SUPPORTING INFORMATION

Benzimidazole inhibitors induce a DFG-out conformation of never in mitosis gene a-related kinase 2 (Nek2) without binding to the back pocket and reveal a non-linear structure-activity relationship

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Nek2 Caliper Methodology. Kinase activity was measured in a microfluidic assay that monitors the separation of a phosphorylated product from its substrate. The assay was run on a Caliper EZ Reader II (Caliper Life Sciences Ltd, Runcorn, UK) using separation buffer (#760367 Caliper LS) containing CR-8 (500nM, #760278, Caliper LS). In 384-well polypropylene plates (#781280, Greiner Bio-One, Gloucestershire, UK), compound stocks (10 mM) were diluted 1:4 in 100% DMSO then sequentially diluted 1:3 in DMSO to make a 8 point dilution curve (2500 µM to 1.1 µM). The compounds were further diluted 1:20 into kinase buffer (HEPES 50 mM, NaN_3 0.02%, BSA 0.01%, sodium orthovanadate 0.1 mM, DTT 1 mM, $MgCl_2$ 5 mM, Tween20 0.1%) before 4 µl was transferred into a 384-well polypropylene assay plate, equivalent to a final concentration range of 50µM to 0.02 µM in 2% DMSO. To this assay plate, Nek2 (2 µL, 4 nM final, #PV3360 Invitrogen), peptide 11 (5-FAM-KKLNRTLSVA-COOH, 2 µL, 1 µM final, #760355 Caliper LS) and ATP (2 µL, 30 µM final) all diluted in kinase buffer were added. The plate was sealed and centrifuged (1 minute, 1000 rpm) before incubation for one hour at room temperature. The reaction was stopped by the addition of separation buffer (90 µL). The plate was read on an EZ Reader II

(Caliper LS) using a 12-sipper chip (760137-0372R, Caliper LS) using instrument settings of pressure -1.8 psi and voltage 1850ΔV. The percentage conversion of product from substrate was generated automatically by the instrument and the percentage inhibition was calculated relative to blank wells (containing no enzyme and 2% DMSO) and total wells (containing all reagents and 2% DMSO). IC_{50} values were calculated in GraphPad Prism 5 using a non linear regression fit of the log (inhibitor) versus response with variable slope equation.

Nek2 Autophosphorylation Assay. Autophosphorylation activity was measured in a DELFIA® assay that monitors autophosphorylation of Nek2 using a specific phospho-Nek2 antibody. This antibody was raised to the T175 autophosphorylation site of Nek2 by immunising rabbits with a CSFAKT(P)FVGTPE peptide (synthesized by J. Metcalfe, ICR) conjugated to KLH and affinity purified prior to use (Open Biosystems, Huntsville, Alabama, USA). In 384-well polypropylene plates (#781280, Greiner Bio-One, Gloucestershire, UK), compound stocks (10 mM) were diluted 1:4 in 100% DMSO then sequentially diluted 1:3 in DMSO to make a 8 point dilution curve (2500 μM to 1.1 μM). The compounds were further diluted 1:20 into kinase buffer (HEPES 50 mM, NaN_3 0.02%, BSA 0.01%, sodium orthovanadate 0.1 mM, DTT 1 mM, $MgCl_2$ 5 mM) before 20 μl was transferred into a 384 well polypropylene assay plate equivalent to a final concentration range of 50 μM to 0.02 μM in 2% DMSO. To this assay plate, Nek2 (10 μL, 4 nM final, #PV3360 Invitrogen) was added, the plate was then briefly shaken and incubated for 5 minutes at room temperature. The reaction was started with the addition of ATP (20 μL, 30 μM final). After 50 seconds incubation time the reaction was stopped by the addition of stop buffer (50 μL, HEPES 50 mM, NaN_3 0.02%, BSA 0.01%, sodium orthovanadate 0.1 mM, DTT 1 mM and 10 mM EDTA). The reaction mixture (50 μL) was transferred to an Immulon 2 high bind plate (#3455, Thermo Scientific, Loughborough, Leicestershire, UK) and left to coat overnight at 4 °C. The plates were washed four times with wash buffer (0.1% Tween 20, WellWash4, Thermo Life Sciences) before being blocked with 5% milk in PBS (100 μl) for 30 minutes at 37 °C. The plate was washed a further two times with wash buffer before the addition of primary phospho T175 antibody (50 μl), the plate was incubated for 2hrs at 37 °C. The plate was washed a further 4 times with wash buffer before the addition of europium labelled secondary antibody (50 μl, #AD0105, anti-rabbit IgG, 0.25 μg/mL final, PerkinElmer Life Sciences, Seer Green, Buckinghamshire, UK) diluted in DELFIA assay buffer (#4002-0010, PerkinElmer Life Sciences). The plates were washed a further four times with wash buffer before the addition of enhancement solution (#4001-0010, 50 μl/well, PerkinElmer Life Sciences). The plate was read on an Envision 2103 multilabel counter (PerkinElmer Life Sciences) using a time-resolved measurement mode reading fluorescence at 615 nM. The percentage inhibition was calculated relative to blank wells (containing no enzyme and 2% DMSO) and total wells (containing all reagents and 2% DMSO). IC_{50} values were calculated in GraphPad Prism 5 using a non linear regression fit of the log (inhibitor) versus response with variable slope equation.

Plk1 Caliper Methodology. In 384-well polypropylene plates (Greiner Bio-One, Gloucestershire, UK), compound stocks (10 mM) were diluted 1:2 in 100% DMSO then sequentially diluted 1:3 in DMSO to make a 8pt dilution curve (5000 μM to 2.3 μM). The compounds were further diluted 1:20 into kinase buffer (MOPS 50 mM pH 6.5, Triton-X-100 0.004%, DTT 1 mM, $MgCl_2$ 5 mM) before 4 μl was transferred into a 384 well polypropylene assay plate equivalent to a final concentration range of 100 μM to 0.046 μM in 2% DMSO. To this assay plate, Plk1 (2 μL, 18 nM final, 05-157, Carna Biosciences- Kinase logistics ApS, Denmark), peptide (5-FAM-RRRAGALMDASFEEQ-CONH2, 2 μL, 2 μM final, Pepceuticals, Nottingham, UK) and ATP (2 μL, 15 μM final) all diluted in kinase buffer were added. The plate was sealed and centrifuged (1 minute, 1000 rpm) before incubation for 75 minutes at room temperature. The reaction was stopped by the addition of separation buffer (70 µL). The plate was read in the same manner as for Nek2.

ProfilerPro selectivity assay. An EZ reader II (Caliper Life Sciences Ltd, Runcorn, UK) was used to run the ProfilerPro selectivity assay kit 1 from Caliper Life Sciences. Plate 1 has 24 kinases (ABL, AKT1, AKT2, AURA, CHK1, CHK2, CK1δ, Erk1, Erk2, FYN, GSK3β, INSR, LCK, LYN, MAPKAP2, MET, MSK1, P38α, PKAcα, PKCz, PKD2, PRAK/MAPKAP5, RSK1 and SRC) which have been optimized to generate a predetermined percent conversion and are run at the apparent ATP Km for each enzyme. The enzyme and fluorescent labelled peptide are provided pre-aliquoted into assay ready plates. Compounds were dissolved in DMSO and diluted to 1.625 x the final assay concentration in assay buffer before addition to the assay giving 3% DMSO. The assay protocol supplied with the kit was followed. The assay was stopped by the addition of termination buffer. Phosphorylated and unphosphorylated peptides were separated by capillary electrophoresis using the EZ reader II. The percentage conversion of product from substrate was generated automatically and the percentage inhibition at each concentration of compound tested was calculated relative to blank wells (containing no ATP) and total wells (containing all reagents) for each enzyme.

PAMPA assay. The parallel artificial membrane permeability assay (PAMPA) was used to determine compound permeability by passive diffusion. The assay used an artificial membrane consisting of 2% phosphatidyl choline (Sigma Aldrich, #P3556) in dodecane. The donor plate was a MultiScreen-IP Plate with 0.45 µm hydrophobic Immobilon-P Membrane (Millipore, #MAIPNTR10)) and the acceptor plate was a MultiScreen 96-well Transport Receiver Plate (Millipore, #MATRNPS50). The permeability was measured at 3 different pH levels: pH 5, 6.5 and pH 7.4. A 10 mM DMSO stock solution of test compound was used to prepare the 50 µM PAMPA donor solutions in each of the three buffers. Calibration curves were prepared by serial dilution from the 50 µM solution. 6 µL of the membrane solution was added to each well of the donor plate. Buffer solutions (200 µL) were added to the appropriate wells of the PAMPA donor plate. 300 µL per well of blank PBS (pH 7.4) was added to the PAMPA acceptor plate. The donor and acceptor plates were then sandwiched together and covered with a lid, followed by incubation at 30 °C in a humid environment for 16 h. After the incubation period the plates were removed from the incubator and the sandwich was dismantled. All the samples were diluted (1/20) and analysed by LC/MS/MS. Permeability values (cm/s) were calculated using the following equation:

$$P_{app} = C \times -\ln\left(1 - \frac{[\mathrm{drug}_{acceptor}]}{[\mathrm{drug}_{equilibrium}]}\right)$$

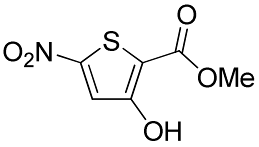
$$\text{where} \qquad C = \frac{V_D \times V_A}{(V_D + V_A) \times \mathrm{area} \times \mathrm{time}}$$

V_D volume of donor
V_A volume of acceptor
Area surface area of the membrane x porosity

Metabolic assay. Compounds (10 µM) were incubated with either male CD1 mouse or mixed gender human liver microsomes (1 mg/mL protein) of in the presence of NADPH (1 mM), UDPGA (2.5 mM) and $MgCl_2$ (3 mM) in phosphate buffered saline (10 mM) at 37 °C. Incubations were conducted for 0, 15 and 30 minutes. Control incubations were generated by the omission of NADPH and UDPGA from the incubation reaction. The percentage compound remaining was determined after analysis by LCMS and major metabolites were quantified.

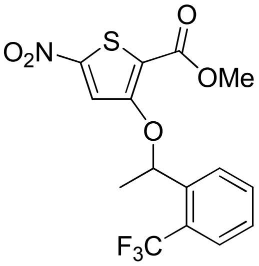
Co-crystallization of Nek2 with Ligands. Structures were experimentally determined as previously described.[1]

Methyl 3-hydroxy-5-nitrothiophene-2-carboxylate (4)



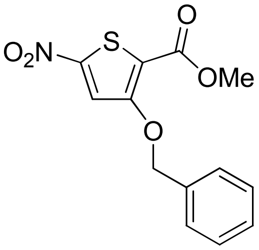
A solution of methyl 3-hydroxythiophene-2-carboxylate **3** (11.05 g, 69.9 mmol) in sulfuric acid (40 mL) was treated with nitric acid (fuming, 4.0 mL, 95.0 mmol) at -10 °C to 0 °C (portionwise, over ca. 1 h). The reaction was stirred for 2 h and dropped carefully onto cold water (ca. 200 mL). The resulting solid was filtrated and washed with water to yield a mixture of 4- and 5-nitroderivatives (ca. 8 g). The aqueous layer was extracted with Et_2O and the combined organics washed with water until neutral, dried (Na_2SO_4) and concentrated (ca. 1.3 g). The combined products were purified by Biotage column chromatography (0 to 15% EtOAc/cyclohexane) to provide thiophene **4** (4.12 g, 29%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.51 (s, 1H), 7.51 (s, 1H), 3.96 (s, 3H).

(±)-Methyl 5-nitro-3-(1-(2-(trifluoromethyl)phenyl)ethoxy)thiophene-2-carboxylate (5a)



A solution of 1-(2-(trifluoromethyl)phenyl)ethanol (2.05 g, 10.78 mmol), thiophene **4** (1.56 g, 7.68 mmol) and triphenylphosphine (2.83 g, 10.79 mmol) in DCM (70 mL) was cooled at 0 °C and treated with di-*tert*-butyl azodicarboxylate (2.48 g, 10.78 mmol). The mixture was allowed to reach room temperature and stirred overnight. The reaction was quenched with water and extracted with DCM. The organics were dried (Na_2SO_4), concentrated and purified by Biotage column chromatography (0 to 15% EtOAc/hexane) to provide thiophene **5a** (2.83 g, 98%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (d, *J* = 7.9 Hz, 1H), 7.67 (d, *J* = 7.9 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.45 (s, 1H), 7.42 (t, *J* = 7.7 Hz, 1H), 5.78 (q, *J* = 6.2 Hz, 1H), 3.93 (s, 3H), 1.73 (d, *J* = 6.3 Hz, 3H).

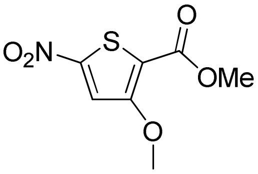
Methyl 3-(benzyloxy)-5-nitrothiophene-2-carboxylate (5b)



A solution of benzyl alcohol (220 μL, 2.13 mmol), nitrothiophene **4** (295 mg, 1.45 mmol) and triphenylphosphine (534 mg, 2.036 mmol) in DCM (14 mL) was cooled at 0 °C and treated with di-

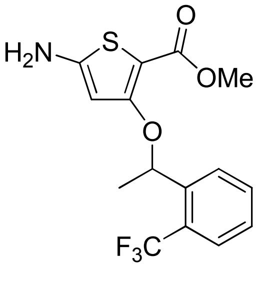
tert-butyl azodicarboxylate (471 mg, 2.05 mmol). The reaction was allowed to reach room temperature and stirred overnight. The mixture was quenched with water (15 mL) and extracted with DCM. The organics were dried (Na_2SO_4), concentrated and purified by Biotage column chromatography (0 to 20% EtOAc/cyclohexane) to afford nitrothiophene **5b** (386 mg, 91%). HRMS (ESI) *m/z* calcd for $C_{13}H_{11}NO_5SNa$ (M+Na) 316.0250, found 316.0246. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.49 – 7.33 (m, 5H), 5.27 (s, 2H), 3.91 (s, 3H).

Methyl 3-methoxy-5-nitrothiophene-2-carboxylate (5c)



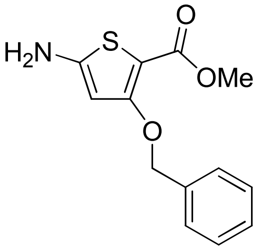
A suspension of thiophene **4** (554 mg, 2.73 mmol) and potassium carbonate (1.88 g, 13.62 mmol) in DMF (27 mL) was treated with iodomethane (275 µL, 4.42 mmol) at room temperature overnight. The mixture was partitioned between water and EtOAc and the aqueous layer extracted with EtOAc. The combined organics were washed with water and brine, dried (Na_2SO_4), concentrated and purified by Biotage column chromatography (0 to 30% EtOAc/cycloexane) to give methyl ether **5c** (562 mg, 95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (s, 1H), 4.04 (s, 3H), 3.90 (s, 3H).

(±)-**Methyl 5-amino-3-(1-(2-(trifluoromethyl)phenyl)ethoxy)thiophene-2-carboxylate (6a)**



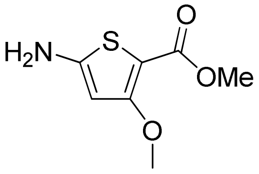
A solution of nitrothiophene **5a** (1.6 g, 4.26 mmol) in AcOH (13 mL) was heated at 65 °C for 2 h in the presence of iron powder (1.2 g, 21.49 mmol). The reaction was diluted with DCM (22 mL), quenched with 6 N NaOH (33 mL) and sat. aqueous $NaHCO_3$ (10 mL). The resulting thick solution was filtrated over celite washing with DCM and sat. aqueous $NaHCO_3$. The organic phase was separated, dried ($MgSO_4$) and concentrated to afford thiophene **6a** (1.18 g, 80%). LCMS (ESI) *m/z* 368 (M+H). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94-7.86 (m, 1H), 7.66-7.53 (m, 2H), 7.42-7.32 (m, 1H), 5.73 (s, 1H), 5.68-5.61 (m, 1H), 3.81 (s, 3H), 1.67 (d, *J* = 6.2 Hz, 3H).

Methyl 5-amino-3-(benzyloxy)thiophene-2-carboxylate (6b)



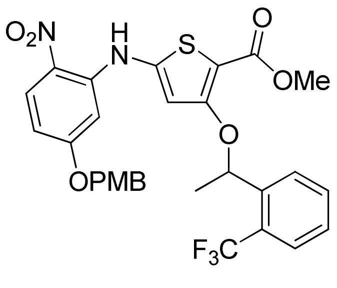
A solution of thiophene **5b** (386 mg, 1.32 mmol) in AcOH (7 mL) was heated at 65 °C for 2 h in the presence of iron powder (367 mg, 6.57 mmol). The reaction was diluted with DCM (12 mL), quenched with 6N NaOH (18 mL) and sat. aqueos $NaHCO_3$ (5 mL), filtered over a sintered disc washing with DCM. The biphasic filtrate was separated and the aqueous layer extracted with DCM. The organics were dried (Na_2SO_4), concentrated and purified by Biotage column chromatography (0 to 40% EtOAc/cyclohexane) to give thiophene **6b** (271 mg, 78%). HRMS (ESI) *m/z* calcd for $C_{13}H_{14}NO_3S$ (M+H) 264.0689, found 264.0701. ^1H NMR (500 MHz, $CDCl_3$) δ 7.47 – 7.27 (m, 5H), 5.87 (s, 1H), 5.17 (s, 2H), 4.30 (br. s, 2H), 3.79 (s, 3H).

Methyl 5-amino-3-methoxythiophene-2-carboxylate (6c)



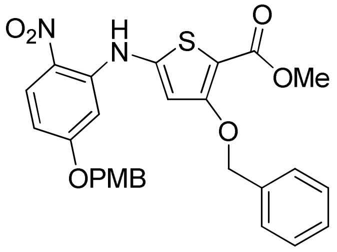
A solution of thiophene **5c** (301 mg, 1.39 mmol) in MeOH/H2O (95/5, 20 mL) was stirred at 35 °C under an atmosphere of hydrogen (40 psi) in the presence of platinum sulfided on charcoal (5% w/w, 200 mg, 0.051 mmol). After 18 hours an additional batch of catalyst was added (100 mg, 0.025 mmol) and the mixture resubjected to the same reaction conditions overnight. Filtration over celite and washing with MeOH gave thiophene **6c** (246 mg, 95%). HRMS (ESI) *m/z* calcd for $C_7H_{10}NO_3S$ (M+H) 188.0376, found 188.0369. ^1H NMR (500 MHz, MeOD) δ 5.88 (s, 1H), 3.85 (s, 3H), 3.69 (s, 3H).

(±)-**Methyl 5-(5-(4-methoxybenzyloxy)-2-nitrophenylamino)-3-(1-(2-(trifluoromethyl)phenyl)ethoxy)thiophene-2-carboxylate (7a)**



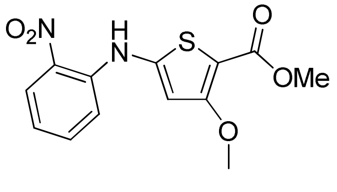
A solution of thiophene **6a** (1.18 g, 3.42 mmol), 2-bromo-4-(4-methoxybenzyloxy)-1-nitrobenzene[2] (1.1 g, 3.25 mmol), tris(dibenzylideneacetone)dipalladium(0), (60 mg, 0.066 mmol), XANTPHOS (75 mg, 0.130 mmol) and cesium carbonate (5.32 g, 16.33 mmol) in 1,4-Dioxane (14 mL, degassed) was heated to 60 °C overnight. The mixture was filtered over celite washing with DCM, the solvent removed under reduced pressure and the residue purified by Biotage column chromatography (0 to 20% EtOAc/cyclohexane) to provide thiophene **7a** (1.02 g, 52%). HRMS(ESI) *m/z* calcd for $C_{29}H_{26}F_3N_2O_7S$ (M+H) 603.1407, found 603.1399. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.85 (s, 1H), 8.17 (d, *J* = 9.5 Hz, 1H), 7.91 (d, *J* = 7.9 Hz, 1H), 7.60 – 7.51 (m, 2H), 7.33 – 7.26 (m, 3H), 6.97 – 6.89 (m, 2H), 6.70 (d, *J* = 2.5 Hz, 1H), 6.49 (dd, *J* = 9.5, 2.6 Hz, 1H), 6.44 (s, 1H), 5.73 (q, *J* = 6.0 Hz, 1H), 4.92 (s, 2H), 3.89 (s, 3H), 3.82 (s, 3H), 1.72 (d, *J* = 6.2 Hz, 3H).

Methyl 3-(benzyloxy)-5-(5-(4-methoxybenzyloxy)-2-nitrophenylamino)thiophene-2-carboxylate (7b)



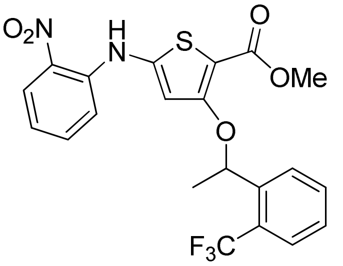
A solution of thiophene derivative **6b** (1.84 g, 6.99 mmol), 2-bromo-4-(4-methoxybenzyloxy)-1-nitrobenzene[2] (2.25 g, 6.65 mmol), tris(dibenzylideneacetone)dipalladium(0) (120 mg, 0.13 mmol), XANTPHOS (154 mg, 0.27 mmol) and cesium carbonate (10.90 g, 33.5 mmol) in 1,4-Dioxane (33 mL, degassed) was heated to 60 °C overnight. The reaction was filtered over celite washing with DCM, concentrated and purified by Biotage column chromatography (0 to 20% EtOAc/cyclohexane) to afford nitroderivative **7b** (785 mg, 23%). HRMS (ESI) *m/z* calcd for $C_{27}H_{25}N_2O_7S$ (M+H) 521.1377, found 521.1382. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.99 (s, 1H), 8.20 (d, *J* = 9.5 Hz, 1H), 7.52 – 7.44 (m, 2H), 7.42 – 7.35 (m, 2H), 7.33 – 7.28 (m, 3H), 6.99 – 6.89 (m, 2H), 6.86 (d, *J* = 2.5 Hz, 1H), 6.60 (s, *J* = 19.9 Hz, 1H), 6.53 (dd, *J* = 9.5, 2.5 Hz, 1H), 5.23 (s, 2H), 4.98 (s, 2H), 3.89 (s, 3H), 3.82 (s, 3H).

Methyl 3-methoxy-5-(2-nitrophenylamino)thiophene-2-carboxylate (7c)



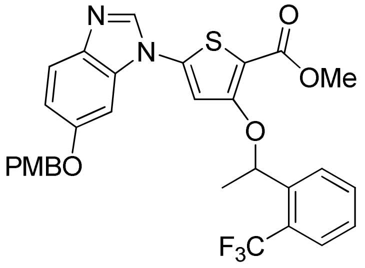
A solution of thiophene **6c** (230 mg, 1.229 mmol), 1-bromo-2-nitrobenzene (238 mg, 1.178 mmol), tris(dibenzylideneacetone)dipalladium(0) (21 mg, 0.023 mmol), XANTPHOS (27 mg, 0.047 mmol) and cesium carbonate (1.91 g, 5.86 mmol) in 1,4-Dioxane (8 mL) was heated to 50 °C. After stirring overnight, an additional batch of catalyst (23mg, 0.025 mmol) was added and the resulting mixture resubmitted to the same reaction conditions for 6 h. The reaction was filtered over celite, washing with DCM, concentrated and purified by Biotage column chromatography (0 to 40% EtOAc/cyclohexane) to give nitroderivative **7c** (135 mg, 37%). HRMS (ESI) *m/z* calcd for $C_{13}H_{13}N_2O_5S$ (M+H) 309.0540, found 309.0549. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.78 (s, 1H), 8.36 – 8.19 (m, 1H), 7.68 – 7.48 (m, 2H), 7.04 – 6.88 (m, 1H), 6.65 (s, 1H), 3.99 (s, 3H), 3.85 (s, 3H).

(±)-Methyl 5-(2-nitrophenylamino)-3-(1-(2-(trifluoromethyl)phenyl)ethoxy)thiophene-2-carboxylate (7d)



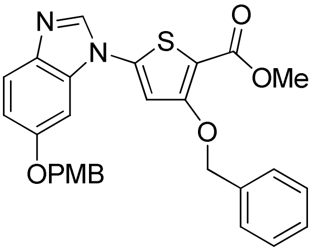
A solution of thiophene **6a** (636 mg, 1.84 mmol), 1-bromo-2-nitrobenzene (356 mg, 1.76 mmol), tris(dibenzylideneacetone)dipalladium(0) (34 mg, 0.037 mmol), XANTPHOS (45 mg, 0.078 mmol) and cesium carbonate (2.87 g, 8.81 mmol) in 1,4-dioxane (7 mL, degassed) was heated to 50 °C overnight. The reaction was filtered over celite (DCM), concentrated and purified by Biotage column chromatography (0 to 35% EtOAc/hexane) to give nitroderivative **7d** (372 mg, 45%). HRMS (ESI) *m/z* calcd for $C_{21}H_{18}F_3N_2O_5S$ (M+H) 467.0883, found 467.0900. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.59 (s, 1H), 8.21 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.93 (d, *J* = 7.9 Hz, 1H), 7.66 (d, *J* = 7.9 Hz, 1H), 7.62 (t, *J* = 7.5 Hz, 1H), 7.47 – 7.39 (m, 2H), 7.30 – 7.26 (m, 1H), 6.93 (ddd, *J* = 8.4, 7.1, 1.2 Hz, 1H), 6.45 (s, 1H), 5.76-5.73 (m, 1H), 3.89 (s, 3H), 1.74 (d, *J* = 6.3 Hz, 3H).

(±)-Methyl 5-(6-(4-methoxybenzyloxy)-1H-benzo[d]imidazol-1-yl)-3-(1-(2-(trifluoromethyl)phenyl)ethoxy)thiophene-2-carboxylate (8a)



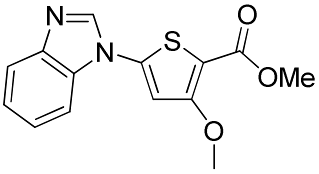
A solution of thiophene **7a** (1.01 g, 1.68 mmol) in EtOAc (17 mL) was treated with platinum sulfided on charcoal (5% w/w, 624 mg, 0.16 mmol), PPTS (84mg, 0.34 mmol) and methyl orthoformate (1.8 mL, 16.47 mmol). The mixture was stirred in an atmosphere of hydrogen overnight. The mixture was filtered over celite washing with EtOAc, the solvent removed under reduced pressure and the residue purified by Biotage column chromatography (0 to 35% EtOAc/hexane) to give benzimidazole **8a** (693 mg, 71%). HRMS (ESI) *m/z* calcd for $C_{30}H_{26}F_3N_2O_5S$ (M+H) 583.1509, found 583.1517. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.92 (d, *J* = 7.9 Hz, 1H), 7.73 (d, *J* = 8.9 Hz, 1H), 7.66 – 7.56 (m, 2H), 7.42 – 7.32 (m, 3H), 7.06 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.99 (d, *J* = 2.3 Hz, 1H), 6.97 – 6.91 (m, 2H), 6.75 (s, 1H), 5.81 (q, *J* = 6.1 Hz, 1H), 4.96 (d, *J* = 1.9 Hz, 2H), 3.94 (s, 3H), 3.83 (s, 3H), 1.77 (d, *J* = 6.2 Hz, 3H).

Methyl 3-(benzyloxy)-5-(6-(4-methoxybenzyloxy)-1H-benzo[d]imidazol-1-yl)thiophene-2-carboxylate (8b)



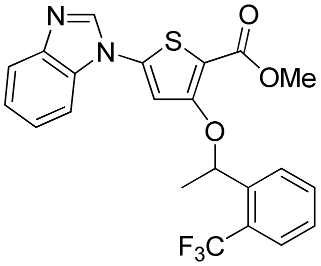
A solution of thiophene **7b** (770 mg, 1.48 mmol) in EtOAc (30 mL) was treated with platinum sulfided on charcoal (5% w/w, 585 mg, 0.15 mmol), PPTS (79 mg, 0.32 mmol) and methyl orthoformate (1.6 mL, 14.64 mmol). The mixture was stirred in an atmosphere of hydrogen overnight. After filtration and washing with EtOAc, the crude was purified by Biotage column chromatography (0 to 50% EtOAc/cyclohexane) to give thiophene **8b** (470 mg, 63%). HRMS (ESI) *m/z* calcd for $C_{28}H_{25}N_2O_5S$ (M+H) 501.1479, found 501.1472. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (br. s, 1H), 7.79 (d, *J* = 9.1 Hz, 1H), 7.52 – 7.48 (m, 2H), 7.43 – 7.38 (m, 4H), 7.35 – 7.30 (m, 1H), 7.16 – 7.08 (m, 2H), 7.02 (br. s, 1H), 6.98 – 6.93 (m, 2H), 5.35 (s, 2H), 5.00 (s, 2H), 3.93 (s, 3H), 3.84 (s, 3H).

Methyl 5-(1H-benzo[d]imidazol-1-yl)-3-methoxythiophene-2-carboxylate (8c)



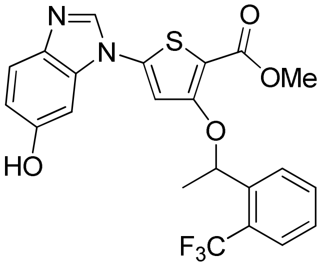
A solution of thiophene **7c** (130 mg, 0.422 mmol) in EtOAc (5 mL) was treated with platinum sulfided on charcoal (5% w/w, 171 mg, 0.044 mmol), PPTS (22 mg, 0.088 mmol) and methyl orthoformate (460 µl, 4.21 mmol). The mixture was stirred in an atmosphere of hydrogen overnight. After filtration, washing with EtOAc and concentration, the residue was purified by Biotage column chromatography (20 to 80% EtOAc/cyclohexane) to afford benzimidazole **8c** (46 mg, 38%). HRMS (ESI) *m/z* calcd for $C_{14}H_{13}N_2O_3S$ (M+H) 289.0641, found 289.0654. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.92 (dd, *J* = 6.7, 1.8 Hz, 1H), 7.76 (dd, *J* = 6.8, 1.8 Hz, 1H), 7.51 – 7.36 (m, 2H), 7.10 (s, 1H), 4.09 (s, 3H), 3.91 (s, 3H).

(±)-Methyl 5-(1H-benzo[d]imidazol-1-yl)-3-(1-(2-(trifluoromethyl)phenyl)ethoxy)thiophene-2-carboxylate (8d)



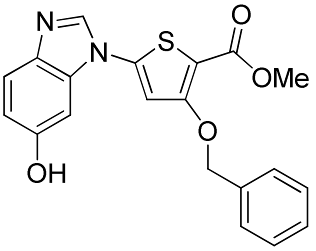
A solution of thiophene **7d** (348 mg, 0.75 mmol) in EtOAc (8 mL) was treated with platinum sulfided on charcoal (5% w/w, 296 mg, 0.076 mmol), PPTS (39 mg, 0.155 mmol) and methyl orthoformate (0.85 mL, 7.78 mmol). The mixture was stirred in an atmosphere of hydrogen overnight. After filtration and washing with EtOAc, the crude was purified by Biotage column chromatography (0 to 45% EtOAc/cyclohexane) to give benzimidazole **8d** (244 mg, 73 %). HRMS (ESI) *m/z* calcd for $C_{22}H_{18}F_3N_2O_3S$ (M+H) 447.0985, found 447.1005. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.94 (d, *J* = 7.9 Hz, 1H), 7.90 – 7.83 (m, 1H), 7.68 (d, *J* = 7.9 Hz, 1H), 7.63 (t, *J* = 7.7 Hz, 1H), 7.52 – 7.46 (m, 1H), 7.46 – 7.36 (m, 3H), 6.79 (s, 1H), 5.84 (q, *J* = 6.1 Hz, 1H), 3.95 (s, 3H), 1.79 (d, *J* = 6.3 Hz, 3H).

(±)-Methyl 5-(6-hydroxy-1H-benzo[d]imidazol-1-yl)-3-(1-(2-(trifluoromethyl)phenyl)ethoxy)thiophene-2-carboxylate (9a)



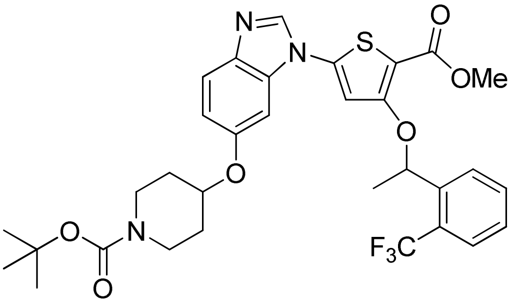
A solution of thiophene derivative **8a** (201 mg, 0.35 mmol) in DCM (2 mL) was treated with trifluoroacetic acid (260 µl, 3.51 mmol) at 0 °C. The mixture was allowed to reach room temperature and stirred for 3h. The reaction was brought to pH ca. 5-6 with 1M NaOH and 1M HCl, the aqueous layer separated and extracted with DCM. The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by Biotage column chromatography (0 to 50% EtOAc:hexane) to afford thiophene **9a** (130 mg, 80%). HRMS (ESI) *m/z* calcd for $C_{22}H_{18}F_3N_2O_4S$ (M+H) 463.0934, found 463.0950. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (d, *J* = 7.9 Hz, 1H), 7.87 (s, 1H), 7.70 – 7.63 (m, 2H), 7.60 (t, *J* = 7.6 Hz, 1H), 7.41 (t, *J* = 7.7 Hz, 1H), 6.94 (d, *J* = 2.3 Hz, 1H), 6.89 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.72 (s, 1H), 5.79 (q, *J* = 6.2 Hz, 1H), 3.92 (s, 3H), 1.76 (d, *J* = 6.2 Hz, 3H).

Methyl 3-(benzyloxy)-5-(6-hydroxy-1H-benzo[d]imidazol-1-yl)thiophene-2-carboxylate (9b)



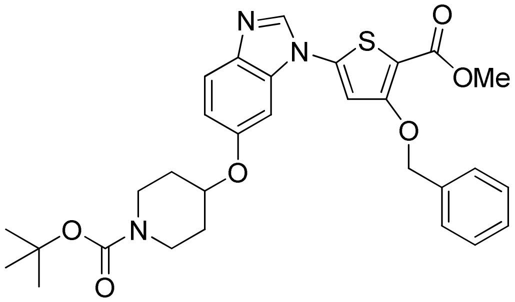
A solultion of thiophene **8b** (460 mg, 0.92 mmol) in DCM (6 mL) was treated with trifluoroacetic acid (680 µl, 9.19 mmol) at 0 °C. The mixture was allowed to reach room temperature and stirred for 3h. The reaction was brought to pH 5-6 with 1M NaOH and 1M HCl, the aqueous layer separated and extracted with DCM. The combined organic layers were concentrated and the residue was purified via Biotage column chromatography (0 to 60% EtOAc/cyclohexane) to afford thiophene **9b** (350 mg, quant.). HRMS (ESI) *m/z* calcd for $C_{20}H_{17}N_2O_4S$ (M+H) 381.0904, found 381.0904. ^{1}H NMR (500 MHz, MeOD) δ 8.32 (s, 1H), 7.59 – 7.51 (m, 3H), 7.44 – 7.32 (m, 4H), 7.12 (d, *J* = 2.2 Hz, 1H), 6.90 (dd, *J* = 8.7, 2.2 Hz, 1H), 5.36 (s, 2H), 3.87 (s, 3H).

(±)-*tert*-Butyl 4-(1-(5-(methoxycarbonyl)-4-(1-(2-(trifluoromethyl)phenyl)ethoxy)thiophen-2-yl)-1H-benzo[d]imidazol-6-yloxy)piperidine-1-carboxylate (10a)



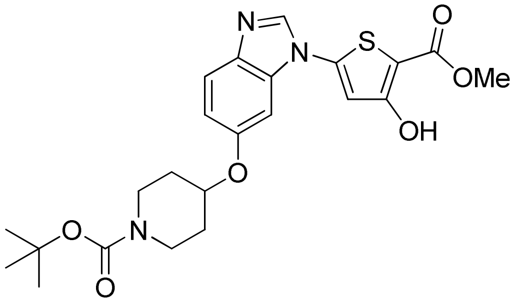
A solution of thiophene **9a** (117 mg, 0.25 mmol) (azeotroped with toluene), *tert*-butyl 4-hydroxypiperidine-1-carboxylate (101 mg, 0.50 mmol) and triphenylphosphine (265 mg, 1.01 mmol) in DCM (2 mL) was cooled at 0 °C and treated with di-*tert*-butyl azodicarboxylate (116 mg, 0.50 mmol). The reaction was allowed to reach room temperature and stirred overnight. The mixture was quenched with water and extracted with DCM. The organics were dried (Na_2SO_4), concentrated and purified by Biotage column chromatography (0 to 60% EtOAc/cyclohexane) to afford thiophene **10a** (155 mg, 96%). HRMS (ESI) *m/z* calcd for $C_{32}H_{35}F_3N_3O_6S$ (M+H) 646.2193, found 646.2200. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.91 (d, *J* = 7.9 Hz, 1H), 7.76 (d, *J* = 8.8 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.62 (t, *J* = 7.7 Hz, 1H), 7.42 (t, *J* = 7.7 Hz, 1H), 7.03 (dd, *J* = 8.9, 2.2 Hz, 1H), 6.97 (d, *J* = 1.9 Hz, 1H), 6.76 (s, 1H), 5.80 (q, *J* = 6.1 Hz, 1H), 4.53 – 4.43 (m, 1H), 3.93 (s, 3H), 3.73 – 3.61 (m, 2H), 3.43 – 3.33 (m, 2H), 1.90 (d, *J* = 7.9 Hz, 2H), 1.76 (t, *J* = 6.8 Hz, 5H), 1.48 (s, 9H).

tert-Butyl 4-(1-(4-(benzyloxy)-5-(methoxycarbonyl)thiophen-2-yl)-1H-benzo[d]imidazol-6-yloxy)piperidine-1-carboxylate (10b)



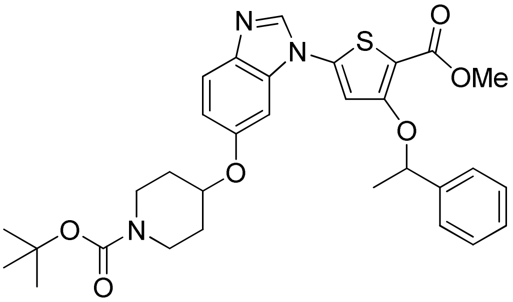
A solution of thiophene **9b** (340 mg, 0.89 mmol), 1-Boc-4-hydroxypiperidine (362 mg, 1.799 mmol) and triphenylphosphine (935 mg, 3.56 mmol) in DCM (8 mL) was cooled at 0 °C and treated with *tert*-butyl azodicarboxylate (409 mg, 1.78 mmol) The reaction was allowed to reach room temperature and stirred overnight. The mixture was quenched with water (8 mL) and extracted with DCM twice. The organics were dried (Na_2SO_4), concentrated and purified by Biotage column chromatography (0 to 60% EtOAc/cyclohexane) to give thiophene **10b** (666 mg, contaminated with minor amounts of coupling reagents, quant.). LCMS (ESI) *m/z* 564 (M+H). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.53 – 7.47 (m, 2H), 7.46 – 7.39 (m, 2H), 7.40 – 7.33 (m, 1H), 7.11 – 7.01 (m, 3H), 5.36 (s, 2H), 4.56 – 4.46 (m, 1H), 3.93 (s, 3H), 3.75 – 3.63 (m, 2H), 3.44 – 3.34 (m, 2H), 1.98 – 1.90 (m, 2H), 1.83 – 1.72 (m, 2H), 1.49 (s, 9H).

tert-Butyl 4-(1-(4-hydroxy-5-(methoxycarbonyl)thiophen-2-yl)-1H-benzo[d]imidazol-6-yloxy)piperidine-1-carboxylate (10c)



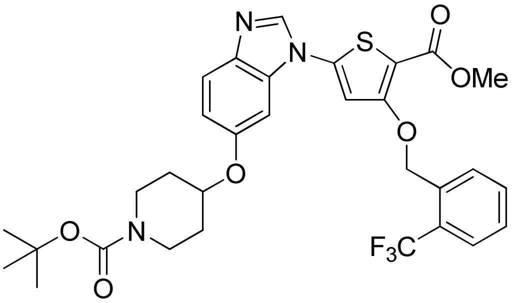
A solution of thiophene **10b** (511 mg, 0.91 mmol) in EtOH/H_2O (95/5, 10 mL) was stirred at room temperature under an atmosphere of hydrogen overnight. The mixture was filtrated over celite washing with EtOAc and the combined organics concentrated to afford thiophene **10c** (364 mg, contaminated with minor amounts of coupling reagents, 85%). LCMS (ESI) *m/z* 474 (M+H). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 8.45 (s, 1H), 7.65 (d, *J* = 8.8 Hz, 1H), 7.29 (d, *J* = 2.2 Hz, 1H), 7.05 – 6.96 (m, 2H), 4.67 – 4.60 (m, 1H), 3.71 (s, 3H), 3.68 – 3.58 (m, 2H), 3.28 – 3.16 (m, 2H), 1.96 – 1.84 (m, 2H), 1.63 – 1.49 (m, 2H), 1.40 (s, 9H).

(±)-*tert*-Butyl 4-(1-(5-(methoxycarbonyl)-4-(1-phenylethoxy)thiophen-2-yl)-1H-benzo[d]imidazol-6-yloxy)piperidine-1-carboxylate (11a)



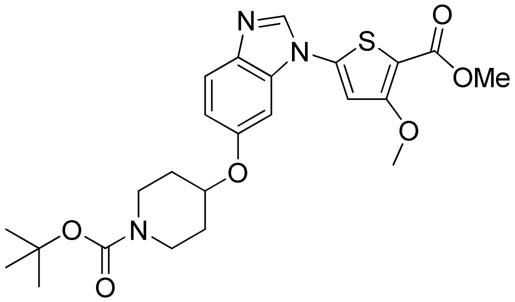
A solution of thiophene **10c** (105 mg, 0.22 mmol), 1-phenylethanol (53 μl, 0.439 mmol) and triphenylphosphine (231 mg, 0.881 mmol) in DCM (2 mL) was cooled at 0 °C and treated with di-*tert*-butyl azodicarboxylate (101 mg, 0.439 mmol) The reaction was allowed to reach room temperature and stirred overnight. The mixture was quenched with water and extracted with DCM. The organics were dried (Na_2SO_4), concentrated and purified by Biotage column chromatography (0 to 50% EtOAc/cyclohexane) to give thiophene **11a** (75 mg, 59%). HRMS (ESI) *m/z* calcd for $C_{31}H_{36}N_3O_6S$ (M+H) 578.2319, found 578.2314. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.79 (d, *J* = 8.9 Hz, 1H), 7.47 – 7.43 (m, 2H), 7.42 – 7.36 (m, 2H), 7.35 – 7.29 (m, 1H), 7.06 (dd, *J* = 8.9, 2.2 Hz, 1H), 6.95 (d, *J* = 2.2 Hz, 1H), 6.82 (s, 1H), 5.42 (q, *J* = 6.4 Hz, 1H), 4.53 – 4.42 (m, 1H), 3.94 (s, 3H), 3.76 – 3.62 (m, 2H), 3.44 – 3.36 (m, 2H), 1.96 – 1.83 (m, 2H), 1.80 – 1.75 (m, 5H), 1.50 (s, 9H).

tert-Butyl 4-(1-(5-(methoxycarbonyl)-4-(2-(trifluoromethyl)benzyloxy)thiophen-2-yl)-1H-benzo[d]imidazol-6-yloxy)piperidine-1-carboxylate (11b)



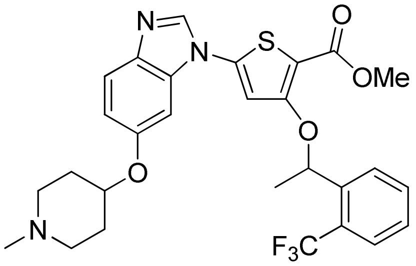
A solution of thiophene **10c** (105 mg, 0.222 mmol), (2-(trifluoromethyl)phenyl)methanol (58 μl, 0.437 mmol) and triphenylphosphine (231 mg, 0.881 mmol) in DCM (2 mL) was cooled at 0 °C and treated with di-*tert*-butyl azodicarboxylate (101 mg, 0.439 mmol). The reaction was allowed to reach room temperature and stirred overnight. The mixture was quenched with water (2 mL) and extracted with DCM. The organics were dried (Na_2SO_4), concentrated and purified by Biotage column chromatography (0 to 50% EtOAc/cyclohexane) to afford thiophene **11b** (70 mg, 50 %). HRMS (ESI) *m/z* calcd for $C_{31}H_{33}F_3N_3O_6S$ (M+H) 632.2037, found 632.2048. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (br. s, 1H), 7.97 (d, *J* = 7.8 Hz, 1H), 7.82 (d, *J* = 8.9 Hz, 1H), 7.72 (d, *J* = 7.8 Hz, 1H), 7.66 (t, *J* = 7.6 Hz, 1H), 7.48 (t, *J* = 7.7 Hz, 1H), 7.12 (br. s, 1H), 7.09 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.01 (s, 1H), 5.52 (s, 2H), 4.56 – 4.46 (m, 1H), 3.95 (s, 3H), 3.76 – 3.64 (m, 2H), 3.46 – 3.31 (m, 2H), 2.00 – 1.87 (m, 2H), 1.86 – 1.74 (m, 2H), 1.49 (s, 9H).

tert-Butyl 4-(1-(4-methoxy-5-(methoxycarbonyl)thiophen-2-yl)-1H-benzo[d]imidazol-6-yloxy)piperidine-1-carboxylate (11c)



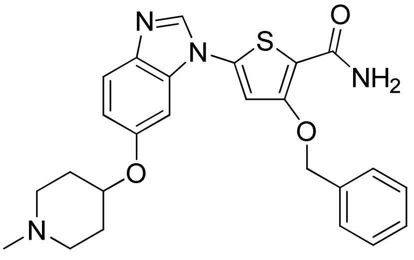
A suspension of thiophene **10c** (60 mg, 0.127 mmol) and potassium carbonate (87 mg, 0.63 mmol) in DMF (2.6 mL) was treated with iodomethane (13 µL, 0.211 mmol) at room temperature overnight. The mixture was partitioned between water and EtOAc and the aqueous layer extracted with EtOAc. The combined organics were washed with brine, dried (Na_2SO_4), concentrated and purified by Biotage column chromatography (0 to 75% EtOAc/cyclohexane) to give thiophene **11c** (34 mg, 56%). LCMS (ESI) *m/z* 488 (M+H), 510 (M+Na). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.48 (br. s, 1H), 7.82 (d, *J* = 8.9 Hz, 1H), 7.23 (d, *J* = 2.2 Hz, 1H), 7.13 (s, 1H), 7.01 (dd, *J* = 8.8, 2.2 Hz, 1H), 4.59 – 4.50 (m, 1H), 4.09 (s, 3H), 3.91 (s, 3H), 3.76 – 3.67 (m, 2H), 3.43 – 3.34 (m, 2H), 2.02 – 1.89 (m, 2H), 1.86 – 1.74 (m, 2H), 1.49 (s, 9H).

(±)-Methyl 5-(6-(1-methylpiperidin-4-yloxy)-1H-benzo[d]imidazol-1-yl)-3-(1-(2-(trifluoromethyl)phenyl)ethoxy)thiophene-2-carboxylate (12a)



A solution of thiophene **10a** (15 mg, 0.023 mmol) in formic acid (0.5 mL) was treated with formaldehyde (37% w/w in water, 50 µl, 0.023 mmol) and heated to 85 °C overnight. The mixture was neutralised with 6M NaOH (to pH 7-8) and extracted with EtOAc. The organics were dried (Na_2SO_4), concentrated and purified by Biotage column chromatography (0 to 10% Et_3N/EtOAc) to afford thiophene **12a** (13 mg, quant.). HRMS (ESI) *m/z* calcd for $C_{28}H_{29}F_3N_3O_4S$ (M+H) 560.1825, found 560.1840. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 8.44 (s, 1H), 7.99 (d, *J* = 7.9 Hz, 1H), 7.78 (t, *J* = 7.7 Hz, 1H), 7.73 (d, *J* = 7.9 Hz, 1H), 7.65 (d, *J* = 8.8 Hz, 1H), 7.54 (t, *J* = 7.7 Hz, 1H), 7.33 (s, 1H), 7.07 (d, *J* = 2.2 Hz, 1H), 7.00 (dd, *J* = 8.8, 2.2 Hz, 1H), 5.99 (q, *J* = 6.1 Hz, 1H), 4.47 – 4.34 (m, 1H), 2.65 – 2.55 (m, 2H), 2.25 – 2.12 (m, 5H), 1.97 – 1.88 (m, 2H), 1.72 – 1.60 (m, 5H).

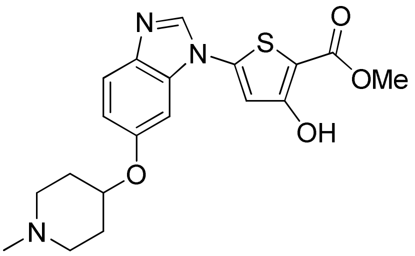
Methyl 3-(benzyloxy)-5-(6-(1-methylpiperidin-4-yloxy)-1H-benzo[d]imidazol-1-yl)thiophene-2-carboxylate (13a)



A solution of thiophene **10b** (44 mg, 0.078 mmol) in formic acid (1.6 mL) was treated with formaldehyde in water (37% w/w, 0.16 mL, 0.078 mmol) and heated to 85 °C overnight. The mixture was brought to pH 7-8 with 6M NaOH and extracted with EtOAc. The organics were dried (Na_2SO_4) and concentrated to afford crude thiophene **12b** (24 mg).

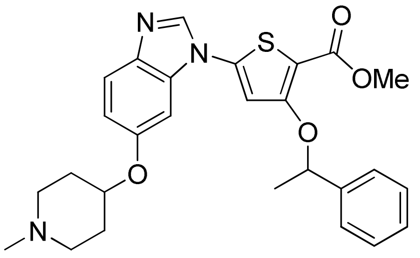
A solution of crude thiophene **12b** (24 mg, 0.050 mmol) was treated with ammonia in methanol (7M, 4 mL) and heated to 70 °C for 6 days. The mixture was concentrated and the residue purified by Biotage column chromatography (0 to 20% (MeOH/Et_3N 2:1)/EtOAc) to afford amide **13a** (10 mg, 28% over two steps). HRMS (ESI) *m/z* calcd for $C_{25}H_{27}N_4O_3S$ (M+H) 463.1798, found 463.1805. ^1H NMR (500 MHz, $(CD_3)_2SO$) δ 8.47 (s, 1H), 7.79 – 7.65 (m, 2H), 7.60 (s, 1H), 7.57 – 7.52 (m, 2H), 7.43 (t, *J* = 7.4 Hz, 2H), 7.38 (dd, *J* = 8.4, 6.2 Hz, 1H), 7.20 (d, *J* = 2.1 Hz, 1H), 7.08 – 6.95 (m, 2H), 5.41 (s, 2H), 4.49 (br. s, 1H), 2.79 (br. s, 2H), 2.34 (br. s, 5H), 1.98 (br. s, 2H), 1.77 (br. s, 2H).

Methyl 3-hydroxy-5-(6-(1-methylpiperidin-4-yloxy)-1H-benzo[d]imidazol-1-yl)thiophene-2-carboxylate (12c)



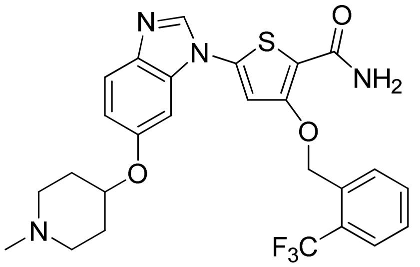
A solution of thiophene **11a** (72 mg, 0.125 mmol) in formic acid (2 mL) was treated with formaldehyde in water (37% w/w, 0.2 mL, 0.125 mmol) and heated to 85 °C overnight. The reaction was neutralised with 6M NaOH and extracted with EtOAc and DCM. The organics were dried (Na_2SO_4) and concentrated to afford the debenzylated product **12c** (45 mg, 94%). LCMS (ESI) *m/z* 388 (M+H). ^1H NMR (500 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.74 (d, *J* = 8.8 Hz, 1H), 7.19 (d, *J* = 2.1 Hz, 1H), 7.01 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.84 (s, 1H), 4.51 (br. s, 1H), 3.95 (s, 3H), 2.94 – 2.84 (br. m, 2H), 2.74 (br. s, 2H), 2.28 – 2.14 (br. m, 2H), 2.10 – 1.96 (br. m, 2H).

(±)-Methyl 5-(6-(1-methylpiperidin-4-yloxy)-1H-benzo[d]imidazol-1-yl)-3-(1-phenylethoxy)thiophene-2-carboxylate (12d)



A solution of thiophene **12c** (45mg, 0.116 mmol), 1-phenylethanol (45 µl, 0.37 mmol) and triphenylphosphine (122 mg, 0.465 mmol) in DCM (1 mL) was cooled at 0 °C and treated with di-*tert*-butyl azodicarboxylate (57 mg, 0.25 mmol) The reaction was allowed to reach room temperature and stirred overnight. The mixture was quenched with water (2 mL) and extracted with DCM. The organics were dried (Na_2SO_4), concentrated and purified by Biotage column chromatography (0 to 5% Et_3N/EtOAc) to give thiophene **12d** (25 mg, 44%). HRMS (ESI) *m/z* calcd for $C_{27}H_{30}N_3O_4S$ (M+H) 492.1952, found 492.1943. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.69 (d, *J* = 8.8 Hz, 1H), 7.46 – 7.36 (m, 4H), 7.36 – 7.29 (m, 1H), 6.98 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.92 (d, *J* = 2.2 Hz, 1H), 6.69 (s, 1H), 5.40 (q, *J* = 6.4 Hz, 1H), 4.32 (br. s, 1H), 3.93 (s, 3H), 2.74 (br. s, 2H), 2.45 – 2.31 (br. m, 5H), 2.05 (br. s, 2H), 1.90 (br. s, 2H), 1.77 (d, *J* = 6.4 Hz, 3H).

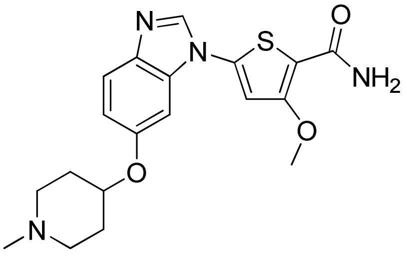
5-(6-(1-Methylpiperidin-4-yloxy)-1H-benzo[d]imidazol-1-yl)-3-(2-(trifluoromethyl)benzyloxy)thiophene-2-carboxamide (13c)



A solution of thiophene **11b** (67 mg, 0.106 mmol) in formic acid (2 mL) was treated with formaldehyde in water (37% w/w, 0.2 mL, 0.106 mmol) and heated to 85 °C overnight. The reaction was neutralised with 6M NaOH and extracted with EtOAc. The organics were dried (Na_2SO_4) and concentrated to afford crude thiophene **12e**.

A solution of crude thiophene **12e** (60 mg, 0.110 mmol) was treated with ammonia in methanol (7M, 4 mL) and heated to 70 °C for 6 days. The reaction was concentrated and purified by Biotage column chromatography (0 to 15% (MeOH/Et_3N 1:1)/EtOAc) to afford amide **13c** (29 mg, 52% over two steps). HRMS (ESI) *m/z* calcd for $C_{26}H_{26}F_3N_4O_3S$ (M+H) 531.1672, found 531.1685. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 8.50 (s, 1H), 7.88 – 7.82 (m, 2H), 7.82 – 7.71 (m, 2H), 7.69 – 7.60 (m, 3H), 7.23 (d, *J* = 2.2 Hz, 1H), 7.01 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.82 (br. s, 1H), 5.55 (s, 2H), 4.45 (br. s, 1H), 2.65 (br. s, 2H), 2.32 – 2.13 (br. m, 5H), 1.94 (br. s, 2H), 1.69 (br. s, 2H).

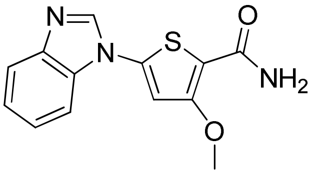
3-Methoxy-5-(6-(1-methylpiperidin-4-yloxy)-1H-benzo[d]imidazol-1-yl)thiophene-2-carboxamide (13d)



A solution of thiophene **11c** (48 mg, 0.098 mmol) in formic acid (1.8 mL) was treated with formaldehyde in water (37% w/w, 0.18 mL, 0.098 mmol) and heated to 85 °C overnight. The reaction was brought to pH 7-8 with 6M NaOH and extracted with EtOAc. The organics were dried (Na_2SO_4) and concentrated to afford crude thiophene **12f**.

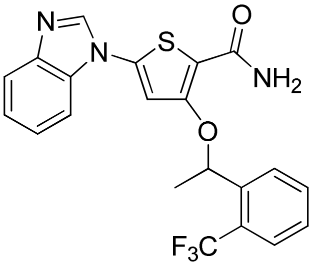
Crude thiophene **12f** was treated with ammonia in methanol (7M, 4 mL) and heated to 70 °C for 6 days. The mixture was concentrated and purified by Biotage column chromatography (0 to 20% (MeOH/Et_3N 2:1)/EtOAc) gave thiophene **13d** (27 mg, 71% over two steps). Purity (HPLC): >90%. HRMS (ESI) *m/z* calcd for $C_{19}H_{23}N_4O_3S$ (M+H) 387.1485, found 387.1501. ^1H NMR (500 MHz, $(CD_3)_2SO$) δ 8.51 (s, 1H), 7.72 – 7.63 (m, 2H), 7.54 (s, 1H), 7.30 (d, *J* = 2.2 Hz, 1H), 7.09 (br. s, 1H), 7.02 (dd, *J* = 8.8, 2.3 Hz, 1H), 4.52 (br. s, 1H), 4.03 (s, 3H), 2.79 (br. s, 2H), 2.47 – 2.26 (br. m, 5H), 1.98 (br. s, 2H), 1.75 (br.s, 2H).

5-(1H-Benzo[d]imidazol-1-yl)-3-methoxythiophene-2-carboxamide (13e)



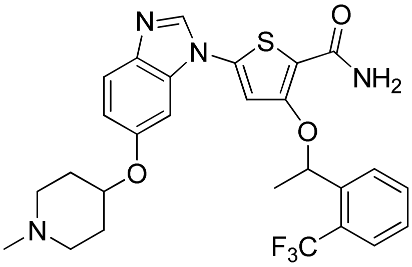
Thiophene **8c** (43 mg, 0.149 mmol) was treated with ammonia in methanol (7M, 4 mL) at 75 °C for 8 days. The mixture was concentrated and purified by Biotage column chromatography (0 to 10% (MeOH/Et_3N 1:1)/EtOAc) to afford amide **13e** (35 mg, 85%). HRMS (ESI) *m/z* calcd for $C_{13}H_{12}N_3O_2S$ (M+H) 274.0645, found 274.0653. ^1H NMR (500 MHz, $(CD_3)_2SO$) δ 8.65 (s, 1H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.62 (br. s, 1H), 7.56 (s, 1H), 7.46 – 7.39 (m, 1H), 7.40 – 7.32 (m, 1H), 7.06 (br. s, 1H), 4.05 (s, 3H).

(±)-5-(1H-Benzo[d]imidazol-1-yl)-3-(1-(2-(trifluoromethyl)phenyl)ethoxy)thiophene-2-carboxamide (13f)



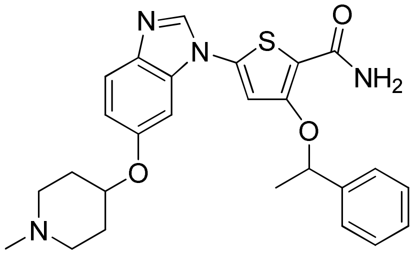
Thiophene **8d** (40 mg, 0.090 mmol) was treated with ammonia in methanol (7M, 3 mL) at 70 °C for 3 days. The mixture was concentrated, and purified by Biotage column chromatography (0 to 65% EtOAc/cyclohexane) to afford amide **13f** (35 mg, 90%). HRMS (ESI) *m/z* calcd for $C_{21}H_{16}F_3N_3O_2S$ (M+H) 432.0988, found 432.0989. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 8.54 (s, 1H), 7.94 (d, *J* = 7.8 Hz, 1H), 7.87 (br. s, 1H), 7.83 – 7.73 (m, 3H), 7.56 (t, *J* = 7.6 Hz, 1H), 7.49 (d, *J* = 7.9 Hz, 1H), 7.42 – 7.29 (m, 2H), 7.15 (br. s, 1H), 7.11 (s, 1H), 5.96 (q, *J* = 6.0 Hz, 1H), 1.75 (d, *J* = 6.2 Hz, 3H).

(±)-5-(6-(1-Methylpiperidin-4-yloxy)-1H-benzo[d]imidazol-1-yl)-3-(1-(2-(trifluoromethyl)phenyl)ethoxy)thiophene-2-carboxamide (*rac*-1)



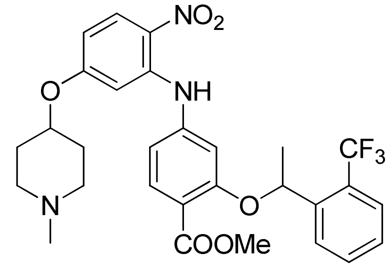
A solution of thiophene **12a** (57 mg, 0.10 mmol) was treated with ammonia in methanol (7M, 3 mL) and heated to 70 °C in a close cap vial for 2 days. The mixture was concentrated and the residue was purified by Biotage column chromatography (0 to10% (95/5 EtOAc/Et_3N)/MeOH) to afford amide ***rac*-1** (38 mg 67%). HRMS (ESI) *m/z* calcd for $C_{27}H_{28}F_3N_4O_3S$ (M+H) 545.1829, found 545.1840. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 8.37 (s, 1H), 7.94 (d, *J* = 7.9 Hz, 1H), 7.85 – 7.71 (m, 3H), 7.64 (d, *J* = 8.7 Hz, 1H), 7.57 (t, *J* = 7.7 Hz, 1H), 7.12 (br. s, 1H), 7.08 (s, 1H), 7.04 – 6.94 (m, 2H), 5.96 (q, *J* = 6.1 Hz, 1H), 4.43 (br. s, 1H), 2.73 (br. s, 2H), 2.44 – 2.21 (br. m, 5H), 1.95 (br. s, 2H), 1.80 – 1.68 (br. m, 5H).

(±)-5-(6-(1-Methylpiperidin-4-yloxy)-1H-benzo[d]imidazol-1-yl)-3-(1-phenylethoxy)thiophene-2-carboxamide (13b)



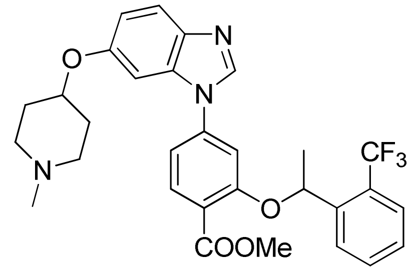
A solution of thiophene **12d** (21 mg, 0.043 mmol) was treated with ammonia in methanol (7M, 4 mL) and heated to 70 °C for 5 days. The mixture was concentrated and purified by Biotage column chromatography (0 to 15% (MeOH/Et$_3$N 1:1)/EtOAc) to afford thiophene **13b** (15 mg 75%). HRMS (ESI) *m/z* calcd for $C_{26}H_{29}N_4O_3S$ (M+H) 477.1955, found 477.1964. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 8.42 (s, 1H), 7.81 (s, 1H), 7.64 (d, *J* = 8.7 Hz, 1H), 7.57 – 7.50 (m, 2H), 7.45 (s, 1H), 7.43 – 7.36 (m, 2H), 7.32 (t, *J* = 7.4 Hz, 1H), 7.11 (s, 1H), 7.03 (s, 1H), 7.00 (dd, *J* = 8.7, 2.2 Hz, 1H), 5.80 – 5.73 (m, 1H), 4.42 (br. s, 1H), 2.66 (br. s, 2H), 2.25 (br. s, 5H), 1.93 (br. s, 2H), 1.75 – 1.63 (br. m, 5H).

(±)-Methyl 4-(5-(1-methylpiperidin-4-yloxy)-2-nitrophenylamino)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzoate (17)



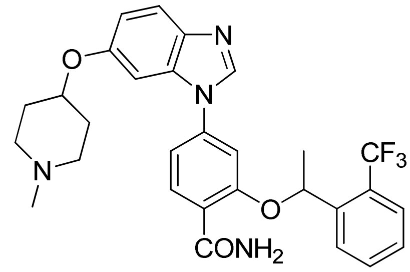
A solution of aniline ***rac*-16** (853 mg, 2.51 mmol), 4-(3-bromo-4-nitrophenoxy)-1-methylpiperidine[3] (0.755 g, 2.39 mmol), tris(dibenzylideneacetone)dipalladium(0) (45 mg, 0.043 mmol), XANTPHOS (73 mg, 0.130 mmol) and cesium carbonate (3.96 g, 12.16 mmol) in 1,4-dioxane (12 mL, degassed) was heated to 60 °C overnight. The mixture was filtered over celite washing with Et$_2$O, the solvent removed under reduced pressure and the residue purified by Biotage column chromatography (0 to 100% EtOAc/cyclohexane) to provide nitroderivative **17** (1.20 g, 87%). HRMS (ESI) *m/z* calcd for $C_{29}H_{31}F_3N_3O_6$ (M+H) 574.2159, found 574.2154. ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.57 (s, 1H), 8.18 (d, *J* = 9.5 Hz, 1H), 7.95 (d, *J* = 7.9 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.64 (d, *J* = 7.9 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.39 (t, *J* = 7.6 Hz, 1H), 6.85 (dd, *J* = 8.4, 1.9 Hz, 1H), 6.72 (d, *J* = 1.9 Hz, 1H), 6.66 (d, *J* = 2.5 Hz, 1H), 6.41 (dd, *J* = 9.5, 2.5 Hz, 1H), 5.78 (d, *J* = 6.2 Hz, 1H), 4.32 – 4.21 (m, 1H), 3.96 (s, 3H), 2.69 – 2.62 (m, 2H), 2.31 – 2.29 (m, 4H), 2.01 - 1.92 (m, 2H), 1.88 – 1.80 (m, 2H), 1.71 (d, *J* = 6.2 Hz, 3H).

(±)-Methyl 4-(6-(1-methylpiperidin-4-yloxy)-1H-benzo[d]imidazol-1-yl)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzoate (18)



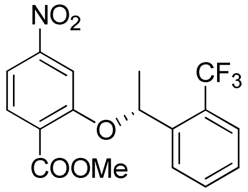
A solution of nitroderivative **17** (500 mg, 0.87 mmol) in EtOAc (8 mL) was treated with platinum sulfided on charcoal (5% w/w, 323 mg, 0.08 mmol), PPTS (263 mg, 1.05 mmol) and methyl orthoformate (0.94 mL, 8.577 mmol). The mixture was stirred in an atmosphere of hydrogen for 48h. The mixture was filtered over celite washing with EtOAc, the solvent removed under reduced pressure and the residue purified by Biotage column chromatography (0 to 10% MeOH/DCM) to give benzimidazole **18** (156 mg, 33%). HRMS (ESI) *m/z* calcd for $C_{30}H_{31}F_3N_3O_4$ (M+H) 554.2261, found 554.2271. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.3 Hz, 1H), 7.92 (d, *J* = 7.9 Hz, 1H), 7.80 (s, 1H), 7.70 – 7.65 (m, 2H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.40 (t, *J* = 7.6 Hz, 1H), 7.06 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.98 – 6.89 (m, 2H), 6.81 (d, *J* = 2.2 Hz, 1H), 5.82 (q, *J* = 6.1 Hz, 1H), 4.33 – 4.27 (m, 1H), 3.99 (s, 3H), 2.78 – 2.72 (m, 2H), 2.42 – 2.32 (m, 5H), 2.06 – 1.98 (m, 2H), 1.92 – 1.84 (m, 2H), 1.74 (d, *J* = 6.2 Hz, 3H).

(±)-4-(6-(1-Methylpiperidin-4-yloxy)-1H-benzo[d]imidazol-1-yl)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzamide (19)



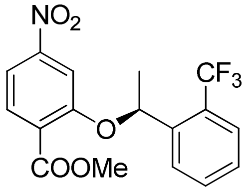
A solution of ester **18** (57 mg, 0.10 mmol) was treated with ammonia in methanol (7M, 4 mL) and heated to 70 °C in a close cap vial for 3 days. The mixture was concentrated and the residue was purified by Biotage column chromatography (0 to 100% (DCM/1M NH_3 in MeOH 9:1)/DCM) to afford amide **19** (48 mg, 88%). HRMS (ESI) *m/z* calcd for $C_{29}H_{30}F_3N_4O_3$ (M+H) 539.2265, found 539.2258. ^{1}H NMR (500 MHz, MeOD) δ 8.12 – 8.09 (m, 2H), 7.84 (d, *J* = 7.9 Hz, 1H), 7.74 (d, *J* = 7.9 Hz, 1H), 7.66 (t, *J* = 7.6 Hz, 1H), 7.60 (d, *J* = 8.9 Hz, 1H), 7.50 (t, *J* = 7.6 Hz, 1H), 7.22 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.01 – 6.99 (m, 2H), 6.67 (d, *J* = 2.0 Hz, 1H), 5.98 (q, *J* = 6.0 Hz, 1H), 4.40 – 4.30 (m, 1H), 2.85 – 2.67 (m, 2H), 2.44 – 2.29 (m, 5H), 2.09 – 1.93 (m, 2H), 1.90 – 1.71 (m, 5H).

(*R*)-Methyl 4-nitro-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzoate ((*R*)-15)



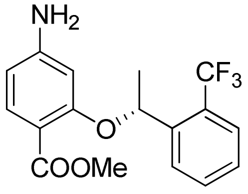
A solution of (*S*)-1-(2-(trifluoromethyl)phenyl)ethanol (910 mg, 4.79 mmol), methyl 2-hydroxy-4-nitrobenzoate **14**[4] (726 mg, 3.68 mmol) and triphenylphosphine (1.25 g, 4.77 mmol) in DCM (30 mL) was cooled at 0 °C and treated with di-*tert*-butyl azodicarboxylate (1.10 g, 4.78 mmol). The mixture was allowed to reach room temperature and stirred overnight. The reaction was quenched with water and extracted with DCM. The organics were dried ($MgSO_4$), concentrated and purified by Biotage column chromatography (0 to 10% EtOAc/cyclohexane) to provide ether **(*R*)-15** (1.26 g, 93%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (dd, *J* = 8.1, 5.5 Hz, 2H), 7.79 – 7.67 (m, 3H), 7.56 (t, *J* = 7.6 Hz, 1H), 7.40 (t, *J* = 7.6 Hz, 1H), 5.89 (q, *J* = 6.2 Hz, 1H), 4.01 (s, 3H), 1.75 (d, *J* = 6.2 Hz, 3H).

(*S*)-Methyl 4-nitro-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzoate ((*S*)-15)



A solution of (*R*)-1-(2-(trifluoromethyl)phenyl)ethanol (974 mg, 5.12 mmol), methyl 2-hydroxy-4-nitrobenzoate **14**[4] (775 mg, 3.93 mmol) and triphenylphosphine (1.35 g, 5.15 mmol) in DCM (30 mL) was cooled at 0 °C and treated with di-*tert*-butyl azodicarboxylate (1.18 g, 5.13 mmol). The mixture was allowed to reach room temperature and stirred overnight. The reaction was quenched with water and extracted with DCM. The organics were dried ($MgSO_4$), concentrated and purified by Biotage column chromatography (0 to 10% EtOAc/cyclohexane) to provide ether **(*S*)-15** (1.35 g, 93%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (dd, *J* = 8.1, 5.5 Hz, 2H), 7.79 – 7.67 (m, 3H), 7.56 (t, *J* = 7.6 Hz, 1H), 7.40 (t, *J* = 7.6 Hz, 1H), 5.89 (q, *J* = 6.2 Hz, 1H), 4.01 (s, 3H), 1.75 (d, *J* = 6.2 Hz, 3H).

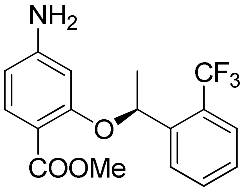
(*R*)-Methyl 4-amino-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzoate ((*R*)-16)



A solution of nitrobenzene **(*R*)-15** (1.23 g, 3.33 mmol) in MeOH/H_2O (95/5, 30 mL) was treated with palladium on charcoal (10% w/w, 192 mg, 0.18 mmol) and stirred in a atmosphere of hydrogen overnight. The mixture was filtered over celite to afford aniline **(*R*)-16** (956 mg, 85%). HRMS (ESI) *m/z* calcd for $C_{17}H_{17}F_3NO_3$ (M+H) 340.1155, found 340.1145. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (d, *J* = 7.9 Hz, 1H), 7.71 (d, *J* = 8.5 Hz, 1H), 7.67 – 7.52 (m, 2H), 7.37 (q, *J* = 7.7

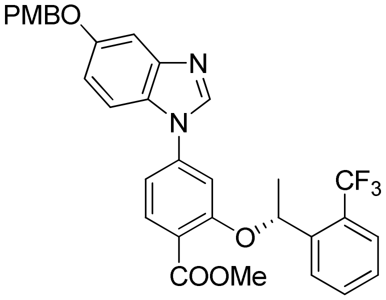
Hz, 1H), 6.17 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.08 (d, *J* = 2.1 Hz, 1H), 5.75 (q, *J* = 6.1 Hz, 1H), 3.89 (s, 3H), 1.69 (d, *J* = 6.1 Hz, 2H).

(*S*)-Methyl 4-amino-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzoate ((*S*)-16)



A solution of nitrobenzene **(*S*)-15** (1.35 g, 3.66 mmol) in MeOH/H_2O (95/5, 35 mL) was treated with palladium on charcoal (10% w/w, 203 mg, 0.19 mmol) and stirred in an atmosphere of hydrogen overnight. The mixture was filtered over celite to afford aniline **(*S*)-16** (1.20 g, 97%). LCMS (ESI) *m/z* 340 (M+H). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (d, *J* = 7.9 Hz, 1H), 7.71 (d, *J* = 8.5 Hz, 1H), 7.67 – 7.52 (m, 2H), 7.37 (q, *J* = 7.7 Hz, 1H), 6.17 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.08 (d, *J* = 2.1 Hz, 1H), 5.75 (q, *J* = 6.1 Hz, 1H), 3.89 (s, 3H), 1.69 (d, *J* = 6.1 Hz, 2H).

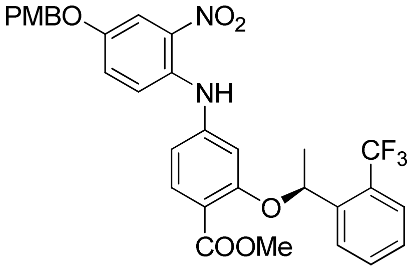
(*R*)-Methyl 4-(5-(4-methoxybenzyloxy)-1H-benzo[d]imidazol-1-yl)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzoate ((*R*)-21)



A solution of aniline (***R***)**-16** (940 mg, 2.77 mmol), 1-iodo-4-(4-methoxybenzyloxy)-2-nitrobenzene[2] (1.17 mg, 3.04 mmol), tris(dibenzylideneacetone)dipalladium(0) (28 mg, 0.031 mmol), XANTPHOS (35 mg, 0.060 mmol) and cesium carbonate (4.52 g, 13.87 mmol) in 1,4-dioxane (20 mL, degassed) was heated to 60 °C overnight. The mixture was filtered over celite washing with THF, the solvent removed under reduced pressure and the residue purified by Biotage column chromatography (0 to 20% EtOAc/cyclohexane) to provide aniline (***R***)**-20** (2.20 g, contaminated with an unknown impurity).

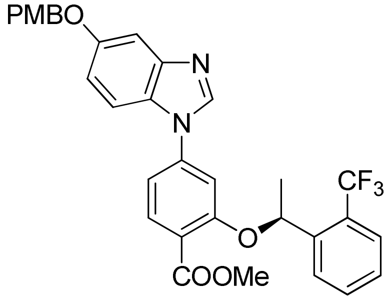
A solution of aniline (***R***)**-20** (2.20 g) in EtOAc (25 mL) was treated with platinum sulfided on charcoal (5% w/w, 556 mg, 0.14 mmol), PPTS (90 mg, 0.36 mmol) and methyl orthoformate (3.20 mL, 29.3 mmol). The mixture was stirred in an atmosphere of hydrogen overnight. The mixture was filtered over celite washing with EtOAc, the solvent removed under reduced pressure and the residue purified by Biotage column chromatography (0 to 65% EtOAc/cyclohexane) to give benzimidazole (***R***)**-21** (791 mg, 50% over two steps). LCMS (ESI) *m/z* 577 (M+H). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.3, Hz 1H), 7.93 – 7.90 (m, 2H), 7.69 (d, *J* = 7.8 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.45 – 7.38 (m, 3H), 7.36 (d, *J* = 2.3 Hz, 1H), 7.04 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.95 – 6.85 (m, 5H), 5.81 (q, *J* = 6.1 Hz, 1H), 5.05 (s, 2H), 4.00 (s, 3H), 3.81 (s, 3H), 1.77 (d, *J* = 6.1 Hz, 3H).

(*S*)-Methyl 4-(4-(4-methoxybenzyloxy)-2-nitrophenylamino)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzoate ((*S*)-20)



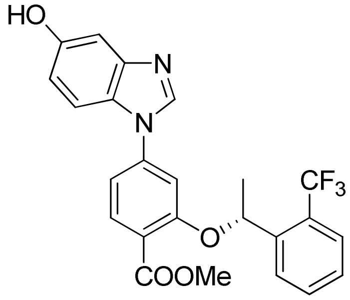
A solution of aniline **(*S*)-16** (1.20 g, 3.54 mmol), 1-iodo-4-(4-methoxybenzyloxy)-2-nitrobenzene[2] (1.50 mg, 3.80 mmol), bis(dibenzylideneacetone)palladium(0) (23 mg, 0.040 mmol), XANTPHOS (46 mg, 0.08 mmol) and cesium carbonate (5.80 g, 17.8 mmol) in 1,4-dioxane (25 mL, degassed) was heated to 60 °C overnight. The mixture was filtered over celite washing with THF, the solvent removed under reduced pressure and the residue purified by Biotage column chromatography (0 to 15% EtOAc/cyclohexane) to provide aniline **(*S*)-20** (2.07 g, 98%.). LCMS (ESI) *m/z* 597 (M+H). ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.02 (s, 1H), 7.92 (d, *J* = 7.9 Hz, 1H), 7.83 (d, *J* = 8.4 Hz, 1H), 7.70 (t, *J* = 1.6 Hz, 1H), 7.65 (d, *J* = 7.9 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.42 – 7.36 (m, 3H), 7.01 – 6.93 (m, 4H), 6.69 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.60 (d, *J* = 2.0 Hz, 1H), 5.72 (q, *J* = 6.1 Hz, 1H), 5.01 (s, 2H), 3.95 (s, 3H), 3.84 (s, 3H), 1.71 (d, *J* = 6.1 Hz, 3H).

(*S*)-Methyl 4-(5-(4-methoxybenzyloxy)-1H-benzo[d]imidazol-1-yl)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzoate ((*S*)-21)



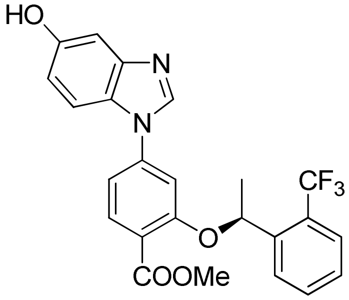
A solution of amine **(*S*)-20** (2.07 g, 3.46 mmol) in EtOAc (30 mL) was treated with platinum sulfided on charcoal (5% w/w, 720 mg, 0.19 mmol), PPTS (99 mg, 0.39 mmol) and methyl orthoformate (3.8 mL, 34.8 mmol). The mixture was stirred in an atmosphere of hydrogen overnight. The mixture was filtered over celite washing with EtOAc, the solvent removed under reduced pressure and the residue purified by Biotage column chromatography (0 to 60% EtOAc/cyclohexane) to give benzimidazole **(*S*)-21** (1.60 g, 80%). LCMS (ESI) *m/z* 577 (M+H). ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.97 (d, *J* = 8.3, Hz 1H), 7.93 – 7.90 (m, 2H), 7.69 (d, *J* = 7.8 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.45 – 7.38 (m, 3H), 7.36 (d, *J* = 2.3 Hz, 1H), 7.04 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.95 – 6.85 (m, 5H), 5.81 (q, *J* = 6.1 Hz, 1H), 5.05 (s, 2H), 4.00 (s, 3H), 3.81 (s, 3H), 1.77 (d, *J* = 6.1 Hz, 3H).

(*R*)-Methyl 4-(5-hydroxy-1H-benzo[d]imidazol-1-yl)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzoate ((*R*)-22)



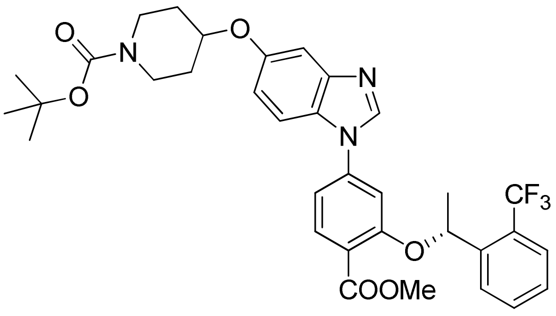
A solultion of benzimidazole (***R*)-21** (747 mg, 1.30 mmol) in DCM (8 mL) was treated with trifluoroacetic acid (1.0 mL, 13.5 mmol) at 0 °C. The mixture was allowed to reach room temperature and stirred for 3h. The reaction was brought to pH 5-6 with 1M NaOH and 1M HCl, the aqueous layer separated and extracted with DCM. The combined organic layers were concentrated and the residue was purified by Biotage column chromatography (0 to 70% EtOAc/cyclohexane) to afford benzimidazole (***R*)-22** (501 mg, 85%). LCMS (ESI) *m/z* 457 (M+H). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 – 7.89 (m, 3H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.42 – 7.39 (m, 2H), 7.02 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.91 (d, *J* = 1.9 Hz, 1H), 6.86 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.77 (d, *J* = 1.9 Hz, 1H), 5.98 (br s, 1H), 5.81 (q, *J* = 6.1 Hz, 1H), 4.00 (s, 3H), 1.76 (d, *J* = 6.1 Hz, 3H).

(*S*)-Methyl 4-(5-hydroxy-1H-benzo[d]imidazol-1-yl)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzoate ((*S*)-22)



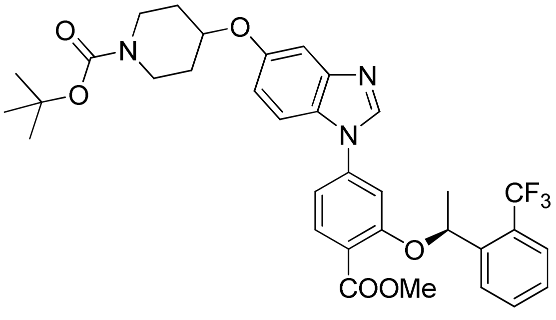
A solultion of benzimidazole (***S*)-21** (1.59 g, 2.76 mmol) in DCM (17 mL) was treated with trifluoroacetic acid (2.1 mL, 28.4 mmol) at 0 °C. The mixture was allowed to reach room temperature and stirred for 2h. The reaction was brought to pH 5-6 with 1M NaOH and 1M HCl, the aqueous layer separated and extracted with DCM. The combined organic layers were concentrated and the residue was purified by Biotage column chromatography (0 to 60% EtOAc/cyclohexane) to afford benzimidazole (***S*)-22** (1.20 mg, 95%). LCMS (ESI) *m/z* 457 (M+H). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 – 7.89 (m, 3H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.42 – 7.39 (m, 2H), 7.02 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.91 (d, *J* = 1.9 Hz, 1H), 6.86 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.77 (d, *J* = 1.9 Hz, 1H), 6.61 (br s, 1H), 5.81 (q, *J* = 6.1 Hz, 1H), 4.00 (s, 3H), 1.76 (d, *J* = 6.1 Hz, 3H).

(*R*)-*tert*-Butyl 4-(1-(4-(methoxycarbonyl)-3-(1-(2-(trifluoromethyl)phenyl)ethoxy)phenyl)-1H-benzo[d]imidazol-5-yloxy)piperidine-1-carboxylate ((*R*)-23a)



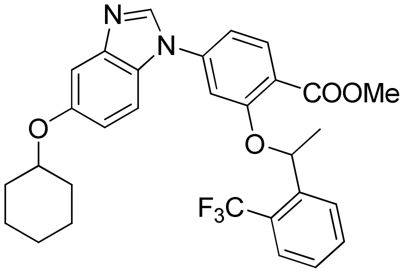
A solution of benzimidazole (***R*)-22** (483 mg, 1.06 mmol), 1-Boc-4-hydroxypiperidine (282 mg, 1.04 mmol) and triphenylphosphine (374 mg, 1.43 mmol) in DCM (6 mL) was cooled at 0 °C and treated with di-*tert*-butyl azodicarboxylate (333 mg, 1.45 mmol). The reaction was allowed to reach room temperature and stirred overnight. The mixture was quenched with water and extracted with DCM twice. The organics were dried (Na_2SO_4), concentrated and purified by Biotage column chromatography (0 to 50% EtOAc/cyclohexane) to give benzimidazole (***R*)-23a** (267 mg, 39%). LCMS (ESI) *m/z* 640 (M+H). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 – 7.94 (m, 1H), 7.91 – 7.87 (m, 2H), 7.59 – 7.55 (m, 1H), 7.53 (dd, *J* = 2.8, 1.4 Hz, 1H), 7.42 – 7.38 (m, 1H), 7.31 (d, *J* = 2.2 Hz, 1H), 7.03 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.91 – 6.82 (m, 3H), 5.80 (q, *J* = 6.1 Hz, 1H), 4.47 (tt, *J* = 7.1, 3.5 Hz, 1H), 3.98 (s, 3H), 3.88 – 3.68 (m, 2H), 3.37 – 3.27 (m, 2H), 1.98 – 1.89 (m, 2H), 1.83 – 1.71 (m, 5H), 1.46 (s, 9H).

(*S*)-*tert*-Butyl 4-(1-(4-(methoxycarbonyl)-3-(1-(2-(trifluoromethyl)phenyl)ethoxy)phenyl)-1H-benzo[d]imidazol-5-yloxy)piperidine-1-carboxylate ((*S*)-23a)



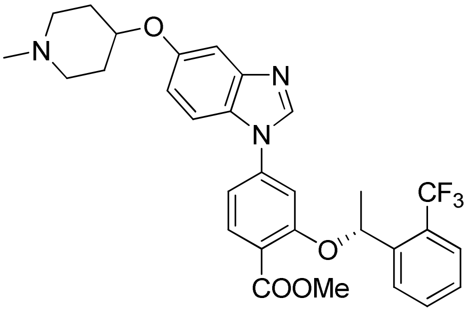
A solution of benzimidazole (***S*)-22** (1.20 mg, 2.63 mmol), 1-Boc-4-hydroxypiperidine (740 mg, 3.68 mmol) and triphenylphosphine (974 mg, 3.71 mmol) in DCM (15 mL) was cooled at 0 °C and treated with di-*tert*-butyl azodicarboxylate (850 mg, 3.70 mmol). The reaction was allowed to reach room temperature and stirred overnight. The mixture was quenched with water and extracted with DCM twice. The organics were dried (Na_2SO_4), concentrated and purified by Biotage column chromatography (0 to 50% EtOAc/cyclohexane) to give benzimidazole (***S*)-23a** (1.78 g, contaminated with coupling reagents, quant.). LCMS (ESI) *m/z* 640 (M+H). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 – 7.94 (m, 1H), 7.91 – 7.87 (m, 2H), 7.59 – 7.55 (m, 1H), 7.53 (dd, *J* = 2.8, 1.4 Hz, 1H), 7.42 – 7.38 (m, 1H), 7.31 (d, *J* = 2.2 Hz, 1H), 7.03 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.91 – 6.82 (m, 3H), 5.80 (q, *J* = 6.1 Hz, 1H), 4.47 (tt, *J* = 7.1, 3.5 Hz, 1H), 3.98 (s, 3H), 3.88 – 3.68 (m, 2H), 3.37 – 3.27 (m, 2H), 1.98 – 1.89 (m, 2H), 1.83 – 1.71 (m, 5H), 1.46 (s, 9H).

(±)-Methyl 4-(5-(cyclohexyloxy)-1H-benzo[d]imidazol-1-yl)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzoate (23b)



A solution of phenol ***rac*-22** (62 mg, 0.14 mmol), cyclohexanol (33 mg, 0.33 mmol) and triphenylphosphine (73 mg, 0.28 mmol) in DCM (1.3 mL) was cooled at 0 °C and treated with di-*tert*-butyl azodicarboxylate (62 mg, 0.27 mmol) The reaction was allowed to reach room temperature and additional batches of reagents were added every day until the reaction reached completion by TLC and LCMS. The mixture was diluted with DCM and quenched with water. The aqueous layer was separated and extracted with DCM twice. The combined organics were dried (Na_2SO_4), concentrated and purified by Biotage column chromatography (0 to 50% EtOAc/cyclohexane) to give ether **23b** (43 mg, 59%). HRMS (ESI) *m/z* calcd for $C_{30}H_{30}F_3N_2O_4$ (M+H) 541.2214, found 541.2228. ^1H NMR (500 MHz, MeOD) δ 8.30 (br. s, 1H), 8.09 – 7.90 (m, 2H), 7.79 (d, *J* = 7.9 Hz, 1H), 7.69 (t, *J* = 7.7 Hz, 1H), 7.55 (t, *J* = 7.7 Hz, 1H), 7.28 – 7.16 (m, 2H), 7.00 (d, *J* = 1.8 Hz, 1H), 6.85 (d, *J* = 8.7 Hz, 1H), 6.75 (br. d, *J* = 8.9 Hz, 1H), 5.89 (q, *J* = 6.3 Hz, 1H), 4.43 – 4.25 (m, 1H), 3.99 (s, 3H), 2.01 (br. s, 2H), 1.83 (br. s, 2H), 1.75 (d, *J* = 6.2 Hz, 3H), 1.65 – 1.34 (m, 6H).

(*R*)-Methyl 4-(5-(1-methylpiperidin-4-yloxy)-1H-benzo[d]imidazol-1-yl)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzoate ((*R*)-23c)

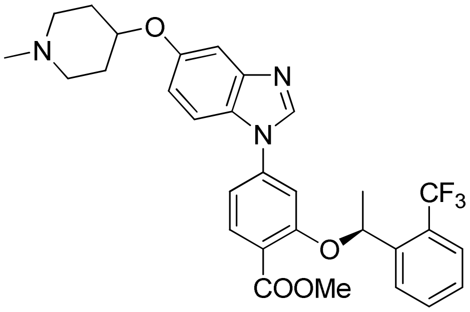


A solution of benzimidazole **(*R*)-23a** (254 mg, 0.40 mmol) in DCM (4 mL) was treated with trifluoroacetic acid (600 µL, 8.1 mmol) at 0 °C for 2h and 30 min. The reaction was brought to pH 8 with 1M NaOH and 1M HCl, and extracted with DCM three times. The combined organic layers were concentrated to afford the crude, unprotected amine **(*R*)-25** (256 mg).

A solution of crude amine **(*R*)-25** (256 mg) in DCM (5 mL) and MeOH (2.4 mL) was treated with formaldehyde in water (37% w/w, 65 µL, 0.81 mmol) and AcOH (30 µL, 0.52 mmol). To this mixture was added sodium triacetoxyborohydride (127 mg, 0.60 mmol) and the reaction mixture stirred for 2h at room temperature. The reaction was quenched with sat. aqueous sodium bicarbonate and the mixture extracted with DCM. The organics were dried (Na_2SO_4) and concentrated to afford benzimidazole **(*R*)-23c** (211 mg, 95% over two steps). LCMS (ESI) *m/z* 554 (M+H). ^1H NMR (500 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.3 Hz, 1H), 7.92 – 7.89 (m, 2H), 7.70 (d, *J* =

7.9 Hz, 1H), 7.59 (t, $J = 7.6$ Hz, 1H), 7.43 (t, $J = 7.6$ Hz, 1H), 7.31 (d, $J = 2.2$ Hz, 1H), 7.05 (dd, $J =$ 8.3, 2.2 Hz, 1H), 6.93 – 6.84 (m, 3H), 5.81 (d, $J = 6.2$ Hz, 1H), 4.40 – 4.33 (m, 1H), 4.00 (s, 3H), 2.85 – 2.72 (m, 2H), 2.39 (d, $J = 19.9$ Hz, 5H), 2.15 – 2.06 (m, 2H), 1.97 – 1.86 (m, 2H), 1.76 (d, J = 6.2 Hz, 3H).

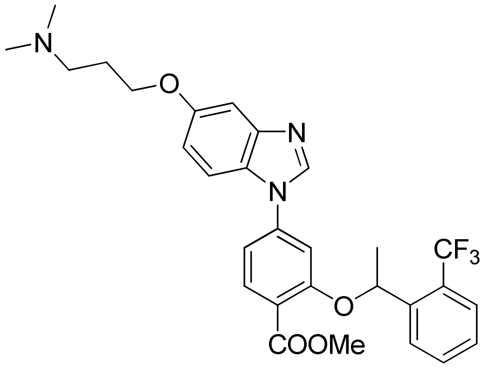
(*S*)-Methyl 4-(5-(1-methylpiperidin-4-yloxy)-1H-benzo[d]imidazol-1-yl)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzoate ((*S*)-23c)



A solution of benzimidazole (***S*)-23a** (498 mg, 0.78 mmol) in DCM (7 mL) was treated with trifluoroacetic acid (1.15 mL, 15.5 mmol) at 0 °C for 2h and 30 min. The reaction was brought to pH 8 with 1M NaOH and 1M HCl, and extracted with DCM twice. The combined organic layers were concentrated to afford the crude amine **(*S*)-25** (437 mg).

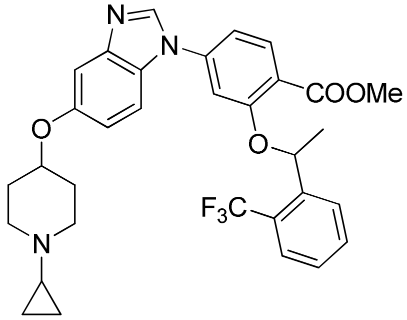
A solution of crude amine **(*S*)-25** (437 mg) in DCM (8 mL) and MeOH (4 mL) was treated with formaldehyde in water (37% w/w, 130 µL, 1.62 mmol) and AcOH (55 µL, 0.96 mmol). To this mixture was added sodium triacetoxyborohydride (252 mg, 1.19 mmol) and the reaction mixture stirred for 1h at room temperature. The reaction was quenched with sat. aqueous sodium bicarbonate and the mixture extracted with DCM. The organics were dried (Na_2SO_4), concentrated and purified by Biotage column chromatography (0 to 20% MeOH/DCM) to gave **(*S*)-23c** (216 mg, 50% over two steps). LCMS (ESI) *m/z* 554 (M+H). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (d, $J = 8.3$ Hz, 1H), 7.92 – 7.89 (m, 2H), 7.70 (d, $J = 7.9$ Hz, 1H), 7.59 (t, $J = 7.6$ Hz, 1H), 7.43 (t, $J = 7.6$ Hz, 1H), 7.31 (d, $J = 2.2$ Hz, 1H), 7.05 (dd, $J = 8.3$, 2.2 Hz, 1H), 6.93 – 6.84 (m, 3H), 5.81 (d, $J = 6.2$ Hz, 1H), 4.40 – 4.33 (m, 1H), 4.00 (s, 3H), 2.85 – 2.72 (m, 2H), 2.39 (d, $J = 19.9$ Hz, 5H), 2.15 – 2.06 (m, 2H), 1.97 – 1.86 (m, 2H), 1.76 (d, $J = 6.2$ Hz, 3H).

(±)-Methyl 4-(5-(3-(dimethylamino)propoxy)-1H-benzo[d]imidazol-1-yl)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzoate (23d)



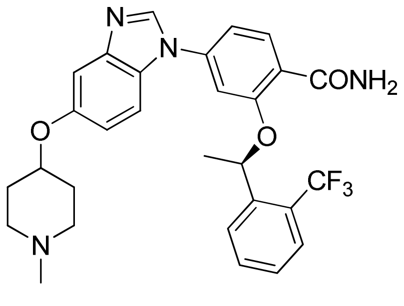
A suspension of alcohol ***rac*-22** (93 mg, 0.204 mmol), 1-dimethylamino-3-chloropropane hydrochloride (81 mg, 0.514 mmol) and cesium carbonate (334 mg, 1.02 mmol) in DMF (3.6 mL) was heated at 50 °C for 16 h. The reaction was partitioned with EtOAc and water and the aqueous layer extracted further with EtOAc. The combined organics were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo* to afford benzimidazole **23d** (839 mg, 75%). HRMS (ESI) *m/z* calcd for $C_{29}H_{31}F_3N_3O_4$ (M+H) 542.2261, found 542.2280. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 – 7.91 (m, 1H), 7.90 – 7.84 (m, 2H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.57 (t, *J* = 7.6 Hz, 1H), 7.41 (t, *J* = 7.6 Hz, 1H), 7.25 (d, *J* = 1.8 Hz, 1H), 7.03 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.88 (d, *J* = 1.8 Hz, 1H), 6.84 – 6.81 (m, 2H), 5.79 (q, *J* = 6.1 Hz, 1H), 4.05 (t, *J* = 6.4 Hz, 2H), 3.98 (s, 3H), 2.51 – 2.45 (m, 2H), 2.26 (s, 6H), 2.02 – 1.94 (m, 2H), 1.74 (d, *J* = 6.1 Hz, 3H).

(±)-Methyl 4-(5-(1-cyclopropylpiperidin-4-yloxy)-1H-benzo[d]imidazol-1-yl)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzoate (23e)



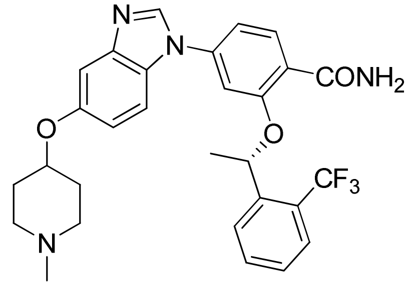
A solution of (1-ethoxycyclopropoxy)trimethylsilane (40 μL, 0.20 mmol) and crude amine ***rac*-25** (68 mg, 0.13 mmol) in THF (1 mL) was treated with acetic acid (22 μL, 0.39 mmol) and sodium cyanoborohydride (13 mg, 0.21 mmol) at rt. The mixture was stirred at 55 °C overnight. The reaction was concentrated and partitioned between sat. aqueous $NaHCO_3$ and EtOAc. The aqueous layer was extracted with EtOAc and the combined organics were washed with brine, dried (Na_2SO_4), concentrated and purified by Biotage column chromatography (0 to 100% EtOAc/cyclohexane) to give cyclopropylamine **23e** (20 mg, 27%). LCMS (ESI) *m/z* 580 (M+H). ^{1}H NMR (500 MHz, CDCl3) δ 8.27 (s, 1H), 8.02 (d, *J* = 8.2 Hz, 1H), 7.88 (d, *J* = 7.9 Hz, 1H), 7.74 (d, *J* = 7.9 Hz, 1H), 7.63 (t, *J* = 7.5 Hz, 1H), 7.40 (d, *J* = 2.2 Hz, 1H), 7.05 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.01 (dd, *J* = 9.1, 2.3 Hz, 1H), 6.93 (d, *J* = 1.9 Hz, 1H), 6.84 (d, *J* = 9.1 Hz, 1H), 5.81 (q, *J* = 6.1 Hz, 1H), 4.53 – 4.45 (m, 1H), 4.04 (s, 3H), 2.99 – 2.86 (br. m, 2H), 2.57 (br. t, *J* = 10.0 Hz, 2H), 2.11 – 2.00 (br. m, 2H), 1.89 – 1.76 (br. m, 5H), 1.71 – 1.64 (m, 1H), 0.54 – 0.39 (m, 4H).

(*R*)-4-(5-(1-Methylpiperidin-4-yloxy)-1H-benzo[d]imidazol-1-yl)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzamide ((*R*)-24a)



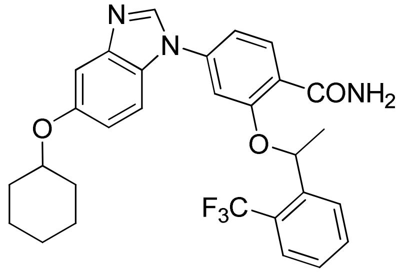
A solution of ester **(*R*)-23c** (107 mg, 0.19 mmol) was treated with ammonia in methanol (7M, 4 mL) and heated to 75 °C in a close cap vial for 6 days. The mixture was concentrated and the residue was purified by Biotage column chromatography (0 to 15% (MeOH/Et_3N 1:1)/EtOAc) to afford amide **(*R*)-24a** (80 mg, 77%). $[\alpha]^{20}_{D}$ –27 (*c* 0.15, MeOH). HRMS (ESI) *m/z* calcd for $C_{29}H_{30}F_3N_4O_3$ (M+H) 539.2265, found 539.2272. ^{1}H NMR (500 MHz, MeOD) δ 8.23 (s, 1H), 8.11 (d, *J* = 8.3 Hz, 1H), 7.82 (t, *J* = 7.0 Hz, 2H), 7.70 (t, *J* = 7.6 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.26 – 7.22 (m, 2H), 6.97 (d, *J* = 1.9 Hz, 1H), 6.84 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.66 (d, *J* = 8.9 Hz, 1H), 5.96 (q, *J* = 6.1 Hz, 1H), 4.49 – 4.41 (m, 1H), 2.82 – 2.74 (m, 2H), 2.49 – 2.38 (m, 2H), 2.35 (s, 3H), 2.11 – 2.02 (m, 2H), 1.90 – 1.80 (m, 5H).

(*S*)-4-(5-(1-Methylpiperidin-4-yloxy)-1H-benzo[d]imidazol-1-yl)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzamide ((*S*)-24a)



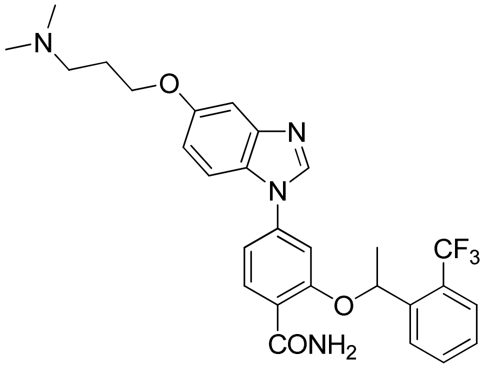
A solution of ester **(*S*)-23c** (89 mg, 0.16 mmol) was treated with ammonia in methanol (7M, 4 mL) and heated to 75 °C in a close cap vial for 7 days. The mixture was concentrated to afford amide **(*S*)-24a** (89 mg, quant.). $[\alpha]^{20}_{D}$ +27 (*c* 0.12, MeOH). HRMS (ESI) *m/z* calcd for $C_{29}H_{30}F_3N_4O_3$ (M+H) 539.2265, found 539.2276. ^{1}H NMR (500 MHz, MeOD) δ 8.23 (s, 1H), 8.11 (d, *J* = 8.3 Hz, 1H), 7.82 (t, *J* = 7.0 Hz, 2H), 7.70 (t, *J* = 7.6 Hz, 1H), 7.58 (t, *J* = 7.6 Hz, 1H), 7.26 – 7.22 (m, 2H), 6.97 (d, *J* = 1.9 Hz, 1H), 6.84 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.66 (d, *J* = 8.9 Hz, 1H), 5.96 (q, *J* = 6.1 Hz, 1H), 4.49 – 4.41 (m, 1H), 2.82 – 2.74 (m, 2H), 2.49 – 2.38 (m, 2H), 2.35 (s, 3H), 2.11 – 2.02 (m, 2H), 1.90 – 1.80 (m, 5H).

(±)-4-(5-(Cyclohexyloxy)-1H-benzo[d]imidazol-1-yl)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzamide (24b)



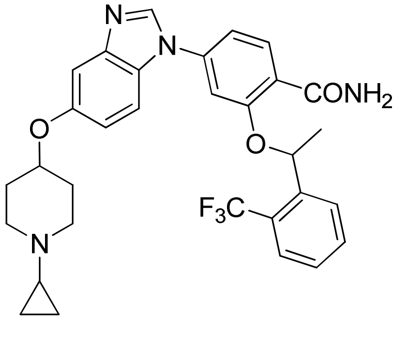
A solution of ester **23b** (41 mg, 0.08 mmol) was treated with ammonia in methanol (7M, 4 mL) and heated to 75 °C for 3 days. The white precipitate formed was collected by filtration to afford amide **24b** (24 mg, 60%). Purity (HPLC): >93%. HRMS (ESI) *m/z* calcd for $C_{29}H_{29}F_3N_3O_3$ (M+H) 525.2187, found 525.2162. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 8.37 (s, 1H), 7.94 – 7.80 (m, 3H), 7.78 – 7.68 (m, 3H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.30 – 7.20 (m, 2H), 6.90 (d, *J* = 1.6 Hz, 1H), 6.76 (dd, *J* = 8.8, 2.2 Hz, 1H), 6.64 (d, *J* = 8.9 Hz, 1H), 5.89 (q, *J* = 6.0 Hz, 1H), 4.42 – 4.28 (m, 1H), 1.92 (br. s, 2H), 1.76 – 1.66 (m, 5H), 1.58 – 1.22 (m, 6H).

(±)-4-(5-(3-(Dimethylamino)propoxy)-1H-benzo[d]imidazol-1-yl)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzamide (24c)



A solution of ester **23d** (42 mg, 0.078 mmol) was treated with ammonia in methanol (7M, 4 mL) and heated to 70 °C in a close cap vial for 3 days. The mixture was concentrated and the residue was purified by Biotage column chromatography (0 to 100% (DCM/1M NH_3 in MeOH 9:1)/DCM) to afford amide **24c** (36 mg, 88%). HRMS (ESI) *m/z* calcd for $C_{28}H_{30}F_3N_4O_3$ (M+H) 527.2265, found 527.2269. ^{1}H NMR (500 MHz, 1:1 $CDCl_3$:MeOD) δ 8.24 (d, *J* = 8.4, 1H), 8.04 (s, 1H), 7.77 (d, *J* = 7.8 Hz, 1H), 7.73 (d, *J* = 7.8 Hz, 1H), 7.65 (t, *J* = 7.6 Hz, 1H), 7.53 (t, *J* = 7.6 Hz, 1H), 7.21 – 7.15 (m, 2H), 6.91 (d, *J* = 1.8 Hz, 1H), 6.80 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.69 (d, *J* = 8.9 Hz, 1H), 5.91 (q, *J* = 6.1 Hz, 1H), 4.05 (t, *J* = 4.9 Hz, 2H), 2.61 – 2.56 (m, 2H), 2.31 (s, 6H), 2.02 (m, 2H), 1.83 (d, *J* = 6.1 Hz, 3H).

(±)-4-(5-(1-Cyclopropylpiperidin-4-yloxy)-1H-benzo[d]imidazol-1-yl)-2-(1-(2-(trifluoromethyl)phenyl)ethoxy)benzamide (24d)



A solution of ester **23e** (20 mg, 0.04 mmol) was treated with ammonia in methanol (7M, 4 mL) and heated to 75 °C for 5 days. The mixture was concentrated and purified by Biotage column chromatography (0 to 5% MeOH/DCM) to give amide **24d** (6 mg, 32%). HRMS (ESI) *m/z* calcd for $C_{31}H_{32}F_3N_4O_3$ (M+H) 565.2421, found 565.2414. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.41 (d, *J* = 8.4 Hz, 1H), 7.96 – 7.83 (m, 2H), 7.77 (d, *J* = 7.7 Hz, 1H), 7.69 (d, *J* = 7.8 Hz, 1H), 7.62 (t, *J* = 7.5 Hz, 1H), 7.50 (t, *J* = 7.6 Hz, 1H), 7.33 (s, 1H), 7.17 (dd, *J* = 8.4, 1.9 Hz, 1H), 6.92 – 6.81 (m, 3H), 5.99 – 5.86 (m, 2H), 4.34 (br. s, 1H), 2.95 (br. s, 2H), 2.45 (br. s, 2H), 2.02 (br. s, 2H), 1.88 – 1.74 (br. m, 5H), 1.65 (br. s, 1H), 0.57 – 0.36 (br. m, 4H).

Table S1. Summary of cocrystallographic analysis of Nek2 with compounds ***rac*-1**, **13f**, **13c** and ***rac*-24a**.[a]

Crystals	Nek2-**rac-1**	Nek2-**13f**	Nek2-**13c**	Nek2-***rac*-24a**
PDB ID	2XNM	2XNN	2XNO	2XNP
Space group	*C*2	*C*2	*C*2	*C*2
Cell dimension				
a, b, c (Å)	100.80, 56.83, 81.30	101.21, 56.85, 80.40	101.05, 56.88, 81.34	101.64, 56.88, 81.84
α, β, γ (°)	90.00,133.21, 90.00	90.00, 133.21, 90.00	90.00, 133.46, 90.00	90.00, 133.63, 90.0000
Data collection				
X-ray source	Diamond I03	ESRF ID14_2	Diamond I03	Diamond I03
Wavelength	0.97630	0.93300	0.97630	0.97630
Resolution range (Å)	46.37-1.79	39.75-2.50	40.15-1.80	46.52-1.90
(Highest resolution shell)	(1.89-1.79)	(2.64-2.5)	(1.90-1.80)	(2.00-1.90)
Unique observations	30991	11653	30461	26646
Completeness (%)	98.1 (99.1)	99.8 (100)	97.7 (96.8)	99.4 (99.7)
Multiplicity	2.6 (2.6)	3.2 (3.2)	3.7 (3.7)	3.6 (3.6)
Rmerge	0.061 (0.395)	0.138 (0.275)	0.078 (0.569)	0.061 (0.395)
$I/\sigma I$	11.1 (2.5)	6.5 (3.6)	13.0 (2.5)	11.1 (2.5)
Refinement				
Resolution range (Å)	36.73-1.85	31.24-2.5	36.67-1.98	36.78-1.98
Reflection used (free R set)	27237 (1391)	11309 (563)	22422 (1136)	22973 (1165)
*R*factor (%)	16.32	17.18	17.03	15.66
*R*free[a] (%)	19.62	23.74	21.41	20.12
Number of atoms (protein/ligand/water)	2117/39/244	2000/30/111	2033/37/207	2101/39/224
B-factor (protein/ligand/water) (Å^2)	30.05/33.29/38.56	33.60/36.70/33.98	29.55/56.63/37.01	30.82/23.90/38.35
Ramachandran (favorable/allowed)	97.52/2.48	97.5/2.50	98.33/1.67	97.48/2.52
Rmsd (bonds, Å; angles, °)	0.007/1.022	0.007/0.955	0.007/0.973	0.010/1.212

[a] Free *R*factor was computed using 5% of the data assigned randomly, and is the same set of reflections for all four structures

Table S2. Selectivity data for ***rac*-24a** against a panel of kinases.[a]

kinase	% of inhibition
MAPKAPK2	1
AurA	16
PKCz	0
RSK1	30
PRAK	1
Erk1	7
PKD2	49
CH1d	55
CHK1	25
ABL	57
FYN	18
LYN	26
CHK2	0
MET	59
LCK	73
SRC	33
GSK3b	41
Erk2	12
PKA	4
AKT2	0
INSR	0
p38a	6
AKT1	10
MSK1	18

[a] 2 µM ***rac*-24a**, 155µM ATP, ~10 nM Nek2 (Millipore), 30min preincubation compound + enzyme; Gini coefficient[5] = 0.534.

Table S3. Metabolic data for compounds ***rac*-24a** and **(*R*)-24a**.[a]

compound	species	% metabolized at 30 min.
***rac*-24a**	HLM	46.1 ± 11.5
(*R*)-24a	HLM	45.0 ± 9.1
verapamil	HML	71.2 ± 4.0
***rac*-24a**	MLM	52.9 ± 6.4
(*R*)-24a	MLM	55.5 ± 0.6
verapamil	MLM	89.8 ± 0.1

[a] HLM, human liver microsomes; MLM, mouse liver microsomes.

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