Discovery and optimization of cyclohexane-1,4-diamines as allosteric MALT1 inhibitors

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abstract

Inhibition of mucosa-associated lymphoid tissue lymphoma translocation protein-1 (MALT1) is a promising strategy to modulate NF-kB signaling, with the potential to treat B-cell lymphoma and autoimmune diseases. We describe the discovery and optimization of (1s,4s)-N,N′-diaryl cyclohexane-1,4-diamines, a novel series of allosteric MALT1 inhibitors, resulting in compound 8 with single digit micromolar cell potency. X-ray analysis confirms that this compound binds to an induced allosteric site in MALT1. Compound 8 is highly selective and has an excellent in vivo rat PK profile with low clearance and high oral bioavailability, making it a promising lead for further optimization.

1. Introduction

Mucosa-associated lymphoid tissue lymphoma translocation protein-1 (MALT1) is the only human paracaspase known to date and is responsible for the cleavage of a number of proteins including A20, RelB, and Regnase [1,2]. Additionally MALT1 has a scaffolding function, and together with CARMA1 and BCL10, forms the so-called CBM-complex. This complex initiates a series of downstream events which mediate the release of NF-kB. Inhibition of the protease and/or scaffolding functions of MALT1 could lead to a suppressed immune response through the downstream modu-lation of signaling from B- and T-cell receptor stimulation, via a reduction in NF-kB activation, and consequently regulation of the activation, survival, and proliferation of B- and T-lymphocytes [3,4].

The potential role of MALT1 inhibition in B-cell lymphoma [5,6] and autoimmune disease has spurred the interest in MALT1 inhibitors as reported, among others [7], by Novartis [8e10], Janssen [11e13], Toray Industries [14], the Helmholtz Zentrum Munich and collab-orators [15], and a collaboration between Weill Cornell Medicine, Dana-Farber Cancer Institute, and Harvard Medical School [16]. Herein we describe our discovery and optimization of a novel series of allosteric MALT1 inhibitors with the most advanced compound showing single digit micromolar cell potency, excellent in vivo PK, and high selectivity in secondary pharmacology panels including 16 proteases.

2. Results and discussion

2.1. Chemistry

Symmetrical hit 1 was accessible in one step from commercially available 4-chloro-2-(trifluoromethyl)pyrimidine 34 and (1s,4s)-cyclohexane-1,4-diamine. Compounds 2e15 were synthesized in three steps (Fig. 1). First 4-chloro-2-(trifluoromethyl)pyrimidine underwent a nucleophilic aromatic substitution (S_NAr) reaction with

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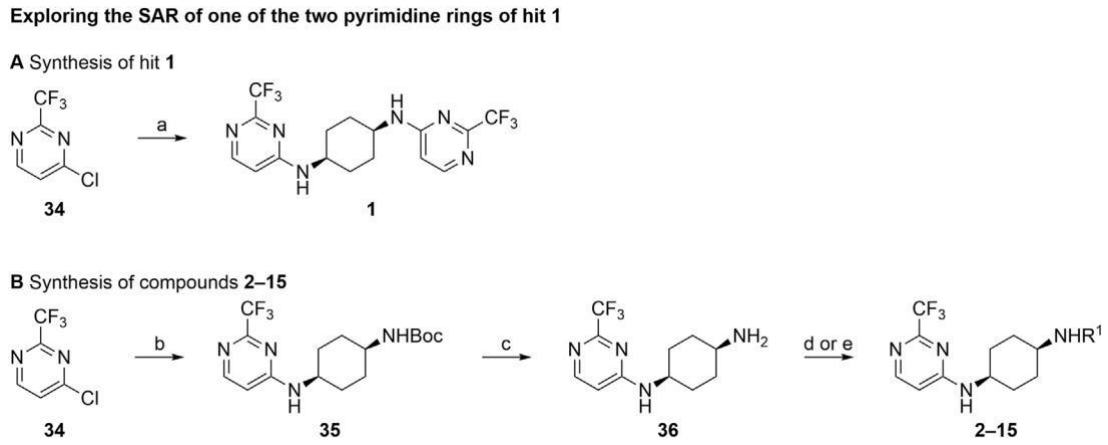


Fig. 1. Reagents and conditions: a) (1s,4s)-cyclohexane-1,4-diamine, DIPEA, ^{i}PrOH, 150 C, mW, 1 h, 51%. b) tert-butyl ((1s,4s)-4-aminocyclohexyl)carbamate, DIPEA, ^{i}PrOH, 150 C, mW, 20 min, 80%. c) AcCl, MeOH, 0 C, 30 min then 36, 0 C to rt, 2 h, 76%. d) corresponding aryl chloride, DIPEA, ^{i}PrOH or DMSO, 140 C or 150 C or 160 C, ± mW, 1e24 h, 1e46%. e) corresponding aryl bromide, Pd_2dba_3, ^{t}BuBrettPhos, Cs_2CO_3, 1,4-dioxane, 111 C, 14e60 h, 1e15%.

mono-Boc-protected (1s,4s)-cyclohexane-1,4-diamine, then the Boc group of compound 35 was cleaved using acidic conditions to obtain compound 36 followed by either an SNAr reaction with the respec-tive aryl chloride (compounds 2, 5e15) or a Buchwald-Hartwig amination with the respective aryl bromide (compounds 3 and 4).

A similar strategy was used to vary the alkyl residue (Fig. 2). In order to obtain both regioisomers when using unsymmetric alkyl residues either 4-chloro-2-(trifluoromethyl)pyrimidine 34 was used in the first SNAr and 3,6-dichloro-[1,2,4]triazolo[4,3-b]pyr-idazine 37 in the second SNAr or vice versa.

For the synthesis of compounds 30, 31, and 33, aryl chloride 37 was reacted with mono-Boc-protected (1s,4s)-cyclohexane-1,4-diamine to obtain compound 38, which then underwent Boc-deprotection (Fig. 3). Compound 39 was then subjected to an SNAr reaction with 4-chloropyrimidine to obtain compound 30 or a Buchwald-Hartwig amination using the respective aryl bromide (compounds 31 and 33). Since neither an SNAr nor a Buchwald-Hartwig amination strategy was successful to transform compound 39 into 32 we introduced the pyrimidine residue of compound 32 in the first step using aryl bromide 40 and copper catalysis to obtain 41, which after Boc-deprotection and SNAr gave the desired compound 32.

2.2. Discovery of hit 1 and optimization to lead 8

2.2.1. Identification and characterization of HTS hit 1

In order to identify suitable, novel chemical starting points for our MALT1 inhibitor program we performed a high-throughput screen (HTS) with the full AstraZeneca compound library (1.8 million compounds in 2014). A FRET assay detecting the cleavage of a four amino acid substrate of MALT1 (LRSR) bearing Rhodamine 110 as the donor and a non-fluorescent quencher (Ac) was used [17]. Horseradish peroxidase and resazurin redox assays were used to eliminate artefacts from the FRET assay [18]. This resulted in the discovery of symmetrical hit 1 with single digit micromolar enzy-matic potency (Table 1).

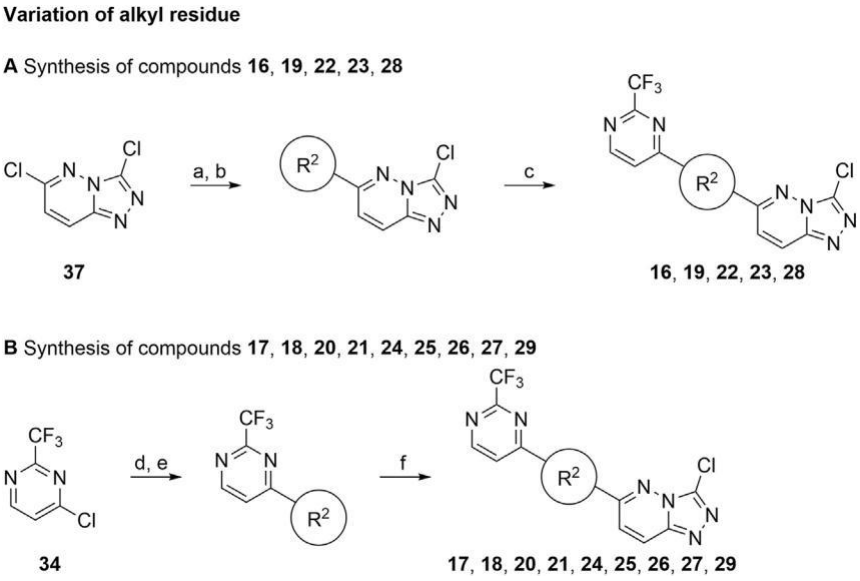


Fig. 2. Reagents and conditions: a) corresponding mono-Boc protected amine, DIPEA or KO^{t}Bu, ^{i}PrOH or 1,4-dioxane, 150 C or 160 C, ± mW, 1.5e24 h. b) HCl, MeOH, or MeO-H/^{i}PrOH 2:7 or 1,4-dioxane/water 1.0:2.7 or ^{i}PrOH/water 1.0:1.3, rt, 1e78 h. c) corresponding aryl chloride, DIPEA, ^{i}PrOH, 150 C, mW, 15e30 min, 3e46% (over three steps). d) corresponding mono-Boc protected amine, DIPEA or KO^{t}Bu, ^{i}PrOH or 1,4-dioxane, 100 C or 150 C, ± mW, 15 mine24 h. e) HCl, MeOH or 1,4-dioxane/water 1.0:2.5 or EtOAc/ water/^{i}PrOH 5.0:1.0:1.3 or ^{i}PrOH/water 1.0:1.3, rt, 5 mine78 h. f) corresponding aryl chloride, DIPEA, ^{i}PrOH, 150 C, mW, 1.5e15 h, 3e38% (over three steps).

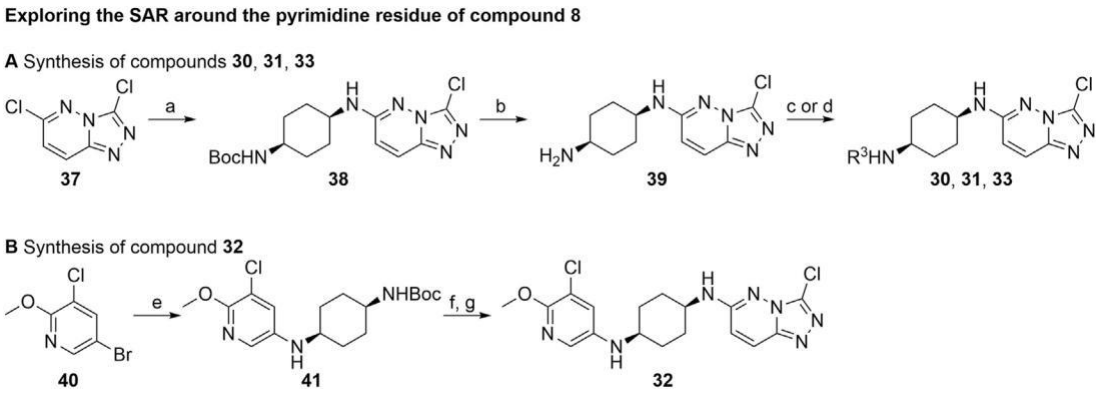


Fig. 3. Reagents and conditions: a) tert-butyl ((1s,4s)-4-aminocyclohexyl)carbamate, DIPEA, ^{i}PrOH, 150 C, 8 h, 31%. b) AcCl, MeOH, 0 C, 30 min then 39, 0 C to rt, 3 h, quant. c) corresponding aryl chloride, ^{i}PrOH, 150 C, mW, 1 h, 10%. d) corresponding aryl bromide, Pd_2dba_3, ^{t}BuBrettPhos, Cs_2CO_3, 1,4-dioxane, 111 C, 12 h or 15 h, 1e7%. e) tert-butyl ((1s,4s)-4-aminocyclohexyl)carbamate, Cu, CsOAc, DMSO, 80 C, 5 h, 4%. f) AcCl, MeOH, 0 C, 1 h then 41, 0 C to rt, 12 h. g) 3,6-dichloro-[1,2,4]triazolo[4,3-b]pyridazine, DIPEA, ^{i}PrOH, 150 C, mW, 5 h, 21% (over two steps).

The trans isomer of compound 1, which was also present in the AstraZeneca compound library, shows an IC_{50} >50 mM in the FRET MALT1 protease assay, showing the importance of the cis-config-uration for MALT1 potency and hinting at specific binding of hit 1.

To elucidate the binding mode we performed an X-ray analysis of hit 1 in complex with a MALT1 construct consisting of the cas-pase and the Ig3 domains. Hit 1 binds to the previously described induced allosteric site [20,21] at the interface of the caspase and Ig3 domain, distant to the active site (Fig. 4A).

The MALT1 dimer is in its inactive conformation [24] with W580 of the interdomain helix being flipped-out [20,21] to accommodate one of the pyrimidine rings of compound 1 (Fig. 4B). The tri-fluoromethyl group of the second pyrimidine binds to a hydro-phobic pocket formed by L383, L386, I712, and the aliphatic side chain of N393 (Fig. 4C). The promising solubility in our high-throughput solubility assay and the relatively high fraction of sp^3 hybridized carbons [25] differentiated hit 1 from compounds in other clusters found in our HTS (unpublished results) and encour-aged us to select this compound as a starting point for lead generation.

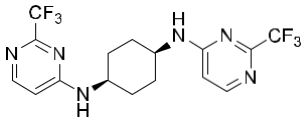
2.2.2. Exploration of structure activity relationship of (1s,4s)-N,N^0-diaryl cyclohexane-1,4-diamines to discover lead 8

Potency SAR understanding was complicated by the symmetry of compound 1, and we knew from unrelated chemical series (unpublished work) that the allosteric site in MALT1 is flexible, making docking and potency predictions challenging. We started our SAR exploration by modifying one of the two pyrimidine resi-dues (Table 2).

Removing the trifluoromethyl group leads to a significantly reduced MALT1 potency (2) whereas taking out both nitrogens retains it (3). Various 6,6- and 6,5-fused bicycles like quinoline (4), 1,6-naphthyridine (5), and thieno[2,3-d]pyrimidine (6) are toler-ated, with a slight decrease in potency. By way of contrast, 3-chloro imidazo[1,2-b]pyridazine analogue 7 has a 6-fold increased potency compared to hit 1. Adding a nitrogen to obtain 3-chloro [1,2,4]-triazolo[4,3-b]pyridazine 8 keeps the promising potency of 0.3 mM while being less lipophilic resulting in a 100-fold increased LLE compared to our initial hit 1 (from an LLE for hit 1 of 1.4 to an LLE for compound 8 of 3.4). Furthermore, compound 8 has a significantly increased metabolic stability in human liver microsomes compared to compound 7. We next investigated the SAR around the newly discovered 3-chloro [1,2,4]-triazolo[4,3-b]pyridazine. Removing the chloro substituent (9) leads to a >100-fold loss in potency, whereas replacing the chloro by a bromo (10) or a thiomethyl group (11) results in only slightly reduced potency. Alkyl residues like methyl (12), cyclopropyl (13), or trifluoromethyl (14) as well as an amino substituent (15) all result in a significant loss of potency, highlighting the importance of the nature of this substituent for MALT1 potency.

X-ray analysis of compound 8 in complex with MALT1 confirms that 8 shares the same binding mode as our initial hit 1 with the 3-

Table 1
Structure, MALT1 potency, and selected in vitro properties of HTS hit 1.



MALT1 IC_{50} [a] [mM]	chromlog$D_{7.4}$ [b]	human liver microsome Cl_{int} [c] [mL/min/mg]	rat hepatocyte Cl_{int} [d] [mL/min/ 10^6]	solubility [e] [mM]
2.2 (0.4)	4.3	<3	<1	37

a Enzymatic potency measured in a FRET assay (for more information see Dumont et al. [17]). Mean and standard deviation of 4 independent measurements.

b ChromlogD$_{7.4}$ obtained by transforming the measured retention time on an LCMS system (with an aqueous mobile phase) into the respective lipophilicity using a cali-bration curve.

c Metabolic stability of the compound measured as disappearance of the parent compound over time when incubated with human liver microsomes.

d Metabolic stability of the compound measured as disappearance of the parent compound over time when incubated with rat hepatocytes.

e Solubility in 0.1 M phosphate buffer (pH 7.4) obtained by drying down a 10 mM DMSO solution of compound 1 and its resolubilization in 0.1 M phosphate buffer (dried DMSO solubility) according to Wernevik et al. [19].

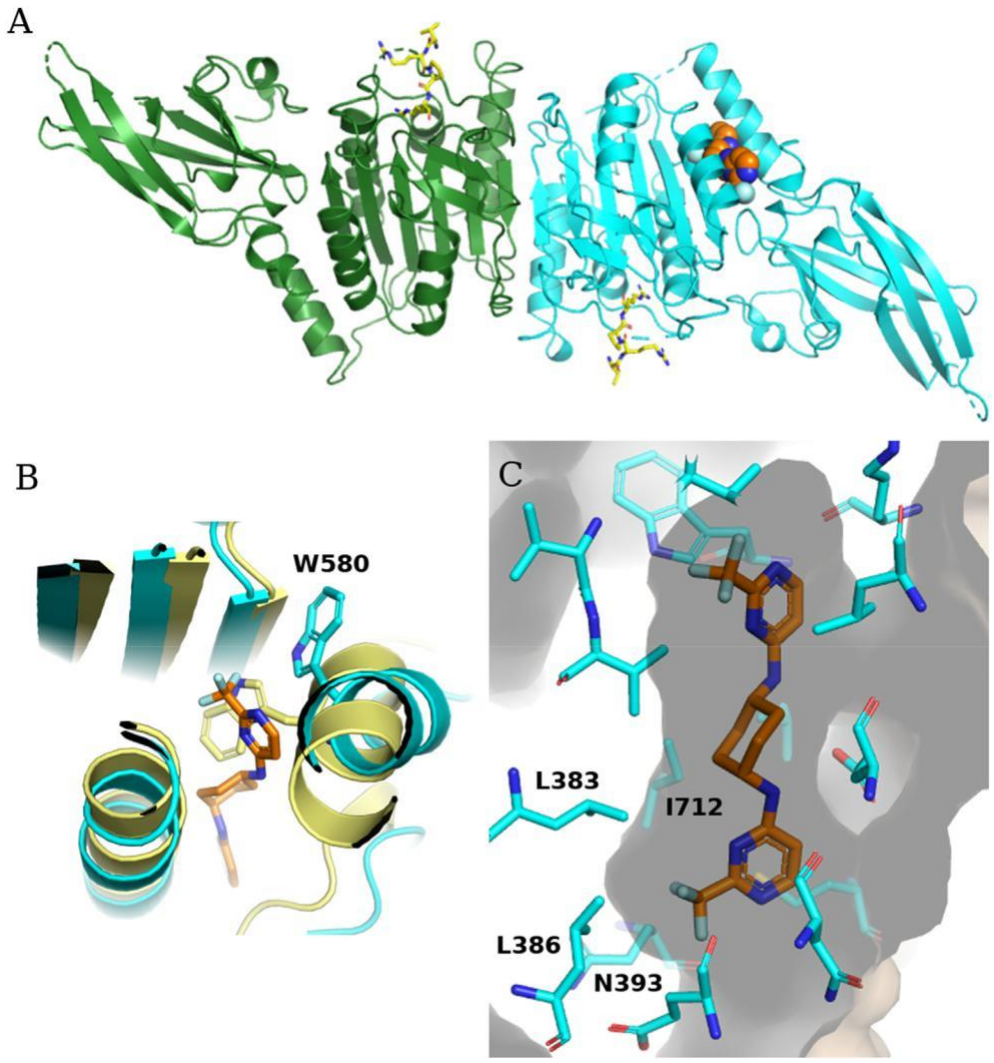


Fig. 4. X-ray analysis of hit 1 in complex with a MALT1 dimer. (A) Hit 1 (orange) bound to an allosteric pocket between the caspase and Ig3 domain of MALT1 with a resolution of 2.2 Å (the coordinates and corresponding structure factors have been deposited to the Protein Data Bank with accession code 7PAV). The active site is highlighted by the tetrapeptide inhibitor VRPR (yellow) from superposition with a reported X-ray [22]. (B) Structure of hit 1 bound to MALT1 (cyan), compared to apo-MALT1 (yellow [23]) shows that a shift of W580 is required to open up the allosteric site. (C) The trifluoromethyl residue of one pyrimidine is occupying a hydrophobic pocket composed of L383, L386, I712, and the aliphatic side chain of N393.

chloro [1,2,4]-triazolo[4,3-b]pyridazine residue of compound 8 displacing W580 (Fig. 5).

In the next step we turned our attention to variations of the central cyclohexyl-1,4-diamine (Table 3). Methylation or substitu-tion by oxygen of either nitrogen leads to decreased potency (compounds 16e19). Bridging the cyclohexyl group (compound 20), rendering either of the nitrogens endocyclic (compounds 21 and 22), shortening the linker (compounds 23e25), or using 5- or 4-membered ring systems (compounds 26e29) all lead to a sig-nificant decrease or in some cases even to a complete loss of po-tency. This data underlines the importance of the cyclohexyl group for the MALT1 potency of this series.

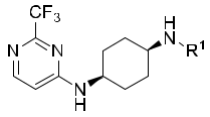
Next we investigated the SAR around the pyrimidine residue of compound 8 (Table 4). We found that unsubstituted pyrimidine 30 has a 100-fold lower potency, confirming the importance of occu-pying the hydrophobic pocket (vide supra). By way of contrast meta-trifluoromethylphenyl analogue 31 has an only slightly reduced potency. Superimposition of compound 8 with the published allo-steric inhibitor TC2 [8] shows that the pyridine substituent of TC2 overlays with the pyrimidine of compound 8 (Fig. 6A and B). Consequently, we replaced our pyrimidine residue with the pyri-dine substituents of TC2 and a second published inhibitor resulting in compounds 32 and 33 respectively [8,9]. In both cases we observed a slightly decreased MALT1 potency compared to com-pound 8.

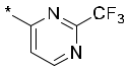
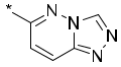
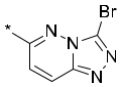
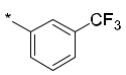
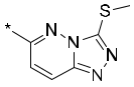
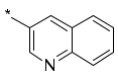
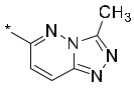
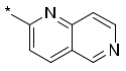
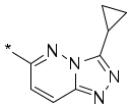
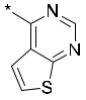
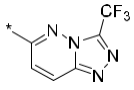
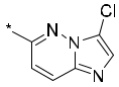
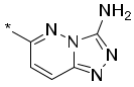
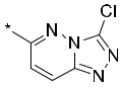
We then determined the cell potency of compounds 8, 10, and 11 against MALT1-mediated cleavage of human A20 in MCF7 cells (Table 5). We were pleased to see that inhibitors 8, 10, and 11 showed single digit micromolar inhibition of MALT1. Encourag-ingly, we observed no reduced cell count for any compound, indi-cating that the detected reduction in MALT1 activity is due to the desired inhibition by our compounds and not to cytotoxicity.

2.2.3. Selectivity, physicochemical properties, and in vivo rat PK of lead 8

Compound 8 was selected for further profiling. It has good mar-gins against hERG and the five most common CYP isoforms, excellent passive permeability across the Caco-2 cell monolayer, good solu-bility (Table 6), and no sign of reactivity with gluthathione after incubation at 37 C for 24 h. Compound 8 showed no significant

Table 2
Initial SAR around one of the two pyrimidine rings.



#	R^1	MALT1 IC_{50}[a] [mM]	chromlogD$_{7.4}$[b]	human liver microsome Cl_{int}[c] [mL/min/mg]	#	R^1	MALT1 IC_{50}[a] [mM]	chromlogD$_{7.4}$[b]	human liver microsome Cl_{int}[c] [mL/min/mg]
1		2.2 (0.4)	4.3	<3	9		35 (2)	1.7	<3
2		>50	1.9	18	10		1.1 (0.8)	2.9	<3
3		1.8 (0.1)	>5.3	7.5	11		0.8 (0.4)	2.8	29
4		7.2 (0.5)	4.6	5.2	12		4.7 (1.2)	2.3	<3
5		7.5 (0.2)	2.8	26	13		4.5 (1.5)	4.5	<3
6		5.0 (1.5)	2.9	22	14		1.8 (0.2)	3.8	<3
7		0.4 (0.1)	4.3	9.9	15		26 (3)	1.4	<3
8		0.3 (0.0)	3.1	<3					

a Enzymatic potency measured in a FRET assay by inhibition of MALT1-mediated cleavage of a tetrapeptide (LRSR, for more information see Dumont et al. [17]). Each experimental value is the mean of at least three independent measurements. The standard deviation is given in brackets.

b ChromlogD$_{7.4}$ obtained by transforming the measured retention time on an LCMS system (with an aqueous mobile phase) into the respective lipophilicity using a cali-bration curve.

c Metabolic stability of the compound measured as disappearance of the parent compound over time when incubated with human liver microsomes.

inhibition of 16 proteases at 30 mM (Eurofins CEREP and Eurofins Panlabs, Table S1). We profiled compound 8 in a kinase panel (ThermoFisher) confirming a >30 fold margin for all 387 kinases investigated (Tables S2 and S3). Compound 8 also showed very good selectivity against 66 additional off-targets in a Eurofins CEREP panel with a margin of >20 fold against all 66 and a 100 fold margin against 49 of the 66 investigated off-targets (Table S4). Moreover, lead 8 has an excellent in vivo rat PK profile with low clearance, high oral bioavailability, and a promising half-life (Table 7).

3. Conclusion

In summary, we have discovered (1s,4s)-N,N^0-diaryl cyclo-hexane-1,4-diamines as novel allosteric inhibitors of MALT1. Starting from HTS hit 1 we rapidly improved LLE by 100-fold to obtain lead 8. The X-ray analyses of 1 and 8 in complex with MALT1 confirm the allosteric binding mode of this novel class of MALT1 inhibitors. X-ray analyses and SAR show that occupying a hydrophobic pocket is crucial for potency in this series. Lead 8 has single digit micromolar cellular activity and a promising secondary pharmacology profile. Additionally, since compound 8 has excellent in vivo rat PK in contrast to some published allo-steric MALT1 inhibitors [10,11], we believe that (1s,4s)-N,N^0-diaryl cyclohexane-1,4-diamines are a promising lead series for further optimization.

4. Experimental section

4.1. Chemistry

All solvents and chemicals were used as purchased without further purification. All reactions were performed in dried reaction vessels under inert atmosphere. Reactions using microwave irra-diation were performed in a Biotage Initiatorþ microwave reactor.

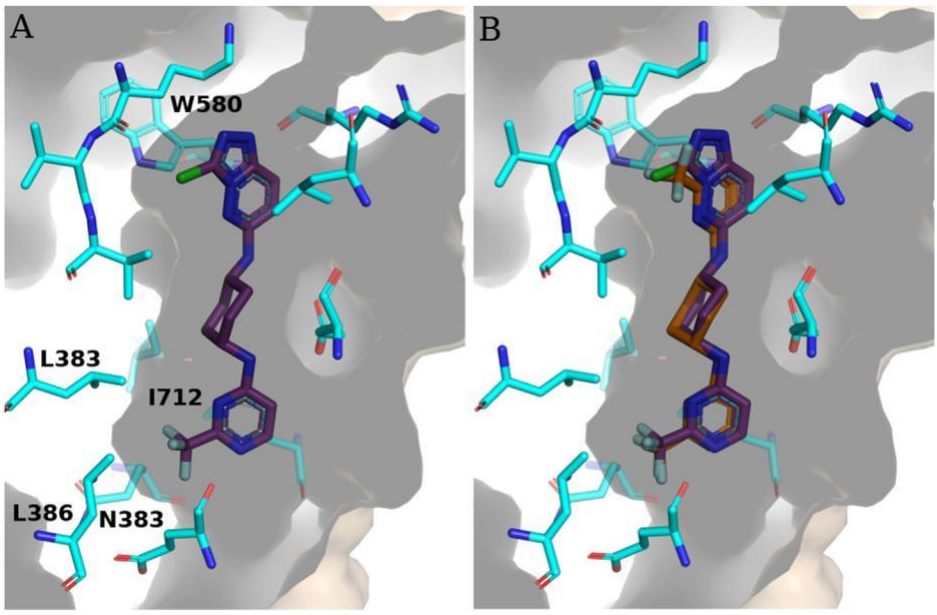


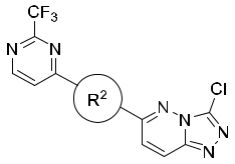
Fig. 5. X-ray analysis of compound 8 in complex with MALT1 with a resolution of 2.2 Å. (A) The 3-chloro [1,2,4]-triazolo[4,3-b]pyridazine residue of 8 displaces W580. (B) Structural overlay of compounds 1 (orange) and 8 (purple). For clarity only MALT1 from the structure of compound 8 is shown in the overlay. The coordinates and corresponding structure factors for the X-ray analysis of compound 8 in complex with MALT1 have been deposited to the Protein Data Bank with accession code 7PAW.

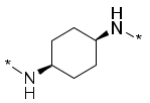
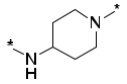
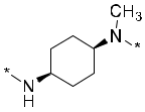
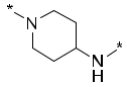
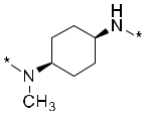
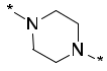
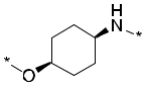
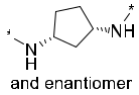
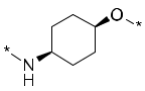
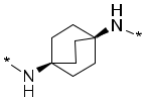
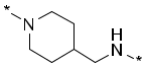
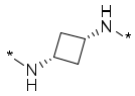
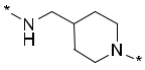
Solvents for reactions were anhydrous (50 ppm water). Solvents for extraction and chromatographic purification were of HPLC grade. Where necessary (so noted) solvents where deoxygenated using three cycles of freeze, pump for 1 min, thaw. Reactions were monitored by LC-LRMS (ESIþ) using a GenTech Scientific Waters ACQ equipped with an Acquity UPLC system, a HSS C18 column (1.8 mm, 2.1 mm 50 mm) and an SQ2 detector. Acetonitrile and water (water modified either with 47 mM ammonia and 6.5 mM ammonium carbonate, pH 10 or with 1 mM ammonium formate and 10 mM formic acid, pH 3) were used as mobile phases. For auto-mated flash column chromatography a Biotage SP-4 system with Biotage pre-packed KP-SIL SNAP cartridges was used. For prepar-ative HPLC a Waters Fraction Lynx system with a Waters Acquity SQD and a Waters binary gradient module 2525, with a flow of 60 mL/min at ambient temperature was used. The HPLC was equipped either with a Waters Xbridge C18 column (5 mm, 19 mm 150 mm, HPLC system A), a Waters Xbridge C18 column (5 mm, 30 mm 150 mm, HPLC system B), a Waters Sunfire C18 column (5 mm, 30 mm 150 mm, HPLC system C), or a Waters Sunfire C18 column (5 mm, 19 mm 150 mm, HPLC system D). For preparative SFC a Waters Prep 100 SFC MS with a Waters mass detector 3100, a TharSFC high pressure pump, and a Waters qua-ternary gradient pump 2545, with a flow of 100 g/min at 40 C and 120 bar was used. The SFC system was equipped either with a Waters BEH column (5 mm, 30 mm 250 mm, SFC system A), a Waters BEH 2-EP column (3.5 mm, 3 mm 100 mm, SFC system B), a Waters BEH 2-EP column (3.5 mm, 30 mm 250 mm, SFC system C), a Waters BEH 2-EP column (5 mm, 30 mm 250 mm, SFC system D), a Waters UPC2 BEH 2-EP column (3.5 mm, 3 mm 100 mm, SFC system E), a Phenomenex Luna Hilic column (5 mm, 30 mm 250 mm, SFC system F), or a Phenomenex Luna Hilic column (3.5 mm, 3 mm 100 mm, SFC system G). Purity analyses (detection at 210 nm) were performed using a Waters Acquity LCT Premiere mass spectrometer coupled to a Waters UPLC, equipped with a BEH C18 column (1.7 mm, 2.1 mm 50 mm) using a gradient from 5 to 90% acetonitrile in water (aqueous phase modified with 40 mM ammonia and 5 mM H_2CO_3, pH 10) at 45 C within 3 min. NMR spectra were recorded at an uncalibrated temperature of 25 C on a Bruker Avance III 500 spectrometer with a 5 mm QNP cryo-probe at a frequency of 500 MHz (^{1}H), 125 MHz (^{13}C) or 471 MHz (^{19}F), a Bruker Avance III with a 5 mm QNP cryoprobe at a frequency of 600 MHz (^{1}H) or 151 MHz (^{13}C). ^{13}C NMRs and ^{19}F NMRs were run in proton decoupled mode. Chemical shifts are reported in parts per million (d) and referenced from the residual protonium for ^{1}H NMR [$CDCl_3$: d 7.26 ($CHCl_3$); DMSO-d_6: d 2.50 (DMSO-d_5)]. ^{13}C NMR are referenced from the carbon reference of the solvent [$CDCl_3$: d 77.2; DMSO-d_6: d 39.5]. A Waters LCT Premiere mass spectrometer coupled to a Waters Acquity UPLC was used to obtain high reso-lution mass spectra (HRMS). The Waters Acquity UPLC was equip-ped with either a BEH C18 column (1.7 mm, 2.1 mm 50 mm, at 45 C using a gradient from 5% to 90% acetonitrile in water, water modified with 40 mM ammonia and 5 mM H_2CO_3, pH 10 within 2.5 min or 3 min) or with a CSH C18 column (1.7 mm, 2.1 mm 50 mm at 45 C using a gradient from 5% to 90% aceto-nitrile in water, water modified with 10 mM formic acid and 1 mM ammonium formate, pH 3, within 2.5 min or 3 min).

4.1.1. Synthesis of compounds 1e15

4.1.1.1. (1s,4s)-N^1,N^4-Bis(2-(trifluoromethyl)pyrimidin-4-yl)cyclo-hexane-1,4-diamine (1). A solution of 4-chloro-2-(trifluoromethyl) pyrimidine (482 mg, 2.64 mmol), N-ethyl-N,N-di-iso-propylamine (1.0 mL, 5.9 mmol) and (1s,4s)-cyclohexane-1,4-diamine (151 mg, 1.32 mmol) in iso-propanol (5 mL) was stirred at 150 C in a single node microwave reactor. After 1 h the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using SFC system A with a gradient from 20% to 25% methanol/water 97:3 (modified with 50 mM ammonia) in 7 min to give (1s,4s)-N^1,N^4-bis(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine as a colourless solid (273 mg, 51%). ^{1}H NMR (500 MHz, DMSO-d_6): d 8.15 (d, J ¼ 5.5 Hz, 2H), 7.96 (d, J ¼ 4.8 Hz, 2H), 6.73 (d, J ¼ 6.1 Hz, 2H), 3.89e4.02 (m, 2H), 1.64e1.82 (m, 8H). ^{13}C NMR (126 MHz, DMSO-d_6): d 161.8 (s), 155.1 (q, J ¼ 34.6 Hz), 153.7 (s), 119.7 (q, J ¼ 275.9 Hz), 108.4 (s), 46.6 (s), 27.2 (s). ^{19}F NMR (471 MHz, DMSO-d_6): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{16}H_{16}F_6N_6$ þ $H^{þ}$ [M þ H]^{þ}: 407.1414. Found: 407.1436.

Table 3
SAR around the alkyl residue.



#	R^2	MALT1 IC_{50}[a] [μM]	chromlogD$_{7.4}$[b]	human liver microsome Cl_{int}[c] [mL/min/mg]	#	R^2	MALT1 IC_{50}[a] [μM]	chromlogD$_{7.4}$[b]	human liver microsome Cl_{int}[c] [mL/min/mg]
8		0.3 (0.0)	3.1	<3	23		6.1 (0.4)	2.6	50
16		0.5 (0.0)	3.5	12	24		>50	2.9	50
17		1.1 (0.1)	3.6	18	25		>50	2.4	<3
18		1.2 (0.2)	3.7	<3	26	 and enantiomer	1.1 (0.5)	3.4	17
19		7.2 (11)	3.5	<3	27		17 (1)	2.5	36
20		4.2 (0.6)	3.0	11	28		18 (1)	2.5	23
21		6.1 (0.8)	2.8	37	29		>50	2.2	6.9
22		23 (2)	2.7	31					

a Enzymatic potency measured in a FRET assay by inhibition of MALT1-mediated cleavage of a tetrapeptide (LRSR, for more information see Dumont et al. [17]). Each experimental value is the mean of at least three independent measurements. The standard deviation is given in brackets.

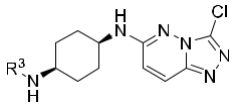
b ChromlogD$_{7.4}$ obtained by transforming the measured retention time on an LCMS system (with an aqueous mobile phase) into the respective lipophilicity using a cali-bration curve.

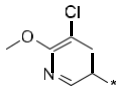
c Metabolic stability of the compound measured as disappearance of the parent compound over time when incubated with human liver microsomes.

4.1.1.2. tert-Butyl ((1s,4s)-4-((2-(trifluoromethyl)pyrimidin-4-yl) amino)cyclohexyl)carbamate (35). A solution of tert-butyl ((1s,4s)-4-aminocyclohexyl)carbamate (527 mg, 2.46 mmol), N-ethyl-N,N-di-iso-propylamine (0.43 mL, 2.5 mmol), and 4-chloro-2-(tri-fluoromethyl)pyrimidine (449 mg, 2.46 mmol) in iso-propanol (10 mL) was stirred at 150 C in a single node microwave reactor. After 20 min the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using automated silica column chromatography with a gradient from 5% to 100% ethyl acetate in heptane to give tert-butyl ((1s,4s)-4-((2-(trifluoromethyl)pyrimidin-4-yl)amino)cyclohexyl)carbamate as a colourless solid (710 mg, 80%). ^{1}H NMR (500 MHz, $CDCl_3$): d 8.26 (s br, 1H), 6.40 (d, J ¼ 6.0 Hz, 1H), 4.48e4.64 (m, 1H), 3.57e3.71 (m, 1H), 1.76e1.88 (m, 4H), 1.65e1.76 (m, 2H), 1.53e1.65 (m, 2H), 1.44 (s, 9H). LRMS (ESI): m/z calcd for $C_{16}H_{23}F_3N_4O_2$ þ H^þ [M þ H]^þ: 361.2. Found: 361.2.

4.1.1.3. (1s,4s)-N^1-(2-(Trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine (36). Acetyl chloride (20 mL, 0.28 mol) was added dropwise to methanol (20 mL) at 0 C within 10 min and the so-lution was stirred for 30 min at 0 C. Then a solution of tert-butyl ((1s,4s)-4-((2-(trifluoromethyl)pyrimidin-4-yl)amino)cyclohexyl) carbamate (35, 4.3 g, 12 mmol) in methanol (20 mL) was added at
0 C, and the resulting solution stirred at room temperature. After
2 h the solvent was removed in vacuo to obtain (1s,4s)-N^1-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine

Table 4
SAR around the pyrimidine ring of compound 8.



#	R^3	MALT1 IC_{50}[a] [mM]	chromlogD$_{7.4}$[b]	human liver microsome Cl_{int}[c] [mL/min/mg]	#	R^3	MALT1 IC_{50}[a] [mM]	chromlogD$_{7.4}$[b]	human liver microsome Cl_{int}[c] [mL/min/mg]
8		0.3 (0.0)	3.1	<3	32		0.6 (0.0)	3.8	<3
30		31 (7)	0.7	25	33		1.5 (0.3)	2.9	11
31		1.4 (0.1)	5.2	9.2					

a Enzymatic potency measured in a FRET assay by inhibition of MALT1-mediated cleavage of a tetrapeptide (LRSR, for more information see Dumont et al. [17]). Each experimental value is the mean of at least three independent measurements. The standard deviation is given in brackets.

b ChromlogD$_{7.4}$ obtained by transforming the measured retention time on an LCMS system (with an aqueous mobile phase) into the respective lipophilicity using a cali-bration curve.

c Metabolic stability of the compound measured as disappearance of the parent compound over time when incubated with human liver microsomes.

hydrochloride as a pale brown powder (2.7 g, 76%). ^{1}H NMR (500 MHz, DMSO-d$_6$): d 11.19 (s, 1H), 8.25 (s, 3H), 8.14 (d, J ¼ 6.1 Hz, 1H), 6.88 (d, J ¼ 6.2 Hz, 1H), 3.85e3.97 (m, 1H), 3.01e3.13 (m, 1H), 1.81e1.94 (m, 2H), 1.68e1.81 (m, 4H), 1.56e1.68 (m, 2H). LRMS (ESI): m/z calcd for $C_{11}H_{15}F_3N_4$ þ H^þ [M þ H]^þ: 261.1. Found: 261.1.

4.1.1.4. (1s,4s)-N^1-(Pyrimidin-4-yl)-N^4-(2-(trifluoromethyl)pyr-imidin-4-yl)cyclohexane-1,4-diamine (2). A solution of 4-chloropyrimidine hydrochloride (58 mg, 0.38 mmol), N-ethyl-N,N-di-iso-propylamine (0.20 mL, 1.2 mmol), and (1s,4s)-N^1-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine hydro-chloride (36, 100 mg, 338 mmol) in iso-propanol (1.7 mL) was stirred at 150 C in a single node microwave reactor. After 1 h the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using HPLC system A with a gradient from 10% to 60% acetonitrile in water (water modified with 0.2% ammonia) in 8.3 min to give (1s,4s)-N^1-(pyrimidin-4-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine as

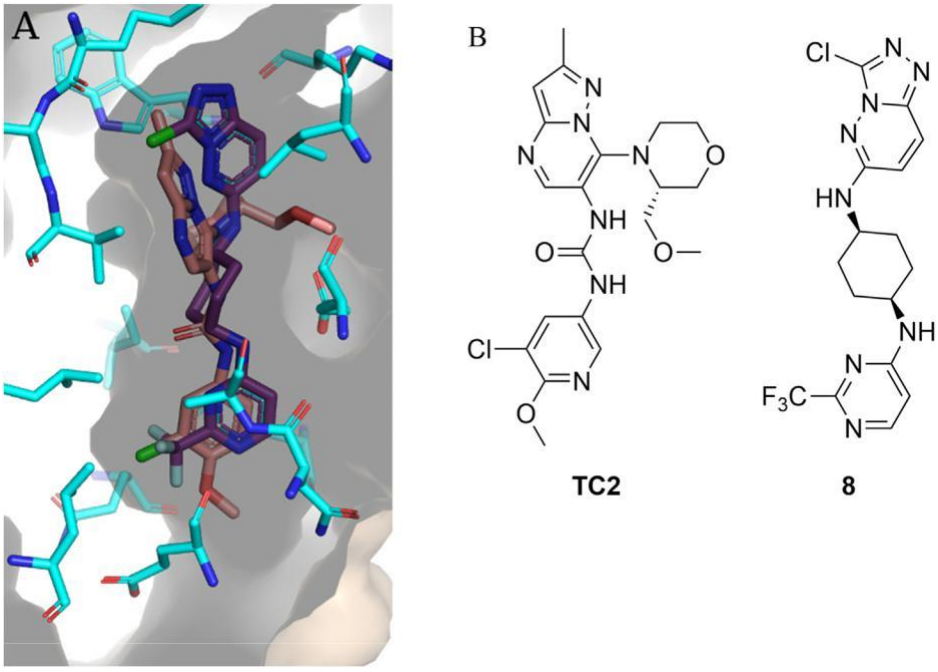


Fig. 6. (A) Structural overlay of 8 (purple) and the published allosteric MALT1 inhibitor TC2 [8] (salmon). For clarity only MALT1 from the structure of compound 8 is shown. (B) Chemical structures of TC2 and our lead 8.

Table 5
Cell potency of selected compounds.

#	MALT1 IC_{50}[a] [mM]	MALT1 IC_{50} in MCF7 cells[b] [mM]
8	0.3 (0.0)	3.2 (0.9)
10	1.1 (0.8)	6.1 (4.0)
11	0.8 (0.4)	5.9 (3.7)

a Enzymatic potency measured in a FRET assay by inhibition of MALT1-mediated cleavage of a tetrapeptide (LRSR, for more information see Dumont et al. [17]). Each experimental value is the mean of at least three independent measurements. The standard deviation is given in brackets.

b MALT1 cell potency assessed via inhibition of MALT1 mediated cleavage of human A20 in MCF7 cells according to Malinverni et al. [26] Each experimental value is the mean of at least three independent measurements. The standard de-viation is given in brackets.

Table 6
Data on inhibition of hERG and the 5 major CYP isoforms, Caco-2 permeability, and solubility of lead 8.

#	IC_{50} hERG [mM]	IC_{50} (CYP1A2, 2C19, 2C9, 2D6, 3A4) [mM]	Caco-2 P_{app}[a] [10^{-6} cm/s]	solubility[b] [mM]
8	24	>30, 14, 10, >30, 18	44	19

[a] Apical to basolateral passive permeability across a Caco-2 cell monolayer in presence of inhibitors against the three major efflux transporters p-gp, BCRP, and MRP2 according to Fredlund et al. [27].

b Solubility in 0.1 M phosphate buffer (pH 7.4) obtained by drying down a 10 mM DMSO solution of compound 8 and its resolubilization in 0.1 M phosphate buffer (dried DMSO solubility) according to Wernevik et al. [19].

a colourless solid (7.7 mg, 7%). ^{1}H NMR (500 MHz, DMSO-d_6): d 8.38 (s, 1H), 8.14 (d, J ¼ 6.0 Hz, 1H), 7.99 (d, J ¼ 6.0 Hz, 1H), 7.95 (d, J ¼ 6.6 Hz, 1H), 7.31 (d, J ¼ 6.8 Hz, 1H), 6.73 (d, J ¼ 6.1 Hz, 1H), 6.51 (d, J ¼ 6.1 Hz, 1H), 3.93e3.99 (m, 1H), 3.84e3.93 (m, 1H), 1.63e1.77 (m, 8H). ^{13}C NMR (126 MHz, DMSO-d_6): d 161.8 (s), 161.2 (s), 158.2 (s), 155.0 (q, J ¼ 34.4 Hz), 153.7 (s, 2C), 119.7 (q, J ¼ 275.9 Hz), 108.4 (s), 106.2 (s), 46.4 (s, 2C), 27.4 (s), 27.4 (s). ^{19}F NMR (471 MHz, DMSO-d_6): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{15}H_{17}F_3N_6$ þ $H^{þ}$ [M þ H]^{þ}: 339.1540. Found: 339.1535.

4.1.1.5. (1s,4s)-N^1 - (3-(Tr ifluo ro methyl)ph enyl)-N^4 -(2-(t ri-fluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine (3). A mixture of (1s,4s)-N^1-(2-(trifluoromethyl)pyrimidin-4-yl)cyclo-hexane-1,4-diamine hydrochloride (36, 100 mg, 338 mmol), 1-iodo-3-(trifluoromethyl)benzene (70 mg, 0.26 mmol), Pd_2dba_3 (5.9 mg, 6.4 mmol), dicyclohexyl(2^{0},4 ^{0},6^{0}-tri-iso-propyl-3,6-dimethoxy-[1,1^{0}-biphenyl]-2-yl)phosphane (14 mg, 26 mmol), and cesium carbonate (334 mg, 1.02 mmol) in degassed 1,4-dioxane (1.9 mL) was stirred at 111 C. After 21 h additional Pd_2dba_3 (5.9 mg, 6.4 mmol) and dicyclohexyl(2^{0},4^{0},6^{0}-tri-iso-pro-pyl-3,6-dimethoxy-[1,1^{0}-biphenyl]-2-yl)phosphane (14 mg, 26 mmol) were added and stirring at 111 C was continued for another 39 h. Then the reaction mixture was allowed to cool to room temperature, the solvent removed in vacuo, and the result-ing residue purified using HPLC system B with a gradient from 40% to 90% acetonitrile in water (water modified with 0.2% ammonia) in 8.3 min to give (1s,4s)-N^1-(3-(trifluoromethyl)phenyl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine as a yellow solid (21 mg, 15%). ^{1}H NMR (500 MHz, DMSO-d_6): d 8.14 (d, J ¼ 6.0 Hz, 1H), 7.95 (d, J ¼ 6.8 Hz, 1H), 7.26 (t, J ¼ 7.9 Hz, 1H), 6.87e6.89 (m, 1H), 6.84 (dd, J ¼ 8.3, 1.9 Hz, 1H), 6.75e6.78 (m, 1H), 6.72 (d, J ¼ 6.0 Hz, 1H), 6.05 (d, J ¼ 6.7 Hz, 1H), 3.93e4.01 (m, 1H), 3.40e3.46 (m, 1H), 1.60e1.80 (m, 8H). ^{13}C NMR (126 MHz, DMSO-d_6): d 161.7 (s), 155.1 (q, J ¼ 34.1 Hz), 153.7 (s), 148.4 (s), 129.9 (s), 129.7 (s), 124.6 (q, J ¼ 272.3 Hz), 119.7 (q, J ¼ 275.7 Hz), 115.3 (s), 111.0 (q, J ¼ 4.6 Hz), 108.4 (s), 108.2 (q, J ¼ 3.9 Hz), 47.9 (s), 46.7 (s), 27.5 (s), 27.2 (s). ^{19}F NMR (471 MHz, DMSO-d_6): d 61.3 (s, 3F), 70.0 (s, 3F). HRMS (ESI): m/z calcd for $C_{18}H_{18}F_6N_4$ þ $H^{þ}$ [M þ H]^{þ}: 405.1509. Found: 405.1490.

4.1.1.6. (1s,4s)-N^1-(Quinolin-3-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine (4). A mixture of (1s,4s)-N^1-(2-(tri-fluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine hydrochlo-ride (36, 20 mg, 68 mmol), 3-bromoquinoline (9.4 mg, 45 mmol), Pd_2dba_3 (1.0 mg, 1.1 mmol), dicyclohexyl(2^{0},4^{0},6^{0}-tri-iso-propyl-3,6-dimethoxy-[1,1^{0}-biphenyl]-2-yl)phosphane (2.2 mg, 4.5 mmol), and cesium carbonate (59 mg, 0.18 mmol) in degassed 1,4-dioxane (0.22 mL) was stirred at 111 C. After 14 h the reaction mixture was allowed to cool to room temperature, the solvent removed in vacuo, and the resulting residue purified using HPLC system C with a gradient from 20% to 70% acetonitrile in water (water modified with 0.1 M formic acid) in 8.3 min to give (1s,4s)-N^1-(quinolin-3-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine as a colourless solid (1.5 mg, 1%). ^{1}H NMR (500 MHz, DMSO-d_6): d 8.54 (s, 1H), 8.15 (d, J ¼ 6.1 Hz, 1H), 7.99 (d, J ¼ 6.6 Hz, 1H), 7.75 (d, J ¼ 8.2 Hz, 1H), 7.65 (d, J ¼ 8.1 Hz, 1H), 7.35e7.41 (m, 1H), 7.27e7.33 (m, 1H), 7.04 (s, 1H), 6.73 (d, J ¼ 5.1 Hz, 1H), 6.23 (d, J ¼ 5.9 Hz, 1H), 4.19e4.24 (m, 1H), 3.97e4.05 (m, 1H), 1.69e1.89 (m, 8H). ^{13}C NMR (126 MHz, DMSO-d_6): d 161.8 (s), 155.1 (q, J ¼ 34.1 Hz), 153.7 (s), 144.1 (s), 141.6 (s), 140.7 (s), 129.7 (s), 128.4 (s), 126.5 (s), 125.8 (s), (q, J 276.3 Hz), 108.4 (s), 107.5 (s), 48.1 (s), 46.8 (s), 123.6 (s), 119.7 ^{19} ¼ 27.3 (s), 27.2 (s). F NMR (471 MHz, DMSO-d_6): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{20}H_{20}F_3N_5$ þ $H^{þ}$ [M þ H]^{þ}: 388.1744. Found: 388.1753.

4.1.1.7. (1s,4s)-N^1-(1,6-Naphthyridin-2-yl)-N^4-(2-(trifluoromethyl)pyr-imidin-4-yl)cyclohexane-1,4-diamine (5). A mixture of (1s,4s)-N^1-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine hydro-chloride (36, 70 mg, 0.27 mmol), N-ethyl-N,N-di-iso-propylamine (0.09 mL, 0.5 mmol), and 2-chloro-1,6-naphthyridine (44 mg, 0.27 mmol) in iso-propanol (1.3 mL) was stirred at 150 C in a single node microwave reactor. After 4 h the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using HPLC system B with a gradient from 22% to 27% acetonitrile in water (water modified with 0.2% ammonia) in 7 min followed by a second purification using HPLC system C with a gradient from 0% to 50% acetonitrile in water (water modified with 0.1 M formic acid) in 8.3 min to give (1s,4s)-N^1-(1,6-naphthyridin-2-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine as a colourless solid (1.5 mg, 1%). ^{1}H NMR (500 MHz, DMSO-d_6): d 8.80 (s, 1H), 8.35 (d, J ¼ 5.8 Hz, 1H), 8.15 (d, J ¼ 6.0 Hz, 1H), 7.98 (d, J ¼ 6.5 Hz, 1H), 7.93 (d, J ¼ 9.0 Hz, 1H), 7.52 (d, J ¼ 6.7 Hz, 1H), 7.31 (d, J ¼ 5.9 Hz, 1H), 6.92 (d, J ¼ 9.1 Hz, 1H), 6.74 (d, J ¼ 6.1 Hz, 1H), 4.08e4.16 (m, 1H), 3.96e4.02 (m, 1H), 1.67e1.85 (m, 8H). ^{13}C NMR (151 MHz, DMSO-d_6): d 161.8 (s), 158.8 (s), 155.3 (q, J ¼ 34.2 Hz), 153.7 (s), 151.5 (s), 150.6 (s), 147.1 (s), 135.2 (s), 119.7 (q, J ¼ 276.0 Hz), 119.2 (s), 119.1 (s), 115.0 (s), 108.4 (s), 46.7 (s), 46.6 (s), 27.5 (s), 27.4 (s). ^{19}F

Table 7
In vivo rat PK of lead 8 (0.9 mg/kg p.o. and 0.5 mg/kg i.v.) in male Han Wistar rats.

#	V_{ss} [L/kg]	clearance [mL/min/kg]	bioavailability [%]	half-life [h]	AUC [nM$h]
8	2.2	6.3	72	4.0	3205

NMR (471 MHz, DMSO-d_6): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{19}H_{19}F_3N_6$ þ Hþ [M þ H]þ: 389.1697. Found: 389.1688.

4.1.1.8. (1s,4s)-N^1-(Thieno[2,3-d]pyrimidin-4-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine (6). A solution of (1s,4s)-N^1-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine hydrochloride (36, 20 mg, 67 mmol), N-ethyl-N,N-di-iso-propylamine (0.05 mL, 0.3 mmol), and 4-chlorothieno[2,3-d]pyrim-idine (17 mg, 0.10 mmol) in DMSO (0.22 mL) was stirred at 140 C. After 12 h the reaction mixture was allowed to cool to room tem-perature, the solvent removed in vacuo, and the resulting residue purified using SFC system B with a gradient from 15% to 20% methanol (modified with 20 mM ammonia) in 7 min as eluent to give (1s,4s)-N^1-(thieno[2,3-d]pyrimidin-4-yl)-N^4-(2-(trifluoromethyl) pyrimidin-4-yl)cyclohexane-1,4-diamine as a pale yellow solid (4.4 mg, 17%). ^{1}H NMR (600 MHz, DMSO-d_6): d 8.50 (s, 1H), 8.16e8.19 (m, 1H), 8.14 (d, J ¼ 5.4 Hz, 1H), 7.96e8.03 (m, 1H), 7.90e7.96 (m, 1H), 7.38 (d, J ¼ 5.4 Hz, 1H), 6.77 (d, J ¼ 6.1 Hz, 1H), 4.16e4.25 (m, 1H), 3.98e4.07 (m, 1H), 1.84e1.96 (m, 2H), 1.75e1.84 (m, 4H), 1.67e1.75 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6): d 161.9 (s), 157.5 (s), 156.4 (s), 155.1 (q, J ¼ 33.9 Hz), 153.7 (s, 2C), 133.8 (s), 123.5 (s), 119.7 (q, J ¼ 275.8 Hz), 114.8 (s), 108.5 (s), 48.3 (s), 45.7 (s), 27.8 (s), 27.0 (s). ^{19}F NMR (471 MHz, DMSO-d_6): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{17}H_{17}F_3N_6S$ þ Hþ [M þ H]þ: 395.1261. Found: 395.1259.

4.1.1.9. (1s,4s)-N^1-(3-Chloroimidazo[1,2-b]pyridazin-6-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine (7). A so-lution of (1s,4s)-N^1-(2-(trifluoromethyl)pyrimidin-4-yl)cyclo-hexane-1,4-diamine hydrochloride (36, 120 mg, 406 mmol), N-ethyl-N,N-di-iso-propylamine (0.16 mL, 0.94 mmol), and 3,6-dichloroimidazo[1,2-b]pyridazine (87 mg, 0.46 mmol) in iso-prop-anol (2.2 mL) was stirred at 150 C in a single node microwave reactor. After 24 h the reaction mixture was cooled to room tem-perature, the solvent removed in vacuo, and the resulting residue purified using SFC system A with a gradient from 22% to 27% methanol/water 97:3 (modified with 50 mM ammonia) in 7 min to give (1s,4s)-N^1-(3-chloroimidazo[1,2-b]pyridazin-6-yl)-N^4-(2-(tri-fluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine as a col-ourless solid (19 mg, 11%). ^{1}H NMR (600 MHz, DMSO-d_6): d 8.14 (d, J ¼ 6.0 Hz, 1H), 7.96 (d, J ¼ 6.7 Hz, 1H), 7.70 (d, J ¼ 9.7 Hz, 1H), 7.45 (s, 1H), 7.06 (d, J ¼ 5.9 Hz, 1H), 6.79 (d, J ¼ 9.7 Hz, 1H), 6.71 (d, J ¼ 6.1 Hz, 1H), 3.91e4.00 (m, 1H), 3.77e3.89 (m, 1H), 1.63e1.89 (m, 8H). ^{13}C NMR (126 MHz, DMSO-d_6): d 161.8 (s), 155.1 (q, J ¼ 33.7 Hz), 153.6 (s), 153.6 (s), 135.3 (s), 126.7 (s), 125.2 (s), 119.7 (q, J ¼ 275.9 Hz), 113.7 (s), 112.6 (s), 108.5 (s), 47.2 (s), 47.0 (s), 27.3 (s), 27.2 (s). ^{19}F NMR (471 MHz, DMSO-d_6): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{17}H_{17}ClF_3N_7$ þ Hþ [M þ H]þ: 412.1259. Found: 412.1270.

4.1.1.10. (1s,4s)-N^1-(3-Chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine (8). A solution of (1s,4s)-N^1-(2-(trifluoromethyl)pyrimidin-4-yl) cyclohexane-1,4-diamine hydrochloride (36, 60 mg, 0.20 mmol), N-ethyl-N,N-di-iso-propylamine (0.08 mL, 0.5 mmol), and 3,6-dichloro-[1,2,4]triazolo[4,3-b]pyridazine (44 mg, 0.24 mmol) in iso-propanol (1.0 mL) was stirred at 150 C in a single node mi-crowave reactor. After 2 h the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the result-ing residue purified using HPLC system D with a gradient from 20% to 70% acetonitrile in water (water modified with 0.1 M formic acid) in 8.3 min to give (1s,4s)-N^1-(3-chloro-[1,2,4] triazolo[4,3-b]pyridazin-6-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine as a colourless solid (17 mg, 20%). ^{1}H NMR (500 MHz, DMSO-d_6): d 8.15 (d, J ¼ 6.0 Hz, 1H), 7.99 (d, J ¼ 6.8 Hz, 1H), 7.94 (d, J ¼ 9.9 Hz, 1H), 7.52 (d, J ¼ 6.1 Hz, 1H), 6.96 (d, J ¼ 9.9 Hz, 1H), 6.72 (d, J ¼ 6.1 Hz, 1H), 3.91e4.01 (m, 1H), 3.77e3.87 (m, 1H), 1.61e1.88 (m, 8H). ^{13}C NMR (126 MHz, DMSO-d_6): d 161.8 (s), 155.1 (d, J ¼ 34.5 Hz), 154.0 (s), 153.7 (s), 143.7 (s), 134.4 (s), 123.6 (s), 119.7 (q, J ¼ 275.3 Hz), 118.3 (s), 108.4 (s), 47.3 (s), 46.9 (s), 27.2 (s), 26.9 (s). ^{19}F NMR (471 MHz, DMSO-d_6): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{16}H_{16}ClF_3N_8$ þ Hþ [M þ H]þ: 413.1212. Found: 413.1229.

4.1.1.11. (1s,4s)-N^1-([1,2,4]Triazolo[4,3-b]pyridazin-6-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine (9). A solution of (1s,4s)-N^1-(2-(trifluoromethyl)pyrimidin-4-yl) cyclohexane-1,4-diamine hydrochloride (36, 50 mg, 0.17 mmol), 6-chloro-[1,2,4]triazolo[4,3-b]pyridazine (26 mg, 0.17 mmol), and N-ethyl-N,N-di-iso-propylamine (0.1 mL, 0.6 mmol) in iso-prop-anol (1.6 mL) was stirred at 160 C in a single node microwave reactor. After 7 h the reaction mixture was cooled to room tem-perature, the solvent removed in vacuo, and the resulting residue purified using SFC system C with a gradient from 20% to 25% methanol (modified with 20 mM ammonia) in 7 min as eluent to give (1s,4s)-N^1-([1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^4-(2-(tri-fluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine as a col-ourless solid (14 mg, 22%). ^{1}H NMR (500 MHz, DMSO-d_6): d 9.11 (s, 1H), 8.15 (d, J ¼ 6.1 Hz, 1H), 7.99 (d, J ¼ 6.7 Hz, 1H), 7.91 (d, J ¼ 9.8 Hz, 1H), 7.28 (d, J ¼ 6.3 Hz, 1H), 6.88 (d, J ¼ 9.9 Hz, 1H), 6.73 (d, J ¼ 6.1 Hz, 1H), 3.93e4.03 (m, 1H), 3.71e3.83 (m, 1H), 1.59e1.84 (m, 8H). ^{13}C NMR (126 MHz, DMSO-d_6): d 161.8 (s), 155.1 (q, J ¼ 33.7 Hz), 153.7 (s), 153.6 (s), 141.9 (s), 138.3 (s), 123.4 (s), 119.7 (q, J 19¼ 275.9 Hz), 117.8 (s), 108.4 (s), 47.2 (s), 46.7 (s), 27.3 (s), 27.0 (s). F NMR (471 MHz, DMSO-d_6): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{16}H_{17}F_3N_8$ þ Hþ [M þ H]þ: 379.1602. Found: 379.1598.

4.1.1.12. (1s,4s)-N^1-(3-Bromo-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine (10). A solution of (1s,4s)-N^1-(2-(trifluoromethyl)pyrimidin-4-yl)cyclo-hexane-1,4-diamine hydrochloride (36, 55 mg, 0.19 mmol), 3-bromo-6-chloro-[1,2,4]triazolo[4,3-b]pyridazine (40 mg, 0.17 mmol) and N-ethyl-N,N-di-iso-propylamine (0.10 mL, 0.57 mmol) in iso-propanol (1.6 mL) was stirred at 150 C in a single node microwave reactor. After 7 h the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using SFC system D with a gradient from 20% to 25% methanol (modified with 20 mM ammonia) in 7 min as eluent to give (1s,4s)-N^1-(3-bromo-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^4-(2-(tri fluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine as a colour-less solid (17 mg, 20%). ^{1}H NMR (500 MHz, DMSO-d_6): d 8.15 (d, J ¼ 6.1 Hz, 1H), 7.98 (d, J ¼ 6.3 Hz, 1H), 7.93 (d, J ¼ 9.9 Hz, 1H), 7.48 (d, J ¼ 6.0 Hz, 1H), 6.95 (d, J ¼ 9.9 Hz, 1H), 6.72 (d, J ¼ 5.9 Hz, 1H), 3.92e4.01 (m, 1H), 3.77e3.87 (m, 1H), 1.59e1.88 (m, 8H). ^{13}C NMR (126 MHz, DMSO-d_6): d 161.7 (s), 155.1 (q, J ¼ 33.8 Hz), 154.0 (s), 153.7 (s), 144.1 (s), 123.5 (s), 123.2 (s), 119.7 (q, J ¼ 276.0 Hz), 118.3 (s), 108.4 (s), 47.3 (s), 46.9 (s), 27.2 (s), 26.9 (s). ^{19}F NMR (471 MHz, DMSO-d_6): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{16}H_{16}BrF_3N_8$ þ Hþ [M þ H]þ: 457.0707/459.0686. Found: 457.0715/459.0697.

4.1.1.13. (1s,4s)-N^1-(3-(Methylthio)-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine (11). A solution of (1s,4s)-N^1-(2-(trifluoromethyl)pyrimidin-4-yl) cyclohexane-1,4-diamine hydrochloride (36, 51 mg, 0.17 mmol), 6-chloro-3-(methylthio)-[1,2,4]triazolo[4,3-b]pyridazine (34 mg, 0.17 mmol) and N-ethyl-N,N-di-iso-propylamine (0.10 mL, 0.57 mmol) in iso-propanol (1.6 mL) was stirred at 150 C for 7 h and at 160 C for another 7 h in a single node microwave reactor. Then the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using SFC system D with a gradient from 20% to 25% methanol (modified with 20 mM ammonia) in 7 min as eluent to give (1s,4s)

-N^1-(3-(methylthio)-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine as a colour-less solid (19 mg, 26%). ^{1}H NMR (500 MHz, DMSO-d_6): d 8.15 (d, J ¼ 6.1 Hz, 1H), 7.98 (d, J ¼ 6.8 Hz, 1H), 7.89 (d, J ¼ 9.8 Hz, 1H), 7.35 (d, J ¼ 5.9 Hz, 1H), 6.87 (d, J ¼ 9.9 Hz, 1H), 6.72 (d, J ¼ 6.1 Hz, 1H), 3.90e4.01 (m, 1H), 3.73e3.83 (m, 1H), 2.66 (s, 3H), 1.60e1.89 (m, 8H). ^{13}C NMR (126 MHz, DMSO-d_6): d 161.7 (s), 155.1 (q, J ¼ 35.4 Hz), 153.7 (s), 153.3 (s), 144.1 (s), 143.8 (s), 123.5 (s), 119.7 (q, J ¼ 275.4 Hz), 117.2 (s), 108.4 (s), 47.3 (s), 46.9 (s), 27.2 (s), 26.9 (s), 13.4 (s). ^{19}F NMR (471 MHz, DMSO-d_6): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{17}H_{19}F_3N_8S$ þ H^þ [M þ H]^þ: 425.1479. Found: 425.1491.

4.1.1.14. (1s,4s)-N^1-(3-Methyl-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine (12). A solution of (1s,4s)-N^1-(2-(trifluoromethyl)pyrimidin-4-yl)cyclo-hexane-1,4-diamine hydrochloride (36, 50 mg, 0.17 mmol), 6-chloro-3-methyl-[1,2,4]triazolo[4,3-b]pyridazine (28 mg, 0.17 mmol), and N-ethyl-N,N-di-iso-propylamine (0.20 mL, 1.1 mmol) in iso-propanol (1.6 mL) was stirred at 150 C in a single node microwave reactor. After 11.5 h the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using HPLC system C with a gradient from 20% to 70% acetonitrile in water (water modified with 0.1 mM formic acid) in 8.3 min to give (1s,4s)-N^1-(3-methyl-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine as a pale yellow solid (4.0 mg, 6%). ^{1}H NMR (600 MHz, DMSO-d_6): d 8.14 (d, J ¼ 6.0 Hz, 1H), 7.98 (d, J ¼ 6.8 Hz, 1H), 7.84 (d, J ¼ 9.8 Hz, 1H), 7.22 (d, J ¼ 5.9 Hz, 1H), 6.84 (d, J ¼ 9.8 Hz, 1H), 6.71 (d, J ¼ 5.9 Hz, 1H), 3.93e3.99 (m, 1H), 3.77e3.85 (m, 1H), 2.50 (s, 3H), 1.64e1.89 (m, 8H). ^{13}C NMR (151 MHz, DMSO-d_6): d 161.7 (s), 155.1 (q, J ¼ 34.5 Hz), 153.7 (s), 153.2 (s), 145.1 (s), 142.1 (s), 123.5 (s), 119.7 (q, J ¼ 276.0 Hz), 116.6 (s), 108.4 (s), 47.1 (s), 46.9 (s), 27.2 (s), 27.0 (s), 9.2 (s). ^{19}F NMR (471 MHz, DMSO-d_6): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{17}H_{19}F_3N_8$ þ H^þ [M þ H]^þ: 393.1758. Found: 393.1754.

4.1.1.15. (1s,4s)-N^1-(3-Cyclopropyl-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine (13). A solution of (1s,4s)-N^1-(2-(trifluoromethyl)pyrimidin-4-yl) cyclohexane-1,4-diamine hydrochloride (36, 53 mg, 0.18 mmol), 6-chloro-3-cyclopropyl-[1,2,4]triazolo[4,3-b]pyridazine (33 mg, 0.17 mmol), and N-ethyl-N,N-di-iso-propylamine (0.10 mL, 0.57 mmol) in iso-propanol (1.6 mL) was stirred at 150 C for 10 h and at 160 C for 4 h in a single node microwave reactor. Then the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using SFC system D with a gradient from 20% to 25% methanol (modified with 20 mM ammonia) in 7 min followed by a second purification using HPLC system B with a gradient from 20% to 70% acetonitrile in water (water modified with 0.2% ammonia)

in 8.3 min to give (1s,4s)-N^1-(3-cyclopropyl-[1,2,4]triazolo [4,3-b]pyridazin-6-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclo hexane-1,4-diamine as a colourless solid (8.4 mg, 12%). ^{1}H NMR (500 MHz, DMSO-d_6): d 8.15 (d, J ¼ 6.0 Hz, 1H), 7.97 (d, J ¼ 6.7 Hz, 1H), 7.81 (d, J ¼ 9.9 Hz, 1H), 7.22 (d, J ¼ 5.8 Hz, 1H), 6.81 (d, J ¼ 9.8 Hz, 1H), 6.71 (d, J ¼ 6.0 Hz, 1H), 3.92e4.03 (m, 1H), 3.75e3.84 (m, 1H), 2.25e2.33 (m, 1H), 1.63e1.90 (m, 8H), 1.11e1.15 (m, 2H), 1.03e1.09 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): d 161.8 (s), 155.1 (q, J ¼ 33.6 Hz), 153.8 (s), 153.1 (s), 149.6 (s), 142.4 (s), 123.6 (s), 119.7 (q, ^{J} ¼ 275.7 Hz), 116.7 (s), 108.4 (s), 47.3 (s), 47.0 (s), 27.2 (s), 27.0 (s), 6.6 (s), 5.2 (s). ^{19}F NMR (471 MHz, DMSO-d_6): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{19}H_{21}F_3N_8$ þ H^þ [M þ H]^þ: 419.1915. Found: 419.1914.

4.1.1.16. (1s,4s)-N^1-(3-(Trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyr-idazin-6-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine (14). A solution of (1s,4s)-N^1-(2-(trifluoromethyl) pyrimidin-4-yl)cyclohexane-1,4-diamine hydrochloride (36, 50 mg, 0.17 mmol), 6-chloro-3-(trifluoromethyl)-[1,2,4]triazolo [4,3-b]pyridazine (38 mg, 0.17 mmol), and N-ethyl-N,N-di-iso-propylamine (0.10 mL, 0.57 mmol) in iso-propanol (1.6 mL) was stirred at 150 C in a single node microwave reactor. After 3 h the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using SFC system E with a gradient from 20% to 25% methanol (modified with 20 mM ammonia) in 7 min to give (1s,4s)-N^1-(3-(trifluoromethyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine as a colourless solid (35 mg, 46%). ^{1}H NMR (500 MHz, DMSO-d_6): d 8.14 (d, J ¼ 6.1 Hz, 1H), 8.08 (d, J ¼ 10.0 Hz, 1H), 7.98 (d, J ¼ 6.2 Hz, 1H), 7.68 (d, J ¼ 5.9 Hz, 1H), 7.09 (d, J ¼ 10.0 Hz, 1H), 6.72 (d, J ¼ 6.1 Hz, 1H),

3.89e4.00 (m, 1H), 3.69e3.82 (m, 1H), 1.58e1.99 (m, 8H). ^{13}C NMR (126 MHz, DMSO-d_6): d 161.7 (s), 155.1 (q, J ¼ 34.0 Hz), 154.3 (s), 153.7 (s), 144.5 (s), 137.3 (q, J ¼ 39.7 Hz), 123.5 (s), 119.7 (q, ¼276.0 Hz), 119.5 (s), 19 ¼ 46.9 (s), 27.1 (s), 26.6 (s). F NMR (471 MHz, DMSO-d_6): d 63.5 (s, 3F), 70.0 (s, 3F). HRMS (ESI): m/z calcd for $C_{17}H_{16}F_6N_8$ þ H^þ ¼ [M þ H]^þ: 447.1475. Found: 447.1465.

4.1.1.17. N^6-((1s,4s)-4-((2-(Trifluoromethyl)pyrimidin-4-yl)amino) cyclohexyl)-[1,2,4]triazolo[4,3-b]pyridazine-3,6-diamine (15). A so-lution of (1s,4s)-N^1-(2-(trifluoromethyl)pyrimidin-4-yl)cyclo-hexane-1,4-diamine hydrochloride (36, 53 mg, 0.18 mmol), 6-chloro-[1,2,4]triazolo[4,3-b]pyridazin-3-amine (29 mg, 0.17 mmol), and N-ethyl-N,N-di-iso-propylamine (0.10 mL, 0.57 mmol) in iso-propanol (1.6 mL) was stirred at 150 C in a single node microwave reactor. After 10 h the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using SFC system F with a gradient from 27% to 32% methanol (modified with 20 mM ammonia) in 7 min to give N^6-((1s,4s)-4-((2-(trifluoromethyl)pyrimidin-4-yl)amino) cyclohexyl)-[1,2,4]triazolo[4,3-b]pyridazine-3,6-diamine as a yel-low solid (4.9 mg, 7%). ^{1}H NMR (600 MHz, DMSO-d_6): d 8.14 (d, J ¼ 5.8 Hz, 1H), 7.97 (d, J ¼ 6.1 Hz, 1H), 7.60e7.63 (m, 1H), 6.99 (d, J ¼ 6.2 Hz, 1H), 6.70e6.76 (m, 1H), 6.61 (d, J ¼ 9.9 Hz, 1H), 5.88 (s, 2H), 3.92e4.01 (m, 1H), 3.77e3.86 (m, 1H), 1.49e1.92 (m, 8H). ^{13}C NMR (126 MHz, DMSO-d_6): d 161.8 (s), 155.2 (q, J ¼ 38.9 Hz), 153.7 (s), 152.3 (s), 149.7 (s), 139.1 (s), 123.5 (s), 119.7 (q, J ¼ 276.3 Hz), 114.9 (s), 108.4 (s), 47.1 (s), 46.8 (s), 27.3 (s), 27.1 (s). ^{19}F NMR (471 MHz, DMSO-d_6): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{16}H_{18}F_3N_9$ þ H^þ [M þ H]^þ: 394.1711. Found: 394.1712.

4.1.2. Synthesis of compounds 16e29

4.1.2.1. (1s,4s)-N^1-(3-Chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^1-methyl-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine (16). A solution of 3,6-dichloro-[1,2,4]triazolo[4,3-b] pyridazine (41 mg, 0.22 mmol), N-ethyl-N,N-di-iso-propylamine (0.08 mL, 0.5 mmol), and tert-butyl ((1s,4s)-4-(methylamino) cyclohexyl)carbamate (50 mg, 0.22 mmol) in iso-propanol (1 mL) was stirred at 150 C in a single node microwave reactor. After 7 h the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using auto-mated silica column chromatography with isocratic 5% ethyl ac-etate in heptane as mobile phase to give tert-butyl ((1s,4s)-4-((3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)(methyl)amino)cyclo-hexyl)carbamate (40 mg, 48%). ^{1}H NMR (500 MHz, $CDCl_3$): d 7.80 (d, J ¼ 10.2 Hz, 1H), 6.92 (d, J ¼ 10.2 Hz, 1H), 4.74 (s, 1H), 3.94e4.08 (m, 1H), 3.83e3.94 (m, 1H), 3.06 (s, 3H), 1.97e2.06 (m, 2H),

1.77e1.90 (m, 2H), 1.64e1.75 (m, 4H), 1.48 (s, 9H). LRMS (ESI): m/z calcd for $C_{17}H_{25}ClN_6O_2$ þ $H^þ$ [M þ H]^þ: 381.2. Found: 381.1. Acetyl chloride (0.15 mL, 2.1 mmol) was added dropwise to methanol (0.15 mL) at 0 C within 10 min and the solution stirred at 0 C for 30 min. Then a solution of tert-butyl ((1s,4s)-4-((3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)(methyl)amino)cyclo-hexyl)carbamate (39 mg, 0.10 mmol) in methanol (0.18 mL) was added at 0 C and the resulting solution stirred at room temper-ature. After 1 h the solvent was removed in vacuo, the residue dissolved in iso-propanol (0.6 mL), 4-chloro-2-(trifluoromethyl) pyrimidine (24 mg, 0.13 mmol) and N-ethyl-N,N-di-iso-propyl-amine (0.05 mL, 0.3 mmol) added, and the reaction mixture stir-red at 150 C in a single node microwave reactor. After 20 min the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using SFC system F with a gradient from 20% to 25% methanol (modified with 20 mM ammonia) in 7 min to give (1s,4s)-N^1-(3-chloro-[1,2,4] triazolo[4,3-b]pyridazin-6-yl)-N^1-methyl-N^4-(2-(trifluoromethyl) pyrimidin-4-yl)cyclohexane-1,4-diamine as a colourless solid (11 mg, 26% over 2 steps). ^{1}H NMR (600 MHz, DMSO-d_6): d 8.19 (d, J ¼ 6.0 Hz, 1H), 8.08 (d, J ¼ 10.2 Hz, 1H), 8.01 (d, J ¼ 7.2 Hz, 1H), 7.46 (d, J ¼ 10.3 Hz, 1H), 6.83 (d, J ¼ 6.0 Hz, 1H), 4.11e4.28 (m, 2H), 3.03 (s, 3H), 1.84e2.01 (m, 4H), 1.71e1.84 (m, 2H), 1.46e1.63 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): d 161.8 (s), 155.2 (q, J ¼ 35.0 Hz), 155.0 (s), 153.8 (s), 143.4 (s), 134.3 (s), 124.1 (s), 119.7 (q, J ¼ 275.5 Hz), 115.4 (s), 108.6 (s), 55.1 (s), 43.9 (s), 30.1 (s), 28.4 (s), 23.9 (s). ^{19}F NMR (471 MHz, DMSO-d_6): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{17}H_{18}ClF_3N_8$ þ $H^þ$ [M þ H]^þ: 427.1368. Found: 427.1373.

4.1.2.2. (1s,4s)-N^1-(3-Chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^4-methyl-N^4-(2-(trifluoromethyl)pyrimidin-4-yl)cyclohexane-1,4-diamine (17). A solution of tert-butyl ((1s,4s)-4-(methylamino) cyclohexyl)carbamate (50 mg, 0.21 mmol), 4-chloro-2-(tri-fluoromethyl)pyrimidine (40 mg, 0.21 mmol) and N-ethyl-N,N-di-iso-propylamine (0.07 mL, 0.4 mmol) in iso-propanol (1.0 mL) was stirred at 150 C in a single node microwave reactor. After 20 min the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using auto-mated silica column chromatography with a gradient from 5% to 50% ethyl acetate in heptane to give tert-butyl ((1s,4s)-4-(methyl(2-(tri-fluoromethyl)pyrimidin-4-yl)amino)cyclohexyl)carbamate (70 mg, 89%). ^{1}H NMR (500 MHz, $CDCl_3$): d 8.26 (d, J ¼ 6.2 Hz, 1H), 6.47 (d, J ¼ 6.3 Hz, 1H), 4.63e4.77 (m, 1H), 3.81e3.93 (m, 1H), 2.98 (s, 3H), 1.93e2.02 (m, 2H), 1.55e1.78 (m, 6H), 1.47 (s, 9H). LRMS (ESI): m/z calcd for $C_{17}H_{25}F_3N_4O_2$ þ $H^þ$ [M þ H]^þ: 375.2. Found: 375.3.

Acetyl chloride (0.27 mL, 3.6 mmol) was added dropwise to methanol (0.3 mL) at 0 C within 10 min and the solution was stirred at 0 C for 30 min. Then a solution of tert-butyl ((1s,4s) -4-(methyl(2-(trifluoromethyl)pyrimidin-4-yl)amino)cyclohexyl) carbamate (70 mg, 0.19 mmol) in methanol (0.3 mL) was added at 0 C and the resulting solution stirred at room temperature. After 1 h the solvent was removed in vacuo, the residue dissolved in iso-propanol (1.0 mL), 3,6-dichloro-[1,2,4]triazolo[4,3-b]pyr-idazine (47 mg, 0.25 mmol) and N-ethyl-N,N-di-iso-propylamine (0.09 mL, 0.5 mmol) added, and the resulting solution stirred at 150 C in a single node microwave reactor. After 5 h the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the residue purified using SFC system D with a gradient from 20% to 25% methanol (modified with 20 mM ammonia) in 7 min to give (1s,4s)-N^1-(3-chloro-[1,2,4]triazolo [4,3-b]pyridazin-6-yl)-N^4-methyl-N^4-(2-(trifluoromethyl)pyrimid in-4-yl)cyclohexane-1,4-diamine as a colourless solid (35 mg, 43% over 2 steps). ^{1}H NMR (600 MHz, DMSO-d_6): d 8.31 (d, J ¼ 6.4 Hz, 1H), 7.97 (d, J ¼ 9.8 Hz, 1H), 7.57 (d, J ¼ 6.5 Hz, 1H), 7.09 (d, J ¼ 9.9 Hz, 1H), 6.74e6.95 (m, 1H), 4.03e4.09 (m, 1H), 3.84e4.01 (m, 1H), 3.02 (s, 3H), 2.04e2.09 (m, 2H), 1.87e1.99 (m, 2H), 1.64e1.84 (m, 2H), 1.44e1.60 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): d 161.0 (s), 156.2 (q, J ¼ 40.6 Hz), 155.4 (s), 154.0 (s), 143.7 (s), 134.5 (s), 123.6 (s), 119.7 (q, J ¼ 275.8 Hz), 118.4 (s), 105.6 (s), 44.6 (s, 2C), 30.0 (s), 27.9 (s), 23.4 (s). ^{19}F NMR (471 MHz, DMSO-d_6): d 69.9 (s). HRMS (ESI): m/z calcd for $C_{17}H_{18}ClF_3N_8$ þ $H^þ$ [M þ H]^þ: 427.1368. Found: 427.1377.

4.1.2.3. 3-Chloro-N-((1s,4s)-4-((2-(trifluoromethyl)pyrimidin-4-yl) oxy)cyclohexyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-amine (18). A mixture of 4-chloro-2-(trifluoromethyl)pyrimidine (88 mg, 0.48 mmol), tert-butyl ((1s,4s)-4-hydroxycyclohexyl)carbamate (0.11 g, 0.51 mmol), and KO^{t}Bu (82 mg, 0.73 mmol) in 1,4-dioxane (1 mL) was stirred at 100 C. After 24 h the reaction mixture was allowed to cool to room temperature, an aqueous HCl solution (3.8 M, 2.5 mL, 9.5 mmol) added, and the resulting solution stirred at room temperature. After 78 h the reaction mixture was adjusted to pH 14 by addition of an aqueous sodium hydroxide solution (4 M), the aqueous phase extracted with dichloromethane (3 10 mL), the combined organic phases dried over magnesium sulfate, and the solvent removed in vacuo to give (1s,4s)-4-((2-(trifluoromethyl)pyrimidin-4-yl)oxy)cyclohexan-1-amine hydro-chloride as a yellow solid (14 mg, 10% over 2 steps). ^{1}H NMR (500 MHz, DMSO-d_6): d 8.71 (d, J ¼ 5.8 Hz, 1H), 7.20 (d, J ¼ 5.9 Hz, 1H), 5.18e5.23 (m, 1H), 2.73e2.82 (m, 1H), 1.92e2.00 (m, 2H), 1.58e1.74 (m, 4H), 1.35e1.50 (m, 2H). LRMS (ESI): m/z calcd for $C_{11}H_{14}F_3N_3O$ þ $H^þ$ [M þ H]^þ: 262.1. Found: 262.3.

A solution of (1s,4s)-4-((2-(trifluoromethyl)pyrimidin-4-yl)oxy) cyclohexan-1-amine hydrochloride (14 mg, 47 mmol), 3,6-dichloro-[1,2,4]triazolo[4,3-b]pyridazine (11 mg, 58 mmol), and N-ethyl-N,N-di-iso-propylamine (0.05 mL, 0.3 mmol) in iso-propanol (0.5 mL) was stirred at 150 C in a single node microwave reactor. After 200 min the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using SFC system E with a gradient from 15% to 20% methanol (modified with 20 mM ammonia) in 7 min to give 3-chloro-N-((1s,4s)-4-((2-(trifluoromethyl)pyrimidin-4-yl)oxy)cyclohexyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-amine as a colourless solid (7.1 mg, 37%). ^{1}H NMR (600 MHz, DMSO-d_6): d 8.74 (d, J ¼ 5.9 Hz, 1H), 7.94 (d, J ¼ 9.9 Hz, 1H), 7.63 (d, J ¼ 7.1 Hz, 1H), 7.23 (d, J ¼ 5.9 Hz, 1H), 6.92 (d, J ¼ 9.9 Hz, 1H), 5.25e5.31 (m, 1H), 3.81e3.89 (m, 1H), 1.94e2.04 (m, 2H), 1.80e1.93 (m, 4H), 1.66e1.77 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): d 169.1 (s), 158.8 (s), 154. 8 (q, J ¼ 35.8 Hz), 153.9 (s), 143. 8 (s), 134.4 (s), 123.7 (s), 119.3 (q, J ¼ 275.4 Hz), 118.3 (s), 111.5 (s), 72.8 (s), 47.4 (s), 27.2 (s), 26.3 (s). ^{19}F NMR (471 MHz, DMSO-d_6): d 69.6 (s). HRMS (ESI): m/z calcd for $C_{16}H_{15}ClF_3N_7O$ þ $H^þ$ [M þ H]^þ: 414.1052. Found: 414.1046.

4.1.2.4. N-((1s,4s)-4-((3-Chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl) oxy)cyclohexyl)-2-(trifluoromethyl)pyrimidin-4-amine (19). A mixture of 3,6-dichloro-[1,2,4]triazolo[4,3-b]pyridazine (92 mg, 0.49 mmol), tert-butyl ((1s,4s)-4-hydroxycyclohexyl)carbamate (0.11 g, 0.51 mmol), and KO^{t}Bu (80 mg, 0.71 mmol) in 1,4-dioxane (1 mL) was stirred at 100 C. After 24 h the reaction mixture was allowed to cool to room temperature, an aqueous HCl solution (3.8 M, 2.7 mL, 10 mmol) added, and the resulting solution stirred at room temperature. After 78 h the reaction mixture was adjusted to pH 14 by addition of an aqueous NaOH solution (4 M), the aqueous phase extracted with dichloromethane (3 10 mL), the combined organic phases dried over magnesium sulfate, and the solvent removed in vacuo. The yellow residue was dissolved in iso-propanol (0.32 mL), 4-chloro-2-(trifluoromethyl)pyrimidine (6.1 mg, 33 mmol) and N-ethyl-N,N-di-iso-propylamine (12 mL, 69 mmol) added, and the mixture stirred at 150 C in a single node microwave

reactor. After 30 min the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the residue puri-fied using SFC system E with a gradient from 15% to 20% methanol (modified with 20 mM ammonia) in 7 min to give N-((1s,4s)-4-((3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)oxy)cyclohexyl)-2-(tri-fluoromethyl)pyrimidin-4-amine a colourless solid (2.2 mg, 1% over 3 steps). ^{1}H NMR (500 MHz, DMSO-d$_6$): d 8.30 (d, J ¼ 9.9 Hz, 1H), 8.15 (d, J ¼ 6.1 Hz, 1H), 8.06 (d, J ¼ 7.5 Hz, 1H), 7.10 (d, J ¼ 9.8 Hz, 1H),
6.67 (d, J ¼ 6.2 Hz, 1H), 5.14e5.22 (m, 1H), 3.96e4.05 (m, 1H), 1.95e2.16 (m, 2H), 1.75e1.93 (m, 4H), 1.61e1.73 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d$_6$): d 161.7 (s), 160.3 (s), 160.3 (s), 153.9 (q, J ¼ 6.1 Hz), 144.4 (s), 135.4 (s), 126.9 (s), 119.7 (q, J ¼ 275.5 Hz), 118.0 (s), 108.4 (s), 73.3 (s), 46.9 (s), 27.1 (s), 26.6 (s). ^{19}F NMR (471 MHz, DMSO-d$_6$): d 69.9 (s). HRMS (ESI): m/z calcd for $C_{16}H_{15}ClF_3N_7O$ þ H^{þ} [M þ H]^{þ}: 414.1052. Found: 414.1063.

4.1.2.5. N^1-(3-Chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^4-(2-(tri-fluoromethyl)pyrimidin-4-yl)bicyclo[2.2.2]octane-1,4-diamine (20).
A solution of tert-butyl (4-aminobicyclo[2.2.2]octan-1-yl)carbamate (50 mg, 0.20 mmol), 4-chloro-2-(trifluoromethyl)pyrimidine (38 mg, 0.20 mmol), and N-ethyl-N,N-di-iso-propylamine (0.07 mL,
0.4 mmol) in iso-propanol (1 mL) was stirred at 150 C in a single node microwave reactor. After 20 min the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using automated silica column chroma-tography with a gradient from 5% to 50% ethyl acetate in heptane. The crude product was dissolved in methanol (0.1 mL) and dropwise added to a freshly prepared solution of HCl in methanol (5 M, pre-pared by dropwise addition of acetyl chloride (0.09 mL, 1 mmol) to methanol (0.11 mL) at 0 C and stirring at 0 C for 10 min). The resulting solution was stirred at room temperature for 1 h. Then the solvent was removed in vacuo, the residue dissolved in iso-propanol (0.5 mL), 3,6-dichloro-[1,2,4]triazolo[4,3-b]pyridazine (9.2 mg, 49 mmol) and N-ethyl-N,N-di-iso-propylamine (17 mL, 98 mmol) added, and the solution stirred at 150 C in a single node microwave reactor. After 15 h the reaction mixture was cooled to room tem-perature, the solvent removed in vacuo, and the residue purified using HPLC system C with a gradient from 20% to 70% acetonitrile in water (water modified with 0.2% ammonia) in 8.3 min to give N^1-(3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^4-(2-(trifluoromethyl) pyrimidin-4-yl)bicyclo[2.2.2]octane-1,4-diamine as a yellow solid (2.2 mg, 3% over three steps). ^{1}H NMR (600 MHz, DMSO-d$_6$): d 8.15 (d, J ¼ 6.1 Hz, 1H), 7.92 (d, J ¼ 9.9 Hz, 1H), 7.67 (s, 1H), 7.20 (s, 1H), 6.88 (d, J ¼ 9.9 Hz, 1H), 6.64e6.73 (m, 1H), 2.09e2.18 (m, 12H). ^{13}C NMR (150 MHz, DMSO-d$_6$): d 162.2 (s), 154.3 (q, J ¼ 31.7 Hz), 153.8 (s), 153.7 (s), 143.2 (s), 134.4 (s), 123.5 (s), 119.0 (s), 118.8 (q, J ¼ 275.9 Hz), 109.1 (s), 50.7 (s), 50.7 (s), 29.5 (s), 29.0 (s). ^{19}F NMR (471 MHz, DMSO-d$_6$):

d 70.2 (s). HRMS (ESI): m/z calcd for $C_{18}H_{18}ClF_3N_8$ þ H^{þ} [M þ H]^{þ}: 439.1368. Found: 439.1361.

4.1.2.6. 3-Chloro-N-((1-(2-(trifluoromethyl)pyrimidin-4-yl)piper-idin-4-yl)methyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-amine (21).
A solution of 4-chloro-2-(trifluoromethyl)pyrimidine (802 mg, 4.39 mmol), tert-butyl (piperidin-4-ylmethyl)carbamate (851 mg,
3.97 mmol), and N-ethyl-N,N-di-iso-propylamine (1.5 mL, 8.6 mmol) in iso-propanol (12.5 mL) was stirred at 150 C in a single node mi-crowave reactor. After 15 min the reaction mixture was cooled to room temperature and the solvent removed in vacuo. The resulting residue was dissolved in ethyl acetate (50 mL), an aqueous HCl solu-tion (3.8 M, 10 mL, 38 mmol) added, and the solution stirred at room temperature. After 5 min the reaction mixture was adjusted to pH 14 by addition of an aqueous sodium hydroxide solution (4 M), the aqueous phase extracted with ethyl acetate (3 50 mL), the com-bined organic phases dried over magnesium sulfate, and the solvent removed in vacuo. The residue was dissolved in iso-propanol (17 mL),
3,6-dichloro-[1,2,4]triazolo[4,3-b]pyridazine (531 mg, 2.81 mmol) and N-ethyl-N,N-di-iso-propylamine (0.92 mL, 5.3 mmol) added, and the solution stirred at 150 C in a single node microwave reactor. After 100 min the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the residue purified using HPLC system B with a gradient from 20% to 70% acetonitrile in water (water modified with 0.2% ammonia) in 8.3 min to give 3-chloro-N-((1-(2-(trifluoromethyl)pyrimidin-4-yl)piperidin-4-yl)methyl)-[1,2,4]triazo lo[4,3-b]pyridazin-6-amine as a colourless solid (447 mg, 27% over three steps). ^{1}H NMR (500 MHz, DMSO-d$_6$): d 8.29 (d, J ¼ 6.3 Hz, 1H), 7.93 (d, J ¼ 9.8 Hz, 1H), 7.65 (t, J ¼ 5.3 Hz, 1H), 7.07 (d, J ¼ 6.4 Hz, 1H), 6.89 (d, J ¼ 9.9 Hz, 1H), 3.97e4.95 (m, 2H), 3.20 (t, J ¼ 6.0 Hz, 2H),
2.91e3.07 (m, 2H),1.93e2.07 (m,1H),1.81e1.90 (m, 2H),1.16e1.30 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d$_6$): d 160.8 (s), 155.7 (s), 154.9 (s), 154.9 (q, J ¼ 34.5 Hz), 143.8 (s), 134.4 (s), 123.6 (s), 119.7 (q, J ¼ 276.1 Hz), 118.0 (s), 105.5 (s), 46.1 (s), 42.4 (s), 34.8 (s), 29.2 (s). ^{19}F NMR (471 MHz, DMSO-d$_6$): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{16}H_{16}ClF_3N_8$ þ H^{þ} [M þ H]^{þ}: 413.1212. Found: 413.1216.

4.1.2.7. N-((1-(3-Chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)piperidin-4-yl)methyl)-2-(trifluoromethyl)pyrimidin-4-amine (22). A solution of tert-butyl (piperidin-4-ylmethyl)carbamate (228 mg, 1.06 mmol), 3,6-dichloro-[1,2,4]triazolo[4,3-b]pyridazine (200 mg, 1.06 mmol), and N-ethyl-N,N-di-iso-propylamine (0.40 mL, 2.3 mmol) in iso-propanol (7 mL) was stirred at 150 C in a single node microwave reactor. After 100 min the reaction mixture was allowed to cool to room temperature, and dropwise added to a freshly prepared solu-tion of HCl in methanol (6 M, prepared by dropwise addition of acetyl chloride (1.5 mL, 21 mmol) to methanol (2 mL) at 0 C and stirring for 10 min at 0 C and for 45 min at room temperature), and the resulting solution stirred at room temperature. After 72 h the solvent was removed in vacuo, the residue dissolved in iso-propanol (8 mL), 4-chloro-2-(trifluoromethyl)pyrimidine (329 mg, 1.80 mmol) and N-ethyl-N,N-di-iso-propylamine (0.68 mL, 3.9 mmol) added, and the solution stirred at 150 C in a single node microwave reactor. After 15 min the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the residue purified using HPLC sys-tem B with a gradient from 10% to 60% acetonitrile in water (water modified with 0.2% ammonia) in 8.3 min to give N-((1-(3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)piperidin-4-yl)methyl)-2-(triflu oromethyl)pyrimidin-4-amine as a yellow solid (202 mg, 46% over three steps). ^{1}H NMR (500 MHz, DMSO-d$_6$): d 8.14 (d, J ¼ 6.0 Hz, 1H), 8.07 (d, J ¼ 10.2 Hz, 1H), 8.06e8.08 (m, 1H), 7.47 (d, J ¼ 10.3 Hz, 1H),

6.67 (d, J ¼ 6.1 Hz, 1H), 4.25e4.32 (m, 2H), 3.29 (t, J ¼ 6.2 Hz, 2H), 2.93e3.02 (m, 2H), 1.72e1.94 (m, 3H), 1.17e1.33 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d$_6$): d 162.6 (s), 155.3 (s), 155.1 (q, J ¼ 34.8 Hz), 153.8 (s), 143.4 (s), 134.4 (s), 124.3 (s), 119.7 (q, J ¼ 276.0 Hz), 115.7 (s), 108.2 (s), 45.2 (s), 45.0 (s), 35.2 (s), 28.9 (s). ^{19}F NMR (471 MHz, DMSO-d$_6$):
d 70.0 (s). HRMS (ESI): m/z calcd for $C_{16}H_{16}ClF_3N_8$ þ H^{þ} [M þ H]^{þ}: 413.1212. Found: 413.1219.

4.1.2.8. N-(1-(3-Chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)piper-idin-4-yl)-2-(trifluoromethyl)pyrimidin-4-amine (23). A solution of 3,6-dichloro-[1,2,4]triazolo[4,3-b]pyridazine (227 mg, 1.20 mmol), tert-butyl piperidin-4-ylcarbamate (265 mg, 1.32 mmol), and N-ethyl-N,N-di-iso-propylamine (0.45 mL, 2.6 mmol) in iso-propanol (5 mL) was stirred at 150 C in a single node microwave reactor. After 100 min the reaction mixture was cooled to room tempera-ture, an aqueous HCl solution (3.8 M, 6.3 mL, 24 mmol) added, and the reaction mixture stirred at room temperature. After 24 h the reaction mixture was washed with ethyl acetate (50 mL) and the aqueous phase evaporated to dryness. The resulting residue was dissolved in iso-propanol (8 mL), 4-chloro-2-(trifluoromethyl)py-rimidine (178 mg, 975 mmol) and N-ethyl-N,N-di-iso-propylamine (0.30 mL, 1.7 mmol) added, and the mixture stirred at 150 C in a

single node microwave reactor. After 15 min the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the residue purified using HPLC system B with a gradient from 10% to 60% acetonitrile in water (water modified with 0.2% ammonia) in 8.3 min to give N-(1-(3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl) piperidin-4-yl)-2-(trifluoromethyl)pyrimidin-4-amine as a yellow solid (132 mg, 28% over three steps). ^{1}H NMR (500 MHz, DMSO-d$_6$): d 8.17 (d, J ¼ 6.0 Hz, 1H), 8.11 (d, J ¼ 10.2 Hz, 1H), 8.04 (d, J ¼ 7.2 Hz, 1H), 7.50 (d, J ¼ 10.3 Hz, 1H), 6.64 (d, J ¼ 6.1 Hz, 1H), 4.06e4.32 (m, 3H), 3.25 (t, J ¼ 12.1 Hz, 2H), 1.93e2.10 (m, 2H), 1.47e1.60 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d$_6$): d 161.6 (s), 155.3 (s), 155.1 (q, J ¼ 34.2 Hz), 154.0 (s), 143.4 (s), 134.5 (s), 124.5 (s), 119.7 (q, J ¼ 275.9 Hz), 115.7 (s), 108.4 (s), 46.7 (s), 44.1 (s), 30.3 (s). ^{19}F NMR (471 MHz, DMSO-d$_6$): d 69.4 (s). HRMS (ESI): m/z calcd for $C_{15}H_{14}ClF_3N_8$ þ H^{þ} [M þ H]^{þ}: 399.1055. Found: 399.1063.

4.1.2.9. 3-Chloro-N-(1-(2-(trifluoromethyl)pyrimidin-4-yl)piperidin-4-yl)-[1,2,4]triazolo[4,3-b]pyridazin-6-amine (24). A solution of tert-butyl piperidin-4-ylcarbamate (270 mg, 1.35 mmol), 4-chloro-2-(trifluoromethyl)pyrimidine (224 mg, 1.23 mmol), and N-ethyl-N,N-di-iso-propylamine (0.45 mL, 2.6 mmol) in iso-propanol (5 mL) was stirred at 150 C in a single node microwave reactor. After 15 min the reaction mixture was cooled to room temperature, an aqueous HCl solution (3.8 M, 6.5 mL, 25 mmol) added, and the re-action mixture stirred at room temperature. After 24 h the solvent was removed in vacuo, the resulting residue dissolved in iso-propanol (8 mL), 3,6-dichloro-[1,2,4]triazolo[4,3-b]pyridazine (168 mg, 889 mmol) and N-ethyl-N,N-di-iso-propylamine (0.30 mL, 1.7 mmol) added, and the reaction mixture stirred at 150 C in a single node microwave reactor. After 100 min the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the residue purified using HPLC system B with a gradient from 10% to 60% acetonitrile in water (water modified with 0.2% ammonia) in 8.3 min to give 3-chloro-N-(1-(2-(trifluoromethyl)pyrimidin-4-yl) piperidin-4-yl)-[1,2,4]triazolo[4,3-b]pyridazin-6-amine as a yellow solid (91 mg, 19% over three steps). ^{1}H NMR (500 MHz, DMSO-d$_6$): d 8.33 (d, J ¼ 6.3 Hz, 1H), 7.95 (d, J ¼ 9.9 Hz, 1H), 7.62 (d, J ¼ 6.9 Hz, 1H), 7.11 (d, J ¼ 6.3 Hz, 1H), 6.85 (d, J ¼ 9.9 Hz, 1H), 3.94e4.07 (m, 1H), 3.27e3.35 (m, 4H), 2.07e2.16 (m, 2H), 1.43e1.55 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d$_6$): d 161.0 (s), 155.9 (s), 154.9 (q, J ¼ 34.5 Hz), 153.9 (s), 143.8 (s), 134.5 (s), 123.9 (s), 119.7 (q, J ¼ 276.0 Hz), 118.2 (s), 105.6 (s), 47.4 (s), 42.2 (s), 30.0 (s). ^{19}F NMR (471 MHz, DMSO-d$_6$): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{15}H_{14}ClF_3N_8$ þ H^{þ} [M þ H]^{þ}: 399.1055. Found: 399.1048.

4.1.2.10. 3-Chloro-6-(4-(2-(trifluoromethyl)pyrimidin-4-yl)piper-azin-1-yl)-[1,2,4]triazolo[4,3-b]pyridazine (25). A solution of 4-chloro-2-(trifluoromethyl)pyrimidine (100 mg, 548 mmol), tert-butyl piperazine-1-carboxylate (106 mg, 569 mmol), and N-ethyl-N,N-di-iso-propylamine (0.20 mL, 1.2 mmol) in iso-propanol (5 mL) was stirred at 150 C in a single node microwave reactor. After 15 min the reaction mixture was cooled to room temperature and the solvent removed in vacuo. The resulting residue was dissolved in methanol (0.5 mL), dropwise added to a freshly prepared HCl solution in methanol (6 M, prepared by dropwise addition of acetyl chloride (0.80 mL, 11 mmol) to methanol (1 mL) at 0 C and stirring at 0 C for 30 min) at 0 C, and the solution stirred at room tem-perature. After 2 h the solvent was removed in vacuo, the residue dissolved in iso-propanol (5.5 mL), 3,6-dichloro-[1,2,4]triazolo[4,3-b]pyridazine (116 mg, 614 mmol) and N-ethyl-N,N-di-iso-propyl-amine (0.35 mL, 2.0 mmol) added, and the solution stirred at 150 C in a single node microwave reactor. After 100 min the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the residue purified using SFC system D with a gradient from 15% to 20% methanol (modified with 20 mM ammonia) in 7 min to give 3-chloro-6-(4-(2-(trifluoromethyl)pyrimidin-4-yl) piperazin-1-yl)-[1,2,4]triazolo[4,3-b]pyridazine as a yellow solid (18 mg, 9% over three steps). ^{1}H NMR (500 MHz, DMSO-d$_6$): d 8.39 (d, J ¼ 6.2 Hz, 1H), 8.17 (d, J ¼ 10.2 Hz, 1H), 7.50 (d, J ¼ 10.2 Hz, 1H), 7.12 (d, J ¼ 6.3 Hz, 1H), 3.82e3.93 (m, 4H), 3.73e3.80 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d$_6$): d 161.2 (s), 156.0 (s), 155.4 (s), 154.7 (q, J ¼ 34.6 Hz), 143.6 (s), 134.6 (s), 124.7 (s), 119.6 (q, J ¼ 276.0 Hz), 115.5 (s), 105.8 (s), 44.3 (s), 42.7 (s). ^{19}F NMR (471 MHz, DMSO-d$_6$): d 69.9 (s). HRMS (ESI): m/z calcd for $C_{14}H_{12}ClF_3N_8$ þ H^{þ} [M þ H]^{þ}: 385.0899. Found: 385.0911.

4.1.2.11. rac-(1R,3S)-N^{1}-(3-Chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^{3}-(2-(trifluoromethyl)pyrimidin-4-yl)cyclopentane-1,3-diamine (26). A solution of rac-tert-butyl ((1R,3S)-3-aminocyclopentyl) carbamate (50 mg, 0.25 mmol), N-ethyl-N,N-di-iso-propylamine (85 mL, 0.50 mmol), and 4-chloro-2-(trifluoromethyl)pyrimidine (46 mg, 0.25 mmol) in iso-propanol (1.2 mL) was stirred at 150 C in a single node microwave reactor. After 20 min the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using automated silica column chromatography with a gradient from 5% to 100% ethyl acetate in heptane to give rac-tert-butyl ((1R,3S)-3-((2-(trifluoromethyl)pyr-imidin-4-yl)amino)cyclopentyl)carbamate (86 mg, 99%). ^{1}H NMR (500 MHz, CDCl$_3$): d 8.26 (s br, 1H), 6.41 (d, J ¼ 6.0 Hz, 1H), 4.66 (s, 1H), 3.94 (s, 1H), 2.48e2.58 (m, 1H), 1.98e2.11 (m, 1H), 1.67e1.79 (m, 2H), 1.47e1.54 (m, 1H), 1.45 (s, 9H), 1.19e1.34 (m, 3H). LRMS (ESI): m/z calcd for $C_{15}H_{21}F_3N_4O_2$ þ H^{þ} [M þ H]^{þ}: 347.2. Found: 347.4.

Acetyl chloride (0.35 mL, 4.9 mmol) was dropwise added to methanol (0.4 mL) at 0 C and the resulting solution stirred at 0 C for 10 min. Then a solution of rac-tert-butyl ((1R,3S)-3-((2-(tri-fluoromethyl)pyrimidin-4-yl)amino)cyclopentyl)carbamate (85 mg, 0.25 mmol) in methanol (0.4 mL) was dropwise added at 0 C and the solution stirred at room temperature. After 1 h the solvent was removed in vacuo, the residue dissolved in iso-propanol (1 mL), 3,6-dichloro-[1,2,4]triazolo[4,3-b]pyridazine (49 mg, 0.26 mmol) and N-ethyl-N,N-di-iso-propylamine (0.09 mL, 0.5 mmol) added, and the solution stirred at 150 C in a single node microwave reactor. After 4 h the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the residue purified using SFC system A with a gradient from 25% to 30% methanol/water 97:3 (modified with 50 mM ammonia) in 7 min to give rac-(1R,3S)-N^{1}-(3-chloro-[1,2,4]triazolo [4,3-b]pyridazin-6-yl)-N^{3}-(2-(trifluoromethyl)pyrimidin-4-yl)cyclo-pentane-1,3-diamine as a colourless solid (38 mg, 38% over two steps). ^{1}H NMR (500 MHz, DMSO-d$_6$): d 8.11e8.18 (m, 2H), 7.93 (d, J ¼ 9.9 Hz, 1H), 7.75 (d, J ¼ 6.0 Hz, 1H), 6.84 (d, J ¼ 9.9 Hz, 1H), 6.64 (d, J ¼ 6.0 Hz, 1H), 4.23e4.31 (m, 1H), 4.05e4.12 (m, 1H), 2.55e2.69 (m, 1H), 1.99e2.18 (m, 2H), 1.61e1.74 (m, 2H), 1.41e1.51 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d$_6$): d 161.9 (s), 155.0 (q, J ¼ 35.4 Hz), 154.2 (s), 153.8 (s), 143.8 (s), 134.5 (s), 123.6 (s), 119.7 (q, J ¼ 275.9 Hz), 118.1 (s), 108.3 (s), 50.7 (s), 50.1 (s), 38.6 (s), 30.3 (s), 30.2 (s). ^{19}F NMR (471 MHz, DMSO-d$_6$): d 70.0 (s). HRMS (ESI): m/z calcd for $C_{15}H_{14}ClF_3N_8$ þ H^{þ} [M þ H]^{þ}: 399.1055. Found: 399.1068.

4.1.2.12. 3-Chloro-N-(((1s,3s)-3-((2-(trifluoromethyl)pyrimidin-4-yl) amino)cyclobutyl)methyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-amine (27). A solution of tert-butyl (((1s,3s)-3-aminocyclobutyl)methyl) carbamate (50 mg, 0.25 mmol), 4-chloro-2-(trifluoromethyl)py-rimidine (46 mg, 0.25 mmol), and N-ethyl-N,N-di-iso-propylamine (0.09 mL, 0.5 mmol) in iso-propanol (1.1 mL) was stirred at 150 C in a single node microwave reactor. After 20 min the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the residue purified using automated silica column chromatography with a gradient from 5% to 95% ethyl acetate in heptane to give tert-butyl (((1s,3s)-3-((2-(trifluoromethyl)pyrimidin-4-yl)

amino)cyclobutyl)methyl)carbamate (75 mg, 87%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.28 (s br, 1H), 6.35 (d, J ¼ 6.0 Hz, 1H), 4.56 (s, 1H), 3.13e3.22 (m, 2H), 2.54e2.63 (m, 2H), 2.20e2.32 (m, 1H), 1.59e1.68 (m, 2H), 1.45 (s, 9H). LRMS (ESI): m/z calcd for $C_{15}H_{21}F_3N_4O_2$ þ $H^{þ}$ $[M þ H]^{þ}$: 347.2. Found: 347.3.

Acetyl chloride (0.35 mL, 4.9 mmol) was dropwise added to methanol (0.4 mL) at 0 C and the resulting solution was stirred at 0 C for 30 min. Then a solution of tert-butyl (((1s,3s)-3-((2-(trifluoromethyl)pyrimidin-4-yl)amino)cyclobutyl)methyl)carbamate (85 mg, 0.25 mmol) in methanol (0.4 mL) was dropwise added at 0 C and the solution was stirred at room temperature. After 1 h the sol-vent was removed in vacuo, the residue dissolved in iso-propanol (1.0 mL), (3,6-dichloro-[1,2,4]triazolo[4,3-b]pyridazine (44 mg, 0.23 mmol) and N-ethyl-N,N-di-iso-propylamine (0.08 mL, 0.5 mmol) added, and the solution stirred at 150 C in a single node microwave reactor. After 4 h the reaction mixture was cooled to room tempera-ture, the solvent removed in vacuo, and the residue purified using SFC system A with a gradient from 25% to 30% methanol/water 97:3 (modified with 50 mM ammonia) in 7 min to give 3-chloro-N-

(((1s,3s)-3-((2-(trifluoromethyl)pyrimidin-4-yl)amino)cyclobutyl) methyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-amine as a colourless solid (32 mg, 32% over two steps). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.24 (d, J ¼ 5.7 Hz,1H), 8.12 (d, J ¼ 5.9 Hz,1H), 7.88 (d, J ¼ 9.9 Hz,1H), 7.57e7.63 (m, 1H), 6.86 (d, J ¼ 9.9 Hz, 1H), 6.58 (d, J ¼ 5.9 Hz, 1H), 4.22e4.32 (m, 1H), 3.29 (t, J ¼ 5.9 Hz, 2H), 2.42e2.48 (m, 2H), 2.27e2.39 (m, 1H), 1.66e1.80 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): 161.3 (s), 155.0 (q, J ¼ 34.6 Hz), 154.8 (s), 154.0 (s), 143.8 (s), 134.4 (s), 123.6 (s), 119.7 (q, J ¼ 275.8 Hz), 118.1 (s), 108.0 (s), 46.4 (s), 41.6 (s), 34.4 (s), 27.3 (s). ^{19}F NMR (471 MHz, DMSO-d_6): δ 70.0 (s). HRMS (ESI): m/z calcd for $C_{15}H_{14}ClF_3N_8$ þ $H^{þ}$ [M þ H]^{þ}: 399.1055. Found: 399.1044.

4.1.2.13. 3-Chloro-N-((1s,3s)-3-(((2-(trifluoromethyl)pyrimidin-4-yl) amino)methyl)cyclobutyl)-[1,2,4]triazolo[4,3-b]pyridazin-6-amine (28). A solution of 3,6-dichloro-[1,2,4]triazolo[4,3-b]pyridazine (47 mg, 0.25 mmol), tert-butyl (((1s,3s)-3-aminocyclobutyl)methyl) carbamate (50 mg, 0.25 mmol), N-ethyl-N,N-di-iso-propylamine (0.09 mL, 0.5 mmol) in iso-propanol (1.2 mL) was stirred at 160 C in a single node microwave reactor. After 3 h the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using automated silica column chroma-tography with a gradient from 5% to 100% ethyl acetate in heptane to give tert-butyl (((1s,3s)-3-((3-chloro-[1,2,4]triazolo[4,3-b]pyr-idazin-6-yl)amino)cyclobutyl)methyl)carbamate (42 mg, 48%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.73 (d, J ¼ 9.8 Hz, 1H), 6.55 (dd, J ¼ 9.8, 0.7 Hz, 1H), 5.38 (d br, J ¼ 5.3 Hz, 1H), 4.53e4.62 (m, 1H), 4.19e4.31 (m, 1H), 3.21 (t, J ¼ 6.7 Hz, 2H), 2.59e2.69 (m, 2H), 2.21e2.34 (m, 1H), 1.64e1.72 (m, 2H), 1.44 (s, 9H).

Acetyl chloride (0.17 mL, 2.4 mmol) was dropwise added to methanol (0.2 mL) at 0 C and the resulting solution was stirred at 0 C for 30 min. Then a solution of tert-butyl (((1s,3s) -3-((3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)amino)cyclo-butyl)methyl)carbamate (42 mg, 0.12 mmol) in methanol (0.19 mL) was dropwise added at 0 C and the solution stirred at room temperature. After 1 h the solvent was removed in vacuo, the res-idue dissolved in iso-propanol (0.7 mL), 4-chloro-2-(trifluoromethyl)pyrimidine (39 mg, 0.15 mmol) and N-ethyl-N,N-di-iso-propylamine (53 mL, 0.30 mmol) added, and the solution stirred at 150 C in a single node microwave reactor. After 20 min the re-action mixture was cooled to room temperature, the solvent removed in vacuo, and the residue purified using SFC system A with a gradient from 25% to 30% methanol/water 97:3 (modified with

50 mM ammonia) in 7 min to give 3-chloro-N-((1s,3s)-3-(((2-(trifluoromethyl)pyrimidin-4-yl)amino)methyl)cyclobutyl)-[1,2,4]tri-azolo[4,3-b]pyridazin-6-amine as a yellow solid (33 mg, 69% over two steps). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.12 (d, J ¼ 6.0 Hz, 1H), 8.04 (t br, J ¼ 5.0 Hz, 1H), 7.88 (d, J ¼ 9.8 Hz, 1H), 7.86 (d, J ¼ 6.4 Hz, 1H), 6.80 (d, J ¼ 9.9 Hz, 1H), 6.65 (d, J ¼ 6.1 Hz, 1H), 4.00e4.10 (m, 1H), 3.35e3.43 (m, 2H), 2.42e2.49 (m, 2H), 2.24e2.35 (m, 1H), 1.63e1.74 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 162.5 (s), 155.1 (q, J ¼ 34.1 Hz), 153.8 (s), 153.7 (s), 143.8 (s), 134.4 (s), 123.8 (s), 119.7 (q, J ¼ 275.7 Hz), 117.9 (s), 108.2 (s), 45.1 (s), 42.5 (s), 33.8 (s), 28.0 (s). ^{19}F NMR (471 MHz, DMSO-d_6): δ 70.0 (s). HRMS (ESI): m/z calcd for $C_{15}H_{14}ClF_3N_8$ þ $H^{þ}$ $[M þ H]^{þ}$: 399.1055. Found: 399.1064.

4.1.2.14. (1s,3s)-N^1-(3-Chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^3-(2-(trifluoromethyl)pyrimidin-4-yl)cyclobutane-1,3-diamine (29). A solution of tert-butyl ((1s,3s)-3-aminocyclobutyl)carbamate (50 mg, 0.26 mmol), 4-chloro-2-(trifluoromethyl)pyrimidine (51 mg, 0.28 mmol), and N-ethyl-N,N-di-iso-propylamine (92 mL, 0.53 mmol) in iso-propanol (1.2 mL) was stirred at 150 C in a single node microwave reactor. After 20 min the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using automated silica column chroma-tography with a gradient from 5% to 50% ethyl acetate in heptane to give tert-butyl ((1s,3s)-3-((2-(trifluoromethyl)pyrimidin-4-yl) amino)cyclobutyl)carbamate (27 mg, 31%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.30 (s br, 1H), 6.37 (d, J ¼ 6.0 Hz, 1H), 5.19e5.69 (m, 1H), 4.71 (s br, 1H), 3.83e4.01 (m, 1H), 2.87e2.96 (m, 2H), 1.74e1.97 (m, 2H), 1.44 (s, 9H). LRMS (ESI): m/z calcd for $C_{14}H_{19}F_3N_4O_2$ þ $H^{þ}$ $[M þ H]^{þ}$: 333.2. Found: 333.2.

Acetyl chloride (77 mL, 2.4 mmol) was dropwise added to methanol (0.09 mL) at 0 C and the resulting solution was stirred at 0 C for 30 min. Then a solution of tert-butyl ((1s,3s)-3-((2-(trifluoromethyl)pyrimidin-4-yl)amino)cyclobutyl)carbamate (18 mg, 54 mmol) in methanol (0.1 mL) was dropwise added at 0 C and the solution stirred at room temperature. After 2 h the sol-vent was removed in vacuo, the residue dissolved in iso-propanol, 3,6-dichloro-[1,2,4]triazolo[4,3-b]pyridazine (32 mg, 0.17 mmol) and N-ethyl-N,N-di-iso-propylamine (58 mL, 0.33 mmol) added, and the solution stirred at 150 C in a single node microwave reactor. After 4 h the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the residue pu-rified using SFC system D with a gradient from 20% to 25% methanol (modified with 20 mM ammonia) in 7 min to give (1s,3s)-N^1-(3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^3-(2-(trifluoromethyl)pyrimidin-4-yl)cyclobutane-1,3-diamine as a colourless solid (20 mg, 96% over two steps). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.37 (d, J ¼ 6.7 Hz, 1H), 8.18 (d, J ¼ 6.0 Hz, 1H), 7.98 (d, J ¼ 6.1 Hz, 1H), 7.95 (d, J ¼ 9.9 Hz, 1H), 6.83 (d, J ¼ 9.9 Hz, 1H), 6.63 (d, J ¼ 6.0 Hz, 1H), 4.17e4.26 (m, 1H), 3.93e4.02 (m, 1H),

2.80e2.89 (m, 2H), 1.85e1.98 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 161.4 (s), 156.4 (s), 155.2 (q, J ¼ 35.5 Hz), 153.8 (s), 143.8 (s), 134.5 (s), 123.9 (s), 119.8 (q, J ¼ 276.0 Hz), 117.7 (s), 108.1 (s), 40.4 (s), 39.4 (s), 37.8 (s). ^{19}F NMR (471 MHz, DMSO-d_6): δ 69.9 (s). HRMS (ESI): m/z calcd for $C_{14}H_{12}ClF_3N_8$ þ $H^{þ}$ $[M þ H]^{þ}$: 385.0899. Found: 385.0897.

4.1.3. Synthesis of compounds 30e33

4.1.3.1. tert-Butyl ((1s,4s)-4-((3-chloro-[1,2,4]triazolo[4,3-b]pyr-idazin-6-yl)amino)cyclohexyl)carbamate (38). A solution of tert-butyl ((1s,4s)-4-aminocyclohexyl)carbamate (460 mg, 2.15 mmol), N-ethyl-N,N-di-iso-propylamine (0.75 mL, 4.3 mmol) and 3,6-dichloro-[1,2,4]triazolo[4,3-b]pyridazine (406 mg, 2.15 mmol) in iso-propanol (10 mL) was stirred at 150 C in a single node mi-crowave reactor. After 8 h the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting res-idue purified using automated silica column chromatography with a gradient from 5% to 100% ethyl acetate in heptane to give tert-

butyl ((1s,4s)-4-((3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl) amino)cyclohexyl)carbamate (247 mg, 31%). ^{1}H NMR (500 MHz,

$CDCl_3$): d 7.77 (d, J ¼ 9.8 Hz, 1H), 6.73 (d, J ¼ 9.8 Hz, 1H), 5.20 (s, 1H), 4.63 (s, 1H), 3.95e4.05 (m, 1H), 3.62e3.73 (m, 1H), 1.75e1.93 (m, 6H), 1.60e1.71 (m, 2H), 1.45 (s, 9H). LRMS (ESI): m/z calcd for $C_{16}H_{23}ClN_6O_2$ þ $H^þ$ [M þ H]^þ: 367.2. Found: 367.4.

4.1.3.2. (1s,4s)-N^1-(3-Chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl) cyclohexane-1,4-diamine (39). Acetyl chloride (3.8 mL, 53 mmol) was dropwise added to methanol (3.5 mL) at 0 C and the resulting so-lution stirred at 0 C. After 30 min a suspension of tert-butyl ((1s,4s)-4-((3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)amino)cyclohexyl) carbamate (38, 963 mg, 2.63 mmol) in methanol (4 mL) was dropwise added at 0 C and the mixture stirred at 0 C for 1 h and at room temperature for additional 2 h. Then the solvent was removed in vacuo to give (1s,4s)-N^1-(3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)cyclohexane-1,4-diamine hydrochloride as a brown solid (941 mg, quant). ^{1}H NMR (500 MHz, DMSO-d_6): d 8.09 (s, 3H), 7.95 (d, J ¼ 9.9 Hz, 1H), 7.65 (d, J ¼ 5.2 Hz, 1H), 7.06 (d, J ¼ 10.0 Hz, 1H), 3.77e3.85 (m, 1H), 3.07e3.15 (m, 1H), 1.95e2.06 (m, 2H), 1.62e1.84 (m, 6H). LRMS (ESI): m/z calcd for $C_{11}H_{15}ClN_6$ þ $H^þ$ [M þ H]^þ: 267.1. Found: 267.3.

4.1.3.3. (1s,4s)-N^1-(3-Chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^4-(pyrimidin-4-yl)cyclohexane-1,4-diamine (30). A solution of (1s,4s)-N^1-(3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)cyclohexa ne-1,4-diamine hydrochloride (39, 0.10 g, 0.33 mmol), 4-chloropyrimidine hydrochloride (57 mg, 0.38 mmol), and N-ethyl-N,N-di-iso-propylamine (0.26 mL, 1.5 mmol) in iso-propanol (1.6 mL) was stirred at 150 C in a single node microwave reactor. After 1 h the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the resulting residue purified using SFC system G with a gradient from 25% to 30% methanol (modified with 20 mM ammonia) in 7 min to give (1s,4s)-N^1-(3-chloro-[1,2,4] triazolo[4,3-b]pyridazin-6-yl)-N^4-(pyrimidin-4-yl)cyclohexane-1,4 -diamine as a yellow solid (11 mg, 10%). ^{1}H NMR (500 MHz, DMSO-d_6): d 8.38 (s, 1H), 7.99 (d, J ¼ 5.6 Hz, 1H), 7.93 (d, J ¼ 9.9 Hz, 1H), 7.50 (d, J ¼ 6.2 Hz, 1H), 7.33 (d, J ¼ 6.9 Hz, 1H), 6.96 (d, J ¼ 9.9 Hz, 1H), 6.50 (dd, J ¼ 6.0, 1.3 Hz, 1H), 3.86e3.98 (m, 1H), 3.80e3.86 (m, 1H), 1.81e1.92 (m, 2H), 1.62e1.80 (m, 6H). ^{13}C NMR (126 MHz, DMSO-d_6): d 161.2 (s), 158.2 (s), 154.0 (s), 153.7 (s), 143.7 (s), 134.4 (s), 123.6 (s), 118.3 (s), 106.2 (s), 47.1 (s), 46.7 (s), 27.5 (s),

27.1 (s). HRMS (ESI): m/z calcd for $C_{15}H_{17}ClN_8$ þ $H^þ$ [M þ H]^þ: 345.1338. Found: 345.1331.

4.1.3.4. (1s,4s)-N^1-(3-Chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^4-(3-(trifluoromethyl)phenyl)cyclohexane-1,4-diamine (31). A mixture (1s,4s)-N^1-(3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl) cyclohexane-1,4-diamine hydrochloride (39, 0.10 g, 0.33 mmol), 3-bromo-5-(trifluoromethyl)pyridine (56 mg, 0.25 mmol), Pd_2dba_3 (5.7 mg, 6.2 mmol), dicyclohexyl(2^0,4^0,6^0-tri-iso-propyl-3,6-dimethoxy-[1,1^0-biphenyl]-2-yl)phosphane (13 mg, 24 mmol), and cesium carbonate (326 mg, 1.00 mmol) in degassed 1,4-dioxane (1.8 mL) was stirred at 111 C. After 15 h the reaction mixture was allowed to cool to room temperature, the solvent removed in vacuo, and the resulting residue purified using SFC system A with a gradient from 22% to 27% methanol/water 97:3 (modified with

50 mM ammonia) in 7 min to give (1s,4s)-N^1-(3-chloro-[1,2,4]tri-azolo[4,3-b]pyridazin-6-yl)-N^4-(3-(trifluoromethyl)phenyl)cyclo-hexane-1,4-diamine as a pale yellow solid (10 mg, 7%). ^{1}H NMR (500 MHz, DMSO-d_6): d 7.93 (d, J ¼ 9.9 Hz, 1H), 7.49 (d, J ¼ 6.4 Hz, 1H), 7.26 (t, J ¼ 7.9 Hz, 1H), 6.95 (d, J ¼ 10.0 Hz, 1H), 6.87e6.89 (m,

1H), 6.84 (dd, J ¼ 8.3, 2.3 Hz, 1H), 6.76 (d, J ¼ 7.9 Hz, 1H), 6.07 (d, J ¼ 6.9 Hz, 1H), 3.80e3.86 (m, 1H), 3.40e3.47 (m, 1H), 1.70e1.88 (m, 6H), 1.58e1.69 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): d 154.0 (s), 148.4 (s), 143.8 (s), 134.4 (s), 129.9 (s), 129.8 (q, J ¼ 30.7 Hz), 124.6 (q,

J ¼ 272.5 Hz), 123.6 (s), 118.3 (s), 115.3 (s), 111.0 (d, J ¼ 4.6 Hz), 108.2 (d, J ¼ 4.6 Hz), 48.2 (s), 47.4 (s), 27.5 (s), 26.9 (s). ^{19}F NMR (471 MHz, DMSO-d_6): d 61.3 (s). HRMS (ESI): m/z calcd for $C_{18}H_{18}ClF_3N_6$ þ $H^þ$ [M þ H]^þ: 411.1307. Found: 411.1311.

4.1.3.5. tert-Butyl ((1s,4s)-4-((5-chloro-6-methoxypyridin-3-yl) amino)cyclohexyl)carbamate (41). A mixture of tert-butyl ((1s,4s)-4-aminocyclohexyl)carbamate (1.4 g, 6.5 mmol) and 5-bromo-3-chloro-2-methoxypyridine (1.0 g, 4.5 mmol), copper (29 mg, 0.45 mmol), and cesium acetate (1.7 g, 8.9 mmol) in DMSO (10 mL) was stirred at 80 C. After 5 h the reaction mixture was allowed to cool to room temperature, an aqueous saturated sodium chloride solution (30 mL) added, the aqueous phase extracted with ethyl acetate (3 10 mL), the combined organic phases dried over magnesium sulfate, filtered, the solvent removed in vacuo, and the resulting residue purified using automated silica column chroma-tography with a gradient from 5% to 50% ethyl acetate in heptane to give tert-butyl ((1s,4s)-4-((5-chloro-6-methoxypyridin-3-yl) amino)cyclohexyl)carbamate (71 mg, 4%). ^{1}H NMR (500 MHz, $CDCl_3$): d 7.43 (d, J ¼ 2.7 Hz, 1H), 7.00 (d, J ¼ 2.6 Hz, 1H), 4.61 (d,

J ¼ 7.1 Hz, 1H), 3.90 (s, 3H), 3.55e3.66 (m, 1H), 3.24e3.33 (m, 1H), 1.65e1.81 (m, 4H), 1.52e1.62 (m, 4H), 1.42 (s, 9H). LRMS (ESI): m/z calcd for $C_{17}H_{26}ClF_3N_3$ þ $H^þ$ [M þ H]^þ: 356.2. Found: 356.4.

4.1.3.6. (1s,4s)-N^1-(5-Chloro-6-methoxypyridin-3-yl)-N^4-(3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)cyclohexane-1,4-diamine (32). To methanol (3.6 mL) was dropwise added acetyl chloride (3.8 mL, 53 mmol) within 10 min at 0 C and the solution stirred at 0 C. After 1 h a solution of tert-butyl ((1s,4s)-4-((5-chloro-6-methoxypyridin-3-yl)amino)cyclohexyl)carbamate (41, 71 mg, 0.20 mmol) in meth-anol (4 mL) was dropwise added at 0 C, and the solution stirred at room temperature. After 12 h the solvent was removed in vacuo and the residue dissolved in iso-propanol (0.9 mL). 3,6-dichloro-[1,2,4] triazolo[4,3-b]pyridazine (38 mg, 0.20 mmol) and N-ethyl-N,N-di-iso-propylamine (68 mL, 0.40 mmol) added and the solution stirred at

150 C in a single node microwave reactor. After 5 h the reaction mixture was cooled to room temperature, the solvent removed in vacuo, and the residue purified using HPLC system D with a gradient from 20% to 70% acetonitrile in water (water modified with 0.1 M formic acid) in 8.3 min to give (1s,4s)-N^1-(5-chloro-6-methoxypyridin-3-yl)-N^4-(3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)cyclohexane-1,4-diamine as a yellow solid (17 mg, 21% over two steps). ^{1}H NMR (600 MHz, DMSO-d_6): d 7.92 (d, J ¼ 9.7 Hz, 1H), 7.51 (d, J ¼ 2.6 Hz, 1H), 7.46 (d, J ¼ 6.3 Hz, 1H), 7.22 (d, J ¼ 2.7 Hz, 1H), 6.94 (d, J ¼ 9.9 Hz, 1H), 5.44 (d, J ¼ 7.1 Hz, 1H), 3.80e3.85 (m, 1H), 3.79 (s, 3H), 3.32e3.36 (m, 1H), 1.68e1.87 (m, 6H), 1.56e1.67 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): d 154.0 (s), 150.0 (s), 143.8(s), 140.3 (s), 134.4 (s), 128.0 (s), 123.9 (s), 123.6 (s), 118.3 (s), 116.6 (s), 53.5 (s), 48.8 (s), 47.4 (s), 27.6 (s), 26.9 (s). HRMS (ESI): m/z calcd for $C_{17}H_{19}Cl_2N_7O$ þ $H^þ$ [M þ H]^þ: 408.1101. Found: 408.1111.

4.1.3.7. (1s,4s)-N^1-(3-Chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-N^4-(2-(trifluoromethyl)pyridin-4-yl)cyclohexane-1,4-diamine (33). A mixture of (1s,4s)-N^1-(3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-6-yl) cyclohexane-1,4-diamine hydrochloride (39, 0.20 g, 0.66 mmol), 4-bromo-2-(trifluoromethyl)pyridine (116 mg, 513 mmol), Pd_2dba_3 (11 mg, 12 mmol), di-tert-butyl(2^0,4^0,6^0-tri-iso-propyl-3,6-dimethoxy-[1,1^0-biphenyl]-2-yl)phosphane (24 mg, 50 mmol), cesium carbonate (651 mg, 2.00 mmol) in degassed 1,4-dioxane (3.6 mL) was stirred at

111 C. After 12 h the reaction mixture was allowed to cool to room temperature, the solvent removed in vacuo, and the resulting residue purified using HPLC system D with a gradient from 10% to 60% acetonitrile in water (water modified with 0.1 M formic acid) in 8.3 min to give (1s,4s)-N^1-(3-chloro-[1,2,4]triazolo[4,3-b]pyridazin-

6-yl)-N^4-(2-(trifluoromethyl)pyridin-4-yl)cyclohexane-1,4-diamine as a pale yellow solid (4 mg, 1%). ^{1}H NMR (500 MHz, DMSO-d_6): d 8.14 (d, J ¼ 5.8 Hz, 1H), 7.94 (d, J ¼ 9.8 Hz, 1H), 7.51 (d, J ¼ 6.2 Hz, 1H), 7.04 (d, J ¼ 7.0 Hz, 1H), 6.98 (s br, 1H), 6.95 (d, J ¼ 9.9 Hz, 1H), 6.73 (dd, J ¼ 5.9, 2.3 Hz, 1H), 3.80e3.88 (m, 1H), 3.51e3.58 (m, 1H), 1.71e1.92 (m, 6H), 1.59e1.71 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): d 154.0 (s), 153.7 (s), 150.0 (s), 147.4 (q, J ¼ 34.0 Hz), 143.8 (s), 134.5 (s), 123.7 (s), 122.1 (q, J ¼ 274.3 Hz), 118.3 (s), 109.0 (s), 104.6 (s), 48.0 (s), 47.1 (s), 27.2 (s), 26.9 (s). ^{19}F NMR (471 MHz, DMSO-d_6): d 67.0 (s). HRMS (ESI): m/z calcd for $C_{17}H_{17}ClF_3N_7$ þ $H^þ$ [M þ H]^þ: 412.1259. Found: 412.1290.

4.2. Animal experiments

The rat pharmacokinetic study of compound 8 was conducted by Pharmaron (AAALAC accredited) in Beijing, China, according to the statutes enforced at state, province, and local levels.

4.3. Physicochemical properties

4.3.1. Caco-2 measurements

Caco-2 measurements were performed by the DMPK depart-ment at Pharmaron as described by Fredlund et al. [27].

4.3.2. ChromlogD$_{7.4}$ measurements

ChromlogD$_{7.4}$ was measured as outlined in Schiesser et al. [28].

4.3.3. Solubility, metabolic stability in human liver microsomes and rat hepatocytes

Solubility and metabolic stability in human liver microsomes and rat hepatocytes were determined according to Wernevik et al. [19].

4.4. MALT1 enzyme and cell potency

The enzymatic and cellular potency against MALT1 was deter-mined according to Dumont et al. [17] and Malinverni et al. [26] respectively.

4.5. X-ray analyses of compounds 1 and 8

Protein of human MALT1 ((L339-R719)-6 His [D595K, S617K, H666A, H681E]) at a concentration of ~10 mg/mL in formulation 25 mM Hepes pH 7.5, 50 mM NaCl, and 1 mM TCEP was set up in crystallization trials. Large enough crystals to use in soaking were obtained by hanging drop crystallization with a 1:1 ratio of protein solution and well solution containing PEG 3350 (17e21%), sodium formate (0.1e0.2 M), and 0.1 M Tris pH 8 at 20 C. For soaking, the compounds were added directly to the crystallization drops to give a final compound concentration of 5 mM for compound 1 and 2 mM for compound 8. PEG 400 and DMSO were included at a concen-tration of 1% and 2% respectively. The compounds were left to soak in 2 h for compound 1 and 4 h for compound 8. Before flash-freezing the crystals, the PEG 400 concentration was gradually increased to 5% while still maintaining the compound concentra-tion and PEG 3350, sodium formate, and Tris concentrations. Data was collected at the European Synchrotron Radiation Facility, France; compound 1 at beamline ID23-1 and compound 8 at beamline ID30B respectively. Both crystals diffracted with a reso-lution of 2.2 Å, but were significantly anisotropic (Table S5). Data was processed using autoPROC with STARANISO and showed that the crystals belong to space group $P2_1$ [29,30]. The structures were solved by molecular replacement using the structure 3UOA as search model [31]. An initial round of refinement was carried out in Refmac [32]. Several rounds of manual rebuilding in Coot and subsequent refinement in autoBUSTER were carried out prior to modelling of the ligand [33,34]. Ligand restraints were generated with the program writedict [35]. Details from the data processing and refinement are reported in Table S5. Pictures were generated with the software PyMol [36].

Author contributions

S.S. led the optimization of this chemical series, designed some of the compounds investigated, and analyzed data. R.J.C. designed some of the compounds investigated and analyzed data. P.H. and H.E.P. synthesized the described compounds and supported the analysis of the data. H.K. solved the crystal structures of MALT1 in complex with compounds 1 and 8. L.O. crystallized MALT1 in complex with compound 1 and 8. All authors contributed to writing the manuscript and have given approval to the final version of the manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: S.S., H.K., L.O., and R.J.C. are employees of AstraZeneca and may own stock or stock options.

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