at 85°C for 10 min. 20-40 μg protein was loaded onto NuPage 4-12% Bis-Tris gels. Proteins were transferred to PVDF membranes and blotted overnight with anti-MEK1 (Cat# 9124, Cell Signaling), anti-pMEK (Ser217/221), (Cat# 9121, Cell Signaling), anti-ERK1/2 (Cell Signaling) and anti-pERK1/2 (Cell Signaling). Membranes were washed 3 times for 15 min each with TBS-T buffer and blotted with HRP-conjugated anti-mouse (Amersham)

or HRP-conjugated anti-rabbit (Amersham) antibody in 5% non-fat milk for 60 min. Membranes were probed with ECL-Plus (Amersham) for 5 min at room temperature and quantitated after scanning using the Typhoon 9400. The ratio of phosphorylation and total MEK and ERK were calculated by the ImageQuant program, and the percentage of compound inhibition was normalized to the vehicle control.

Efficacy Studies: The effects of compounds on tumor growth were determined in female athymice nude mice bearing established MDA-MB-231 tumors. The tumors were staged at an average size of 92 ± 4 mg, at which time they were randomized into treatment groups, each containing 10 mice. A transponder was implanted into each mouse for identification and data tracking, and animals were monitored daily for clinical symptoms and survival. Compounds were administered at the dosing regimens summarized. No significant loss of body weight was observed during the study. During the dosing period, the tumor weight of each animal was determined twice weekly and the body weight of each animal was measured daily. Tumor weight (TW) was determined by measuring perpendicular diameters with a caliper, using the following formula:

$$TW\ (mg) = [\text{tumor volume} = \text{length (mm)} \times \text{width}^2\ (mm^2)]/2$$

These data were recorded for tumors in each experimental group and group mean ± SEM values were determined and plotted on a tumor weight vs. days post-implantation line graph to display tumor growth rates. Percent inhibition of tumor growth from size at staging relative to the average growth of the vehicle group (TGI) is determined with the following formula:

$$\left[1 - \left[\frac{(X_f - X_0)}{(Y_f - X_0)}\right]\right] * 100$$

where X_0 = average TW of all tumors on day of staging/grouping

X_f = average TW of tumors from treatment group on Day f

Y_f = average TW of vehicle control group tumors on Day f

Statistical significance was determined using the 2-tailed Student's t-test (significance defined as $p < 0.05$).

MBA-MD-231T Protocol and Tumor Growth Inhibition Summary:

Test Article	Dose	Schedule	TGI (%)	P value (vs. Vehicle)	Regression (%)	P value (vs. start)
Vehicle	10 mL/kg	qd x 14	-	-	-	-
(**1**)	0.3 mg/kg	qd x 14	32	1.26E-01	NA	NA
(**1**)	1 mg/kg	qd x 14	60	1.37E-03	NA	NA
(**1**)	3 mg/kg	qd x 14	93	1.18E-05	NA	NA
(**1**)	10 mg/kg	qd x 14	>100	1.42E-06	32	2.05E-04
(**1**)	30 mg/kg	qd x 14	>100	1.20E-06	78	1.74E-12

Plasma Concentrations for (1) and Metabolite (4):

Test Article Group	Time (h)	Plasma Concentration (1) (μM)		Plasma Concentration (4) (μM)	
		Mean±SD[a]	% CV	Mean±SD[b]	% CV
0.3 mg/kg qd	1	0.016±0.004	26	<LQ	NA
	4	0.01±0.002	17	<LQ	NA
	26	<LQ	NA	<LQ	NA
1.0 mg/kg qd	1	0.035±0.008	23	<LQ	NA
	4	0.04±0.01	19	<LQ	NA
	26	<LQ	NA	<LQ	NA
3.0 mg/kg qd	1	0.11±0.01	13	<LQ	NA
	4	0.13±0.011	89	<LQ	NA
	26	<LQ	NA	<LQ	NA
10 mg/kg qd	1	0.61±0.27	44	<LQ	NA
	4	0.45±0.36	80	<LQ	NA
	26	0.01±0.01	173	<LQ	NA
30 mg/kg qd	1	2.01±0.64	32	<LQ	NA
	4	2.74±0.37	14	<LQ	NA
	26	0.85±0.87	103	<LQ	NA

[a]Compound (**1**) LQ = 0.004 μM. [b]Compound (**4**) LQ = 0.005 μM.

MEK1:AMP-PCP Co-Crystal Structure Determinations:

Crystallization: Crystals were grown at 10^{o}C in sitting drops using vapor diffusion. MEK1 at 8 mg/mL in 20 mM Tris (pH 7.5), 250 mM NaCl, 0.5 mM EDTA, 10% glycerol, 5 mM DTT, and 3% DMSO was incubated with 300mM compound for two hours on ice. Subsequently, adenosine 5'-(β,γ-methylenetriphosphonate) (AMP-PCP) was added to a final concentration of 2.8 mM and $MgCl_2$ to a final concentration of 7 mM. Crystals of the ternary complex were obtained by mixing 1.2 μL of this stock solution with 1.8 μL of a reservoir solution consisting of 25.5% PEG-2000 MME, 0.1 M trimethyleneamine N-oxide, 0.1 M Tris (pH 8.9). Crystals were grown to full size in approximately 1-3 weeks. As required crystals were soaked for 5 hours in a solution consisting of the reservoir solution supplemented with 400 mM compound , 0.1 mM NaCl, 1 mM AMP-PCP, and 2.5 mM $MgCl_2$ to ensure optimal occupancy. Crystals were then transferred to a cryoprotectant and subsequently flash cooled in liquid nitrogen.

Data Collection and Refinement: Data were collected from a single crystal at 100 K at the Advanced Light Source (ALS) and measured on a Quantum 210 ADSC area detector. Data were collected using 1.0o scans in Φ with an exposure time of 10 s per image. The crystal to detector distance was 190 mm. Diffraction data were integrated with MOSFLM version 6.2.4 and scaled with SCALA from the CCP4 suite, version 5.0.2. The structure of the MEK1:AMP-PCP:compound ternary complex was solved by molecular replacement using all protein atoms of a previously solved structure as a search model. A single unambiguous solution was found using MOLREP, with one molecule in the asymmetric unit. The resulting model was subsequently refined in REFMAC5 using rigid body and maximum likelihood procedures. At this stage fourier syntheses yielded clearly interpretable electron density for all atoms and ligand. The ligand was built and minimized in Chem3D, imported into QUANTA/X-Ligand, and fit into the difference electron density. The complex was subsequently refined using maximum likelihood procedures in REFMAC5. Portions of the protein were adjusted by iterative cycles of manual rebuilding in QUANTA and refinement in REFMAC5 and waters were built and manually inspected in QUANTA. Atomic coordinates for all structures have been

deposited in the Protein Data Bank (PDB), accessible at http://www.rcsb.org/. Final refinement statistics are summarized in the table below:

	MEK1 Complex			
Inhibitor	(**2**)	(**7**)	(**8**)	(**1**)
Nucleotide	AMP-PCP	AMP-PCP	AMP-PCP	AMP-PCP
Maximum resolution (Å)	2.10 *(2.02)*	2.80	2.20	2.50 *(2.40)*
Space group	P61	P61	P61	P61
PDB accession code	4an3	4an9	4anb	4an2
Unit cell dimensions (Å, °)	a = 108.659 b = 108.659 c = 50.133 α = 90.0 β = 90.0 γ = 120.0	a = 108.653 b = 108.653 c = 49.394 α = 90.0 β = 90.0 γ = 120.0	a = 109.001 b = 109.001 c = 48.076 α = 90.0 β = 90.0 γ = 120.0	a = 108.844 b = 108.844 c = 47.710 α = 90.0 β = 90.0 γ = 120.0
Total reflections	71968	45070	88831	56704
Unique reflections	22248	8366	16691	12819
Completeness (%)[a]	99.5 (100.0)	99.8 (100.0)	99.6 (100.0)	99.8 (99.3)
I/σ(I) [a]	8.1 (2.0)	11.3 (2.3)	14.7 (2.0)	15.2 (1.1)
Multiplicity [a]	3.2 (3.1)	5.4 (5.5)	5.3 (5.4)	4.4 (4.1)
Rmerge (I) (%)[a]	7.3 (50.3)	12.2 (82.1)	5.4 (82.5)	7.3 (111.2)
	d*TREK	Mosflm/SCALA	Mosflm/SCALA	Mosflm/SCALA
Refinement:				
Rwork (%)	22.87	20.52	22.98	21.49
Rfree (%)	28.31	31.26	29.62	29.89
Protein atoms	2191	2198	2209	2214
Inhibitor atoms	26	24	26	30
Nucleotide atoms	31	31	31	31
Magnesium ion	1	1	1	1
Water molecules	88	5	57	38
RMS deviation from ideality				
Bond lengths (Å)	0.020	0.020	0.019	0.021
Bond angles (°)	2.100	2.260	2.125	2.178

[a] Value in parentheses: highest resolution shell.