Electronic supplementary information for paper

Novel p38α MAP kinase inhibitors identified from yoctoReactor DNA-encoded small molecule library

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Supporting information

Biology of MAP kinases

Since several biological therapies based on cytokine blockade have proven highly potent in treatment of chronic inflammatory disorders, identification of small molecules blocking key regulators in such cytokine cascades have been a major focus for the pharmaceutical industry. Thus, p38 inhibitors have applications in a number of inflammatory disorders including rheumatoid arthritis, inflammatory bowel disease, psoriasis and chronic obstructive pulmonary disease.[1,2] Several p38 inhibitors have already been developed and tested in clinical trials but initially the progression to phase III studies and beyond for many p38 inhibitors was delayed and many trials targeting p38 were concluded unsuccessfully.[3] For many early clinical failures a major problem of existing inhibitors may be related to structure-based toxicity.[4] Indeed, due to the existence of 518 human protein kinases with structural similarity in the adenosine-binding pocket, the design of potent and specific p38 kinase inhibitors remains a major challenge.[5] However, novel p38 inhibitors such as PH-797804 have recently demonstrated efficacy in phase II/III trials for chronic obstructive pulmonary disease.[6] Interestingly, a recent phase II study for losmapimod has instead suggested some improved outcome after acute coronary syndromes.[7]

Lib022 synthesis

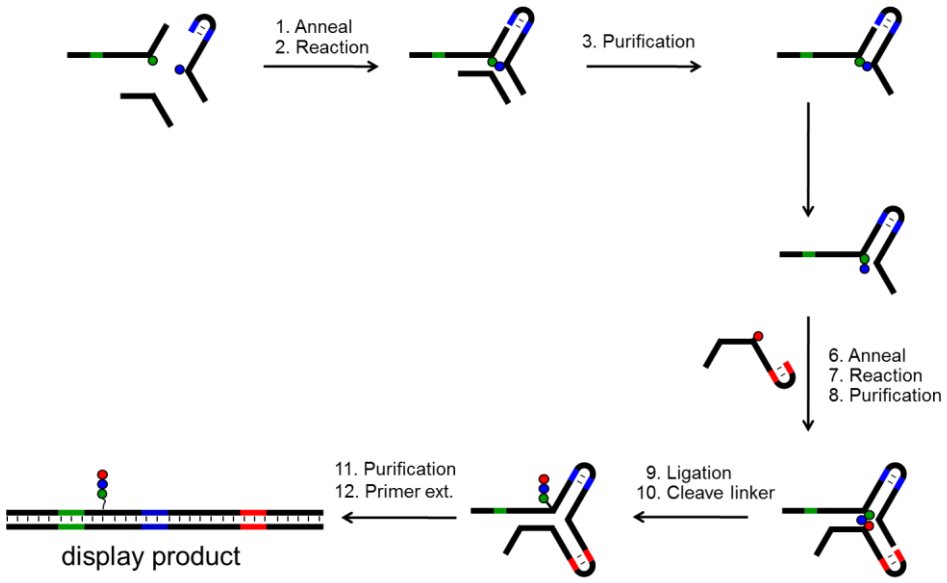


Fig. S1 Stepwise library synthesis using yoctoReactor technology (see text for details)

The library was synthesized sequentially in two DNA-directed chemical reaction steps employing three types of reactions (acylation, reductive amination and urea formation) in each step (Figure S1). yoctoReactor DNA was divided into pools for each step according to distribution of chemistries and the reaction conditions used were the following (same conditions for steps 2 and 7):

Acylation: Crosslink was mediated by the yoctoReactor carrying amines and carboxylic acids using EDC (50 mM, Aldrich E6383) and Oxyma (25 mM, ethyl (hydroxyimino)cyanoacetate, Aldrich 233412) in 100 mM MOPS buffer pH 6.5 containing 1M NaCl and 22.5 vol% acetonitrile for 16h at 40°C.

Reductive amination: Crosslink was mediated by the yoctoReactor carrying amines and aldehydes using sodium cyanoborohydride (25 mM, Aldrich 71435) in 100 mM MOPS buffer pH 6.0 containing 1M NaCl and 22.5 vol% acetonitrile for 16h at 40°C.

Urea formation: Crosslink was mediated by the yoctoReactor carrying two amines using bis(4-nitrophenyl carbonate (2.5 vol% of a 200 mM stock solution in *N*-methylpyrrolidinone yielding nominally 5 mM; Aldrich 161691) in 100 mM HEPBS buffer pH 9.0 containing 1M NaCl and 22.5 vol% acetonitrile for 16h at 40°C.

The BBs were linked to DNA via a non-cleavable linker in position 1 (P1, green building block) and cleavable linkers in P2 and P3 (blue and red building blocks, respectively). In the first step, a P1 repertoire of 232 amino acids were reacted with a P2 repertoire of 189 carboxylic acids, aldehydes or amines linked through amines or phenols. After purification and linker cleavage, an amine was liberated, which was reacted with a P3 repertoire of 298 carboxylic acids, aldehydes and amines. Purification and cleavage of the P3 linker completed the compound synthesis. Finally, a primer extension reaction converts the yR to double-stranded DNA and the chemical product displayed and ready for selection experiments.

The library synthesis was validated by ligation ability to target DNA, test of PCR amplification, determination of absolute final amount of DNA isolated, and DNA sequencing. After decoding of DNA codons into building blocks, the relative building block distribution could be assessed. No minimal building block reactivity was required prior to library synthesis, nor was target selection data sets corrected for building block frequencies.

Lib022 description

Based on the building blocks entering library synthesis, the corresponding products were enumerated *in silico* and descriptors for physical/chemical and for drug-likeness were calculated (Instant JChem software package; www.chemaxon.com). From this database was extracted a random set of 30.000 library molecules, which were used to assess descriptor distributions. Below are given average values and histogram plots for selected descriptors of Lib022.

Lib022	Average	Filter	Total compliance
Molecular weight	485	<500	**Lipinski filter - 94% compliance**
H-bond acceptors	5.1	<10	
H-bond donors	3.7	<5	
cLogP	2.0	<5	
Polar surface area	128.2	<140	**Veber filter – 76% compliance**
Rotatable bonds	9.8	≤10	

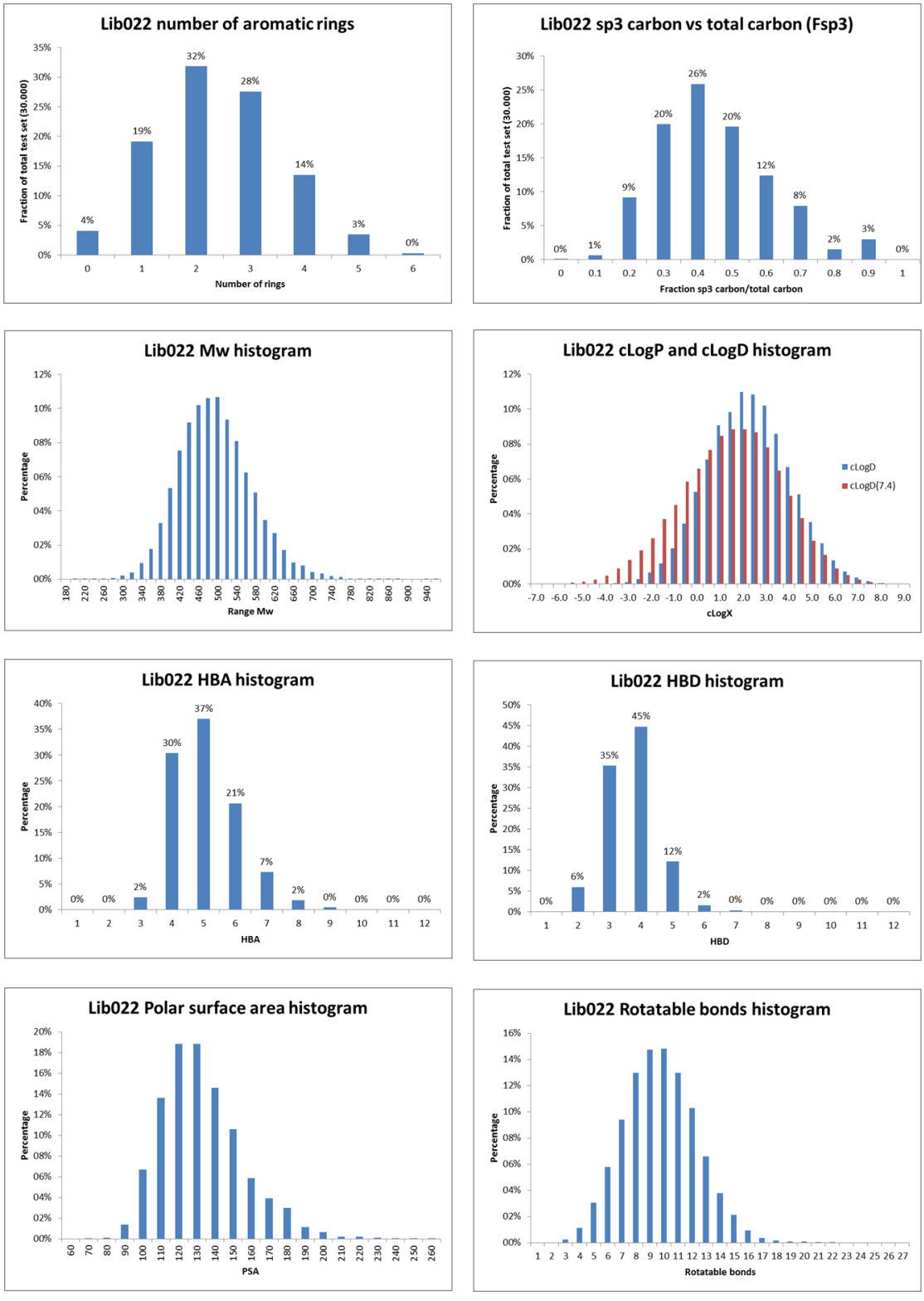
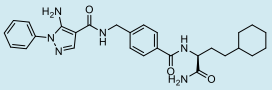
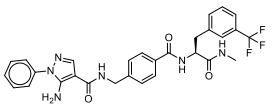
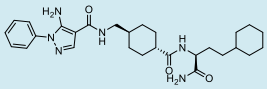
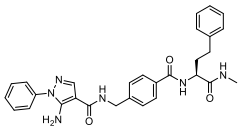
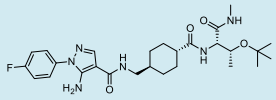
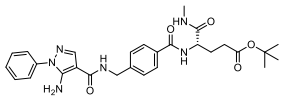
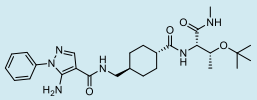
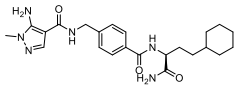
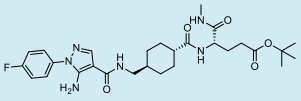


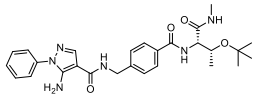
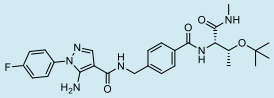
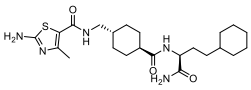
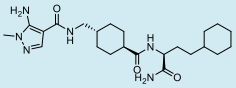
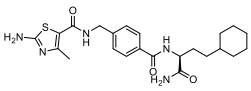
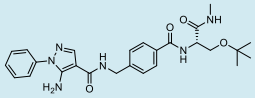
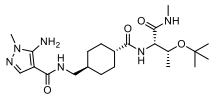
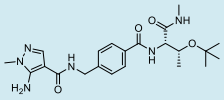
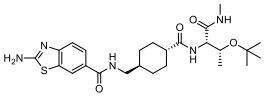
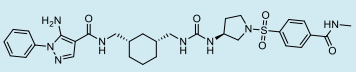
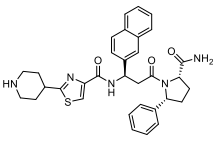
Fig. S2. Selected structural and physical/chemical descriptors for a 30.000 membered random test set from Lib022

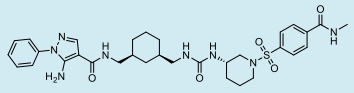
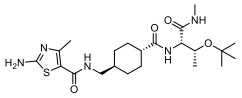
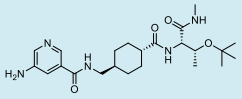
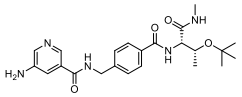
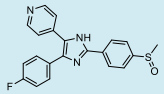
Hits synthesized as inhibitors for p38α

Based on 97x97 heat map, 24 compounds were selected for synthesis by conventional means (*off*-DNA). All were tested in ADP quest assay for inhibition of enzymatic ADP generation.[8]

Table S1 Structures, descriptors, and IC_{50} values from compounds synthesized and tested in biochemical and cellular assays.

Vipergen ID	Structure	Series	Enzymatic assay IC_{50} (μM)	Cellular assay IC_{50} (nM; N=2)	MW (Da)	logD (pH 7.4)	LLE
vpc00628 *)		1	<0.025	7 ± 0.9	502.6	3.7	4.0
vpc00257		1	0.14	46 ± 2.5	564.6	3.8	3.0
vpc00630		1	0.17	44 ± 4.4	508.7	3.6	3.2
vpc00256		1	0.37	-	510.6	3.4	3.0
vpc00251		1	0.39	-	530.6	2.5	3.9
vpc00262		1	0.49	-	534.6	2.2	4.2
vpc00249		1	0.51	-	512.7	2.3	4.0
vpc00626		1	0.69	-	440.5	2.0	4.2
vpc00261		1	0.86	-	558.7	2.3	3.8

vpc00254		1	1.9	-	506.6	2.4	3.3
vpc00258		1	2.2	-	524.6	2.5	3.1
vpc00629		4	2.9	-	463.6	2.4	3.2
vpc00627		1	3.3	-	446.6	1.9	3.5
vpc00631		4	7.3	-	457.6	2.4	2.7
vpc00263		1	15	-	492.6	2.0	2.9
vpc00250		1	16	-	450.6	0.7	4.1
vpc00255		1	31	-	444.5	0.7	3.8
vpc00259		0	34	-	503.7	2.3	2.1
vpc00424		3	41	-	636.8	1.6	2.8
vpc00632		0	51	-	581.7	1.1	3.2

vpc00425		3	74	-	650.8	2.1	2.0
vpc00253		4	75	-	467.6	1.1	3.0
vpc00252		2	>200	-	447.6	0.3	3.4
vpc00260		2	>200	-	441.5	0.3	2.9
SB203580 (ref)		1	0.087	-	377.4	3.1	4.0

***)** K_d for VPC00628 was determined to 2.5 nM (K_d was measured by DiscoverX using proprietary KINOMEscan^{TM} technology)

For the three hits taken further into cellular assay, synthetic details and additional characterizations are given below.

General conditions and abbreviations

Standard washing procedure for resins used in solid-phase synthesis: DMF (2 x 3.0 mL), THF (2 x 3.0 mL) and DCM (2 x 3.0 mL)

DCM = dichloromethane

DBU = 1,8-diazabicycloundec-7-ene

DIPEA = N,N-diisopropylethylamine

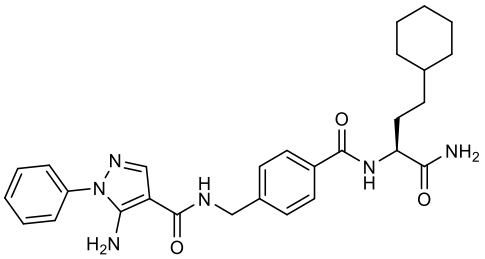
DMF = N,N-dimethylformamide

HCTU = O-(6-chlorobenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate

TBTU = O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate

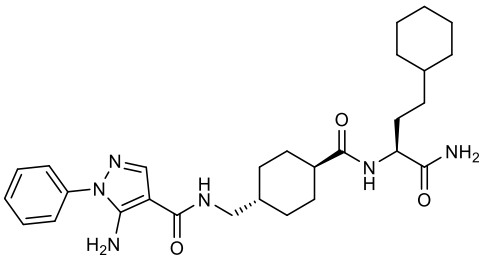
THF = tetrahydrofuran

VPC00628: (S)-5-amino-N-(4-((1-amino-4-cyclohexyl-1-oxobutan-2-yl)carbamoyl)benzyl)-1-phenyl-1H-pyrazole-4-carboxamide



Rink amide resin 100-200 mesh 0.64 mmol/g (125.0 mg, 0.08 mmol) was left to swell in DCM for 15 minutes. Fmoc group was removed by treatment with solution of 2 % DBU, 2% piperidine in DMF (3 x 5 min, 3 x 3.0 mL). The standard washing of the resin was carried out. A solution of Fmoc-homocyclohexyl-alanine (97.8 mg, 0.24 mmol), HCTU (99.3 mg, 0.24 mmol) in DMF (2.5 mL) was added to resin and then DIPEA (84 µL, 0.48 mmol) in DMF (0.5 mL) was added. The mixture was shaken for 1 hour. The standard washing of the resin was carried out. Fmoc group was removed by treatment with 20% piperidine in DMF (3 x 10 min, 3 x 3.0 mL). The standard washing of the resin was carried out. Solution of 4-(Fmoc-aminomethyl)benzoic acid (89.6 mg, 0.24 mmol), HCTU (99.3 mg, 0.24 mmol) in DMF (2.5 mL) was added to resin and then DIPEA (84.0 µL, 0.48 mmol) in DMF (0.5 mL) was added. The mixture was shaken for 1 hour. The standard washing of the resin was carried out. Fmoc group was removed by treatment with 20% piperidine in DMF (3 x 10 min, 3 x 3.0 mL). The standard washing of the resin was carried out. Solution of 5-amino-1-phenyl-1H-pyrazole-4-carboxylic acid (48.8 mg, 0.24 mmol), TBTU (77.1 mg, 0.24 mmol) in DMF (2.5 mL) was added to resin and then DIPEA (84 µL, 0.48 mmol) in DMF (0.5 mL) was added. The mixture was shaken overnight. The standard washing of the resin was carried out. The product was cleaved from the resin by the treatment with 90 % trifluoroacetic acid in 1,2-dichloroethane (2.0 mL) for 1 hour and the resin was washed with DCM. The product was purified by LCMS on RP column (Sunfire C18, 19x100 mm, 5 µ) with a gradient of water+0.1% formic acid/acetonitrile+0.1% formic acid from 95:5 to 30:70. Evaporation of the fraction with the addition of 80 µl of 1 M aqueous HCl afforded the title product (4.1 mg, 10 %). ^{1}H-NMR (500 MHz, DMSO-d6, 300K): δ = 8.52 (t, ^{3}J = 6.1 Hz, 1H), 8.24 (t, ^{3}J = 8.2 Hz, 1H), 7.99 (s, 1H), 7.85 (d, ^{3}J = 8.3 Hz, 2H), 7.58–7.50 (m, 4H), 7.41–7.36 (m, 4H), 6.99 (s, 1H), 6.38 (br s, 2H), 4.46 (d, ^{3}J = 6.0 Hz, 2H), 4.36–4.31 (m, 1H), 1.81–1.74 (m, 1H), 1.71–1.56 (m, 6H), 1.28–1.05 (m, 6H), 0.90–0.78 (m, 2H); ^{13}C-NMR (125 MHz, DMSO-d6, 300K): δ = 174.0, 166.0, 164.1, 149.2, 143.6, 138.4, 138.2, 132.7, 129.4, 127.5, 127.1, 126.7, 123.1, 97.4, 53.4, 41.3, 32.9, 32.7, 29.2, 26.2, 25.8, 25.8; MS (ESI pos.): *m/z* (%) =503.3 (100) ([M+H]$^{+}$, calcd. 503.3).

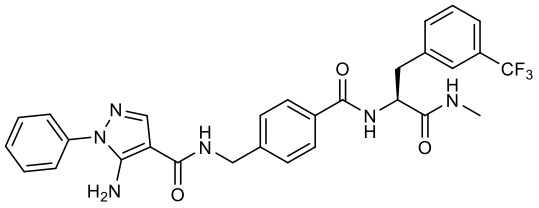
VPC00630: 5-amino-N-(((1S,4R)-4-(((S)-1-amino-4-cyclohexyl-1-oxobutan-2-yl)carbamoyl)cyclohexyl)methyl)-1-phenyl-1H-pyrazole-4-carboxamide



Rink amide resin 100-200 mesh 0.64 mmol/g (125.0 mg, 0.08 mmol) was left to swell in dichloromethane for 15 minutes. Fmoc group was removed by treatment with solution of 2 % DBU, 2% piperidine in DMF (3 x

5 min, 3 x 3.0 mL). The standard washing of the resin was carried out. A solution of Fmoc-homocyclohexyl-alanine (97.8 mg, 0.24 mmol), HCTU (99.3 mg, 0.24 mmol) in DMF (2.5 mL) was added to resin and then DIPEA (84 µL, 0.48 mmol) in DMF (0.5 mL) was added. The mixture was shaken for 1 hour. The standard washing of the resin was carried out. Fmoc group was removed by treatment with 20% piperidine in DMF (3 x 10 min, 3 x 3.0 mL). The standard washing of the resin was carried out. Solution of trans-(Fmoc-aminomethyl)cyclohexane carboxylic acid(91.1 mg, 0.24 mmol), HCTU (99.3 mg, 0.24 mmol) in DMF (2.5 mL) was added to resin and then DIPEA (84.0 µL, 0.48 mmol) in DMF (0.5 mL) was added. The mixture was shaken for 1 hour. The standard washing of the resin was carried out. Fmoc group was removed by treatment with 20% piperidine in DMF (3 x 10 min, 3 x 3.0 mL). The standard washing of the resin was carried out. Solution of 5-amino-1-phenyl-1H-pyrazole-4-carboxylic acid (48.8 mg, 0.24 mmol), TBTU (77.1 mg, 0.24 mmol) in DMF (2.5 mL) was added to resin and then DIPEA (84 µL, 0.48 mmol) in DMF (0.5 mL) was added. The mixture was shaken overnight. The standard washing of the resin was carried out. The product was cleaved from the resin by the treatment with 90 % trifluoroacetic acid in 1,2-dichloroethane (2.0 mL) for 1 hour and the resin was washed with DCM. The product was purified by LCMS on RP column (Sunfire C18, 19x100 mm, 5 µ) with a gradient of water+0.1% formic acid/acetonitrile+0.1% formic acid from 95:5 to 30:70. Evaporation of the fraction with the addition of 80 µl of 1 M aqueous HCl afforded the title product (4.0 mg, 10 %). ^{1}H-NMR (500 MHz, DMSO-d6, 300K): δ = 7.96 (s, 1H), 7.86 (t, ^{3}J = 7.9 Hz, 1H), 7.76 (d, ^{3}J = 7.7 Hz, 1H), 7.56–7.49 (m, 4H), 7.56–7.49 (m, 1H), 7.26 (s, 1H), 6.92 (s, 1H), 6.33 (br s, 2H), 4.14–4.08 (m, 1H), 3.05 (t, ^{3}J = 6.3 Hz, 2H), 1.78–1.72 (m, 4H), 1.67–1.57 (m, 6H), 1.50–1.40 (m, 2H), 1.35–1.05 (m, 8H), 0.97–0.76 (m, 4H); ^{13}C-NMR (125 MHz, DMSO-d6, 300K): δ = 175.1, 174.0, 164.1, 149.0, 138.3, 138.3, 129.4, 127.0, 123.0, 97.7, 52.1, 44.3, 43.8, 37.4, 36.7, 32.7, 29.5, 29.1, 28.5, 26.2, 25.8, 25.8; MS (ESI pos.): *m/z* (%) =509.3 (100) ([M+H]$^{+}$, calcd. 509.3).

VPC00257: (S)-5-amino-N-(4-((1-(methylamino)-1-oxo-3-(3-(trifluoromethyl)phenyl)propan-2-yl)carbamoyl)benzyl)-1-phenyl-1H-pyrazole-4-carboxamide



DFPE resin 100-200 mesh 0.82 mmol/g (NovaBiochem 01-64-0360; 219 mg, 0.18 mmol) was left to swell in DCM for 15 minutes. A solution of methylamine hydrochloride (121 mg, 1.8 mmol) and DBU (270 µL, 1.8 mmol) in DMF (1.8 mL) was added to resin and mixed for 30 minutes. Then a freshly prepared solution of sodium triacetoxyborohydride (305 mg, 1.44 mmol) in DMF (3.0 mL) was added followed by acetic acid (530 µL). The mixture was shaken overnight. An overpressure had to be released after 1 hour of mixing. The resin was washed by methanol (5.0 mL) for 20 minutes and a 5% solution of DIPEA in DMF (2 x 5 min, 2 x 5 mL). Then the standard washing of the resin was carried out and resin was dried *in vacuo*.

A solution of Fmoc-L-(3-trifluoromethyl)phenylalanine (73 mg, 0.16 mmol) and HCTU (66 mg, 0.16 mmol) in DMF (0.75 mL) was added to a part of the above resin (0.08 mmol) and then DIPEA (56 µL, 0.32 mmol) in DMF (0.25 mL) was added. The mixture was shaken for 1 hour. The standard washing of the resin was carried out. Fmoc group was removed by treatment with 20% piperidine in DMF (3 x 10 min, 3 x 3.0 mL).

The standard washing of the resin was carried out. A solution of 4-(Fmoc-aminomethyl)benzoic acid (60 mg, 0.16 mmol) and HCTU (66 mg, 0.16 mmol) in DMF (0.75 mL) was added to the resin and then DIPEA (56 µL, 0.32 mmol) in DMF (0.25 mL) was added. The mixture was shaken for 1 hour. The standard washing of the resin was carried out. Fmoc group was removed by treatment with 20% piperidine in DMF (3 x 10 min, 3 x 3.0 mL). The standard washing of the resin was carried out. A solution of 5-amino-1-phenyl-1*H*-pyrazole-4-carboxylic acid (49 mg, 0.24 mmol), TBTU (77 mg, 0.24 mmol) in DMF (0.75 mL) was added to resin and then DIPEA (84 µL, 0.48 mmol) in DMF (0.5 mL) was added. The mixture was shaken overnight. The standard washing of the resin was carried out. The product was cleaved from the resin by the treatment with 25 % trifluoroacetic acid in 1,2-dichloroethane (2.0 mL) for 1 hour and the resin was washed with DCM. The product was purified by LCMS on RP column (Sunfire C18, 19x100 mm, 5 µ) with a gradient of water+0.1% formic acid/acetonitrile+0.1% formic acid from 95:5 to 30:70. Evaporation of the fraction with the addition of 80 µl of 1 M aqueous HCl afforded the title product (19.5 mg, 43 %). ^{1}H-NMR (500 MHz, DMSO-d6, 300K): δ =8.06 (d, ^{3}J = 8.6 Hz, 1H), 8.51 (t, ^{3}J = 6.1 Hz, 1H), 8.09–8.05 (m, 1H), 7.99 (s, 1H), 7.75 (d, ^{3}J = 8.3 Hz, 2H), 7.71, (s, 1H), 7.62 (d, ^{3}J = 7.4 Hz, 1H), 7.59–7.46 (m, 6H), 7.41–7.37 (m, 1H), 7.74 (d, ^{3}J = 8.3 Hz, 2H), 6.26 (br s, 2H), 4.69–4.62 (m, 1H), 4.46 (d, ^{3}J = 5.9 Hz, 2H), 3.21–3.15 (m, 1H), 3.11–3.03 (m, 1H), 2.62 (d, ^{3}J = 4.6 Hz, 3H); ^{13}C-NMR (125 MHz, DMSO-d6, 300K): δ =171.4, 166.1, 164.1, 149.2, 143.7, 138.4, 138.2, 133.3, 132.4, 129.4, 129.1, 128.9, 128.6, 127.5, 127.1, 126.7, 125.8, 123.2, 123.1, 123.0, 97.4, 54.6, 41.3, 37.0, 25.6; MS (ESI pos.): *m/z* (%) = 565.2 (100) ([M+H]$^{+}$, calcd. 565.2).

X-ray crystallography data

Table S2: Data collection and refinement *statistics*

Complex	**p38α-VPC00628**
PDB accession code	**5LAR**
Data Collection	
Beamline	Diamond Light Source, I04
Wavelength (Å)	0.97949
Resolution[a] (Å)	25.70-1.50 (1.58-1.50)
Spacegroup	$P2_12_12_1$
Cell dimensions	*a*=69.0, *b*=70.6, *c*=75.0 Å
	α=*β*=*γ*=90.0˚
No. unique reflections[a]	58,904 (8,182)
Completeness[a] (%)	99.3 (96.6)
I/σI[a]	9.7 (3.1)
R_{merge}[a] (%)	0.102 (0.428)
Redundancy[a]	5.8 (4.7)
Refinement	
No. atoms in refinement (P/L/O)[b]	2,853/37/407
B_f (P/His/O)[b] (Å^2)	24/33/19
R_{fact} (%)	16.9
R_{free} (%)	20.5
rms deviation bond[c] (Å)	0.016

rms deviation angle[c] (°)	1.6

[a] Values in brackets show the statistics for the highest resolution shells.

[b] P/L/O indicate protein, ligand, and other (water and other molecules), respectively.

[c] rms indicates root-mean-square

[1] D. M. Goldstein and T. Gabriel, *Curr. Top. Med. Chem.*, 2005, **5(10)**, 1017-1029.
[2] R. J. Hill, K. Dabbagh, D. Phippard, C. Li, R. T. Suttmann, M. Welch, E. Papp, K. W. Song, K. C. Chang, D. Leaffer, Y. N. Kim, R. T. Roberts, T. S. Zabka, D. Aud, J. Dal Porto, A. M. Manning, S. L. Peng, D. M. Goldstein, and B. R. Wong, *J. Pharmacol. Exp. Ther.*, 2008, **327(3)**, 610-609.
[3] H. Watz, H. Barnacle, B. F. Hartley, and R. Chan, *Lancet Respir. Med.*, 2014, **2(1)**, 63-72.
[4] J. Zhang, B. Shen, and A. Lin, *Trends Pharmacol. Sci.*, 2007, **28(6)**, 286-295.
[5] M. A. Fabian, W. H. 3rd Biggs, D. K. Treiber, C. E. Atteridge, M. D. Azimioara, M. G. Benedetti, T. A. Carter, P. Ciceri, P. T. Edeen, M. Floyd, J. M. Ford, M. Galvin, J. L. Gerlach, R. M. Grotzfeld, S. Herrgard, D. E. Insko, M. A. Insko, A. G. Lai, J. M. Lélias, S. A. Mehta, Z. V. Milanov, A. M. Velasco, L. M. Wodicka, H. K. Patel, P. P. Zarrinkar, and D. J. Lockhart, *Nat. Biotechnol.*, 2005, **23(3)**, 329-336.
[6] W. MacNee, R. J. Allan, I. Jones, M. C. De Salvo, L. F. Tan, *Thorax.*, 2013, **68(8)**, 738-745.
[7] L. K. Newby, M. S. Marber, C. Melloni, L. Sarov-Blat, L. H. Aberle, P. E. Aylward, G. Cai, R. J. de Winter, C. W. Hamm, J. F. Heitner, R. Kim, A. Lerman, M. R. Patel, J. F. Tanguay, J. J. Lepore, H. R. Al-Khalidi, D. L. Sprecher, C. B. Granger, SOLSTICE Investigators, *Lancet*, 2014, **384(9949)**, 1187-1195.
[8] http://www.discoverx.com