Supplementary Information for

Aminoacyl-triazine derivatives are isoform-selective PI3Kβ inhibitors that target a non-conserved aspartyl residue, D862 of PI3Kβ.

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Supplementary Table 1 Biochemical characterization of WT PI3Kβ, WT PI3Kα, D862Q PI3Kβ and Q859D PI3Kα.

	Km ATP (μM)	Km PI (μM)
WT PI3Kα	24	9.2
Q859D PI3Kα	24	6.5
WT PI3Kβ	70	26
D862Q PI3Kβ	25	3.3

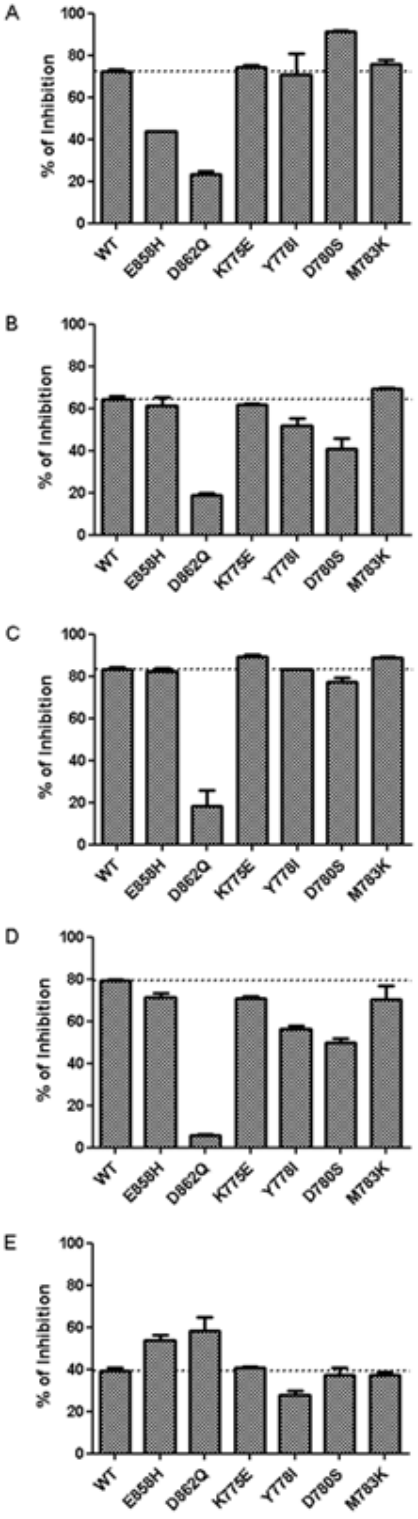
Supplementary Table 2 Inhibition of PI3K isoforms and in vitro mutants by ZSTK474 analogues.

IC_{50} (nM)	PI3Kα WT	PI3Kα Q859D	PI3Kβ WT	PI3Kβ D862Q	Fold change αWT→ αQ859D	Fold change βWT→ βD862Q
19	8200±1200	2300±440	62±11	890±15	↓3.5	↑14
21	12000±1400	8100±450	74±7.0	6000±1700	↓1.4	↑82
23	9200±760	2800±710	140±26	1400±190	↓3.3	↑10

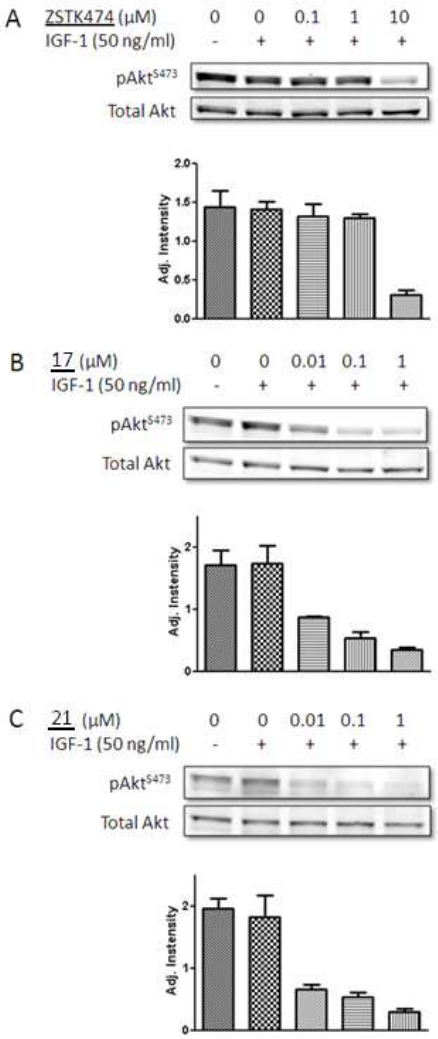
Supplementary Table 3 KinomeScan® screen of **21** (10μM) against 96 kinases. (www.discoverx.com)

KINOMEscan Gene Symbol	Percent Control
ABL1(T315I)-phosphorylated	100
ABL1-nonphosphorylated	100
ABL1-phosphorylated	100
ACVR1B	87
ADCK3	53
AKT1	93
AKT2	74
ALK	61
AURKA	100
AURKB	100
AXL	96
BMPR2	100
BRAF	63
BRAF(V600E)	54
BTK	100
CDK11	100
CDK2	76
CDK3	86
CDK7	100
CDK9	78
CHEK1	100
CSF1R	85
CSNK1D	29
CSNK1G2	94
DCAMKL1	95
DYRK1B	55
EGFR	91
EGFR(L858R)	77
EPHA2	89
ERBB2	53
ERBB4	100
ERK1	95
FAK	82
FGFR2	90
FGFR3	75
FLT3	74
GSK3B	91
IGF1R	100
IKK-alpha	100
IKK-beta	100
INSR	93
JAK2(JH1domain-catalytic)	100
JAK3(JH1domain-catalytic)	100
JNK1	49
JNK2	68
JNK3	80
KIT	100
KIT(D816V)	85
KIT(V559D,T670I)	79
LKB1	59
MAP3K4	99
MAPKAPK2	100
MARK3	67

MEK1	100
MEK2	100
MET	100
MKNK1	100
MKNK2	100
MLK1	100
MTOR	9.1
p38-alpha	100
p38-beta	100
PAK1	77
PAK2	53
PAK4	76
PCTK1	100
PDGFRA	82
PDGFRB	100
PDPK1	82
PIK3C2B	0.6
PIK3CB	0.5
PIK4CB	100
PIM1	62
PIM2	46
PIM3	41
PKAC-alpha	88
PLK1	85
PLK3	100
PLK4	100
PRKCE	11
RAF1	74
RET	96
RIOK2	21
ROCK2	76
RSK2(Kin.Dom.1-N-terminal)	97
SNARK	100
SRC	91
SRPK3	100
TGFBR1	94
TIE2	21
TRKA	100
TSSK1B	75
TYK2(JH1domain-catalytic)	95
ULK2	100
VEGFR2	83
YANK3	80
ZAP70	94



Supplementry Figure 1 Effect of PI3K isoform mutations (β-α) on compound inhibition profile at selected concentrations using 10μM ATP: (A) **17,** 70nM**,** (B) **19,** 70nM**,** (C) **23,** 200nM (D) **21**, 80nM (E) **20** 1μM. The percentage inhibition was calculated using the activity determined in the absence of inhibitor as 0% inhibition and the activity in the absence of enzyme as 100% inhibition. Results are the average of at least 3 separate determinations. Error bars represent the standard deviation from the mean.



Supplementary Fig. 2 pAkt-S473 inhibition by selective compounds in MDA-MB-468 cells. (A) ZSTK474 (B) 17 (C) 21. Cells were serum-starved overnight before exposing to various concentrations of compounds for 2h, followed by IGF-1 (50ng/ml) induction for 15 min. Western blots for each compound are an example of three independent experiments. Image-J analysis is reported as Mean±SD of three independent experiments.

Experimental

Chemistry

All chemical reagents acquired from Sigma-Aldrich, Fluka, Merck, BDH laboratories, CSL, Ajax Finechem, Merck Schuhardt, ChemSupply, Auspep, Prolabo, Lancaster, TCI, Matrix Scientific, Boron Molecular, Alfa Aesar, Chem-Impex and May and Baker were used without further purification. 1H-NMR spectra were recorded with either a 300 MHz Varian widebore NMR spectrometer or a 400 MHz Bruker Ultrashield-Avance NMR spectrometer. ^{13}C-NMR spectra were recorded with a 400 MHz Bruker Ultrashield-Avance NMR spectrometer.

Results were recorded as follows: chemical shift values are expressed units acquired in either $CDCl_3$ (7.26 ppm), $(CD_3)_2SO$ (2.50 ppm) or CD_3OD (3.31 ppm) as references, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration and coupling constants (*J*) in Hertz. Mass spectra were acquired in the positive and negative mode using an atmospheric pressure (ESI/APCI) ion source on a Micromass Platform ESI/APCI single quadrupole mass spectrometer with sample management facilitated by an Agilent 1100 series HPLC system using MassLynx version 3.5 software. High Resolution Mass Spectrometry analyses were collected on a Waters Micromass LCT Premier XE Orthogonal Acceleration Time-of-Flight Mass Spectrometer coupled to an Alliance 2795 Separation Module using MassLynx version 4.1 software. LCMS analysis was performed using an Agilent 6100 series Single Quad (Series 1200 HPLC, Column: Luna C8(2), 50 x 4.6 mm; solvent system: 5-100% acetonitrile in water with 0.1% Formic acid over 10 minutes; flow rate 0.5 ml/min; molecular weight range 100-1000; cone Voltage ; column temperature 30 °C). All masses were reported as the protonated parent ions. Analytical RP-HPLC was obtained on a Waters Millenium 2690 system, with UV detection at 254 nM. 28Method used gradient elution through a Supelco C8 column (150 x 2.1 mm ID) 20-100% Buffer B (Buffer A: H_2O, 0.1% TFA; Buffer B: 80% CH_3CN, 0.1% TFA, 19.9% H_2O or Buffer B: 80% CH_3OH, 0.1% TFA, 19.9% H_2O) over 10 minutes at 1.0 ml/min. Preparative RP-HPLC was obtained on a Waters 600 HPLC system and Waters 486 tunable absorbance detector, with UV detection at 254 nM. Gradient elution through a Phenomonex Luna C8 (2) 10 µ column (50 x 21.2 mm ID), 20-100% Buffer B (Buffer A: H_2O, Buffer B: 80% CH_3CN, 20% H_2O) over 30 minutes at 5 ml/min. Melting point determination was performed uncorrected using a Mettler Toledo MP50 melting point apparatus. Microwave chemistry was performed using a Biotage Initiator Microwave Reactor.

Synthesis of Precursors

N-Boc-piperazine

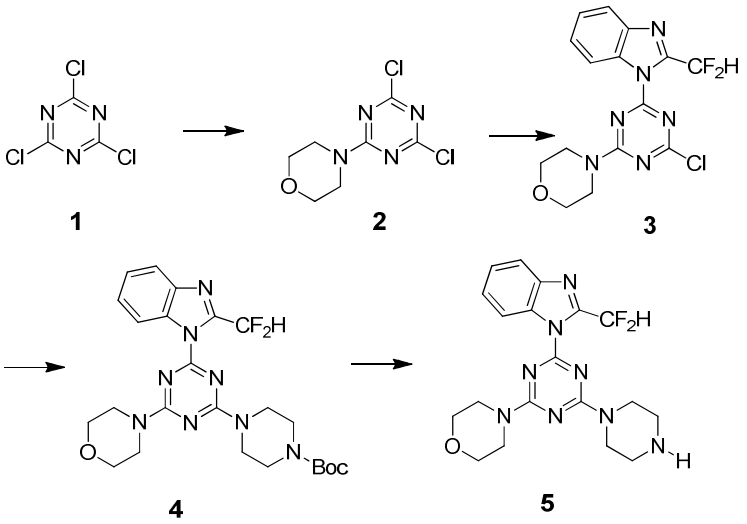
To a stirred solution of piperazine (5.0 g, 58 mmol, 2 equiv) in DCM (145 ml) at 0 °C, was added dropwise a solution of di-tert-butyldicarbonate (6.3 g, 1 equiv) in DCM (58 ml) over 20 minutes, then stirred a further 1 hour at 0 °C. The reaction mixture was filtered and remaining filtrate was evaporated under reduced pressure, with remaining oil diluted with H_2O, refiltered, saturated with K_2CO_3, extracted with ether, evaporated under reduced pressure to yield the product as white crystals; yield: 3.5 g, 65.2%; 1H-NMR (300 MHz, CD_3OD), 3.40 (t, *J* = 4.8 Hz, 4H), 2.77 (t, *J* = 5.4 Hz, 4H), 1.46 (s, 9H); ESI-MS, *m/z* 187.3 [M+H]+.

2-Difluoromethylbenzimidazole

A mixture of 1,2-phenylenediamine (0.50 g, 4.6 mmol, 1 equiv) and difluoroacetic acid (2.2 g, 5 equiv) in H_2O (10 ml) in a sealed tube was exposed to MW irradiation (90 W,

130 °C) for 10-15 minutes. The reaction mixture was diluted with H_2O (50 ml), neutralized with 50% NaOH with resulting precipitate filtered, washed with H_2O, then dried to yield the product as orange powder. Remaining product was extracted into EtOAc, evaporated under reduced pressure and dried to yield the product as brown crystals; yield: 0.70 g, 90.3%; 1H-NMR (300 MHz, CD_3OD), 7.65 (s, 2H), 7.35 (dd, *J* = 6.1, 3.1 Hz, 2H), 7.02 (t, *J* = 53.1 Hz, 1H); ESI-MS, *m/z* 169.2 [M+H]+.

Synthesis of 4-(4-(2-(Difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (5)



Scheme S1 - Synthesis of key intermediate 5

4-(4,6-dichloro-1,3,5-triazin-2-yl)morpholine (2)

To a stirred solution of cyanuric chloride (10.0 g, 54 mmol, 1.4 equiv) in acetone (100 ml) was added dropwise a solution of morpholine (3.4 g, 1 equiv) and triethylamine (3.9 g, 1 equiv) in acetone (100 ml) at -20 °C, then quenched with H_2O, stirred for a few minutes, filtered, washed with MeOH and dried to yield the product **2** ashite powder,
yield: 8.4 g, 93.0%; 1H-NMR (300 MHz, CD_3OD), 3.87 (t, *J* = 4.8 Hz, 4H, OCH_2), 3.73 (t, *J* = 4.8 Hz, 4H, NCH_2); LCMS (ESI): (m/z) = 236.1 [M+H]+; HPLC (MeCN): 5.99 min.

4-(4-Chloro-6-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-1,3,5-triazin-2-yl)morpholine (3)

A mixture of 4-(4,6-dichloro-1,3,5-triazin-2-yl)morpholine (**2**) (0.74 g, 3.2 mmol, 1 equiv), 2-difluoromethylbenzimidazole (0.53 g, 1 equiv) and K_2CO_3 (0.44 g, 1 equiv) in DMF (10 ml) was stirred at room temperature for 4 hours. The reaction mixture was poured onto H_2O, filtered, washed with H_2O and small amount of MeOH, then dried to yield the product **3** as off-white powder, yield: 0.49 g, 42.6%; 1H-NMR (300 MHz, $CDCl_3$), 8.44 (dd, *J* = 7.4, 1.4 Hz, 1H), 7.91 (dd, *J* = 7.1, 1.4 Hz, 1H), 7.58 (t, *J* = 53.4 Hz, 1H), 7.47 (m, 2H), 3.97 (m, 4H), 3.83 (m, 4H); LCMS (ESI): (m/z) = 367.2 [M+H]+; ESI-MS, *m/z* 367.8 [M+H]+; HPLC (MeCN): 8.30 min.

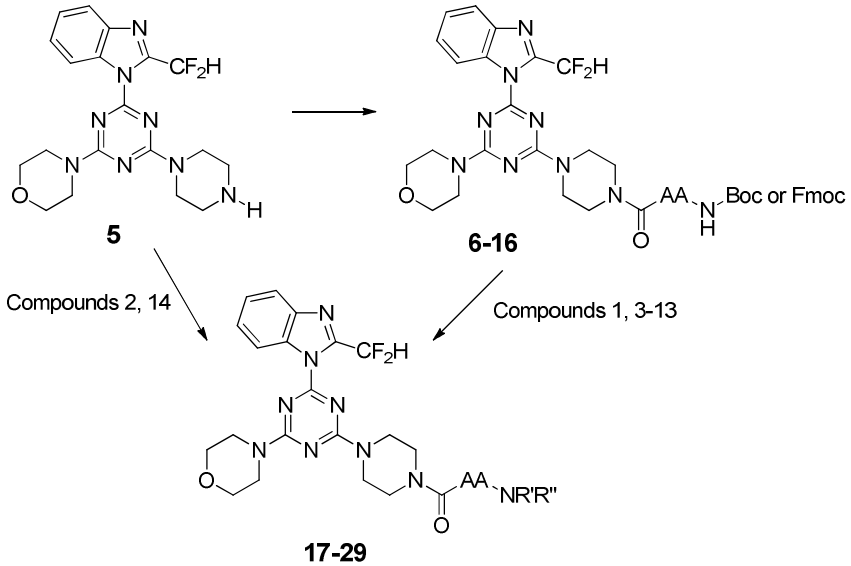
Tert-butyl-4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazine-1-carboxylate (4)

A mixture of 4-(4-chloro-6-(2-(difluoromethyl)-1*H*-benzo[*d*]imidazol-1-yl)-1,3,5-triazin-2-yl)morpholine (**3**) (0.48 g, 1.3 mmol, 1 equiv), K_2CO_3 (0.36 g, 2 equiv) and boc-piperazine (0.49 g, 2 equiv) in DMF (15 ml) in a sealed tube was exposed to microwave irradiation (90W, 140 °C) for 30 – 40 min. The reaction mixture was cooled, taken up into H_2O, extracted into EtOAc, dried over Na_2SO_4, evaporated under reduced pressure with product crystallising overnight as off-white crystals which were filtered, washed with ether and $CHCl_3$, yielding **4**: 0.27 g, 39.5%; 1H-NMR (300 MHz, $CDCl_3$), 8.33 (dd, *J* = 7.1, 1.7 Hz, 1H), 7.89 (dd, *J* = 6.7, 1.5 Hz, 1H), 7.56 (t, *J* = 53.0 Hz, 1H), 7.42 (m, 2H), 3.87 (t, *J* = 4.5 Hz, 8H), 3.79 (t, *J* = 4.2 Hz, 4H), 3.54 (br s, 4H), 3.35 (t, *J* = 4.5 Hz, 4H), 1.50 (s, 9H); LCMS (ESI): (m/z) = 517.1 [M+H]+; ESI-MS, *m/z* 517.4 (100%), 417.4 (85%) [M+H]+; HPLC (MeCN): 9.75 min.

4-(4-(2-(Difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (5)

A solution of tert-butyl 4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazine-1-carboxylate (**4**) (0.13 g, 0.25 mmol) in DCM:TFA (1:1, 2 ml) was stirred at room temperature for 20 minutes. The mixture was evaporated under reduced pressure, diluted with H_2O, neutralized with 2 N NaOH, filtered, washed with H_2O, then dried to yield product (**5**) as off-white crystals, yield: 0.13 g, 100%, Mp: 250-252 °C; 1H-NMR (300 MHz, CD_3OD), 8.44 (d, *J* = 7.5 Hz, 1H), 7.81 (d, *J* = 7.4 Hz, 1H), 7.69 (t, *J* = 53.0 Hz, 1H), 7.47 (m, 2H), 4.14 (t, *J* = 6.1 Hz, 4H), 3.91 (t, *J* =7.8 Hz, 4H), 3.76 (br s, 4H), 3.35 (t, *J* = 13.1 Hz, 4H); ^{13}C-NMR (101 MHz, CD_3OD), 166.30, 166.22, 163.28, 147.72, 142.43, 134.48, 127.25, 125.85, 121.31, 117.42, 109.82 (t, *J* = 240 Hz), 67.58, 45.42, 45.34, 44.27; LCMS (ESI): (m/z) = 417.1 [M+H]+; ESI-MS, *m/z* 417.6 [M+H]+; HR-MS calculated for $C_{19}H_{22}N_8OF_2$ [M+H]+: 417.1957; found 417.1975; HPLC (MeCN): 5.57 min.

Synthesis of Final compounds



Scheme S2 - Synthesis of final products

3a. General methods

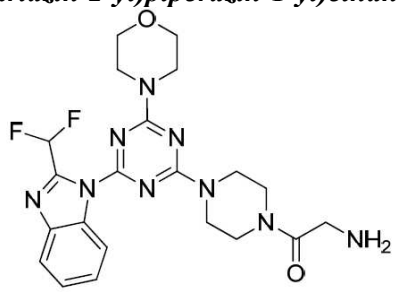
General method for amino acid coupling – Method A

To a solution of a Boc-protected or Fmoc protected amino acid (0.24 mmol, 2 equiv) in DMF (3 ml) was added HBTU or HCTU (0.091 g, 2 equiv) followed by DIPEA (0.062 g, 4 equiv). This solution was added to 4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**5**) (0.050 g, 1 equiv) in DMF (1 ml) then stirred at room temperature for 2 hr. The reaction mixture was diluted with MeCN:H_2O (1:1, 50 ml) then placed on freezedrier overnight resulting in a solid residue. The product was purified by elution through silica (100% EtOAc) or crystallization from MeOH and H_2O.

General procedure for Boc-deprotection – Method B
A solution of the Boc-protected aminoacylpiperazinyltriazine (1.3 mmol) in DCM:TFA (1:1, 2 ml) was stirred at room temperature for 20 minutes. The mixture was evaporated under reduced pressure, diluted with H_2O, neutralized with 1 or 2 N NaOH, filtered, washed with H_2O, then dried to yield the product which was purified as described.

General procedure for Fmoc deprotection- Method C
A solution of the Fmoc-protected aminoacylpiperazinyltriazine (0.062 mmol) in THF (4 ml) was added 1-octanethiol (0.091 g, 10 equiv) followed by DBU (0.003 g, 0.03 equiv) under N_2, then stirred at room temperature for 4 hr. The reaction mixture was evaporated under reduced pressure then triturated with ether. This was left to stand for approximately 1 hr, filtered, washed with ether then dried to yield the product.

***2-Amino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)ethanone* 17 (AA = Gly)**

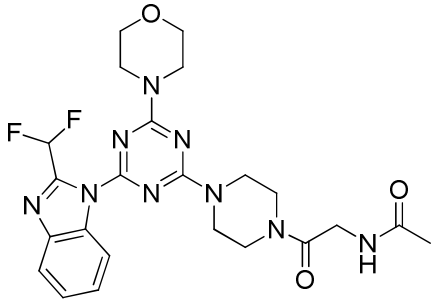


Tert-butyl-(2-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-2-oxoethyl)carbamate (6) was prepared via Method A from 4-(4-(2-(difluoromethyl)-1*H*-benzo[*d*]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**5**) (0.050 g, 0.12 mmol, 1 equiv) and Boc-Gly-OH (0.042 g, 2 equiv) then purified using silica plug (100% EtOAc) to yield product as yellow powder, yield: 0.011 g, 16.4%; LCMS (ESI): (m/z) = 574.2 [M+H]+; ESI-MS, *m/z* 574.4 [M+H]+; HPLC (MeCN): 8.67 min.

2-Amino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)ethanone (17) was prepared via Method B from *tert*-butyl (2-(4-(4-(2-(difluoromethyl)-1*H*-benzo[*d*]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-2-oxoethyl)carbamate (**6**) (0.011 g, 0.02 mmol) to yield product as yellow powder, yield: 0.010 g, 100%; Mp: 154-157 °C; 1H-NMR (300 MHz, CD_3OD), 8.44 (d, *J* = 8.2 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.72 (t, *J* = 53.7 Hz, 1H), 7.50 (dd, *J* = 18.1, 8.5 Hz, 2H), 4.62 (br s, 2H), 3.98 (br s, 4H), 3.92 (br s, 4H), 3.78 (br s, 6H), 3.59 (br s, 2H); ^{13}C-NMR (101 MHz, CD_3OD), 166.56, 166.28, 163.58, 142.64, 141.41, 134.78, 127.37, 125.93, 121.45, 117.49, 109.91 (t, *J* = 248 Hz), 68.72, 67.65, 45.36, 42.87, 41.11, 27.12; LCMS (ESI): (m/z) = 474.1 [M+H]+; ESI-MS, *m/z* 474.4 [M+H]+; HR-MS

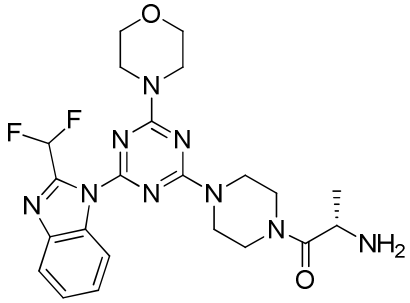
calculated for $C_{21}H_{25}N_9O_2F_2$ [M+H]+: 474.2172; found 474.2179; HPLC (MeCN): 6.29 min.

N-(2-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-2-oxoethyl)acetamide **18 (AA = Ac-Gly)**



N-(2-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-2-oxoethyl)acetamide (18) was prepared via Method A from 4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**5**) (0.050 g, 0.12 mmol, 1 equiv) and N-acetylglycine (0.028 g, 2 equiv) then purified by semi preparative RP-HPLC to yield product as white powder, yield: 0.005 g, 10.0%; 1H-NMR (400 MHz, CD_3OD), 8.46 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 7.6 Hz, 1H), 7.73 (t, J = 52.8 Hz, 1H), 7.48 (m, 2H), 4.13 (d, J = 11.2 Hz, 2H), 3.99 (t, J = 4.8 Hz, 2H), 3.93 (t, J = 4.8 Hz, 6H), 3.78 (m, 4H), 3.71 (t, J = 4.8 Hz, 2H), 3.67 (s, 2H), 2.03 (s, 3H); LCMS (ESI): (m/z) = 516.1 [M+H]+; HR-MS calculated for $C_{23}H_{27}N_9O_3F_2$ [M+H]+: 516.2278; found 516.2291; HPLC (MeCN): 7.26 min.

(S)-2-amino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)propan-1-one **19 (AA = L-Ala)**

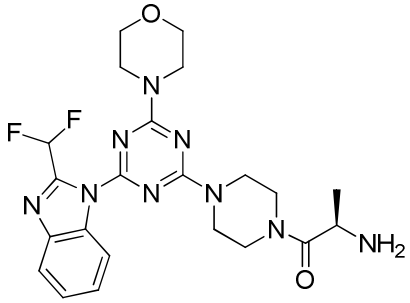


(S)-tert-butyl (1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-1-oxopropan-2-yl)carbamate (7) was prepared via Method A from 4-(4-(2-(difluoromethyl)-1*H*-benzo[*d*]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**5**) (0.050 g, 0.12 mmol, 1 equiv) and Boc-L-Ala-OH (0.045 g, 2 equiv) then purified by column chromatography using 100% EtOAc to yield product as an oil, yield: 0.066 g, 92.9%; LCMS (ESI): (m/z) = 588.2 [M+H]+; HPLC (MeCN): 8.83 min.

(S)-2-amino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)propan-1-one (19) was prepared from (S)-tert-butyl (1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-1-oxopropan-2-yl)carbamate via Method B from (**7**) (0.066 g, 0.11 mmol) to yield product as white crystals, yield: 0.004 g, 7.7%; 1H-NMR (400 MHz, CD_3OD), 8.42 (t, J = 8.0 Hz, 1H), 7.80 (d, J = 7.7 Hz, 1H), 7.71 (t, J = 53.2 Hz, 1H), 7.46 (m, 2H), 4.23 (m, 1H), 4.03 (m, 2H), 3.98 (m, 2H), 3.90 (t, 6H), 3.78 (s, 4H), 3.69 (s, 2H), 1.40 (d, J = 6.9

Hz, 3H); LCMS (ESI): (m/z) = 488.1 [M+H]+; HR-MS calculated for $C_{22}H_{27}N_9O_2F_2$ [M+H]+: 488.2329; found 488.2320; HPLC (MeCN): 6.15 min.

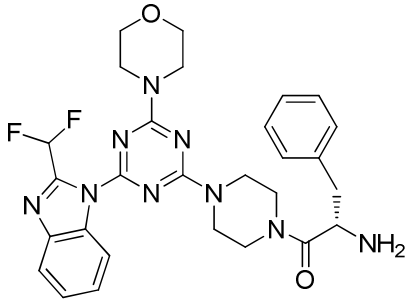
***(R)-2-amino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)propan-1-one* 20 (AA = D-Ala)**



(R)-tert-butyl (1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-1-oxopropan-2-yl)carbamate (8) was prepared via Method A from 4-(4-(2-(difluoromethyl)-1*H*-benzo[*d*]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**5**) (0.050 g, 0.12 mmol, 1 equiv) and Boc-D-Ala-OH (0.045 g, 2 equiv) then purified by column chromatography using 100% EtOAc to yield product as an oil, yield: 0.066 g, 93.6% LCMS (ESI): (m/z) = 588.2 [M+H]+; HPLC (MeCN): 8.78 min.

(R)-2-amino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)propan-1-one (20) was prepared from (R)-tert-butyl (1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-1-oxopropan-2-yl)carbamate via Method B from (**8**) (0.066 g, 0.11 mmol) to yield product as off-white powder, yield: 0.007 g, 12.8%; 1H-NMR (400 MHz, CD_3OD), 8.43 (d, *J* = 8.2 Hz, 1H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.70 (t, *J* = 53.2 Hz, 1H), 7.46 (m, 2H), 4.22 (dd, *J* = 13.5, 6.5 Hz, 1H), 4.02 (m, 2H), 3.98 (s, 2H), 3.88 (dd, *J* = 18.8, 13.7 Hz, 6H), 3.75 (s, 4H), 3.69 (s, 2H), 1.39 (d, *J* = 6.9 Hz, 3H); LCMS (ESI): (m/z) = 488.1 [M+H]+; HR-MS calculated for $C_{22}H_{27}N_9O_2F_2$ [M+H]+: 488.2329; found 488.2329; HPLC (MeCN): 5.96 min.

***(S)-2-amino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-3-phenylpropan-1-one* 21 (AA = L-Phe)**

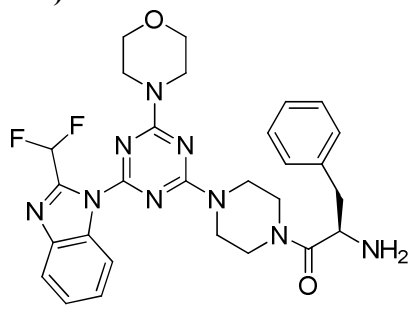


(S)-tert-butyl (1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate (9) was prepared via Method A from 4-(4-(2-(difluoromethyl)-1*H*benzo[*d*]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**5**) (0.050 g, 0.12 mmol, 1 equiv) and Boc-L-Phe-OH (0.064 g, 2 equiv) then purified by column chromatography using 100% EtOAc to yield product as an oil, yield: 0.070 g, 87.9%; LCMS (ESI): (m/z) = 664.2 [M+H]+; HPLC (MeCN):

9.79 min.

(S)-2-amino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-3-phenylpropan-1-one (21) was prepared via Method B from (S)-tert-butyl (1-(4-(4-(2-(difluoromethyl)-1Hbenzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate (**9**) (0.087 g, 0.13 mmol) to yield product as yellow crystals, yield: 0.024 g, 35.8%, yellow crystals; 1H-NMR (400 MHz, CD_3OD), 8.21 (m, 2H), 7.85 (d, 2H), 7.39 (m, 6H), 4.70 (br s, 1H), 3.80 (s, 8H), 3.67 (s, 2H), 3.54 (m, 2H), 3.36 (m, 2H), 3.06 (m, 3H), 2.80 (t, J = 8.0 Hz, 1H); LCMS (ESI): (m/z) = 564.2 [M+H]+; HR-MS calculated for $C_{28}H_{31}N_9O_2F_2$ [M+H]+: 564.2642; found 564.2647; HPLC (MeCN): 6.94 min.

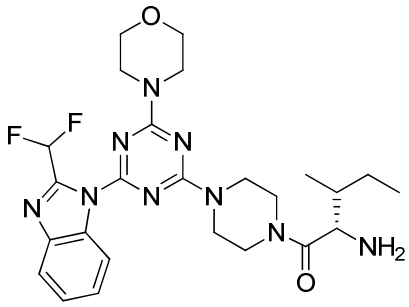
(R)-tert-butyl (1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate **22 (AA = D-Phe)**



(R)-tert-butyl (1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate (10) was prepared via Method A from 4-(4-(2-(difluoromethyl)-1*H*-benzo[*d*]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**5**) (0.050 g, 0.12 mmol, 1 equiv) and Boc-D-Phe-OH (0.064 g, 2 equiv) then purified by column chromatography using 100% EtOAc to yield product as an oil, yield: 0.11 g, > 100% ; LCMS (ESI): (m/z) = 664.2 [M+H]+; HPLC (MeCN): 9.62 min.

(R)-2-amino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-3-phenylpropan-1-one (22) was prepared via Method B from (R)-tert-butyl (1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate (**10**) (0.080 g, 0.12 mmol) then purified by semi preparative RP-HPLC to yield product as off-white powder, yield: 0.042 g, 62.4%; 1H-NMR (400 MHz, CD_3OD), 8.38 (s, 1H), 7.78 (d, J = 7.6 Hz, 1H), 7.66 (d, J = 9.6 Hz, 1H), 7.40 (m, 7H), 4.72 (m, 1H), 3.90 (s, 4H), 3.77 (s, 4H), 3.70 (dd, J = 8.9, 4.8 Hz, 4H), 3.45 (br s, 2H), 3.10 (m, 4H); LCMS (ESI): (m/z) = 564.1 [M+H]+; HR-MS calculated for $C_{28}H_{31}N_9O_2F_2$ [M+H]+: 564.2642; found 564.2632; HPLC (MeCN): 7.09 min.

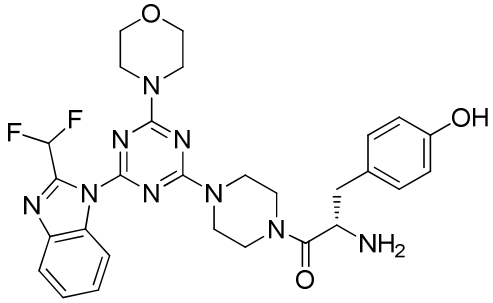
(2S,3S)-2-amino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-3-methylpentan-1-one **23 (AA = L-Ile)**



tert-butyl ((2S,3S)-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-3-methyl-1-oxopentan-2-yl)carbamate (11) was prepared via Method A from 4-(4-(2-(difluoromethyl)-1*H*-benzo[*d*]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**5**) (0.050 g, 0.12 mmol, 1 equiv) and Boc-L-Ile-OH (0.056 g, 2 equiv) then purified by column chromatography using 100% EtOAc to yield product as an oil, yield: 0.066 g, 95.2%; LCMS (ESI): (m/z) = 630.2 [M+H]+; HPLC (MeCN): 9.97 min.

(2S,3S)-2-amino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-3-methylpentan-1-one (23) was prepared from tertbutyl ((2S,3S)-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-3-methyl-1-oxopentan-2-yl)carbamate via Method B from (**11**) (0.068 g, 0.11 mmol) to yield product as off-white crystals, yield: 0.017 g, 29.0%; 1H-NMR (400 MHz, CD_3OD), 8.44 (d, J = 8.1 Hz, 1H), 7.81 (d, J = 7.5 Hz, 1H), 7.72 (t, J = 53.2 Hz, 1H), 7.46 (m, 2H), 4.39 (d, J = 5.0 Hz, 1H), 4.10 (m, 2H), 3.90 (m, 8H), 3.78 (s, 4H), 3.67 (s, 2H), 1.95 (m, 1H), 1.60 (m, 1H), 1.26 (m, 2H), 1.12 (m, 3H), 0.97 (m, 3H); LCMS (ESI): (m/z) = 530.2 [M+H]+; HR-MS calculated for $C_{25}H_{33}N_9O_2F_2$ [M+H]+: 530.2798; found 530.2813; HPLC (MeCN): 6.73 min.

(S)-2-amino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-3-(4-hydroxyphenyl)propan-1-one **24 (AA = L-Tyr)**

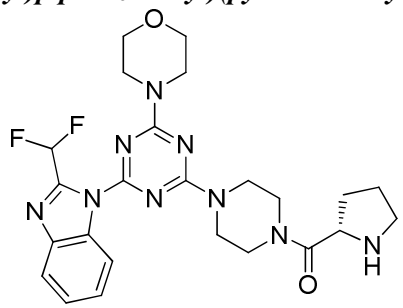


(S)-tert-butyl (1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamate (12) was prepared via Method A from 4-(4-(2-(difluoromethyl)-1*H*-benzo[*d*]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**5**) (0.050 g, 0.12 mmol, 1 equiv) and Boc-L-Tyr-OH (0.068 g, 2 equiv) then purified by column chromatography using 100% EtOAc to yield product as an oil, yield: 0.091 g, > 100% ; LCMS (ESI): (m/z) = 680.2 [M+H]+; HPLC (MeCN): 8.82 min.

(S)-2-amino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-3-(4-hydroxyphenyl)propan-1-one (24) was prepared from (S)-tert-butyl (1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamate via Method

B from (**12**) (0.091 g, 0.13 mmol) to yield product as off-white powder yield: 0.020 g, 25.4%; 1H-NMR (400 MHz, CD_3OD), 8.36 (s, 1H), 7.77 (d, *J* = 7.6 Hz, 1H), 7.63 (d, *J* = 10.4 Hz, 1H), 7.43 (m, 2H), 7.07 (d, *J* = 8.4 Hz, 1H), 6.71 (m, 2H), 4.70 (s, 1H), 4.41 (s, 1H), 3.86 (s, 4H), 3.71 (s, , 6H), 3.57 (m, 1H), 3.52 (m, 1H), 3.46 (m, 2H), 3.05 (m, 2H), 2.85 (m, 2H); LCMS (ESI): (m/z) = 580.1 [M+H]+; HR-MS calculated for $C_{28}H_{31}N_9O_3F_2$ [M+H]+: 580.2591; found 580.2590; HPLC (MeCN): 6.31 min.

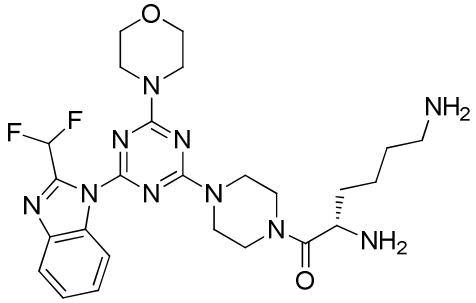
***(S)-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)(pyrrolidin-2-yl)methanone* 25 (AA = L-Pro)**



(S)-tert-butyl 2-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazine-1-carbonyl)pyrrolidine-1-carboxylate (13) was prepared via Method A from 4-(4-(2-(difluoromethyl)-1*H*-benzo[*d*]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**5**) (0.050 g, 0.12 mmol, 1 equiv) and Boc-L-Pro-OH (0.048 g, 2 equiv) then purified by column chromatography using EtOAc:MeOH (95:5) to yield product as an oil, yield: 0.075 g, 100%; LCMS (ESI): (m/z) = 614.2 [M+H]+; HPLC (MeCN): 9.63 min.

(S)-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)(pyrrolidin-2-yl)methanone (25) was prepared from (S)-tert-butyl 2-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazine-1-carbonyl)pyrrolidine-1-carboxylate via Method B from (**13**) (0.059 g, 0.1 mmol) then purified by semi preparative RP-HPLC to yield product as white powder, yield: 0.004 g, 8.2%; 1H-NMR (400 MHz, CD_3OD), 8.41 (d, *J* = 8.0 Hz, 1H), 7.80 (m, 1H), 7.71 (t, *J* = 52.8 Hz, 1H), 7.42 (m, 3H), 4.76 (dd, *J* = 8.8, 7.0 Hz, 1H), 4.00 (m, 2H), 3.90 (m, 6H), 3.77 (s, 4H), 3.65 (s, 2H), 3.45 (m, 2H), 2.59 (m, 1H), 2.10 (m, 2H), 2.00 (m, 1H); LCMS (ESI): (m/z)= 514.1 [M+H]+; HR-MS calculated for $C_{24}H_{29}N_9O_2F_2$ [M+H]+: 514.2485; found 514.2502; HPLC (MeCN): 6.59 min.

***(S)-2,6-diamino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)hexan-1-one* 26 (AA = Lys)**

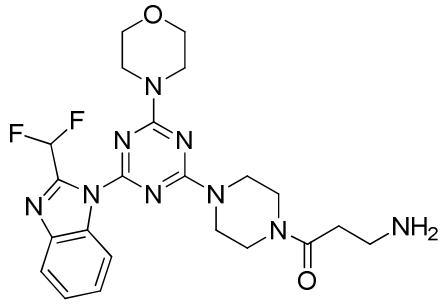


(S)-di-tert-butyl (6-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-6-oxohexane-1,5-diyl)dicarbamate (14) was prepared

via Method A from 4-(4-(2-(difluoromethyl)-1*H*-benzo[*d*]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**5**) (0.050 g, 0.12 mmol, 1 equiv) and Boc-L-Lys(Boc)-OH.DCHA (0.13 g, 2 equiv) then purified by column chromatography using 100% EtOAc to yield product as an oil, yield: 0.080 g, 89.0%; LCMS (ESI): (m/z) = 745.1 [M+H]+; HPLC (MeCN): 9.67 min.

(S)-2,6-diamino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)hexan-1-one (26) was prepared from (S)-di-tert-butyl(6-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-6-oxohexane-1,5-diyl)dicarbamate via Method B from (**14**) (0.089 g, 0.12 mmol) then purified by semi preparative RP-HPLC to yield product as a white powder, yield: 0.006 g, 9.2%; 1H-NMR (400 MHz, CD_3OD), 8.44 (d, *J* = 8.1 Hz, 1H), 7.80 (d, *J* = 7.9 Hz, 1H), 7.71 (t, *J* = 52.8 Hz, 1H), 7.51 (d, *J* = 8.2 Hz, 1H), 7.42 (m, 1H), 4.46 (t, *J* = 6.1 Hz, 1H), 4.12 (m, 2H), 3.91 (m, 6H), 3.78 (s, 6H), 3.64 (s, 2H), 2.96 (m, 2H), 1.90 (m, 2H), 1.71 (m, 2H), 1.52 (m, 2H); LCMS (ESI): (m/z) = 545.2 [M+H]+; HR-MS calculated for $C_{25}H_{34}N_{10}O_2F_2$ [M+H]+: 545.2907; found 545.2921; HPLC (MeCN): 5.17 min.

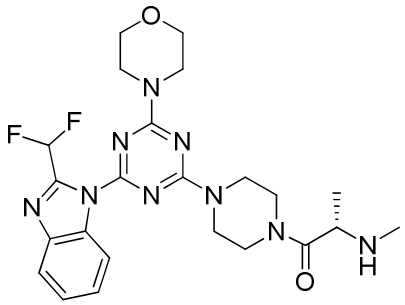
***3-amino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)propan-1-one* 27 (AA = β-Ala)**



(9H-fluoren-9-yl)methyl (3-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-3-oxopropyl)carbamate (15) was prepared via Method A from 4-(4-(2-(difluoromethyl)-1*H*-benzo[*d*]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**5**) (0.050 g, 0.12 mmol, 1 equiv) and Fmoc-β-Ala-OH (0.075 g, 2 equiv) then purified by column chromatography using EtOAc:MeOH (95:5) to yield product as an oil, yield: 0.044 g, 51.6%; LCMS (ESI): (m/z) = 710.1 [M+H]+; HPLC (MeCN): 8.93 min.

3-amino-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)propan-1-one (27) was prepared via Method C from (9Hfluoren-9-yl)methyl (3-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-3-oxopropyl)carbamate (**15**) (0.044 g, 0.1 mmol) to yield product as off-white powder, yield: 0.010 g, 33.1%; 1H-NMR (400 MHz, CD_3OD) 8.45 (d, *J* = 8.0 Hz, 1H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.72 (t, *J* = 52.8 Hz, 1H), 7.45 (m, , 2H), 3.96 (t, *J* = 5.2 Hz, 2H), 3.89 (t, *J* = 4.0 Hz, 4H), 3.76 (s, 4H), 3.71 (s, 2H), 3.61 (t, *J* = 5.2 Hz, 2H), 3.55 (t, *J* = 6.0 Hz, 2H), 3.35 (t, *J* = 6.0 Hz, 2H), 2.96 (t, *J* = 6.4 Hz, 1H), 2.65 (t, *J* = 6.4 Hz, 2H), 1.75 (br s, 2H); LCMS (ESI): (m/z) = 488.1 [M+H]+; HR-MS calculated for $C_{22}H_{27}N_9O_2F_2$ [M+H]+: 488.2329; found 488.2343; HPLC (MeCN): 6.07 min.

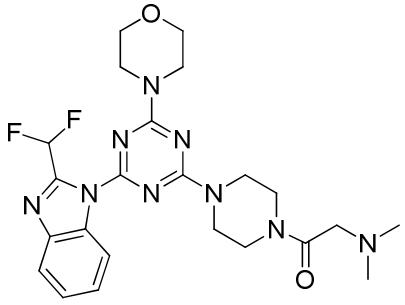
***(S)-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-2-(methylamino)propan-1-one* 28 (AA = N-Me-L-Ala)**



(S)-tert-butyl (1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-1-oxopropan-2-yl)(methyl)carbamate (16) was prepared via Method A from 4-(4-(2-(difluoromethyl)-1*H*-benzo[*d*]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**5**) (0.050 g, 0.12 mmol, 1 equiv) and Boc-N-Me-Ala-OH (0.049 g, 2 equiv) then purified by column chromatography using 100% EtOAc to yield product as an oil, yield: 0.066 g, 92.0%; LCMS (ESI): (m/z) = 602. 2 [M+H]+; HPLC (MeCN): 9.29 min.

(S)-1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-2-(methylamino)propan-1-one (28) was prepared via Method B from (S)-tert-butyl (1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate (**16**) (0.066 g, 0.11 mmol) then purified by semi preparative RP-HPLC to yield product as off-white powder, yield: 0.010 g, 18.2%; 1H-NMR (400 MHz, CD_3OD), 8.43 (d, J = 8.0 Hz, 1H), 7.80 (d, J = 7.5 Hz, 1H), 7.71 (t, J = 53.2 Hz, 1H), 7.46 (m, 2H), 4.57 (s, 1H), 4.39 (q, J = 6.9 Hz, 1H), 4.03 (m, 2H), 3.89 (t, J = 4.8 Hz, 6H), 3.78 (s, 4H), 3.67 (s, 4H), 2.69 (s, 3H), 1.51 (d, J = 6.8 Hz, 3H); LCMS (ESI): (m/z) = 502.1 [M+H]+; HR-MS calculated for $C_{23}H_{29}N_9O_2F_2$ [M+H]+: 502.2485; found 502.2494; HPLC (MeCN): 6.14 min.

1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-2-(dimethylamino)ethanone **29 (AA = *N,N*-dimethylglycine)**



1-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-2-(dimethylamino)ethanone (29) was prepared via Method A from 4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**5**) (0.050 g, 0.12 mmol, 1 equiv) and dimethylglycine.HCl (0.034 g, 2 equiv) to yield product as white powder, yield: 0.026 g, 17.2%; 1H-NMR (400 MHz, CD_3OD), 8.44 (d, J = 7.8 Hz, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.72 (t, J = 52.8 Hz, 1H), 7.48 (m, 2H), 4.28 (s, 2H), 4.00 (dt, J = 15.6, 1.6 Hz, 4H), 3.92 (t, J = 4.8 Hz, 4H), 3.74 (s, 6H), 3.54 (s, 2H), 2.96 (s, 6H); LCMS (ESI): (m/z) = 502.3 [M+H]+; HR-MS calculated for $C_{23}H_{29}N_9O_2F_2$ [M+H]+: 502.2485; found 502.2473; HPLC (MeCN): 6.20 min.

Materials and Methods
Computational Modelling

Computer modelling was performed using the using Maestro 9.3 suite (Schrödinger). The PI3Kβ X-ray structure (2Y3A) was obtained from the PDB (http://www.rcsb.org). All solvents and small molecules were removed from structures. For 2Y3A Q862D mutant structure, the residue was mutated prior to refinement. Protein preparation and refinement was performed Protein Preparation Wizard using default parameters. Receptor grid generation was confined to 20 Å from the binding site ligand. Ligands were prepared using LigPrep 2.5. Docking calculations were performed in Glide 5.8 using extra precision (XP) mode. Sampling was limited to 10000 ligand poses per docking run and 10 poses per ligand were retained for analysis.

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Generation of baculovirus containing p110β mutant DNA

The methods used here have been described previously (Frazzetto et al., 2008; Zheng et al., 2011) with the pFastBac™ system (Invitrogen, U.S.A.) used to generate recombinant baculovirus. In brief, mutant plasmids were generated using the appropriate primer pair and Pfu DNA polymerase (Promega, U.S.A.) with the template DNA being pFastBac™ wild-type p110β as appropriate. The DNA sequence was then confirmed as containing the correct mutation with the remaining DNA sequence re-confirmed as being identical to wild-type. Mutant plasmids were then transformed into DH10Bac *E.coli* for transposition into the bacmid. Blue/white selection was used to select for colonies containing recombinant bacmids with presence of the recombinant DNA in the bacmid confirmed using PCR. Recombinant bacmid DNA was then transfected, using lipofectin (Invitrogen, U.S.A.), into Sf21 cells and supernatant containing recombinant virus was collected after 3-5 days at 27^{o}C. High titre virus stock was then produced by amplification through two cycles of infection. Production of p110 protein was confirmed by western blotting of cell extracts separated by SDS-PAGE using a p110α or p110β specific antibody.

Protein expression and purification

p110 virus (20 ml) and p85 virus (5 ml) were added to each 200 ml of SF21 cells (2 x 10^6 cells/ml) and incubated shaking at 140 rpm for 48 h at 27 °C, after which time the cells were collected by centrifugation and stored at -80 °C until ready for extraction. The p110/p85 PI3K protein complex was extracted from the cells and purified using Ni-agarose chromatography. Fractions containing the PI3K protein were pooled and dialysed against 50 mM TrisHCl pH 7.5, 300 mM NaCl at 4 °C. PI3K protein was then made 20% (v/v) glycerol and 2 mM dithiothreitol and stored at -80°C.

Inhibition assays

The PI3K inhibitors were dissolved at 10 mM in dimenthyl sulphoxide (DMSO) and stored at -20°C until use. PI3K enzyme activity was determined using a luminescence assay measuring ATP consumption. PI3K enzyme activity was determined in 50 µl of 20 mM HEPES pH 7.5, 5 mM $MgCl_2$ with PI and ATP at the indicated concentrations. After a 60 min incubation at room temperature the reaction was stopped by the addition of 50 µl of Kinase-Glo (Promega) followed by a further 15 min incubation. Luminescence was then read using a Fluostar plate reader (BMG Labtech). Inhibitors were diluted in 20% (v/v) DMSO at the indicated concentrations in order to generate a concentration versus inhibition of enzyme activity curve which was then analysed using GraphPad Prism version 5.00 for Windows, (GraphPad Software, San Diego California USA) in order to calculate the IC_{50}. Standard deviations for the calculated IC50's are within 25% of the mean.

Assay of Akt phosphorylation in MB-MDA-468 cells

Cells were seeded in 6-well plates at a density of 5 x 10^5 cells per well and allowed to grow for 32 h. Cells were then serum-starved overnight before exposing to various concentrations of compounds for 2 h, followed by IGF-1 (50ng/ml) stimulation for 15 min. The cell lysates were subsequently subjected to western blot analysis. Blots were detected by Odyssey infrared imaging system (LI-COR) and bands were quantified by Image-J.

Assay of cell growth in MB-MDA-468 cells

Cell viability was assessed using the Cell Titer 96 Aqueous One Solution Cell Proliferation Assay according to Gozgit JM, Wong MJ, Wardwell S, Tyner JW, Loriaux MM, et al. 2011. *Mol Cancer Ther* 10: 1028-35. Exponentially growing cell lines were plated into 96-well plates and incubated for twenty-four hours at 37°C. Wells were treated with compound or vehicle (dimethyl sulfoxide) for 48 hours. Absorbance was measured using a VERSA max microplate reader (Molecular Devices), data were analysed by GraphPadPrism 5 and are the results of three independent experiments, each tested in duplicate.