Journal of
Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.9b00681 • Publication Date (Web): 10 Jul 2019

Just Accepted

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Development of Dual Chitinases Inhibitors as Potential New Treatment for Respiratory System Diseases

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ABSTRACT Acidic mammalian chitinase (AMCase) and chitotriosidase-1 (CHIT1) are two enzymatically active proteins produced by mammals and capable of cleaving glycosidic bond in

chitin. Based on the clinical findings and animal models studies, involvement of chitinases has been suggested in several respiratory system diseases including asthma, COPD and idiopathic pulmonary fibrosis. Exploration of structure-activity relationships within the series of 1-(3-amino-1H-1,2,4-triazol-5-yl)-piperidin-4-amines, that was earlier identified as a scaffold of potent AMCase inhibitors, led us to discovery of highly active dual (i.e. AMCase and CHIT1) inhibitors with very good pharmacokinetic properties. Among them, compound **30** was shown to reduce the total number of cells in bronchoalveolar lavage fluid (BALf) of mice challenged with house dust mite (HDM) extract after oral administration (50 mg/kg, qd). In addition, affinity towards hERG potassium channel of compound **30** was significantly reduced when compared to the earlier reported chitinases inhibitors.

INTRODUCTION

Asthma is a chronic inflammatory disease that affects over 330 million people worldwide [1]. Despite availability of rapidly acting bronchodilators and anti-inflammatory drugs such as steroids, asthma remains an unmet medical need due to variable pathogenesis, side-effects of current therapeutics, resistance to treatment developing in subsets of patients, and poor patients' compliance to inhaled drugs [2]. There is compelling evidence implicating type 2 immune response in development of asthma pathogenesis [3]. A chronic pulmonary inflammation leads to development of airway hyperresponsiveness, airways remodeling and fibrosis. Remarkably, increasing number of studies report changes in the expression and activity of chitinases in patients with asthma [4-7] and other chronic lung diseases [8-15]. Chitinases are chitin-degrading enzymes that

belong to the 18 glycosyl hydrolase family, which consists of two enzymatically active molecules (chitotriosidase [CHIT1] and acidic mammalian chitinase [AMCase]) as well as several chitinase-like proteins, that lack enzymatic activity [4, 7, 12, 16-19]. Chitinases were initially hypothesized to play a significant role in immune response modulation in asthma, owing to the observation that predominant aeroallergen sources, such as house dust mites, cockroaches and fungi, are chitin-containing organisms [20]. However, several reports have clearly shown their critical role in facilitating immune response and tissue repair processes in chitin-independent manner. For example, both enzymes were shown to actively participate in interleukin-13-driven development of type 2 immune response in asthma [7, 16, 21] or tissue repair in pulmonary fibrosis [15] in animal models induced by non-chitin containing insults, such as ovalbumin and bleomycin.

AMCase is produced by lung epithelial cells and macrophages at sites of Th2 inflammation in animal models of asthma, induced by HDM, *Aspergillus sp.*, ovalbumin (OVA), and IL-13 [7, 20, 22]. Moreover, this enzyme is believed to be the dominant true chitinase in mouse lungs [16]. AMCase expression and activity was mostly described to modulate type 2 immune response in mouse models of asthma [7, 16, 17, 20-23] and was linked to human asthma pathogenesis [5, 7]. However, the increase in overall chitinolytic activity observed in the lungs of patients with chronic and interstitial lung diseases has recently been mostly attributed to CHIT1 enzymatic activity [6, 8, 11-15, 24].

Studies in animal models revealed that CHIT1 deficiency results in decreased TGFβ and IL-13-driven fibrotic responses in the lungs [15]. Also other observations support the central role of CHIT1 in human lungs and in expanding spectrum of human lungs disorders ranging from asthma throughout granulomatous diseases such as sarcoidosis [11-13, 18], tuberculosis [25, 26] to interstitial lung fibrosis [15]. All these observations suggests that dual chitinase inhibition could potentially be

considered as the therapeutic strategy not only for asthma patients, where AMCase is considered to be a crucial modulator of Th2 inflammatory response, but also for chronic interstitial lung diseases dominated by chronic inflammation and ongoing pulmonary fibrosis.

Our group has recently described compound **3b** [21] that displays AMCase *vs* CHIT1 selectivity (150-times and 15-times in murine and human enzymes, respectively), has good pharmacokinetic profile in mice and shows significant anti-inflammatory efficacy in HDM-induced asthma model after oral administration. Starting from compound **3b** we sought to identify dual specificity inhibitor targeting both chitinases simultaneously and capable of ameliorating both pulmonary inflammation and bronchial fibrosis.

RESULTS AND DISSCUSSION

We have previously reported [21] that shifting the position of the central nitrogen atom from piperazine ring to the 4-aminopiperidine system in a moderately potent dual chitinase inhibitor (compound **1**), discovered by the Wyeth group (Wyeth 1, Figure 1) [27], led to a significant, over one order of magnitude increase in the inhibitory potency against both chitinases (compound **2**, R = Me). Initial exploration of SAR revealed however that increasing the size of the hydrophobic substituent on the central nitrogen atom exemplified by compounds **3a** and **3b-d**, leads to a further increase in potency against hAMCase, *but not* hCHIT1.

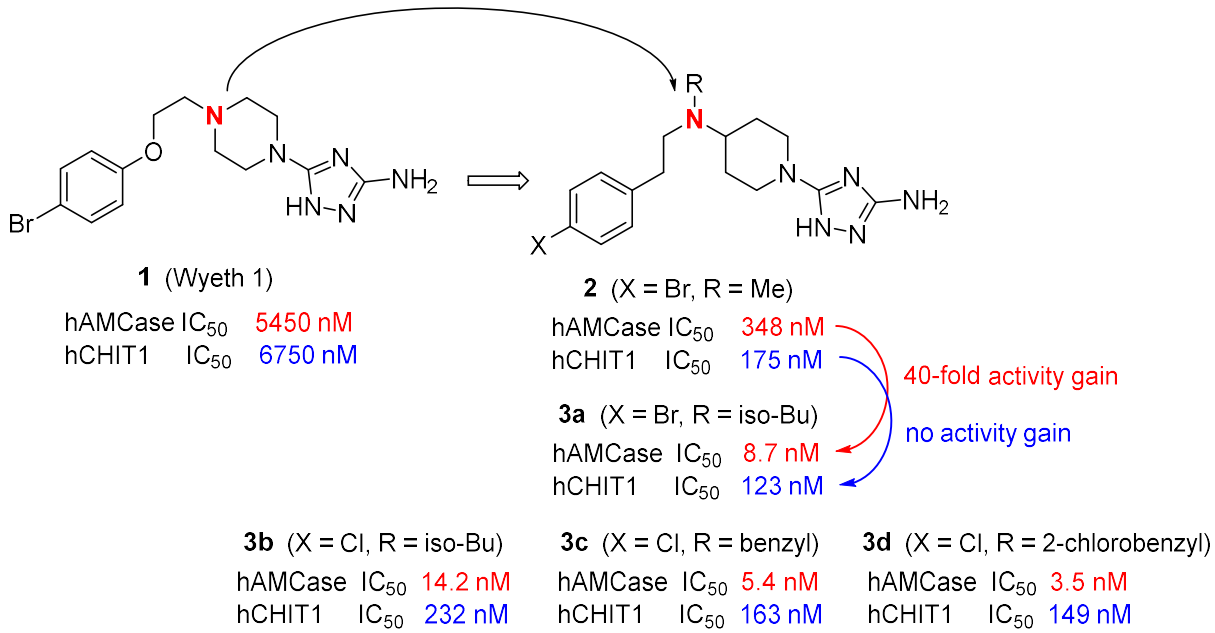


Figure 1. Early optimization of Wyeth 1 – moderately potent dual chitinase inhibitor (replacement of bromine by chlorine atom in the phenyl ring has no major impact on inhibitory potency) [21].

Examination of the published X-ray structures of complexes of hCHIT1 and hAMCase with their small-molecule inhibitors [27,28] indicated that the active sites of both enzymes are very similar and differ by only two amino acids: Met300 and Arg269 in hCHIT1 are replaced by Ile300 and His269 in hAMCase. Our previously reported compounds bind to the acidic binding site of the enzymes through the network of the hydrogen bonds between 3-aminotriazole moiety and Tyr27, Asp138 and Glu140 residues [21]. Critical for the high activity of those inhibitors is the salt bridge (length of 2.8 Å) between the central basic nitrogen of 4-aminopiperidine fragment and carboxylic group of Asp213. The hydrophobic interactions of para-halogenated phenethyl group and hydrophobic pocket created by Tyr267, Glu297 and Met300 (hCHIT1) or Ile300 (hAMCase) residues appear to be well optimized. As shown in Figure 1, with enlargement of the size of the hydrophobic substituent on the central nitrogen atom from methyl to isobutyl or (ring-substituted)benzyl, the inhibitory activity against hAMCase is increased by two orders of

magnitude while no analogous effect is observed for hCHIT1. We hypothesized that the key factor determining this high activity against hAMCase are the hydrophobic interactions of the aliphatic substituent with Ile300 and His269 in the enzyme binding pocket. On the other hand, such a bulky N-substituent causes the steric clash with the positively charged, guanidine-containing Arg269 side chain in hCHIT1, which is reflected in no activity gain.

Another difference between the analyzed structures was the orientation of Trp99 side-chain. While in the hAMCase-compound **1** complex (PDB ID: 3RM4) the indole ring of this amino acid residue is directed slightly outside of the binding pocket (Figure 2A), it is directed towards the pocket in hCHIT1 complexed with compound **3a** (PDB ID: 5NRA; Figure 2B). As this residue plays an important role in binding of both the oligosaccharide substrate and small molecule inhibitors, we performed analysis of the experimentally solved structures of hAMCase (see: Figure 5 in Supporting Information). In six structures, both apo and with ligands, the Trp99 side-chain is placed in the same position as in the hAMCase-compound **1** complex. In one complex (PDB ID: 3RM9), Trp99 can adopt a double conformation and only in one case (PDB ID: 3RM8) the Trp99 is in the same position as in hCHIT1 complexed with compound **3b**. It may be concluded that although for hAMCase, the Trp99 side-chain may adopt both conformations, the preferred state is towards *outside of the binding pocket*. It is also not fully clear what structural features of the ligand induce the flip of this residue.

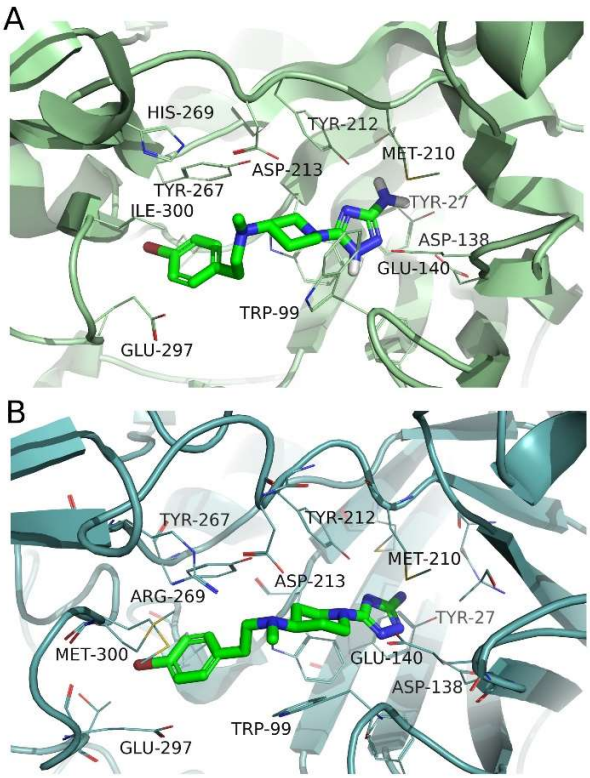


Figure 2. (A) Docking of compound **2** to the binding pocket of hAMCase (PDB ID: 3RM4 [29]) and (B) structure of hCHIT1-compound **2** complex (PDB ID: 5NR8). For ligands, carbon atoms are colored in green, nitrogen atoms in blue, oxygen atoms in red, sulfur atoms in yellow and bromine atoms in dark red.

In order to design the equally active inhibitor for these two enzymes, during optimization of the structure of compound **2** we explored only these fragments of the binding pockets, which are conserved among binding sites of both proteins. Therefore, our initial design strategy to improve interactions of the inhibitor molecule with the hCHIT1 active site (while maintaining high hAMCase affinity) was based on the introduction of the *small* N-substituents containing polar functional groups that might be capable of creating the additional binding interactions with the guanidine moiety of Arg269 in hCHIT1. Such an approach was also driven by the fact that our first generation inhibitors displayed considerable activity against hERG potassium channel and

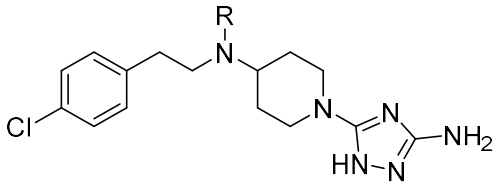
dopamine and serotonin transporters [21]. While we realized that the presence of the central basic nitrogen atom and phenethylamine fragment are the possible structural features of our inhibitors that trigger those two off-targets activities, we also assumed that we could not afford losing the strong interaction between the central amine and the Asp213 residue and therefore decided that this structural motif would be kept intact. On the other hand, it is known from the previous studies[30] that implementation of a polar fragment into the peripheral part of the inhibitor resulting in the overall decrease of the lipophilicity of the final molecule, oftentimes attenuates interaction with the hERG potassium channel. In a similar manner, introduction of the alkoxy/hydroxyl group at the β-position with respect to the amino group, causing reduction of its basicity could provide yet another measure of destabilizing interactions with hERG. We also hoped that the presence of the polar moiety in the inhibitor molecule would help to reduce the monoamine transporters off-target activity.

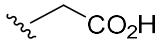
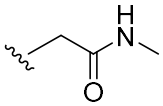
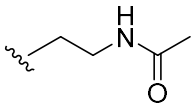
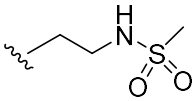
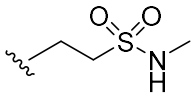
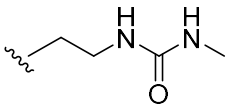
FIRST APPROACH – COMPOUNDS WITH SMALL N-SUBSTITUENTS CONTAINING POLAR GROUPS

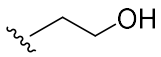
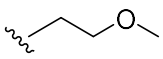
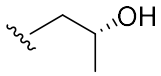
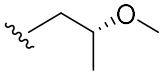
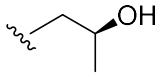
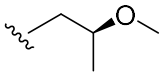
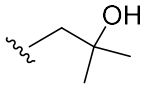
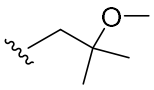
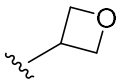
As seen in Table 1 introduction of carboxylic group (compound **4**) led to a significant decrease in activity against both human chitinases in comparison with the parent compound **2**. Amidation of the carboxylic group (compound **5**) partially restored the inhibitory potency against hAMCase, but the activity *vs* hCHIT1 remained at over 1 micromolar level. Reversal of the sequence of the amide fragment (compound **6**) and especially replacement of the acetyl group with methanesulfonyl group (compound **7**) led to a further increase in activity *vs* hAMCase with only marginal improvement of hCHIT1 inhibitory potency. Sulfonamide **8** exhibited analogous profile as its parent counterpart **7**, but introduction of N-methylcarbamoyl moiety effected in significantly

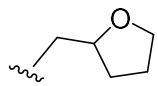
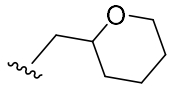
less active inhibitor (compound **9**). In contrast to analogs **5** – **8** that were either hAMCase-selective inhibitors or only moderately active dual inhibitors (compounds **4** and **9**), compound **10** containing hydroxyethyl group on the central nitrogen atom displayed only ca. 3-fold hAMCase/hCHIT1 selectivity and reasonable activity against both enzymes (IC_{50} 111 nM and 384 nM respectively). Methylation of the hydroxyl group provided compound **11** that approached the desired dual inhibitor profile even more closely. Introduction of additional methyl groups of the defined stereochemistry into the hydroxy/methoxyethyl substituent (analogs **12** – **15**) resulted in general in no positive impact on the inhibitory activity with the exception of compound **15** (IC_{50} 103 nM and 44 nM against hAMCase and hCHIT1, respectively). Worth noting is the low hERG inhibition of analog **15** that was achieved by quite minor structural change (as compared to the parent compound **11**: IC_{50} 17.6 μM *vs* 2.8 μM respectively). However, 20-fold selectivity in murine enzymes and 33% inhibition at 10 μM of dopamine transporter appeared to be a serious shortcomings of compound **15**. Adding yet one more methyl group afforded compounds **16** and **17** displaying distinct AMCase-selectivity both in human and murine enzymes. Similar selectivity was found for oxetane-containing inhibitor **18**, while, conversely, the other two compounds (**19** and **20**) incorporating the cyclic ether moieties (tetrahydrofuran and tetrahydropyran respectively) showed very promising potency against both human proteins. However, these two analogs suffered from the undesired mAMCase-selectivity and were quite active against both off-targets investigated.

Table 1. Structure-activity relationship of the series of polar group – containing inhibitors [a]



Cmpd #	R	hAMCase (IC_{50} nM)	hCHIT1 (IC_{50} nM)	mAMCase (IC_{50} nM)	mCHIT1 (IC_{50} nM)	hERG[b] (IC_{50} μM)	DAT[c] %inh. @ 10 μM
4		1040 ± 233	1900 ± 424	395 ± 64	21000 ± 2830	8.0	NT
5		690 ± 42	1800 ± 141	1150 ± 71	48000 ± 1414	2.6	NT
6		163 ± 71	495 ± 21	35 ± 9.2	8100 ± 141	3.3	NT
7		37 ± 5.7	1200 ± 141	22 ± 2.1	12500 ± 707	7.2	4
8		28 ± 1.4	870 ± 141	21 ± 0.7	7550 ± 1343	16.0	13
9		425 ± 35	1430 ± 35	130 ± 14	9100 ± 424	1.8	NT

10		111 ± 28	384 ± 48	135 ± 21	4800 ± 566	6.3	NT
11		98 ± 17	84 ± 11	55 ± 1.4	1700 ± 424	2.8	NT
12		185 ± 7.1	330 ± 14	66 ± 16	6800 ± 848	6.1	NT
13		135 ± 35	530 ± 156	110 ± 28	12500 ± 2121	6.4	NT
14		135 ± 64	145 ± 21	180 ± 42	2350 ± 212	14	NT
15		103 ± 9.9	44 ± 1.4	53 ± 7.1	1150 ± 212	17.6	33
16		40 ± 3.5	395 ± 106	73 ± 16	4700 ± 990	19	NT
17		28 ± 2.1	505 ± 7.1	28 ± 7.1	3650 ± 353	3.5	NT
18		83 ± 9.8	180 ± 28	27 ± 2.8	5500 ± 1273	4.4	NT

19		21 ± 6.4	27 ± 2.8	17 ± 4.2	1080 ± 170	15	74
20		68 ± 1.4	21 ± 1.4	56 ± 24	750 ± 14	11	50

[a] IC_{50} data of each compound were determined in 2−3 independent assays, and mean IC_{50} ± SD values are shown in the table. Cmpd, abbreviation of compound. SD, standard deviation.

[b] data from Predictor™ hERG Fluorescence Polarization assay [31]

[c] data from DAT Human Dopamine Transporter Binding (Antagonist Radioligand) Assay, Cerep[32]

It is worth noting that while we were able to achieve good activity against three out of four enzymes tested, even best compounds of this series (analogs **11**, **19** and **20**) only moderately inhibited murine CHIT1. As no crystal structures of the murine chitinases are known, we compared their sequences and three-dimensional models of binding pockets in order to explain observed differences in activities of our inhibitors between human and murine enzymes.

With regards to AMCase, the only differences within binding pockets observed on the structural level between human and murine proteins are residues 297 (Glu for human, Gln for murine) and 300 (Ile and Phe, respectively). Glu and Gln are similar in size, and they may form analogous interactions with ligand. Ile and Phe are both hydrophobic amino acids, and despite Phe is more bulky, its rotation allows for accommodation of even relatively big substituents (like tetrahydrofuran and tetrahydropyran fragments in compounds **19** and **20**).

Human and murine CHIT1 binding pockets differ in residues at the same positions as in AMCase. Residue 297 is Glu for human enzyme and Asp for murine protein, and residue 300 is Met and Val, respectively. The observed lower activity of our compounds against mCHIT1 may

indicate importance of interactions of Met300 of human protein with haloaromatic ring of inhibitors. Also, while formation of the hydrogen bonds between ligands and Glu297 of human enzyme could be predicted by molecular modeling, it appears that the carboxylic group of Asp297 in mCHIT1 is too distant to form this type of interactions with inhibitors.

SECOND APPROACH – CONFORMATIONALLY CONSTRAINED COMPOUNDS WITH OXYGEN ATOM CONTAINING FUNCTIONALITY IN BETA-POSITION WITH RESPECT TO THE CENTRAL AMINE GROUP

Analysis of the crystallographic structures of complexes of hCHIT1 with inhibitors **2** and **3a** supported by molecular modeling studies indicated that two linear substituents present on the central nitrogen atom in all of the analogs we synthesized so far could be joined one to another to form new four, five or six-membered ring (Figure 3). We predicted, that for both human chitinases, the binding mode of the resulting compounds would be similar to their noncyclic analogs. The calculated binding score values suggested that the benzylic position as the site of cyclization resulting in considerably constrained 3-aryl-substituted nitrogen-containing heterocycle is slightly preferred (Figure 3A). However, the alternative mode of cyclization at the homo-benzylic position (Figure 3B), while providing less effective conformational constraint, allowed for better alignment of the benzyl fragment to the hydrophobic pockets of the enzymes.

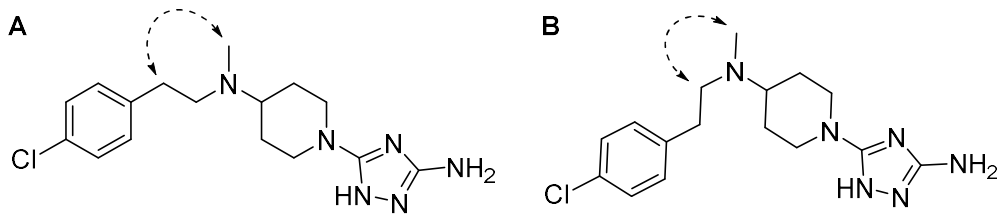


Figure 3. Two possible modes of cyclization of two linear N-substituents leading to (A) more and (B) less rigidified (4-chlorophenyl)-N-heterocycles.

3-(4-chlorophenyl)pyrrolidine and 3-(4-chlorophenyl)piperidine required for the synthesis of the more conformationally constrained inhibitors were commercially available, what prompted us to synthesize the corresponding analogs in their racemic forms. On the other hand, the 2-(4-chlorobenzyl)pyrrolidine and 2-(4-chlorobenzyl)piperidine required for the synthesis of more flexible inhibitors had to be synthesized. Careful examination of the experimentally solved structure of hCHIT1 complexed with compound **2** (PDB ID: 5NR8) clearly indicated that only one of the enantiomers can fulfill the constraints of the binding site (Figure 4) and thus we decided to prepare these cyclic amines in the optically pure form.

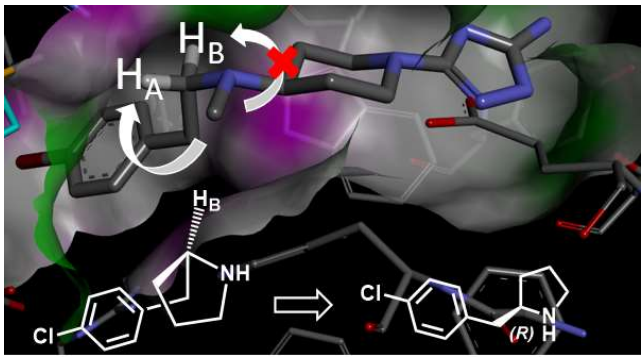


Figure 4. Binding site of hCHIT1 complexed with compound **2** (PDB ID: 5NR8); homobenzylic hydrogens marked as H_A and H_B. H_A can be substituted by the incoming alkyl linker joining the homobenzylic position with the N-methyl group (the resulting 2-(4-chlorobenzyl)pyrrolidine of the *R* configuration shown). On the other hand, H_B can not be displaced in the same way without disrupting conformation of the p-halophenetyl substituent.

As seen in Table 2, comparison of the biochemical potencies of inhibitors containing 3-(4-chlorophenyl)-substituted N-heterocycles (compounds **21** and **22**) with more conformationally

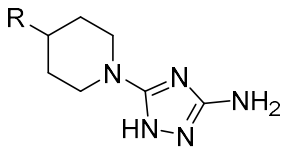
flexible analogues **23** and **24** clearly indicated the favored mode of cyclization. Introduction of 3-(4-chlorophenyl)pyrrolidine fragment resulted in poorly active compound **21** (IC_{50} 6 μM and 19.5 μM *vs* hAMCase and hCHIT1, respectively), and the 3-(4-chlorophenyl)piperidine moiety provided only modest improvement of activity against both human chitinases. On the other hand, analogue **23**, and especially **24**, approached the targeted *in vitro* pharmacological profile very closely, being potent, non-selective chitinases inhibitors against both human and murine enzymes. However, the single-digit micromolar level of hERG inhibition displayed by those two compounds was perceived as a serious disadvantage and the introduction of the oxygen-containing functionality placed in the β-position with respect to the amino functionality was attempted as a countermeasure to this undesired side-activity. The first attempt was not very successful, as the 2-(4-chlorobenzyl)-3-methoxyazetidine containing compound **25** was not only the most potent hERG inhibitor we synthesized, but, moreover, its inhibitory activities against chitinases were greatly diminished. Conversely, introduction of oxygen-containing functionality into position 4 of pyrrolidine ring provided analogs **26** – **29**, whose inhibition ability of chitinases was (regardless the configuration of the additional chiral center) retained by larger part, while their hERG activity was significantly attenuated. The *in vitro* pharmacological profile of the best compound of this series **28** was further improved two-fold, by replacement of 2-(4-chlorobenzyl)-4-hydroxypyrrolidine fragment with 2-(4-chlorobenzyl)morpholine resulting in inhibitor **30** that displayed only two- and four-fold selectivity AMCase *vs* CHIT1 in murine and human enzymes, respectively. The so obtained analog **30** represented the optimum combination of structural features to achieve the desired dual inhibition both in human and murine enzymes while maintaining acceptable hERG channel affinity level of 39 μM, and therefore meeting the selected

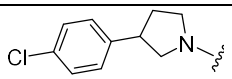
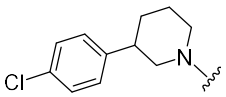
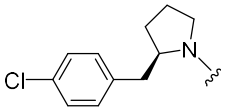
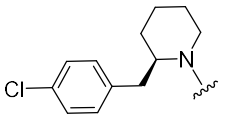
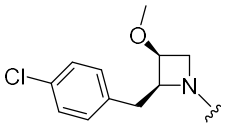
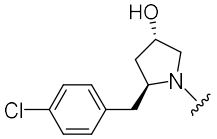
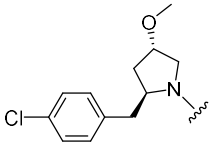
criteria of the primary pharmacological parameters and justifying its further *in vitro* and *in vivo* profiling.

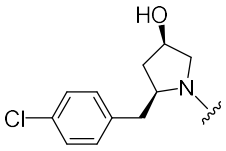
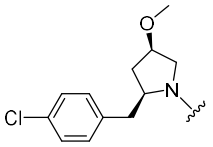
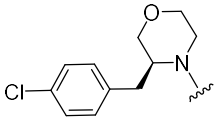
Since lowering of lipophilicity and basicity of the compound is considered to be the method of controlling the inhibition of the hERG channel, the pK_a values were calculated and $logD_{7.4}$ values were measured for the most interesting compounds (**23** – **30**). Unfortunately, based on this valuations no simple conclusions could be drawn in relation to how do these parameters affect the affinity of our inhibitors for hERG. Two compounds of comparable (and low – pK_a of ca. 7.5) basicity of the central amine, **25** and **30**, were the most potent (IC_{50} 0.34 μM) and second least potent (IC_{50} 39 μM) hERG inhibitors, respectively, spanning over two orders of magnitude of activity. Also a pair of stereoisomers, compounds **26** and **28**, sharing the same calculated value of pK_a, showed significant, 4-fold, difference in hERG activity (IC_{50} 13.2 μM and 55 μM, respectively). Lipophilicity of the inhibitors within this series of analogues spanned only 1.3 unit of magnitude ($logD_{7.4}$ = 0.68 for **28** and $logD_{7.4}$ = 1.96 for **30**; solubility of compound **30** in water was measured to be 0.31 mg/mL), so the significant variability of impact of this parameter on the hERG inhibition should not be expected. Nevertheless, it was these two compounds that proved to be the best and the second best analogs, respectively, in terms of avoiding the potassium channel binding.

In summary, it can be concluded that the structural factors rather than the physicochemical properties govern the activity against hERG in this series of the conformationally constrained chitinases inhibitors.

Table 2. Structure-activity relationship of the conformationally constrained analogs [a]



Cmpd #	R	hAMCase (IC_{50} nM)	hCHIT1 (IC_{50} nM)	mAMCase (IC_{50} nM)	mCHIT1 (IC_{50} nM)	hERG[b] (IC_{50} μM)	DAT[c] %inh. @ 10 μM	pK_a[d]	$logD_{7.4}$[e]
21		5950 ± 212	19500 ± 2120	2600 ± 141	37000 ± 4240	NT	NT	NT	NT
22		1350 ± 71	1850 ± 354	335 ± 49	19000 ± 2828	NT	NT	NT	NT
23		73 ± 5.1	59 ± 1.4	120 ± 0	430 ± 42	4.0	NT	9.86	1.15
24		23 ± 2.1	41 ± 8.5	81 ± 12	87 ± 7.8	8.3	NT	9.74	1.41
25		395 ± 64	580 ± 85	485 ± 78	5550 ± 919	0.34	NT	7.49	1.55
26		39 ± 7.1	82 ± 3.5	170 ± 28	520 ± 70	13.2	NT	9.08	0.81
27		19 ± 0.7	47 ± 5.7	65 ± 7.1	345 ± 64	19	59	11.73	1.60

28		50 ± 9.2	87 ± 7.1	68 ± 9.9	135 ± 7.1	55	NT	9.08	0.68
29		26 ± 6.4	44 ± 1.4	21 ± 1.4	255 ± 49	15.8	NT	11.73	1.56
30		22 ± 2.1	48 ± 8.2	30 ± 12.7	74 ± 13.4	39	95	7.58	1.96

[a] IC_{50} data of each compound were determined in 2−3 independent assays, and mean IC_{50} ± SD values are shown in the table. Cmpd, abbreviation of compound. SD, standard deviation.

[b] data from Predictor™ hERG Fluorescence Polarization assay [31]

[c] data from DAT Human Dopamine Transporter Binding (Antagonist Radioligand) Assay, Cerep[32]

[d] pK_a values were calculated online using chemicalize.com freeware by ChemAxon

[e] measured values

PHARMACOKINETIC AND ANTI-INFLAMMATORY EFFECTS OF COMPOUND **30**.

Good oral bioavailability of compound **30** and low clearance provided sufficient exposure for efficacy examination following once daily oral administration in mice (Table 3 and Figure 5). Considerably higher clearance value in rats (accompanied by still very good bioavailability, though) suggests that more frequent dosing of inhibitor **30** may be required for *in vivo* studies in higher species.

Table 3. *In vivo* pharmacokinetic profile of compound **30** in mice and rats after 3 mg/kg IV and 10 mg/kg PO administration

	Mouse IV	Mouse PO	Rat IV	Rat PO
$AUC_{0\text{-inf}}$ (mg*h/L)	4.76	11.1	4.98	8.01
C_{max} (mg/L)	1.59	2.39	16.5	1.56
CL (mL/min/kg)	10.5	n/a	33.7	n/a
$t_{1/2}$ (h)	2.50	2.68	7.95	2.14
t_{max} (h)	n/a	1.0	n/a	1.5
F (%)	n/a	70	n/a	48

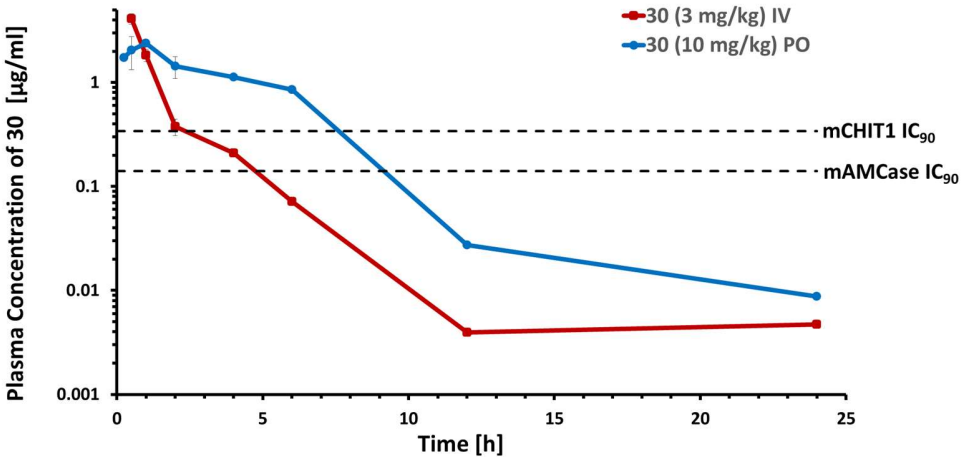


Figure 5. Plasma total concentration – time course for compound **30** after 3 mg/kg IV bolus and after 10 mg/kg oral administration (PO) to female BALB/c mice.

Compound **30** passed the test for potential genotoxicity (AMES test [33] was negative) and was evaluated in the CEREP Diversity Panel [34] in order to identify the possible off-target activities.

hERG potassium channel was found to be inhibited with IC_{50} of 12 μM in the automated patch-clamp assay [35] and also considerable dopamine transporter (DAT) inhibition was revealed showing IC_{50} of 7.9 μM and 0.3 μM in DAT cell-based assay [36] and DAT radioligand binding assay [32], respectively. This result prompted us to study the plasma and brain concentration of inhibitor **30** following oral administration in mice in the context of possible occurrence of the CNS-related side-effects. By comparison of AUCs in plasma and brain it was found that approximately 6.8% of **30** crosses the blood-brain barrier approaching the DAT IC_{50} radioligand assay value, during first 2 hours after administration (Figure 6).

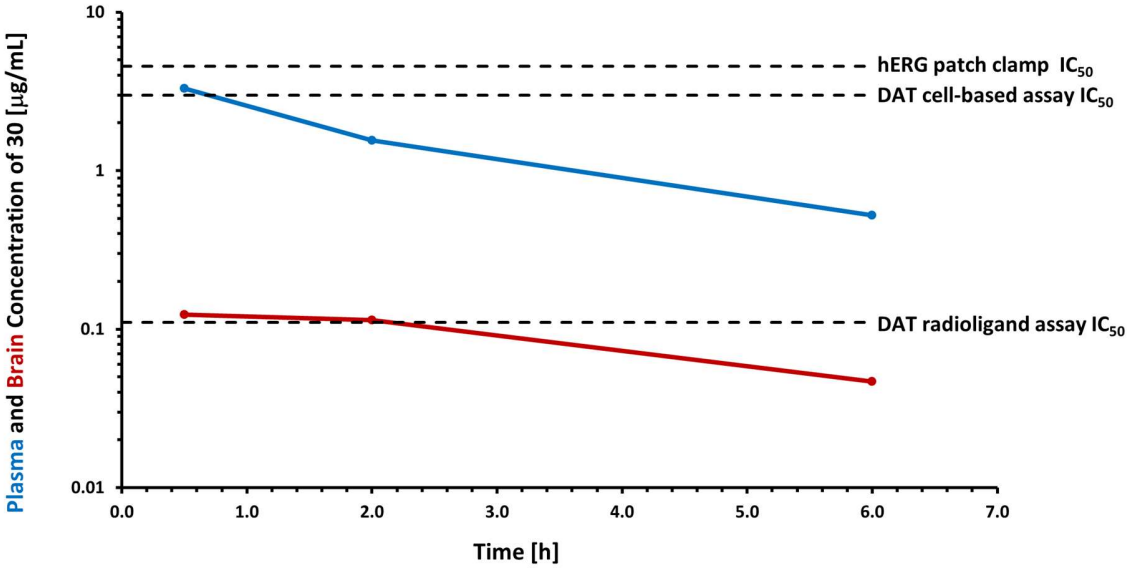


Figure 6. Plasma and brain total concentrations of **30** after 10 mg/kg oral administration in female BALB/c mice compared to the observed off-target activities.

Common, clinically relevant aeroallergen – house dust mite (HDM) extract was used to induce allergic airway inflammation in C57BL/6 mice which was represented by the increased CD45-positive leukocytes influx to the lungs and chitinolytic activity in BALf of mice challenged with

HDM as compared to PBS challenged mice (control group) (Figure 7). Oral administration of compound **30** (50 mg/kg, qd) starting from day 7 after the beginning of the HDM challenge led to a significant reduction of total CD45-positive leukocyte number (37%) as compared to the HDM vehicle group.

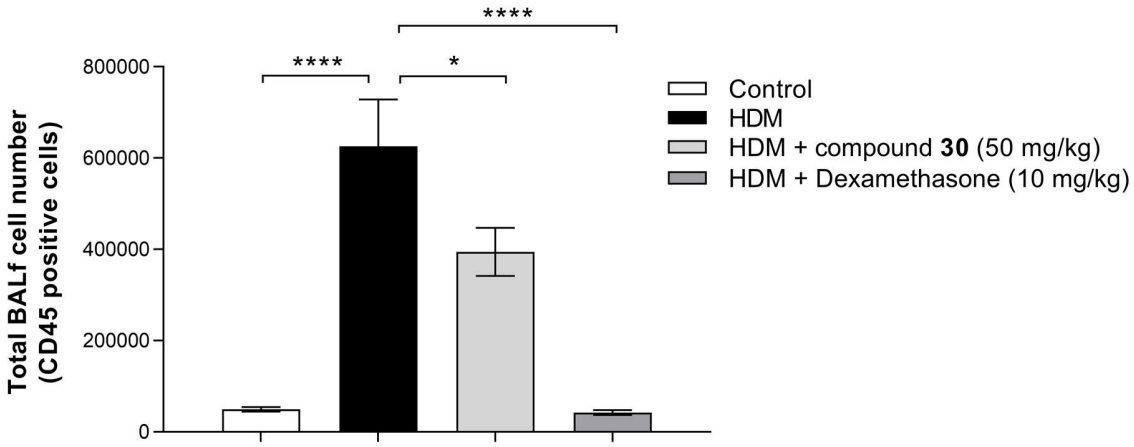


Figure 7. Dual AMCase/CHIT1 inhibition with compound **30** results in significant anti-inflammatory effects in HDM-induced asthma mouse model. Flow cytometry analysis of total infiltrating leukocytes numbers in BALf recovered from naïve mice challenged with PBS or HDM with or without compound **30** (50 mg/kg; PO; qd) or dexamethasone (10 mg/kg, IP, qd) treatment.

Molecular docking of **30** to binding pockets of hAMCase and hCHIT1 (Figure 8 A, C and B, D, respectively) revealed that, indeed - morpholine ring is too distant to interact with residues at positions 269 and 300. The interaction network formed by the 2-aminotriazole ring of **30** in both proteins is analogous to that described earlier [21]: direct hydrogen bonds are formed with Tyr27, Asp138 and the water-mediated hydrogen bond is possibly formed with Glu140. Heterocyclic ring is further stabilized by the Met - π interaction formed by Met210. Similarly to the earlier described

structures of hCHIT1 complexes with compounds **2** and **3a**, the nitrogen of the central 4-aminopiperidine ring may form hydrogen bond with Asp213, however in the hCHIT1-**30** and hAMCase-**30** complexes the distances between carboxylic group of aspartic acid residue and amino group of the ligand appear to be longer (3.7 Å and 3.5 Å respectively). 4-Chlorobenzyl fragment is located within hydrophobic pocket formed by Leu362, Ala302, Leu301, Tyr267 (in both enzymes), Ile300 (hAMCase), and Met300 (hCHIT1) residues. This last residue may further stabilize the phenyl ring by Met - π interaction in hCHIT1 binding pocket. Oxygen atom in the morpholine ring of **30** may also form a weak hydrogen bond with Trp99 of hCHIT1 and also in hAMCase provided the side-chain of this residue is directed toward the binding pocket (Figure 8C).

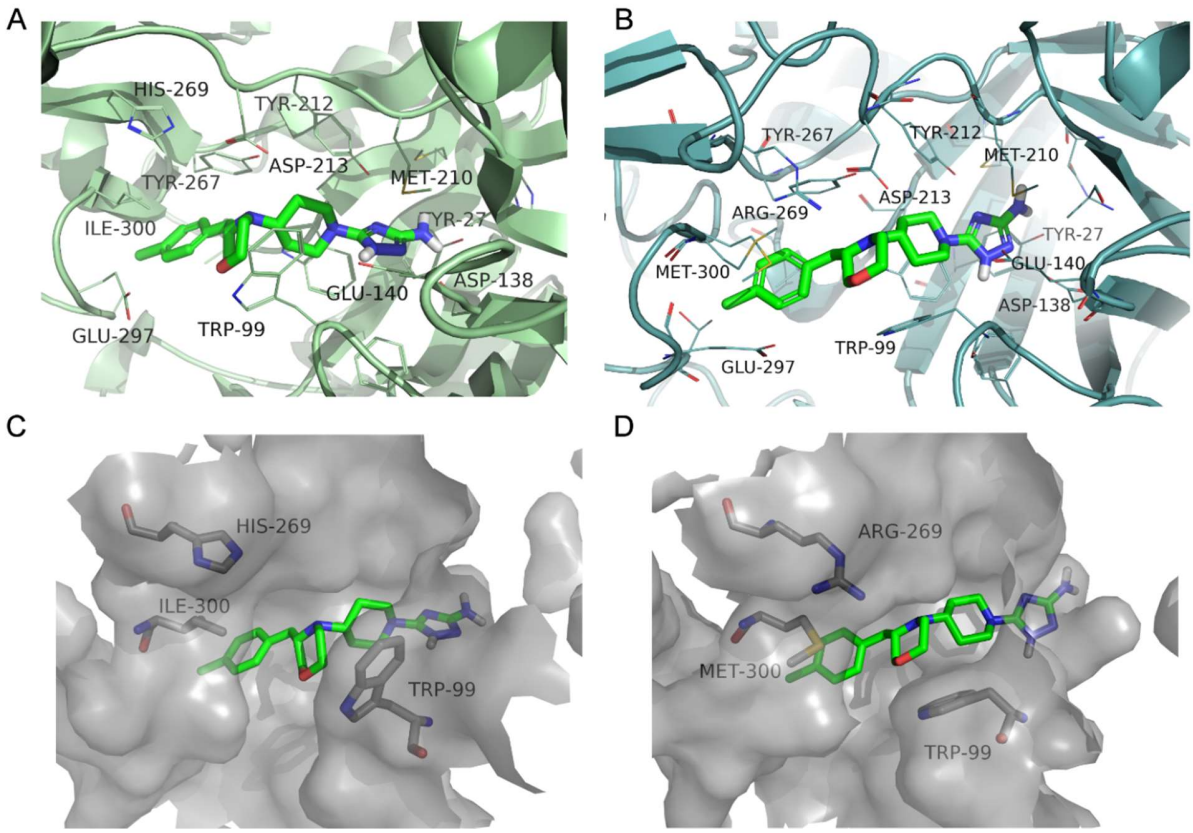
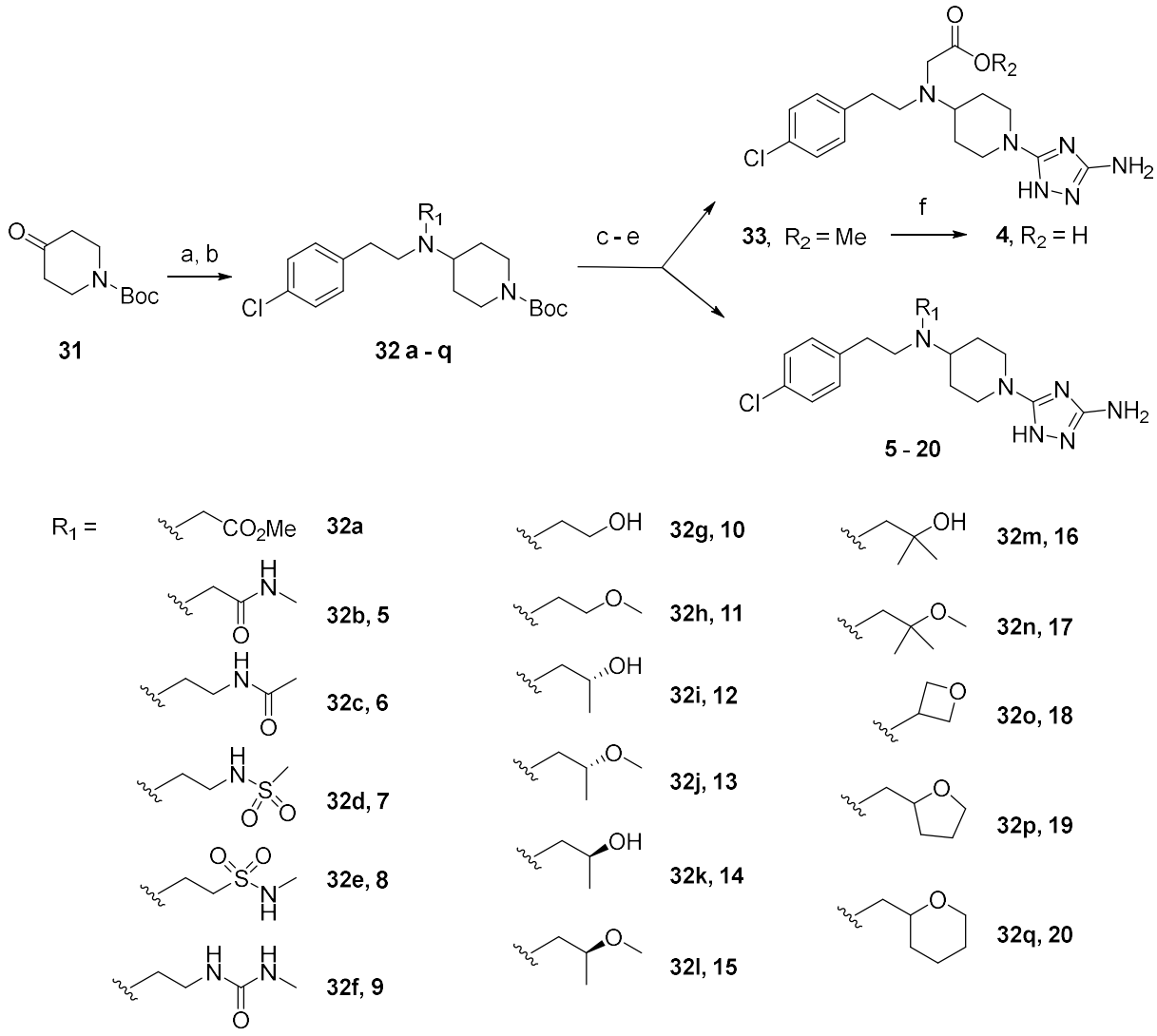


Figure 8. Compound **30** docked to the hAMCase (PDB ID: 3RM4, panes A and C) and hCHIT1 (PDB ID: 5NR8, panes B and D) binding pockets. Upper panes (A, B) show key residues forming the binding pockets of both proteins. Bottom panes (C, D) show a sphere representation of binding pockets of proteins; distinct residues (269 and 300) and Trp99 are shown as sticks. For ligands, carbon atoms are coloured in green, nitrogen atoms in blue, oxygen atoms in red and sulphur atoms in yellow.

SYNTHESIS

Scheme 1. General Synthesis of Compounds **4** – **20** [a]



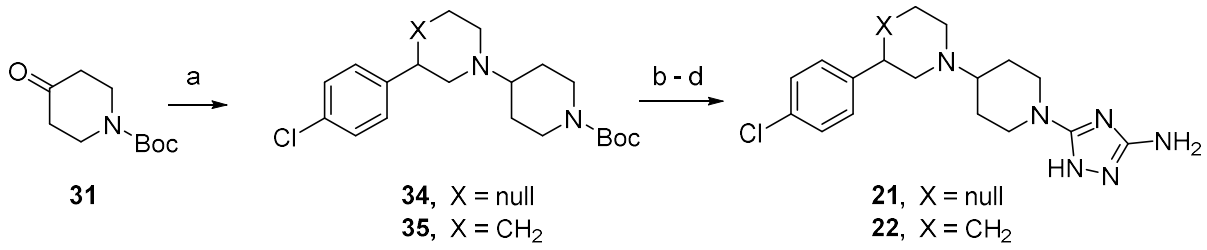
[a]Reagents and conditions: (a) R_1-NH_2, $NaBH(OAc)_3$, DCE, rt; then (b) 4-chlorophenylacetaldehyde, $NaBH(OAc)_3$, DCE, rt, 45-88% over 2 steps (c) HCl, AcOEt, rt; (d) S,S'-dimethyl N-cyanodithioiminocarbonate, K_2CO_3, MeCN, 82 °C; (e) hydrazine, MeCN, 82 °C, 15-55% over 3 steps; (f) NaOH, H_2O, reflux, 10%.

Synthesis of analogs **4** – **20** was accomplished in a similar fashion as described previously [21] for compounds **2**, **3a** and **3b** (Scheme 1). N-Boc-4-piperidone **31**, was reductively aminated with a set of commercially available primary amines and the resulting intermediate amines were *in situ*

subjected to the second reductive alkylation with 4-chlorophenylacetaldehyde to provide compounds **32a** - **q**. Acidic deprotection of the piperidine followed by a two-step 3-aminotriazole ring formation completed synthesis of the target compounds. An additional step of the hydrolysis of the methyl ester was required for the synthesis of compound **4**.

Analogues **21** and **22** were prepared in a straightforward manner from commercially available racemic 3-(4-chlorophenyl)pyrrolidine and 3-(4-chlorophenyl)piperidine respectively, by the reductive amination of N-Boc-4-piperidone that yielded intermediates **34** and **35**. N-deprotection and 3-aminotriazole ring installation afforded the final analogues.

Scheme 2. Synthesis of analogues **21** and **22** [a]

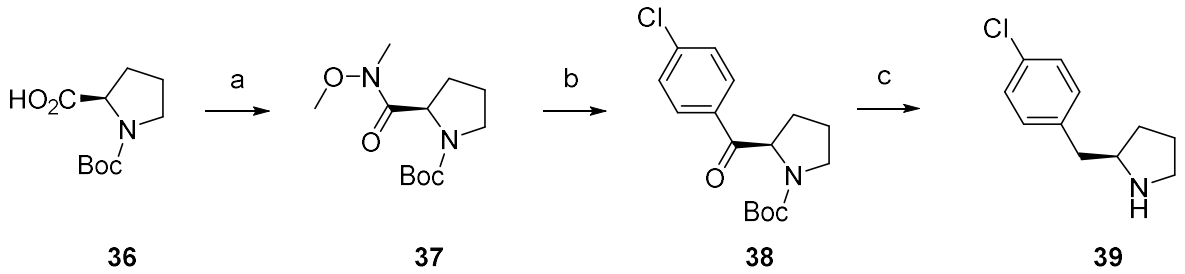


[a]Reagents and conditions: (a) 3-(4-chlorophenyl)pyrrolidine (for **34**) or 3-(4-chlorophenyl)piperidine (for **35**), $NaBH(OAc)_3$, AcOH, DCE, rt, 45% and 23% for **34** and **35**, respectively; (b) HCl, AcOEt, rt; (c) S,S'-dimethyl N-cyanodithioiminocarbonate, K_2CO_3, MeCN, 82 °C; (d) hydrazine, MeCN, 82 °C, 55% and 14% over 3 steps for **21** and **22**, respectively.

The syntheses of compounds **23** – **30** required the prerequisite cyclic secondary amines that would be incorporated into the molecules of the final inhibitors to be prepared. Accordingly, Boc-D-proline **36** was coupled with N,O-dimethylhydroxylamine and the resulting Weinreb amide **37** was reacted with 4-chlorophenyl magnesium bromide affording ketone **38** that was reduced with

concomitant removal of the Boc- group by treatment with $AlCl_3$ / Et_3SiH system to give the desired (S)-2-(4-chlorobenzyl)pyrrolidine **39**.

Scheme 3. Synthesis of (R)-2-(4-chlorobenzyl)pyrrolidine **39** [a]

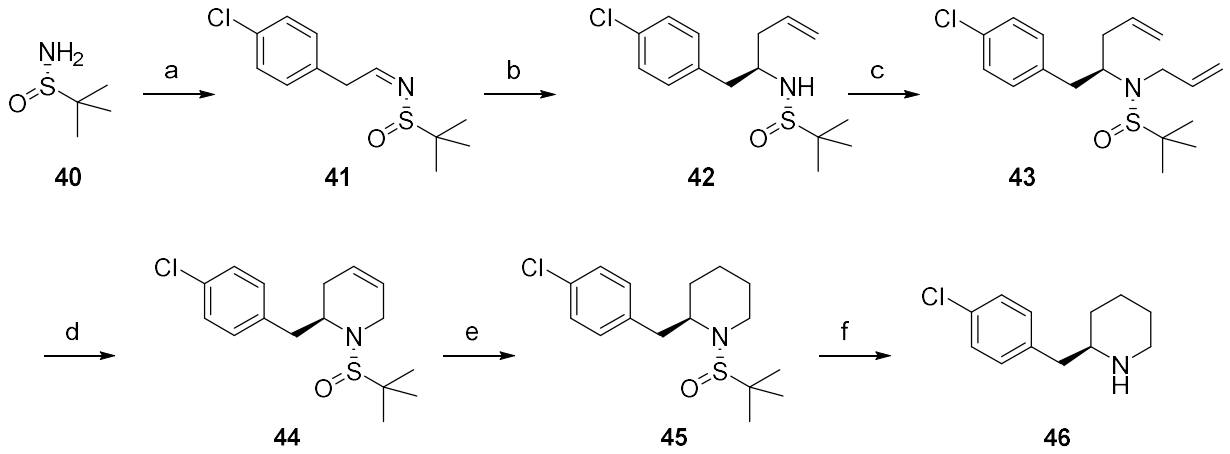


36 **37** **38** **39**

[a]Reagents and conditions: (a) HN(Me)OMe, CDI, DCM, rt, 52%; (b) 4-chlorophenymagnesium bromide, Et_2O, -70 °C to rt, 69%; (c) $AlCl_3$, Et_3SiH, DCM, 0 °C to rt, 99%; (d) N-Boc-piperid-4-one, $NaBH(OAc)_3$, AcOH, DCE, rt, 45%; (e) HCl, AcOEt, rt; (f) S,S'-dimethyl N-cyanodithioiminocarbonate, K_2CO_3, MeCN, 82 °C; (g) hydrazine, MeCN, 82 °C, 39% over 3 steps

(S)-2-methyl-2-propanesulfinamide **40** was condensed with 4-chlorophenylacetaldehyde providing sulfinimine **41**, to which allyl Grignard reagent was added yielding sulfinamide **42**. Nitrogen atom was deprotonated with sodium hydride followed by allylation with allyl bromide and the resulting diene **43** was subjected to the ring-closing metathesis reaction using Grubbs catalyst (1st generation). Hydrogenation of the double bond in the resulting (R)-6-(4-chlorobenzyl)-3,4-dehydropiperidine **44** followed by the removal of the *tert*-butyl sulfonamide group in acidic conditions from intermediate **45** completed the synthesis of (R)-2-(4-chlorobenzyl)piperidine **46**.

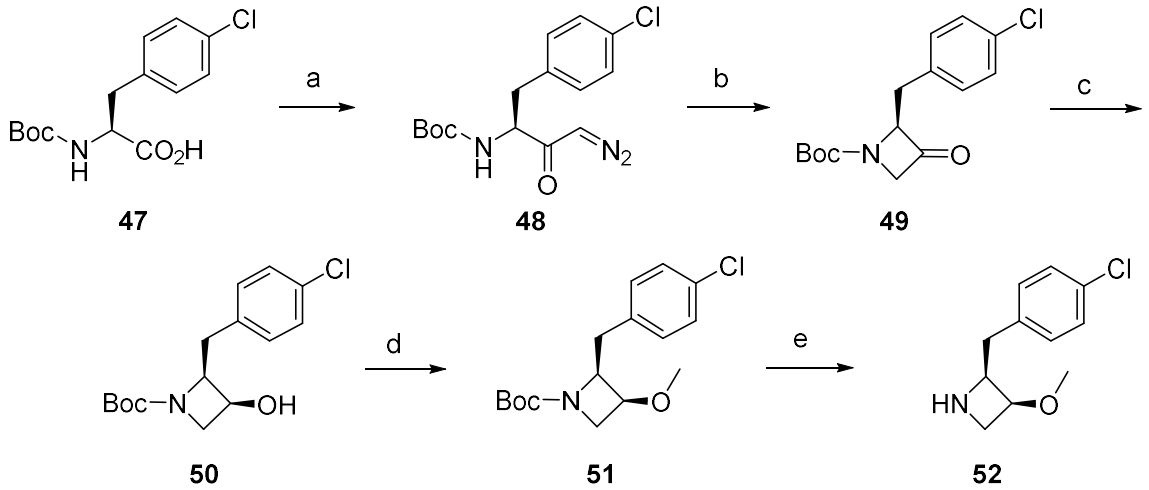
Scheme 4. Synthesis of (R)-2-(4-chlorobenzyl)piperidine **46** [a]



[a]Reagents and conditions: (a) 4-chlorophenylacetaldehyde, $Ti(OEt)_4$, DCM, reflux, then rt; (b) allylmagnesium bromide, -20 °C to rt, 23% over two steps; (c) allyl bromide, NaH, DMF, rt, 71%; (d) Grubbs catalyst 1st generation, DCM, reflux, 94%; (e) H_2, Pd/C (cat.), MeOH, rt, 77%; (f) HCl, MeOH, rt, 72%.

Boc-L-(4-chloro)phenylalanine **47** was transformed into the corresponding α-diazoketone **48** *via* reaction of the preformed mixed anhydride with an excess of diazomethane. Rhodium acetate catalyzed cyclization afforded S-2-(4-chlorophenyl)azetidinone **49** that was stereoselectively reduced to the secondary alcohol **50** (stereoselectivity > 10:1; minor isomer was rejected by crystallization from ethyl acetate/hexanes mixture, ca. 1 : 5 ratio; the configuration of the newly created chiral center was confirmed by single-crystal X-ray diffraction – see Supporting Information). Williamson etherification of the hydroxyl group and removal of the N-Boc protection from intermediate **51** afforded the desired azetidine **52**.

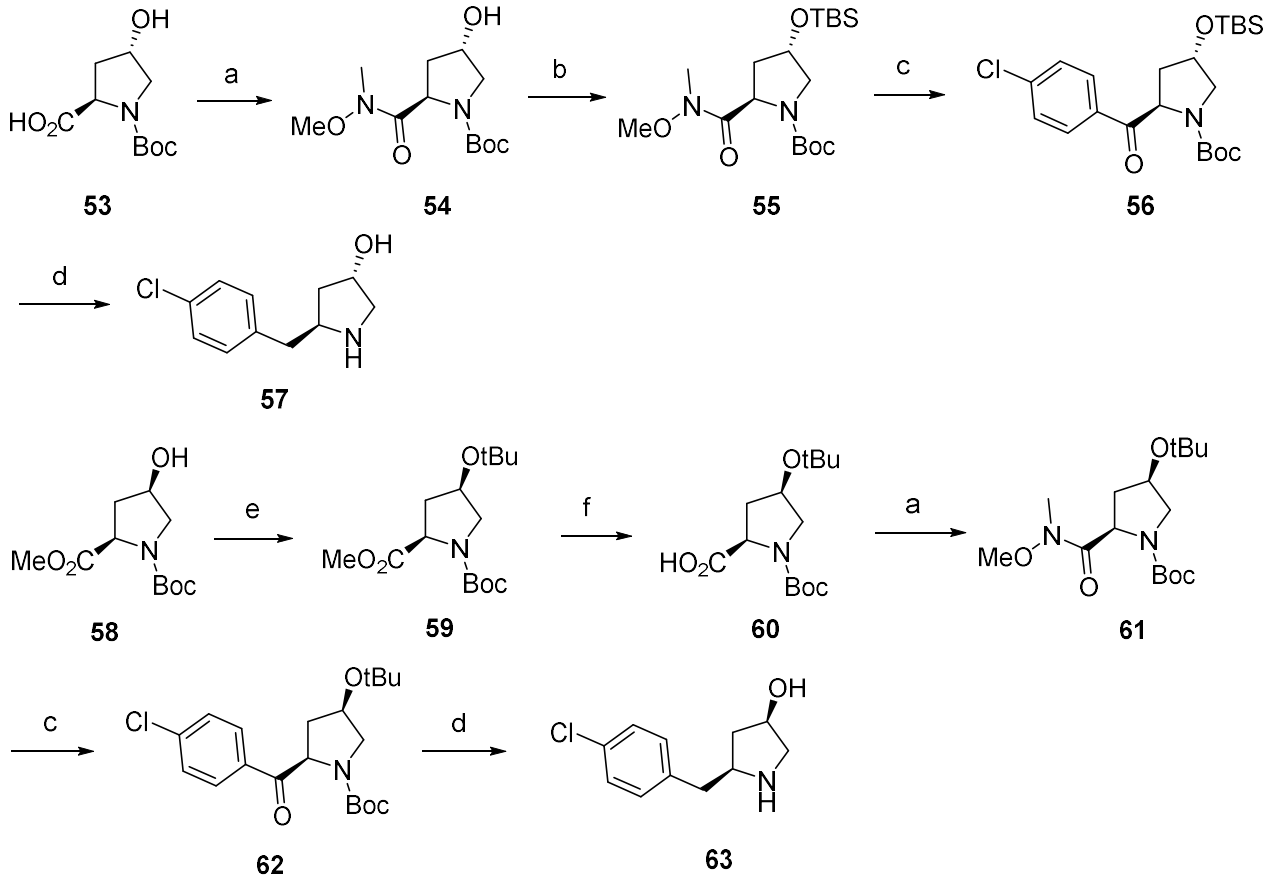
Scheme 5. Synthesis of (2S,3S)-2-(4-chlorobenzyl)-3-methoxyazetidine **52** [a]



[a]Reagents and conditions: (a) methyl chloroformate, Et_3N, THF, -15 °C, then CH_2N_2 solution in Et_2O, -30 °C to rt, 96%; (b) $Rh_2(OAc)_4$ (0.5% mol), Et_3N (1% mol), DCM, 0 °C to rt, 46%; (c) $NaBH_4$, MeOH, 0 °C to rt, 86%; (d) NaH, MeI, DMF, rt, 100%; (e) HCl, 1,4-dioxane, rt, 77%.

Fully protected Weinreb amides **55** and **61** were prepared in few straightforward synthetic steps starting from commercially available *trans*- and *cis*- D-4-hydroxyproline derivatives **53** and **58**, respectively, and reacted with 4-chlorophenyl magnesium bromide yielding ketones **56** and **62**. Reduction of a carbonyl groups in a strongly acidic conditions accompanied by concomitant removal of protecting groups afforded the desired (2S,4S)-2-(4-chlorobenzyl)-4-hydroxypyrrolidine **57** and (2S,4R)-2-(4-chlorobenzyl)-4-hydroxypyrrolidine **63**.

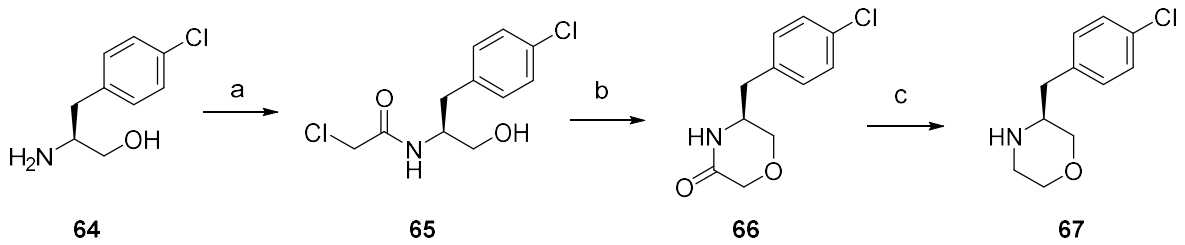
Scheme 6. Synthesis of (2S,4S)-2-(4-chlorobenzyl)-4-hydroxypyrrolidine **57** and (2S,4R)-2-(4-chlorobenzyl)-4-hydroxypyrrolidine **63** [a]



[a]Reagents and conditions: (a) HN(Me)OMe, CDI, DCM, rt, 49% for **54**, 67% for **61**; (b) TBSCl, imidazole, DMF, rt, 92%; (c) 4-chlorophenymagnesium bromide, Et_2O, -70 ºC to rt, 86% for **56**, 40% for **62**; (d) $AlCl_3$, Et_3SiH, DCM, 0 ºC to rt, 81% for **57**, 98% for **63**; (e) Boc_2O, $Mg(ClO_4)_2$, DCM, reflux, 68%; (f) LiOH, THF/MeOH/H_2O, rt, 94%.

(S)-2-amino-(4-chlorophenyl)propan-1-ol **64** was reacted with chloroacetyl chloride and the resulting amide **65** was cyclized in basic conditions. The so obtained S-5-(4-chlorophenyl)morpholin-3-one **66** was reduced with borane dimethyl sulfide complex affording morpholine **67**.

Scheme 7. Synthesis of (S)-3-(4-chlorobenzyl)morpholine **67** [a]

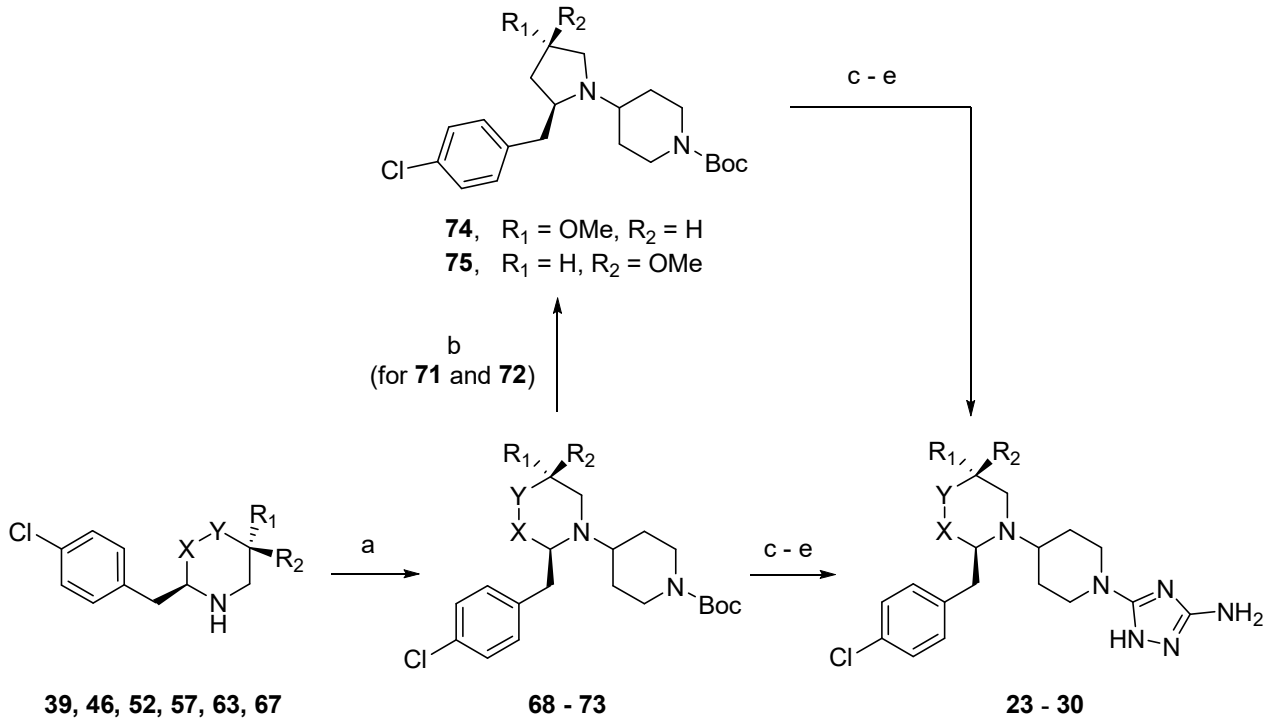


64 **65** **66** **67**

[a]Reagents and conditions: (a) chloroacetyl chloride, Et_3N, THF, 0 °C, 85%; (b) NaH, THF, 0 °C to rt, 59%; (c) BH_3·DMS complex, THF, reflux, 73%

N-Boc-piperid-4-one **31** was subjected to the reductive amination reaction with the heterocyclic amines **39**, **46**, **52**, **57**, **63** and **67** in slightly modified conditions as compared to the usual procedure (see the Experimental Section for details). Out of so obtained intermediates **67** – **72** portions of compounds **70** and **71**, bearing free hydroxylic group in position 4 of the pyrrolidine ring, were methylated affording intermediates **73** and **74**. Acidic removal of the Boc- group followed by the usual two-step 3-aminotriazole ring formation completed the syntheses of the target analogues **23** – **30**.

Scheme 8. General Synthesis of Compounds **23** – **30** [a]



	X	Y	R_1	R_2
39, 68, 23	null	CH_2	H	H
46, 69, 24	CH_2	CH_2	H	H
52, 70, 25	null	null	H	OMe
57, 71, 26	null	CH_2	OH	H
27	null	CH_2	OMe	H
63, 72, 28	null	CH_2	H	OH
29	null	CH_2	H	OMe
68, 73, 30	CH_2	O	H	H

[a]Reagents and conditions: (a) N-Boc-piperid-4-one **31**, $NaBH(OAc)_3$, AcOH, DCE, rt, 45 – 85%; (b) MeI, NaH, DMF, rt, 95% and 77% for **74** and **75**, respectively; (c) HCl, AcOEt, rt; (d) S,S'-dimethyl N-cyanodithioiminocarbonate, K_2CO_3, MeCN, 82 °C; (e) hydrazine, MeCN, 82 °C, 15 – 84% over three steps.

CONCLUSIONS

Structure-based drug design directed to the optimization of previously described chitinase inhibitors led us to discovery of compound **30** – a highly potent inhibitor, similarly active against both human and murine enzymes. Given once daily at a dose of 50 mg/kg orally *in vivo* compound **30** showed significant reduction of HDM-induced pulmonary inflammation in mice. Thus generation of a dual chitinase inhibitor seems to be a rational approach for the management of incurable chronic pulmonary diseases by targeting ongoing AMCase-dependent pulmonary inflammation and potentially modulating CHIT1-dependent excessive tissue repair processes in severe asthma or ILDs such as sarcoidosis and IPF. Moreover, significant activity of compound **30** towards CHIT1 in addition to AMCase could serve as great tool to understand the profit of chitinase inhibition in animal models of chronic lung diseases, but also to evaluate the role of individual chitinase in the area of lung inflammation and fibrotic remodeling. The hERG activity was brought to the acceptable level from the further preclinical development standpoint, but the inhibition of dopamine transporter still remains an unresolved issue and needs further optimization. Inhibitor **30** is an advanced lead compound that paved the way to the discovery of our clinical candidate OATD-01 – currently in phase Ib, as a new therapy against chronic lung diseases relying on the simultaneous inhibition of both human chitinases.

EXPERIMENTAL SECTION

Chemical Methods. All solvents, substrates and reagents that were commercially available were used without further purification. TLC analysis was performed using pre-coated glass plates (0.2 ± 0.03 mm thickness, GF-254, particle size 0.01–0.04 mm) from Fluorochem Ltd, UK. Column

chromatography was performed using high-purity grade silica gel (pore size 60 Å, 220-440 mesh particle size, 35-75 μm particle size) from Fluka. Preparative HPLC was performed on LC-20AP Shimadzu with ELSD-LTII detector equipped with Hypersil GOLD 21.2/250 mm, 5 μm C18 column. ^{1}H NMR spectra were recorded on Bruker AVANCE DRX500, AVANCE DRX600 or Bruker AVANCE II PLUS (respectively at 500, 600 or 700 MHz) NMR spectrometers. All spectra were recorded in appropriate deuterated solvents ($CDCl_3$, DMSO-d_6, D_2O and CD_3OD). Resonances are given in parts per million relative to tetramethylsilane. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, br = broad), coupling constants (J in Hz) and integration. LC-MS spectra were recorded on a Shimadzu LC-20AD LPG separation module with a SPD-M20A UV detector and LCMS-2020 mass detector equipped with Kinetex 2.1/50 mm, 2.6 μm C18 column eluted with 0.5 mL/min flow of 10-90% gradient (over 6 min) of acetonitrile in water. Purities of all final reported compounds were greater than 95% based on HPLC chromatograms. Purification of the final compounds by preparative HPLC was accomplished on C-18 250x21 mm column in 0.05% TFA in water / acetonitrile 95:5 → 45:55 gradient over 30 minutes followed by freeze-drying of the pooled fractions containing pure products. In some cases, the so obtained trifluoroacetate salts of the final compounds were judged to be of insufficient quality for biological testing due to their physical appearance (oils with a distinguished scent of trifluoroacetic acid). Therefore, they were re-dissolved in a small amount of 0.1 M HCl and subjected to the second lyophilization providing well-behaving hydrochloride salts. HRMS-ESI spectra were recorded on Bruker Maxis Impact instrument. Purities of all reported compounds were greater than 95% based on HPLC chromatograms. HPLC analyses were performed on a Waters Acquity UPLC system fitted with Phenomenex 5 μm, 100A, PFP(2) column (4.6 mm × 150 mm) and with UV detection (220 nm), gradient 10−90% of acetonitrile in water,

flow rate 1.5 mL/min over 10 minutes, then 90% acetonitrile over 5 minutes. Analysis of optical purity of compound **30** was accomplished on the same HPLC system fitted with Phenomenex Luna Cellulose-4 column (150 x 4.6 mm; 5 μm) in isocratic flow of isopropanol : hexanes (70 : 30, 1 mL/min) over 20 minutes.

General Procedure A for compounds 32 a–q (one-pot double reductive amination procedure). To a stirred solution of N-Boc-4-piperidone (**31**, 0.4 g, 2 mmol) and appropriate primary amine or its hydrochloride salt (depending on the availability) (2 mmol) in 1,2-dichloroethane (6 mL), sodium triacetoxyborohydride (0.85 g, 4 mmol) was added. The reaction mixture was stirred at ambient temperature overnight and 4-chlorophenylacetaldehyde (0.3 g, 2 mmol) followed by the second portion of sodium triacetoxyborohydride (0.85 g, 4 mmol) were added. Stirring was continued for 1-2 h (TLC control) after which time the reaction was stopped by addition of 5% solution of $NaHCO_3$ (10 mL) and the biphasic mixture was stirred for 30 min. The layers were separated and the aqueous layer was additionally extracted with DCM twice. The combined organic extracts were then dried over $MgSO_4$, filtered and solvent was removed *in vacuo*. The crude residue was purified by flash chromatography to afford compounds **32a-q**.

General Procedure B for compounds 34, 35 and 68 – 73 (reductive amination of N-Boc-4-piperidone with cyclic secondary amines: 3-(4-chlorophenyl)pyrrolidine, 3-(4-chlorophenyl)piperidine and compounds: 39, 46, 52, 57, 63 and 67). To a stirred solution of N-Boc-4-piperidone (**31**, 0.4 g, 1 mmol) and secondary amine or its hydrochloride salt (1 mmol) in 1,2-dichloroethane (3 mL), acetic acid (0.36 mL, 3 mmol) was added and the resulting mixture was stirred in room temperature until clear solution was obtained (overnight, if neccessary). It was concentrated *in vacuo* to about 30-50% of its initial volume and sodium triacetoxyborohydride (0.42 g, 2 mmol) was added. The thick reaction mixture was stirred at ambient temperature

overnight after which time it was diluted with 3 mL of DCM and quenched by addition of 5% solution of $NaHCO_3$ (6 mL). Compounds **34**, **35** and **68** – **73** were isolated by usual aqueous work-up and purified by flash chromatography in appropriate solvent system.

General Procedure C for compounds 4 – 30 (N-Boc- group removal followed by 3-aminotriazole ring formation). To a solution of Boc-protected compounds **32a-q**, **34**, **35** and **68** – **75** in EtOAc (0.5 mL/mmol), 4M HCl in EtOAc solution was added (3 mL/mmol) and reaction was stirred in room temperature until chromatography (either TLC or LC-MS) indicated complete consumption of a starting material (typically 30 min – 2 hours). Volatiles were removed *in vacuo* and the residue was triturated thrice with diethyl ether and dried under high vacuum for 1 hour. The crude hydrochloride salt was suspended in acetonitrile (4 mL/mmol, assuming quantitative yield of the deprotection step) and solid K_2CO_3 (3 equivalents) followed by *S*,*S*`-dimethyl *N*-cyanodithioiminocarbonate (1.1 equivalent) were added and the reaction mixture was refluxed until complete consumption of the starting material was achieved as judged by chromatography (typically 2-3 hours). Hydrazine hydrate (4 equivalents) was then added and reaction was further refluxed for 3-5 hours, after which time it was cooled, and poured into the vigorously stirred biphasic mixture of 5% $NaHCO_3$ and ethyl acetate. After the separation of the phases, the organic layer was additionally washed by water and brine, dried with anhydrous $MgSO_4$, filtered and concentrated. Depending on the amount of the compound synthesized, the final products were purified by crystallization, flash chromatography or reversed-phase chromatography.

N-(1-(3-amino-1H-1,2,4-triazol-5-yl)piperidin-4-yl)-N-(4-chlorophenethyl)glycine 2,2,2-trifluoroacetate (4). *Step 1: Synthesis of tert-butyl 4-((4-chlorophenethyl)(2-methoxy-2-oxoethyl)amino)piperidine-1-carboxylate (**32a**).* Title compound was prepared according to the General Procedure A on 2 mmol scale from N-Boc-4-piperidone and glycine methyl ester

hydrochloride. 600 mg (1.46 mmol, yield 73%) of **32a** was obtained in a form of transparent thick oil after purification by flash column chromatography (gradient elution 1 – 5 % MeOH in DCM). LC-MS (ES+): m/z 410.8/412.7 $[M + H]^+$.

*Step 2: Synthesis of methyl N-(1-(3-amino-1H-1,2,4-triazol-5-yl)piperidin-4-yl)-N-(4-chlorophenethyl)glycinate bis-2,2,2-trifluoroacetate (**33**).* Starting with 300 mg (0.73 mmol) of compound **32a**, 200 mg (0.32 mmol, yield 44%) of methyl N-(1-(3-amino-1H-1,2,4-triazol-5-yl)piperidin-4-yl)-N-(4-chlorophenethyl)glycinate in a form of bis-trifluoroacetate salt (**33**) was obtained according to the General Procedure C after purification by reversed-phase chromatography. LC-MS (ES+): m/z 392.9/394.8 $[M + H]^+$. ^{1}H NMR (700 MHz, CD_3OD) δ 7.35 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 8.4 Hz, 2H), 4.18 (s, 2H), 3.97 – 3.95 (m, 2H), 3.86 (s, 3H), 3.61 – 3.56 (m, 1H), 3.41 – 3.39 (m, 2H), 3.04 – 3.02 (m, 2H), 2.99 – 2.95 (m, 2H), 2.08 (br d, *J* = 12.6 Hz, 2H), 1.83 – 1.78 (m, 2H).

*Step 3: Synthesis of N-(1-(3-amino-1H-1,2,4-triazol-5-yl)piperidin-4-yl)-N-(4-chlorophenethyl)glycine 2,2,2-trifluoroacetate salt (**4**).* Solution of compound **33** (180 mg, 0.29 mmol) in MeOH (2 mL) was treated with aqueous solution of NaOH (56 mg, 1.4 mmol dissolved in 0.5 mL of water) and heated at 50 °C for 2 hours. The reaction was then cooled to room temperature, acidified with 2 M HCl to the neutral pH and solvents were removed *in vacuo*. The residue was purified by the reversed-phase chromatography affording compound **4** (15 mg, 0.03 mmol, yield 10%). LC-MS (ES+): m/z 379.0/381.0 $[M + H]^+$. ^{1}H NMR (700 MHz, CD_3OD) δ 7.36 (d, *J* = 8.4 Hz, 2H), 7.31 (d, *J* = 8.4 Hz, 2H), 4.06 (s, 2H), 3.98 – 3.95 (m, 2H), 3.66 (tt, *J* = 12.6, 3.5 Hz, 1H), 3.47 – 3.44 (m, 2H), 3.09 – 3.07 (m, 2H), 3.04 – 3.00 (m, 2H), 2.13 – 2.11 (m, 2H), 1.89 – 1.83 (m, 2H).

2-((1-(3-amino-1H-1,2,4-triazol-5-yl)piperidin-4-yl)(4-chlorophenethyl)amino)-N-methylacetamide dihydrochloride (5). Obtained from 2-amino-N-methylacetamide hydrochloride in two steps according to the General Procedures A (yield 55%) and C (yield 15%). 23 mg (0.05 mmol) of the final compound was obtained as a dihydrochloride salt after reversed-phase purification and lyophilization from 0.1 M HCl solution. LC-MS (ES+): m/z 392.1/394.1 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.34 (d, J = 8.4 Hz, 2H), 7.30 (d, J = 8.4 Hz, 2H), 3.99 (s, 2H), 3.88 (brd, J = 13.4 Hz, 2H), 3.65 (ddd, J = 15.3, 7.7, 3.4 Hz, 1H), 3.40 – 3.37 (m, 2H), 3.02 – 2.97 (m, 4H), 2.69 (s, 3H), 2.07 (brd, J = 11.9 Hz, 2H), 1.73 (qd, J = 12.2, 4.0 Hz, 2H).

N-(2-((1-(3-amino-1H-1,2,4-triazol-5-yl)piperidin-4-yl)(4-chlorophenethyl)amino)ethyl)acetamide dihydrochloride (6). Obtained from N-(2-aminoethyl)acetamide in two steps according to General Procedures A (yield 51%) and C (yield 27%). 10 mg (0.02 mmol) of the final compound was obtained as a dihydrochloride salt after reversed-phase purification and lyophilization from 0.1 M HCl solution. LC-MS (ES+): m/z 406.0/408.0 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.36 – 7.33 (m, 4H), 3.89 (d, J = 13.3 Hz, 2H), 3.66 (tt, J = 11.9, 3.4 Hz, 1H), 3.50 – 3.48 (m, 2H), 3.35 – 3.33 (m, 2H), 3.29 – 3.27 (m, 2H), 3.10 – 3.08 (m, 2H), 3.04 – 3.00 (m, 2H), 2.14 – 2.12 (m, 2H), 1.87 (s, 3H), 1.78 (qd, J = 12.2, 4.1 Hz, 2H).

N-(2-((1-(3-amino-4H-1,2,4-triazol-5-yl)piperidin-4-yl)(4-chlorophenethyl)amino)ethyl)methanesulfonamide dihydrochloride (7). Obtained from N-(2-aminoethyl)methanesulfonamide hydrochloride in two steps according to General Procedures A (yield 49%) and C (yield 30%). 55 mg (0.11 mmol) of the final compound was obtained as a dihydrochloride salt after reversed-phase purification and lyophilization from 0.1 M HCl solution.

LC-MS (ES+): m/z 442.0/444.0 $[M + H]^+$. ^1H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.36 (brd, J = 8.6 Hz, 2H), 7.33 (d, J = 8.6 Hz, 2H), 3.87 (d, J = 13.3 Hz, 2H), 3.64 (tt, J = 11.9, 3.4 Hz, 1H), 3.42 (t, J = 6.2 Hz, 2H overlapping with water), 3.37 – 3.31 (m, 4H), 3.08 – 3.04 (m, 2H), 3.01 – 2.95 (m, 5H), 2.10 (brd, J = 12.0 Hz, 2H), 1,77 (qd, J = 12.2, 4.3 Hz, 2H).

2-((1-(3-amino-1H-1,2,4-triazol-5-yl)piperidin-4-yl)(4-chlorophenethyl)amino)-N-methylethane-1-sulfonamide (8). Obtained from 2-amino-N-methylethanesulfonamide hydrochloride in two steps according to General Procedures A (yield 63%) and C (yield 48%). 360 mg (0.81 mmol) of the final compound was obtained after purification by silica-gel chromatography in $CHCl_3$: MeOH solvent system (gradient elution from 100:1 to 20:1) followed by trituration of the so obtained white solid with diethyl ether and drying under high vacuum. LC-MS (ES+): m/z 442.1/444.1 $[M + H]^+$. ^1H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.37 (d, J = 8.5 Hz, 2H), 7.33 (d, J = 8.5 Hz, 2H), 3.86 (brd, J = 12.8 Hz, 2H), 3.53 – 3.22 (m, 7H), 2.96 – 2.94 (m, 2H), 2.78 (t, J = 12.0 Hz, 2H), 2.60 (s, 3H), 1.95 – 1.93 (m, 2H), 1.65 – 1.59 (m, 2H).

1-(2-((1-(3-amino-1H-1,2,4-triazol-5-yl)piperidin-4-yl)(4-chlorophenethyl)amino)ethyl)-3-methylurea dihydrochloride (9). Obtained from 1-(2-aminoethyl)-3-methylurea hydrochloride in two steps according to General Procedures A (yield 67%) and C (yield 34%). 85 mg (0.17 mmol) of the final compound was obtained as a dihydrochloride salt after reversed-phase purification and lyophilization from 0.1 M HCl solution. LC-MS (ES+): m/z 421.2/423.1 $[M + H]^+$. ^1H NMR (700 MHz, CD_3OD) δ 7.36 – 7.33 (m, 4H), 4.00 – 3.97 (m, 2H), 3.74 (tt, J = 11.9, 3.5 Hz, 1H), 3.54 – 3.47 (m, 4H), 3.35 – 3.32 (m, 1H), 3.28 – 3.24 (m, 1H), 3.15 – 3.11 (m, 2H), 3.07 – 3.03 (m, 2H), 2.71 (s, 3H), 2.16 – 2.07 (m, 2H), 1.92 – 1.87 (m, 2H).

2-((1-(3-amino-4H-1,2,4-triazol-5-yl)piperidin-4-yl)(4-chlorophenethyl)amino)ethan-1-ol dihydrochloride (10). Obtained from 2-aminoethanol in two steps according to General Procedures A (yield 45%) and C (yield 25%). 45 mg (0.10 mmol) of the final compound was obtained as a dihydrochloride salt after reversed-phase purification and lyophilization from 0.1 M HCl solution. LC-MS (ES+): m/z 365.0/367.0 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O) δ 7.40 – 7.35 (m, 2H), 7.33 – 7.29 (m, 2H), 3.86 – 3.81 (m, 2H), 3.74 – 3.68 (m, 2H), 3.60 (tt, J = 12.0, 3.6 Hz, 1H), 3.39 – 3.27 (m, 3H), 3.24 – 3.16 (m, 1H), 3.02 (q, J = 9.8 Hz, 2H), 2.96 – 2.88 (m, 2H), 2.10 – 1.97 (m, 2H), 1.77 – 1.65 (m, 2H).

1-(3-amino-4H-1,2,4-triazol-5-yl)-N-(4-chlorophenethyl)-N-(2-methoxyethyl)piperidin-4-amine dihydrochloride (11). Obtained from 2-methoxyethylamine in two steps according to General Procedures A (yield 67%) and C (yield 32%). 50 mg (0.11 mmol) of the final compound was obtained as a dihydrochloride salt after reversed-phase purification and lyophilization from 0.1 M HCl solution. LC-MS (ES+): m/z 379.0/381.0 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O) δ 7.40 – 7.36 (m, 2H), 7.34 – 7.30 (m, 2H), 3.86 – 3.80 (m, 2H), 3.69 – 3.64 (m, 2H), 3.59 (tt, J = 12.1, 3.7 Hz, 1H), 3.46 (d, J = 15.8 Hz, 1H), 3.36 – 3.25 (m, 6H), 3.04 – 2.90 (m, 4H), 2.09 – 1.98 (m, 2H), 1.81 – 1.67 (m, 2H).

(R)-1-((1-(3-amino-1H-1,2,4-triazol-5-yl)piperidin-4-yl)(4-chlorophenethyl)amino)propan-2-ol bis-2,2,2-trifluoroacetate (12). Obtained from (R)-1-amino-2-propanol in two steps according to General Procedures A (yield 51%) and C (yield 19%). 72 mg (0.12 mmol) of the final compound was obtained as a bis-trifluoroacetate salt after reversed-phase purification. LC-MS (ES+): m/z 379.2/381.2 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.38 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 4.03 – 4.01 (m, 1H), 3.83 (brd, J = 14.2 Hz, 2H), 3.59 (tt, J = 12.0, 3.3 Hz, 1H), [3.35 – 3.29 (m, 2.5H), 3.08 – 2.99 (m, 3.5H)

rotamers], 2.94 (t, *J* = 12.6 Hz, 2H), 2.09 – 1.99 (m, 2H), 1.80 – 1.65 (m, 2H), 1.15 (d, *J* = 4.1 Hz, 3H).

(R)-1-((1-(3-amino-1H-1,2,4-triazol-5-yl)piperidin-4-yl)(4-chlorophenethyl)amino)propan-2-ol bis-2,2,2-trifluoroacetate (13). Obtained from (R)-2-methoxypropylamine hydrochloride in two steps according to General Procedures A (yield 66%) and C (yield 33%). 25 mg (0.04 mmol) of the final compound was obtained as a bis-trifluoroacetate salt after reversed-phase purification. LC-MS (ES+): m/z 393.0/395.0 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.37 (d, *J* = 8.5 Hz, 2H), 7.31 (d, *J* = 8.4 Hz, 2H), 3.88 (brd, *J* = 13.2 Hz, 2H), 3.76 – 3.72 (m, 1H), 3.58 – 3.53 (m, 1H), 3.36 – 3.31 (m, 2H), 3.29 (s, 3H), 3.25 – 3.22 (m, 1H), 3.18 – 3.14 (m, 1H), 3.05 – 3.00 (m, 2H), 2.92 – 2.88 (m, 2H), 2.06 – 2.01 (m, 2H), 1.79 – 1.69 (m, 2H), 1.15 (d, *J* = 6.1 Hz, 3H).

(S)-1-((1-(3-amino-1H-1,2,4-triazol-5-yl)piperidin-4-yl)(4-chlorophenethyl)amino)propan-2-ol bis-2,2,2-trifluoroacetate (14). Obtained from (S)-1-amino-2-propanol in two steps according to General Procedures A (yield 60%) and C (yield 21%). 80 mg (0.13 mmol) of the final compound was obtained as a bis-trifluoroacetate salt after reversed-phase purification. LC-MS (ES+): m/z 379.2/381.2 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 300K) δ 7.37 (d, *J* = 8.0 Hz, 2H), 7.32 (d, *J* = 7.3 Hz, 2H), 4.02 – 4.00 (m, 1H), 3.83 – 3.81 (m, 2H), 3.58 (tt, *J* = 12.8, 3.3 Hz, 1H), [3.33 – 3.28 (m, 2.3H), 3.10 – 2.99 (m, 3.7H) rotamers], 2.92 (t, *J* = 12.4 Hz, 2H), 2.07 – 1.98 (m, 2H), 1.79 – 1.64 (m, 2H), 1.14 (d, *J* = 4.1 Hz, 3H).

(S)-1-(3-amino-1H-1,2,4-triazol-5-yl)-N-(4-chlorophenethyl)-N-(2-methoxypropyl)piperidin-4-amine bis-2,2,2-trifluoroacetate (15). Obtained from (S)-2-methoxypropylamine hydrochloride in two steps according to General Procedures A (yield 72%)

and C (yield 32%). 50 mg (0.08 mmol) of the final compound was obtained as a bis-trifluoroacetate salt after reversed-phase purification. LC-MS (ES+): m/z 393.0/395.0 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.37 (d, J = 8.5 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 3.88 (brd, J = 13.2 Hz, 2H), 3.76 – 3.72 (m, 1H), 3.58 – 3.53 (m, 1H), 3.35 – 3.32 (m, 2H), 3.29 (s, 3H), 3.25 – 3.21 (m, 1H), 3.18 – 3.14 (m, 1H), 3.05 – 3.00 (m, 2H), 2.93 – 2.89 (m, 2H), 2.06 – 2.01 (m, 2H), 1.79 – 1.69 (m, 2H), 1.15 (d, J = 6.1 Hz, 3H).

1-((1-(3-amino-1H-1,2,4-triazol-5-yl)piperidin-4-yl)(4-chlorophenethyl)amino)-2-methylpropan-2-ol (16). Obtained from 1-amino-2-methypropan-2-ol in two steps according to General Procedures A (yield 54%) and C (yield 32%). 197 mg (0.50 mmol) of the final compound was obtained after purification by silica-gel chromatography in $CHCl_3$: MeOH solvent system (gradient elution from 100:1 to 15:1) followed by trituration of the so obtained white solid with diethyl ether and drying under high vacuum. LC-MS (ES+): m/z 393.0/395.0 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.39 – 7.34 (m, 2H), 7.33 – 7.29 (m, 2H), 3.92 – 3.85 (m, 2H), 3.64 (tt, J = 12.1, 3.6 Hz, 1H), 3.44 – 3.40 (m, 2H), 3.18 (brs, 2H), 3.12 – 3.06 (m, 2H), 2.98 – 2.89 (m, 2H), 2.14 – 2.05 (m, 2H), 1.82 – 1.70 (m, 2H), 1.28 (s, 6H).

1-(3-amino-1H-1,2,4-triazol-5-yl)-N-(4-chlorophenethyl)-N-(2-methoxy-2-methylpropyl)piperidin-4-amine (17). Obtained from 2-methoxy-2-methylpropylamine in two steps according to General Procedures A (yield 75%) and C (yield 41%). 158 mg (0.39 mmol) of the final compound was obtained after purification by silica-gel chromatography in $CHCl_3$: MeOH solvent system (gradient elution from 100:1 to 25:1) followed by trituration of the so obtained white solid with diethyl ether and drying under high vacuum. LC-MS (ES+): m/z 407.0/409.1 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.38 – 7.35 (m, 2H), 7.32 – 7.29 (m, 2H), 3.90 – 3.88 (m, 1H), 3.88 – 3.86 (m, 1H), 3.59 (tt, J = 11.9, 3.5 Hz, 1H), 3.41 – 3.36 (m, 2H), 3.27

– 3.21 (m, 2H), 3.18 (s, 3H), 3.10 – 3.04 (m, 2H), 2.96 – 2.90 (m, 2H), 2.11 – 2.04 (m, 2H), 1.81 – 1.70 (m, 2H), 1.26 (s, 6H).

1-(3-amino-1H-1,2,4-triazol-5-yl)-N-(4-chlorophenethyl)-N-(oxetan-3-yl)piperidin-4-amine (18). Obtained from oxetan-3-amine hydrochloride in two steps according to General Procedures A (yield 44%) and C (yield 30%). 18 mg (0.04 mmol) of the final compound was obtained as a dihydrochloride salt after reversed-phase purification and lyophilization from 0.1 M HCl solution. LC-MS (ES+): m/z 377.0/379.0 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.32 (d, J = 8.4 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 4.64 – 4.59 (m, 4H), 4.46 – 4.44 (m, 1H), 3.84 (brd, J = 13.1 Hz, 2H), 3.12 – 3.05 (m, 3H), 2.82 – 2.80 (m, 2H), 2.76 (td, J = 13.0, 2.1 Hz, 2H), 1.77 (d, J = 11.9 Hz, 2H), 1.48 (qd, J = 12.0, 4.1 Hz, 2H).

1-(3-amino-4H-1,2,4-triazol-5-yl)-N-(4-chlorophenethyl)-N-((tetrahydrofuran-2-yl)methyl)piperidin-4-amine bis-2,2,2-trifluoroacetate (19). Obtained from 2-(aminomethyl)tetrahydrofuran in two steps according to General Procedures A (yield 79%) and C (yield 40%). 93 mg (0.15 mmol) of the final compound was obtained as a bis-trifluoroacetate salt after reversed-phase purification. LC-MS (ES+) m/z 421.0/423.0 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.36 (d, J = 8.5 Hz, 2H), 7.31 (d, J = 8.5 Hz, 2H), 4.25 – 4.20 (m, 1H), 3.89 – 3.85 (m, 2H), 3.83 (dt, J = 8.2, 7.0 Hz, 1H), 3.75 (td, J = 7.9, 6.1 Hz, 1H), 3.60 (tt, J = 12.2, 3.4 Hz, 1H), 3.37 (t, J = 8.5 Hz, 2H), 3.33 (brd, J = 12.6 Hz, 1H), 3.21 – 3.14 (m, 1H), 3.07 – 3.00 (m, 2H), 2.94 – 2.88 (m, 2H), 2.11 – 2.03 (m, 3H), 1.93 – 1.81 (m, 2H), 1.74 (pd, J = 12.2, 4.4 Hz, 2H), 1.56 (ddt, J = 12.4, 8.6, 7.1 Hz, 1H).

1-(3-amino-4H-1,2,4-triazol-5-yl)-N-(4-chlorophenethyl)-N-((tetrahydro-2H-pyran-2-yl)methyl)piperidin-4-amine bis-2,2,2-trifluoroacetate (20). Obtained from tetrahydropyran-2-

ylmethylamine in two steps according to General Procedures A (yield 88%) and C (yield 55%). 141 mg (0.22 mmol) of the final compound was obtained as a bis-trifluoroacetate salt after reversed-phase purification. LC-MS (ES+) m/z 419.1/421.1 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.36 (d, J = 8.4 Hz, 2H), 7.30 (d, J = 8.4 Hz, 2H), 3.94 – 3.90 (m, 1H), 3.89 – 3.84 (m, 2H), 3.70 – 3.65 (m, 1H), 3.56 (tt, J = 12.0, 3.5 Hz, 1H), 3.43 – 3.39 (m, 1H, overlapping with water signal), 3.36 – 3.31 (m, 2H), 3.26 – 3.14 (m, 2H), 3.04 – 3.00 (m, 2H), 2.91 (tt, J = 12.8, 3.1 Hz, 2H), 2.07 – 1.99 (m, 2H), 1.82 – 1.67 (m, 3H), 1.60 (d, J = 12.9 Hz, 1H), 1.53 – 1.43 (m, 3H), 1.25 – 1.19 (m, 1H).

5-(4-(3-(4-chlorophenyl)pyrrolidin-1-yl)piperidin-1-yl)-1H-1,2,4-triazol-3-amine bis-2,2,2-trifluoroacetate (21). Obtained from 3-(4-chlorophenyl)pyrrolidine in two steps according to General Procedures B (yield 45%) and C (yield 55%). 19 mg (0.03 mmol) of the final compound was obtained as a bis-trifluoroacetate salt after reversed-phase purification. LC-MS (ES+): m/z 346.9/348.9 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.40 – 7.37 (m, 2H), 7.37 – 7.33 (m, 2H), 3.85 (dq, J = 11.6, 2.6 Hz, 3H), 3.71 – 3.49 (m, 3H), 3.46 – 3.39 (m, 2H), 2.90 (dddd, J = 15.1, 13.2, 5.8, 2.6 Hz, 2H), 2.49 – 2.38 (m, 1H), 2.17 – 2.10 (m, 2H), 2.09 – 1.95 (m, 1H), 1.65 (qt, J = 12.2, 4.3 Hz, 2H).

5-(3-(4-chlorophenyl)-[1,4'-bipiperidin]-1'-yl)-1H-1,2,4-triazol-3-amine bis-2,2,2-trifluoroacetate (22). Obtained from 3-(4-chlorophenyl)piperidine in two steps according to General Procedures B (yield 23%) and C (yield 14%). 8 mg (0.01 mmol) of the final compound was obtained as a bis-trifluoroacetate salt after reversed-phase purification. LC-MS (ES+): m/z 360.9/362.9 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O) δ 7.42 – 7.37 (m, 2H), 7.35 – 7.31 (m, 2H), 3.50 – 3.44 (m, 1H), 3.45 – 3.38 (m, 2H), 3.10 (t, J = 12.0 Hz, 1H), 3.07 – 2.97 (m, 2H),

2.97 – 2.90 (m, 2H), 2.12 – 2.04 (m, 2H), 2.03 – 1.97 (m, 1H), 1.90 – 1.78 (m, 2H), 1.76 – 1.62 (m, 3H), 2 protons overlapping with water signal.

(R)-3-(4-(2-(4-chlorobenzyl)pyrrolidin-1-yl)piperidin-1-yl)-4H-1,2,4-triazol-5-amine bis-2,2,2-trifluoroacetate (23). *Step 1: Synthesis of tert-butyl (R)-2-(methoxy(methyl)carbamoyl)pyrrolidine-1-carboxylate (**37**)*. To a solution of N-Boc-D-proline (10 g, 46.5 mmol) in DCM (200 mL) carbonyldiimidazole (11.3 g, 69.7 mmol) was added. After 30 min of activation of the carboxylic group *N*,*O*-dimethylhydroxylamine (6.8 g, 69.7 mmol) was added and reaction was stirred overnight. The reaction mixture was washed with 1M HCl, 5% solution of $NaHCO_3$ and brine. Organic layer was dried over $MgSO_4$ and concentrated. Product was purified by column chromatography in hexanes : AcOEt (4 : 1) to afford **37** as a colorless oil (6.19 g, 52%). LC-MS (ES+): m/z 259.1 $[M + H]^+$, 281.0 $[M + Na]^+$. ^1H NMR (600 MHz, $CDCl_3$; two rotamers in 1:1 ratio) δ 4.73 – 4.55 (m, 1H), 3.78 (s, 1st rotamer), 3.72 (s, 2nd rotamer; signals at 3.78 and 3.72 summarizing to 3H), 3.63 – 3.52 (m, 1H), 3.51 – 3.44 (m, 1H, rotamer), 3.44 – 3.35 (m, 1H, rotamer), 3.19 (s, 3H), 2.26 – 2.10 (m, 1H), 2.04 – 1.92 (m, 1H), 1.92 – 1.77 (m, 2H), 1.46 (s, 1st rotamer), 1.41 (s, 2nd rotamer; signals at 1.46 and 1.41 summarizing to 9H).

*Step 2: Synthesis of tert-butyl (R)-2-(4-chlorobenzoyl)pyrrolidine-1-carboxylate (**38**).* Amide **37** (3.0 g, 11.61 mmol) was dissolved in dry diethyl ether (30 mL) under atmosphere of argon and cooled to -70 ºC. *p*-Chlorophenylmagnesium bromide, previously generated from magnesium (875 mg, 36.0 mmol) and *p*-bromochlorobenzene (6.67 g, 34.8 mmol) in 150 mL of diethyl ether, was added dropwise to the solution of amide **37** at -70 ºC. After addition of Grignard reagent reaction was allowed to warm to room temperature and stirred for 2 h more. The reaction was quenched with saturated NH_4Cl solution and extracted with diethyl ether. Organic layer was washed with 1M HCl and brine. Solvent was evaporated and oily residue was purified by column

chromatography in hexanes : EtOAc (10 : 1) affording compound **38** as a white crystalline solid (2.47 g, 69%). LC-MS (ES+): m/z 310.0/312.0 $[M + H]^+$. ^1H NMR (600 MHz, $CDCl_3$; two rotamers in 1:3 ratio) δ 7.96 – 7.92 (m, 2H, minor rotamer), 7.92 – 7.88 (m, 2H, major rotamer), 7.49 – 7.45 (m, 2H, major rotamer), 7.45 – 7.42 (m, 2H, minor rotamer), 5.28 (dd, J = 9.2, 3.6 Hz, 1H, minor rotamer), 5.14 (dd, J = 9.1, 4.0 Hz, 1H, major rotamer), 3.74 – 3.65 (m, 1H, major rotamer), 3.66 – 3.59 (m, 1H, minor rotamer), 3.60 – 3.52 (m, 1H, major rotamer), 3.52 – 3.44 (m, 1H, minor rotamer), 2.39 – 2.23 (m, 1H), 2.01 – 1.84 (m, 3H), 1.46 (s, 9H, minor rotamer), 1.26 (s, 9H, major rotamer).

*Step 3: Synthesis of (R)-2-(4-chlorobenzyl)pyrrolidine (**39**).* To a solution of ketone **38** (1 g, 3.23 mmol) in DCM anhydrous aluminum chloride (1.29 g, 9.68 mmol) and triethylsilane (1.55 mL, 9.68 mmol) were added. After 1 h of stirring LC-MS indicated completion of reaction. The reaction was quenched with mixture of 4 M NaOH and brine. The aqueous phase was extracted with DCM, dried over $MgSO_4$, filtered and concentrated to afford **39** as a yellow oil (632 mg, 99%) that was used without further purification in the next step. LC-MS (ES+): m/z 195.9/197.9 $[M + H]^+$.

*Step 4: Synthesis of tert-butyl (R)-4-(2-(4-chlorobenzyl)pyrrolidin-1-yl)piperidine-1-carboxylate (**68**).* N-Boc-4-piperidone (0.74 g, 3.17 mmol) was reductively aminated with 0.62 g (3.17 mmol) of (R)-2-(4-chlorobenzyl)pyrrolidine (**39**) according to the General Procedure B. Product was purified by flash chromatography using DCM : MeOH (50 : 1) solvent system affording **68** as a yellow oil (540 mg, 45%). LC-MS (ES+): m/z 379.1/381.1 $[M + H]^+$. ^1H NMR (600 MHz, $CDCl_3$) δ 7.26 – 7.22 (m, 2H), 7.13 – 7.09 (m, 2H), 4.29 – 4.03 (m, 3H), 3.07 – 3.00 (m, 1H), 2.98 – 2.89 (m, 1H), 2.90 – 2.81 (m, 1H), 2.81 – 2.63 (m, 3H), 2.63 – 2.54 (m, 1H), 2.48 – 2.37 (m, 1H), 1.92 – 1.81 (m, 1H), 1.81 – 1.75 (m, 1H), 1.75 – 1.59 (m, 3H), 1.59 – 1.47 (m, 2H), 1.47 (s, 9H).

*Step 5: Synthesis of (R)-3-(4-(2-(4-chlorobenzyl)pyrrolidin-1-yl)piperidin-1-yl)-4H-1,2,4-triazol-5-amine bis-2,2,2-trifluoroacetate (**23**).* The title compound was prepared from **68** according to the General Procedure C and was purified by preparative reversed-phase chromatography. 201 mg (0.34 mmol) of compound **23** was obtained in a form of bis-trifluoroacetate salt. LC-MS (ES+): m/z 361.0/363.0 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O) δ 7.40 – 7.37 (m, 2H), 7.36 – 7.32 (m, 2H), 3.89 – 3.78 9 (m, 3H, overlapping with water signal), 3.54 – 3.48 (m, 1H), 3.42 (dt, *J* = 11.6, 5.9 Hz, 1H), 3.24 – 3.15 (m, 2H), 2.89 (q, *J* = 11.9 Hz, 2H), 2.75 (dd, *J* = 13.4, 11.3 Hz, 1H), 2.12 (d, *J* = 12.3 Hz, 1H), 2.04 (d, *J* = 12.2 Hz, 1H), 1.94 – 1.83 (m, 3H), 1.75 – 1.69 (m, 1H), 1.65 (qd, *J* = 12.6, 5.3 Hz, 2H).

(R)-3-(2-(4-chlorobenzyl)-[1,4'-bipiperidin]-1'-yl)-4H-1,2,4-triazol-5-amine bis-2,2,2-trifluoroacetate (24). *Step 1: Synthesis of (S)-N-((R)-1-(4-chlorophenyl)pent-4-en-2-yl)-2-methylpropane-2-sulfinamide (**42**).* A solution of *p*-chlorophenylacetaldehyde (6.5 g, 42 mmol), (S)-2-methyl-2-propanesulfinamide (5.09 g, 42 mmol) and $Ti(OEt)_4$ (9.2 mL, 84 mmol) in anhydrous DCM (85 mL) was refluxed for 2 h, then slightly heated (ca. 30 °C) overnight with stirring. $MgSO_4$ was added and after 15 min the reaction was filtered through a pad of Celite. Filtrate was cooled to -20 °C and a solution of allyl magnesium bromide (63 mL, 63 mmol, 1M in diethyl ether) was added dropwise. Upon completion of the addition, the cooling bath was removed and the reaction was allowed to warm to room temperature. After this reaction mixture was poured into a saturated solution of NH_4Cl and extracted three times with diethyl ether. Combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography in hexanes : AcOEt (4 : 1) solvent system affording compound **42** as yellow oil (5.0 g, 23%). LC-MS (ES+): m/z 300.1/302.1 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24 – 7.21(m, 2H), 7.11 – 7.07 (m, 2H), 5.81 – 5.71 (m, 1H), 5.18 – 5.10 (m, 2H),

3.54 – 3.46 (m, 1H), 3.33 – 3.28 (m, 1H), 2.78 (dd, J = 7.1, 13.7 Hz, 1H), 2.69 (dd, J = 6.4, 13.7 Hz, 1H), 2.40 – 2.32 (m, 1H), 2.31 – 2.23 (m, 1H), 1.08 (s, 9H).

*Step 2: Synthesis of (S)-N-allyl-N-((R)-1-(4-chlorophenyl)pent-4-en-2-yl)-2-methylpropane-2-sulfinamide (**43**).* To a solution of sulfinamide **24** (1 g, 3.33 mmol) in DMF (10 mL), sodium hydride (0.32 g, 6.66 mmol) was added. After 20 min a solution of allyl bromide (0.57 mL, 6.66 mmol) in DMF (12 mL), was added dropwise and the reaction mixture was allowed to stir for 1 h. After this time the reaction mixture was poured into a saturated solution of NH_4Cl and extracted several times with diethyl ether. Combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography in hexanes : AcOEt (3 : 1) gave title compound **43** (0.78 g, 71%). LC-MS (ES+): m/z 340.2/342.2 $[M + H]^+$. ^1H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.30 – 7.27 (m, 2H), 7.24 – 7.22 (m, 2H), 5.86 – 5.77 (m, 1H), 5.74 – 5.65 (m, 1H), 5.23 (dq, J = 17.2, 1.6 Hz, 1H), 5.16 (dq, J = 10.2, 1.5 Hz, 1H), 5.01 – 4.93 (m, 2H), 3.80 (ddt, J = 16.5, 5.4, 1.7 Hz, 1H), 3.36 (tt, J = 8.2, 5.7 Hz, 1H), 3.22 (dd, J = 16.5, 7.0 Hz, 1H), 2.99 (dd, J = 13.8, 5.7 Hz, 1H), 2.78 (dd, J = 13.8, 8.3 Hz, 1H), 2.38 – 2.30 (m, 1H), 2.23 – 2.15 (m, 1H), 1.07 (s, 9H).

*Step 3: Synthesis of (R)-1-((S)-tert-butylsulfinyl)-2-(4-chlorobenzyl)-1,2,3,6-tetrahydropyridine (**44**).* A solution of starting diene **43** (0.78 g, 2.29 mmol) and Grubbs catalyst 1st generation (90 mg, 0.1 mmol) in DCM (40 mL) was refluxed for 1.5 h. After this time, reaction mixture was cooled down and concentrated. The desired product was isolated by silica-gel column chromatography in hexanes : AcOEt (10 : 1) providing **44** (0.67 g, 94%). LC-MS (ES+): m/z 312.2/314.2 $[M + H]^+$. ^1H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.35 – 7.32 (m, 2H), 7.32 – 7.29 (m, 2H), 5.76 – 5.68 (m, 2H), 3.77 – 3.72 (m, 1H), 3.63 (dt, J = 8.9, 6.2 Hz, 1H), 3.49 –

3.42 (m, 1H), 2.91 (dd, *J* = 13.6, 9.1 Hz, 1H), 2.75 (dd, *J* = 13.6, 6.3 Hz, 1H), 2.37 – 2.29 (m, 1H), 1.93 – 1.88 (m, 1H), 0.80 (s, 9H).

*Step 4: Synthesis of (R)-1-((S)-tert-butylsulfinyl)-2-(4-chlorobenzyl)piperidine (**45**).* To a solution of olefin **44** (0.62 g, 1.98 mmol) in MeOH (5 mL) a catalytic amount of 10% palladium on activated carbon was added. The reaction mixture was stirred under hydrogen overnight. After this time, the catalyst was filtered off through a Celite pad and the filtrate was concentrated to dryness and purified by column chromatography using hexanes : AcOEt (4 : 1), affording piperidine **45** (0.48 g, 77%). LC-MS (ES+): m/z 314.2/316.2 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.32 – 7.26 (m, 2H), 7.26 – 7.20 (m, 2H), 3.53 – 3.47 (m, 1H), 3.17 – 3.11 (m, 2H), 3.04 (dd, *J* = 13.6, 6.6 Hz, 1H), 2.84 (dd, *J* = 13.6, 8.5 Hz, 1H), 1.75 – 1.66 (m, 1H), 1.60 – 1.52 (m, 1H), 1.54 – 1.45 (m, 3H), 1.44 – 1.37 (m, 1H), 0.97 (s, 9H).

*Step 5: Synthesis of (R)-2-(4-chlorobenzyl)piperidine (**46**).* Compound **45** (0.48 g, 1.5 mmol) was treated with 2M HCl(gas) in MeOH at room temperature for 1 h and then the reaction was concentrated to dryness. The residue was taken between DCM and 1M NaOH and phases were separated. Aqueous phase was extracted with DCM and the organics were combined, dried and concentrated to give unprotected piperidine **46** (0.23 g, 72%). LC-MS (ES+): m/z 210.2/212.2 $[M + H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 – 7.21 (m, 2H), 7.14 – 7.08 (m, 2H), 3.00 (dq, *J* = 11.8, 2.0 Hz, 1H), 2.71 – 2.60 (m, 2H), 2.54 (m, 2H), 1.80 – 1.73 (m, 1H), 1.66 (s, 1H), 1.61 – 1.53 (m, 1H), 1.43 (m, 1H), 1.34 – 1.23 (m, 1H), 1.22 – 1.13 (m, 1H).

*Step 6: Synthesis of tert-butyl (R)-2-(4-chlorobenzyl)-[1,4'-bipiperidine]-1'-carboxylate (**69**).* N-Boc-4-piperidone (**31**) was reductively aminated with of (R)-2-(4-chlorobenzyl)piperidine (**46**)

according to the General Procedure B. Product was purified by column chromatography using hexanes : AcOEt (4 : 1), providing **69** (0.17 g, 40%). LC-MS (E+): m/z 393.1/395.1 $[M + H]^+$.

*Step 7: Synthesis of (R)-3-(2-(4-chlorobenzyl)-[1,4'-bipiperidin]-1'-yl)-4H-1,2,4-triazol-5-amine bis-2,2,2-trifluoroacetate (**24**).* The title compound was prepared according to the General Procedure C and purified by preparative HPLC to give **24** as a bis-trifluoroacetate salt (40 mg, 28%). LC-MS (ES+): m/z 375.1/377.1 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6) δ 7.42 – 7.38 (m, 2H), 7.35 – 7.28 (m, 2H), 3.65 – 3.56 (m, 1H), 3.56 – 3.48 (m, 1H), 3.45 – 3.33 (m, 1H), 3.15 – 3.02 (m, 1H), 3.02 – 2.86 (m, 2H), 2.88 – 2.80 (m, 1H), 2.71 (dd, J = 13.7, 9.8 Hz, 1H), 2.27 – 2.15 (m, 1H), 1.98 – 1.92 (m, 1H), 1.92 – 1.76 (m, 2H), 1.69 – 1.56 (m, 4H), 1.50 – 1.40 (m, 1H), 1.40 – 1.31 (m, 1H), 2 protons overlapping with water signal.

3-(4-((2S,3S)-2-(4-chlorobenzyl)-3-methoxyazetidin-1-yl)piperidin-1-yl)-4H-1,2,4-triazol-5-amine 2,2,2-trifluoroacetate (25). *Step 1: (S)-(1-(4-chlorophenyl)-4-diazo-3-oxobutan-2-yl)carbamate (**48**).* To a solution of (S)-2-((tert-butoxycarbonyl)amino)-3-(4-chlorophenyl)propanoic acid (17.2 g, 57 mmol) in THF (200 mL) was added TEA (17 mL, 120 mmol) and methyl chloroformate (4.87 mL, 63 mmol) at -10°C. After 15 min a solution of diazomethane (342 mmol) in diethyl ether (400 mL) was added at -30°C. The reaction mixture was allowed to warm and stirred overnight at room temperature. The excess of diazomethane was destroyed by a dropwise addition of AcOH (15 mL). The mixture was diluted with diethyl ether and washed with 5% $NaHCO_3$, saturated NH_4Cl, and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give title product **48** as an orange solid (18.0 g, 96%). LC-MS (ES+): m/z 325.8/327.8 $[M + H]^+$. ^{1}H NMR (700 MHz, $CDCl_3$) 7.28 (d, J = 8.4 Hz, 2H), 7.13(d, J = 8.3 Hz, 2H), 5.26 (brs, 1H), 5.07 (brs, 1H), 4.40 (brs, 1H), 3.03 (dd, J = 14.0, 7.0 Hz, 1H), 2.97 (dd, J = 13.5, 6.1 Hz, 1H), 1.42 (s, 9H).

*Step 2: Tert-butyl (S)-2-(4-chlorobenzyl)-3-oxoazetidine-1-carboxylate (**49**).* To a solution of compound **48** (3.6 g, 11 mmol) in DCM (55 mL), triethylamine (16 µL, 0.11 mmol) was added. The reaction mixture was cooled to 0°C and rhodium acetate (25 mg, 0.056 mmol) was added. Cooling bath was removed and the reaction was stirred at room temperature overnight. The mixture was diluted with water (50 mL), phases were separated and the aqueous layer was extracted with DCM. The combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash chromatography using hexanes : AcOEt (15 : 1) solvent system to give title product **49** as colorless oil (1.5 g, 46%). LC-MS (ES+): m/z 296.1/298.1 $[M + H]^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 7.28 – 7.23 (m, 2H), 7.14 – 7.09 (m, 2H), 5.10 (dt, *J* = 6.4, 4.2 Hz, 1H), 4.55 (d, *J* = 16.6 Hz, 1H), 4.07 (dd, *J* = 16.6, 4.4 Hz, 1H), 3.16 (dd, *J* = 14.4, 6.4 Hz, 1H), 3.07 (dd, *J* = 14.3, 4.1 Hz, 1H), 1.47 (s, 9H).

*Step 3: Tert-butyl (2S,3S)-2-(4-chlorobenzyl)-3-hydroxyazetidine-1-carboxylate (**50**).* Boc-protected azetidinone **49** (1.5 g, 5.07 mmol) was dissolved in MeOH (20 mL) and sodium borohydride (0.23 g, 6.1 mmol) was added at 0°C. The reaction mixture was allowed to warm and stirred at room temperature for 1 h after which time it was quenched with 15 mL of 1M NaOH. MeOH was evaporated under reduced pressure and the aqueous layer was extracted with AcOEt. The combined organic layers was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified by flash chromatography using hexanes : AcOEt (5 : 1) solvent system yielding mixture of diastereoisomers in 10:1 ratio. Minor diasteroisomer was rejected by crystallization from DCM / hexanes to give the title product **50** (1.3 g, 86%). LC-MS (ES+): m/z 320.2/322.2 $[M + Na]^+$. ^1H NMR (700 MHz, $CDCl_3$) δ 7.26 – 7.24 (m, 2H), 7.21 (d, *J* = 8.1 Hz, 2H), 4.59 (qd, *J* = 6.6, 4.0 Hz, 1H), 4.45 – 4.39 (m, 1H), 4.14 (dd, *J* = 9.8, 7.0 Hz, 1H),

3.70 (ddd, *J* = 9.8, 4.0, 1.1 Hz, 1H), 3.20 (dd, *J* = 13.9, 9.6 Hz, 1H), 3.14 (brs, 1H), 1.83 (d, *J* = 5.8 Hz, 1H), 1.41 (s, 9H).

*Step 4: tert-butyl (2S,3S)-2-(4-chlorobenzyl)-3-methoxyazetidine-1-carboxylate (**51**).* To a solution of compound **50** (0.4 g, 1.34 mmol) in DMF (4 mL) was added sodium hydride (0.16 g, 4.04 mmol). The mixture was stirred for 5 min and then methyl iodide (250 µL, 4.04 mmol) was added and stirring was continued at room temperature for 1 h after which time the reaction mixture was diluted with water (15 mL) and extracted several times with diethyl ether. The combined organic layers were washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure to give title product **51** as colorless oil (418 mg, 100%). LC-MS (ES+): m/z 334.1/336.1 $[M + Na]^+$. ^1H NMR (700 MHz, $CDCl_3$) δ 7.25 – 7.21 (m, 2H), 7.19 (d, *J* = 8.1 Hz, 2H), 4.54 – 4.33 (m, 1H), 4.09 (q, *J* = 6.1 Hz, 1H), 4.02 (t, *J* = 8.1 Hz, 1H), 3.75 (ddd, *J* = 9.5, 4.2, 1.0 Hz, 1H), 3.23 (s, 3H), 3.17 (dd, *J* = 13.8, 9.1 Hz, 1H), 3.06 (d, *J* = 14.0 Hz, 1H), 1.39 (s, 9H).

*Step 5: (2S,3S)-2-(4-chlorobenzyl)-3-methoxyazetidine hydrochloride (**52**).* Compound **51** (418 mg, 1.34 mmol) was treated with 4M HCl (gas) in 1,4-dioxane (2.2 mL, 8.8 mmol). The reaction mixture was stirred for 1 h at room temperature. The volatiles were evaporated under reduced pressure and the residue was triturated with diethyl ether to give title product **52** as white solid in a form of hydrochloride salt (256 mg, 77%). LC-MS (ES+): m/z 212.1/214.1 $[M + H]^+$. ^1H NMR (700 MHz, DMSO-d_6 + D_2O) δ 7.36 – 7.33 (m, 2H), 7.30 – 7.27 (m, 2H), 4.74 (dt, *J* = 8.5, 6.5 Hz, 1H), 4.24 (td, *J* = 6.1, 3.5 Hz, 1H), 4.10 (dd, *J* = 11.5, 5.9 Hz, 1H), 3.70 (dd, *J* = 11.5, 3.5 Hz, 1H), 3.26 (s, 3H), 3.18 (dd, *J* = 14.8, 6.8 Hz, 1H), 3.08 (dd, *J* = 14.9, 8.7 Hz, 1H).

*Step 6: tert-butyl 4-((2S,3S)-2-(4-chlorobenzyl)-3-methoxyazetidin-1-yl)piperidine-1'-carboxylate (**70**).* The title compound was prepared according to the General Procedure B starting

from 250 mg (1 mmol) of azetidine **52**. The crude post-reaction residue was loaded on silica-gel and eluted with hexanes : AcOEt (1 : 1) solvent system yielding 209 mg (0.53 mmol, 53%) of pure compound **70** in a form of colorless oil. LC-MS (ES+): m/z 395.1/397.0 $[M + H]^+$. ^{1}H NMR (700 MHz, $CDCl_3$) δ 7.24 – 7.20 (m, 2H), 7.15 – 7.11 (m, 2H), 4.08 – 3.91 (m, 2H), 3.82 (td, *J* = 5.6, 1.3 Hz, 1H), 3.44 (d, *J* = 8.8 Hz, 1H), 3.38 – 3.31 (m, 2H), 3.30 (s, 3H), 2.84 (dd, *J* = 8.8, 5.4 Hz, 1H), 2.79 – 2.72 (m, 2H), 2.59 (dd, *J* = 12.8, 2.4 Hz, 1H), 2.24 (tt, *J* = 10.1, 3.8 Hz, 1H), 1.72 (d, *J* = 12.3 Hz, 1H), 1.60 (d, *J* = 12.7 Hz, 1H), 1.45 (s, 9H), 1.41 – 1.32 (m, 1H), 1.30 – 1.22 (m, 1H).

*Step 7: 3-(4-((2S,3S)-2-(4-chlorobenzyl)-3-methoxyazetidin-1-yl)piperidin-1-yl)-4H-1,2,4-triazol-5-amine 2,2,2-trifluoroacetate (**25**).* The title compound was prepared according to the General Procedure C and purified by preparative HPLC to give **25** as a bis-trifluoroacetate salt (80 mg, 25%). LC-MS (ES+): m/z 377.0/378.9 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O) δ 7.39 – 7.35 (m, 2H), 7.33 – 7.27 (m, 2H), 4.84 (dt, *J* = 10.9, 5.5 Hz, 1H), 4.13 – 4.05 (m, 2H), 3.96 (dd, *J* = 11.4, 2.0 Hz, 1H), 3.75 – 3.71 (m, 2H), 3.41 – 3.30 (m, 2H), 3.26 (s, 3H), 2.95 (dd, *J* = 14.0, 5.0 Hz, 1H), 2.82 (dddd, *J* = 13.0, 10.3, 7.6, 2.7 Hz, 2H), 1.96 – 1.86 (m, 2H), 1.36 (dqd, *J* = 20.1, 12.1, 4.5 Hz, 2H).

(3S,5S)-1-(1-(3-amino-4H-1,2,4-triazol-5-yl)piperidin-4-yl)-5-(4-chlorobenzyl)pyrrolidin-3-ol 2,2,2-trifluoroacetate (26). *Step 1: Synthesis of tert-butyl (2R,4S)-4-hydroxy-2-(methoxy(methyl)carbamoyl)pyrrolidine-1-carboxylate (**54**)*. The title compound was prepared by a similar procedure as that used for compound **37** starting from Boc-D-hydroxyproline. Purification by column chromatography (hexanes : AcOEt, 2 : 1) provided **54** as a colorless oil (4.15 g, 49%). LC-MS (ES+) m/z 274.9. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 4.67 (t, *J* = 7.3 Hz, 1H), 4.28 – 4.23 (m, 1H), 3.68 (s, 3H), 3.41 – 3.36 (m, 1H), 3.29 (dt, *J* = 11.2, 1.9 Hz,

1H), 3.10 (s, 3H), 2.18 – 2.06 (m, 1H), 1.87 – 1.79 (m, 1H), 1.37 (s), 1.32 (s) (integrations of signals at 1.37 and 1.32 ppm summarize to 9H)

*Step 2: Synthesis of tert-butyl (2R,4S)-4-((tert-butyldimethylsilyl)oxy)-2-(methoxy(methyl)carbamoyl)pyrrolidine-1-carboxylate (**55**)*. To a solution of compound **54** (5 g, 18.22 mmol) in DMF (60 mL), imidazole (6.2 g, 91.13 mmol) was added followed by TBDMSCl (4.1 g, 27.33 mmol) and reaction was stirred overnight. After this time reaction mixture was diluted with water (250 mL) and extracted several times with AcOEt. Combined organics were washed with water, brine, dried over anhydrous $MgSO_4$, filtered and concentrated. Crude product was purified by column chromatography (hexanes : AcOEt, 8 : 1) to give 6.50g of **55** as a colorless oil (16.7 mmol, 92%). LC-MS (ES+) m/z 289.7 $[M - Boc + H]^+$, 411.1 $[M + Na]^+$. ^1H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 4.70 – 4.63 (m, 1H), 4.42 (qd, J = 4.2, 1.7 Hz, 1H), 3.44 – 3.41 (m, 4H), 3.31 – 3.24 (m, 1H), 3.10 (s, 3H), 2.13 – 2.05 (m, 1H), 1.90 – 1.83 (m, 1H), 1.35 (bs), 1.31 (bs) (integrations of signals at 1.35 and 1.31 ppm summarize to 9H), 0.84 (s, 9H), 0.04 (s, 6H).

*Step 3: Synthesis of (2R,4S)-tert-butyl 4-((tert-butyldimethylsilyl)oxy)-2-(4-chlorobenzoyl)pyrrolidine-1-carboxylate (**56**)*. The title compound was prepared by a similar procedure as that used for compound **38** and purified by column chromatography (hexanes : AcOEt, 10 : 1) to give **56** as a colorless oil (970 mg, 86%). LC-MS (ES+): m/z 340.0/341.9 $[M - Boc + H]^+$. ^1H NMR (700MHz, DMSO-d_6 + D_2O, presence of 2 rotamers) δ 7.97 – 7.95 (m, 2H), 7.61 – 7.59 (m, 2H), 5.27 – 5.22 (m, 1H), 4.44 – 4.39 (m, 1H), 3.52 (dd, J = 11.4, 4.4 Hz, 1H, rotamer), 3.48 (dd, J = 11.4, 4.4 Hz, 1H, rotamer), 3.34 (m, 1H, rotamer) , 3.30 (m, 1H, rotamer), 2.24 – 2.19 (m, 1H), 1.92 (ddd, J = 12.9, 7.8, 4.8 Hz, 1H, rotamer), 1.85 (ddd, J = 13.0, 7.7, 4.8

Hz, 1H, rotamer), 1.34 (s, 9H, rotamer) , 1.11 (s, 9H, rotamer), 0.83 (s, 9H), 0.04 (s, 6H, rotamer), 0.03 (s, 6H, rotamer).

*Step 4: Synthesis of (3S,5S)-5-(4-chlorobenzyl)pyrrolidin-3-ol (**57**).* Starting from 883 mg (2 mmol) of **56**, the title compound was prepared by a similar procedure as that used for compound **39**. 0.35g (1.65 mmol, 81%) of crude **57** was obtained in a form of a yellowish oil that was sufficiently pure to be used in the next step without further purification. LC-MS (ES+): m/z 212.0 /213.9 $[M + H]^+$.

*Step 5: Synthesis of tert-butyl 4-((2S,4S)-2-(4-chlorobenzyl)-4-hydroxypyrrolidin-1-yl)piperidine-1-carboxylate (**71**).* The title compound was prepared according to the General Procedure B starting from 176 mg (0.83 mmol) of **57**. It was purified by silica-gel column chromatography using hexanes : AcOEt (4 : 1) solvent system yielding 147 mg of **70** (0.37 mmol, 45%). LC-MS (ES+): m/z 295.4/297.3 $[M – Boc + 1]^+$, 395.4/397.3 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.29 – 7.26 (m, 2H), 7.21 – 7.18 (m, 2H), 4.08 – 4.02 (m, 1H), 3.97 – 3.91 (m, 2H), 2.99 (dd, J = 9.7, 5.8 Hz, 1H), 2.82 (dd, J = 13.4, 4.3 Hz, 1H), 2.80 – 2.73 (m, 1H), 2.72 – 2.62 (m, 2H), 2.43 (dd, J = 9.6, 4.7 Hz, 1H), 2.42 – 2.38 (m, 2H), 1.74 – 1.67 (m, 2H), 1.58 (dt, J = 13.0, 6.6 Hz, 1H), 1.53 – 1.46 (m, 1H), 1.38 (s, 9H), 1.35 – 1.21 (m, 2H).

*Step 6: Synthesis of (3S,5S)-1-(1-(3-amino-1H-1,2,4-triazol-5-yl)piperidin-4-yl)-5-(4-chlorobenzyl)pyrrolidin-3-ol bis-2,2,2-trifluoroacetate (**26**).* The title compound was prepared according to the General Procedure C and purified by preparative HPLC to give **26** as a bis-trifluoroacetate salt (80 mg, 44%). LC-MS (ES+) m/z 377.2/379.2 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O) δ 7.40 – 7.38 (m, 2H), 7.34 (d, J = 8.4 Hz, 2H), 4.33 (s, 1H), 4.02 – 3.94 (m, 1H), 3.64 – 3.56 (m, 1H), 3.48 (dd, J = 12.8, 4.3 Hz, 1H), 3.31 (dd, J = 12.9, 4.0 Hz, 1H), 3.23 (d,

J = 12.7 Hz, 1H), 2.90 (t, *J* = 11.9 Hz, 2H), 2.79 (t, *J* = 12.0 Hz, 1H), 2.10 – 2.03 (m, 2H), 1.85 – 1.78 (m, 1H), 1.77 – 1.72 (m, 1H), 1.72 – 1.60 (m, 2H).

5-(4-((2S,4S)-2-(4-chlorobenzyl)-4-methoxypyrrolidin-1-yl)piperidin-1-yl)-4H-1,2,4-triazol-3-amine 2,2,2-trifluoroacetate (27). *Step 1: Synthesis of tert-butyl 4-((2S,4S)-2-(4-chlorobenzyl)-4-methoxypyrrolidin-1-yl)piperidine-1-carboxylate (**74**).* Starting from 101 mg of compound **71** (0.25 mmol), the title compound was prepared by a similar procedure as that used for compound **50**. Purification by column chromatography (hexanes : AcOEt, 1 : 1) gave 120 mg of **73** as a yellowish oil (0.24 mmol, 95%). LC-MS (ES+) m/z 409.1/411.1 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.31 – 7.28 (m, 2H), 7.22 – 7.19 (m, 2H), 3.98 – 3.89 (m, 2H), 3.76 – 3.70 (m, 1H), 3.11 – 3.07 (m, 1H), 3.08 (s, 3H), 2.99 (dt, J = 9.0, 4.5 Hz, 1H), 2.85 (dd, J = 13.2, 4.1 Hz, 1H), 2.78 (td, J = 11.7, 10.9, 5.6 Hz, 1H), 2.75 – 2.55 (m, 2H), 2.47 (dd, J = 9.6, 3.9 Hz, 1H), 2.34 (dd, J = 13.2, 9.2 Hz, 1H), 1.72 – 1.65 (m, 2H), 1.58 – 1.51 (m, 1H), 1.53 – 1.46 (m, 1H), 1.37 (s, 9H), 1.33 – 1.24 (m, 1H), 1.23 – 1.16 (m, 1H).

*Step 2: Synthesis of 5-(4-((2S,4S)-2-(4-chlorobenzyl)-4-methoxypyrrolidin-1-yl)piperidin-1-yl)-1H-1,2,4-triazol-3-amine bis-2,2,2-trifluoroacetate (**27**).* The title compound was prepared according to the General Procedure C and purified by preparative HPLC to give **27** as a bis-trifluoroacetate salt (11 mg, 8%). LC-MS (ES+): m/z 391.2/393.2 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O) δ 7.40 (d, *J* = 8.5 Hz, 2H), 7.35 (d, *J* = 8.5 Hz, 2H), 4.01 (s, 1H), 3.94 – 3.80 (m, 3H), 3.56 – 3.41 (m, 3H), 3.31 (d, *J* = 10.9 Hz, 1H), 3.17 (s, 3H), 2.89 – 2.77 (m, 3H), 2.02 (brs, 2H), 1.92 (brs, 1H), 1.83 – 1.76 (m, 1H), 1.65 (dd, *J* = 5.1, 9.7 Hz, 2H).

(3R,5S)-1-(1-(3-amino-1H-1,2,4-triazol-5-yl)piperidin-4-yl)-5-(4-chlorobenzyl)pyrrolidin-3-ol 2,2,2-trifluoroacetate (28). *Step 1: Synthesis of (2R,4R)-1-tert-butyl 2-methyl 4-(tert-*

*butoxy)pyrrolidine-1,2-dicarboxylate (**59**).* To a solution of 16.06 g (65.5 mmol) of 1-tert-butyl 2-methyl (2R,4R)-4-hydroxypyrrolidine-1,2-dicarboxylate (**58**) in DCM (100 mL), $Mg(ClO_4)_2$ (1.46 g, 6.55 mmol) was added, followed by Boc_2O (32.87 g, 150.6 mmol) and reaction was stirred overnight. The following day another portion of $Mg(ClO_4)_2$ (1.46 g, 6.55 mmol) and Boc_2O (44.30 g, 203 mmol) were added and reaction was stirred overnight after which time LC-MS control indicated completion of the reaction. Reaction mixture was filtrated and evaporated to dryness. Purification by column chromatography (hexanes : AcOEt, 9 : 1) afforded 13.51g (44.8 mmol, 68%) of compound **59** as a white crystalline solid. LC-MS (ES+): m/z 302.4 $[M + H]^+$, 324.3 $[M + Na]^+$. ^1H NMR (700 MHz DMSO-d_6 + D_2O, presence of 2 rotamers in 2:1 ratio) δ 4.24 – 4.19 (m, 1H), 4.17 – 4.13 (m, 1H), 3.61 (s, 3H, major rotamer), 3.58 (s, 3H, minor rotamer), 3.54 – 3.51 (m, 1H), 2.99 – 2.94 (m, 1H), 2.38 – 2.30 (m, 1H), 1.75 – 1.71 (m, 1H), 1.36 (s, 9H, minor rotamer), 1.29 (s, 9H, major rotamer), 1.08 (s, 9H, major rotamer), 1.07 (s, 9H, minor rotamer).

*Step 2: Synthesis of (2R,4R)-4-(tert-butoxy)-1-(tert-butoxycarbonyl)pyrrolidine-2-carboxylic acid (**60**).* Compound **59** (13.5 g, 44.8 mmol) was dissolved in a mixture of 200 mL THF and 100 mL MeOH. Solution of lithium hydroxide hydrate in 100 mL of water was added to the reaction mixture and resulting mixture was stirred overnight. After LC-MS control had indicated completion of the reaction, the reaction mixture was concentrated and the aqueous residue was carefully acidified to pH 4 by dropwise addition of 2M HCl at 0 °C and product was extracted with AcOEt. Organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated affording 12.1 g (42.1 mmol, 94%) of the title compound **60** as a yellowish oil, which was used in the next step without further purification. LC-MS (ES+): m/z 310.3 $[M + Na]^+$, 286.0 $[M - H]^-$.

*Step 3: Synthesis of (2R,4R)-tert-butyl 4-(tert-butoxy)-2-(methoxy(methyl)carbamoyl)pyrrolidine-1-carboxylate (**61**).* Starting from 12.1 g (42.2 mmol) of

the crude carboxylic acid **60**, the title compound was prepared by a similar procedure as that used for compound **37**. Purification by column chromatography (hexanes : AcOEt, 5 : 1) afforded 9.34 g of **61** as a white crystalline solid (28.3 mmol, 67%). LC-MS (ES+): m/z 331.4 $[M + H]^+$. ^1H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 4.49 (t, J = 8.4 Hz, 1H), 4.20 (q, J = 7.7 Hz, 1H), 3.66 – 3.63 (m, 4H), 3.09 (s, 3H), 2.91 (brs, 1H), 2.47 (dt, J = 2.3, 7.9 Hz, 1H), 1.54 – 1.50 (m, 1H), 1.31 (brs, 9H), 1.11 (s, 9H).

*Step 4: Synthesis of (2R,4R)-tert-butyl 4-(tert-butoxy)-2-(4-chlorobenzoyl)pyrrolidine-1-carboxylate (**62**).* Starting from 4.55 g (13.8 mmol) of the Weinreb amide **61**, the title compound was prepared by a similar procedure as that used for compound **38**. Purification by column chromatography (hexanes : AcOEt, 9 : 1) afforded 2.11 g of **62** (5.52 mmol, 40%) as a white solid. LC-MS (ES+): m/z 326.3/328.3 $[M - {}^t\text{Bu} + 1]^+$. ^1H NMR (700 MHz, DMSO-d_6 + D_2O, presence of two rotamers in 1:1 ratio) δ 7.96 – 7.86 (m, 2H), 7.59 – 7.53 (m, 2H), 5.13 – 5.06 (m, 1H), 4.30 – 4.22 (m, 1H), 3.67 – 3.59 (m, 1H), 3.04 – 2.97 (m, 1H), 2.62 – 2.53 (m, 1H), 1.57 – 1.49 (m, 1H), 1.33 (s, 3H, rotamer), 1.11 (s, 6H, rotamer), 1.04 (s, 6H, rotamer), 1.01 (s, 3H, rotamer).

*Step 5: Synthesis of (3R,5S)-5-(4-chlorobenzyl)pyrrolidin-3-ol (**63**).* Starting from 359 mg (0.94 mmol) of the ketone **62**, the title compound was prepared by a similar procedure as that used for **39** to give 195 mg of crude **63** (0.92 mmol, 98%) as a yellow oil that was used in the following reductive amination step without further purification. LC-MS (ES+) m/z 212.2/214.2 $[M + H]^+$.

*Step 6: Synthesis of tert-butyl 4-((2S,4R)-2-(4-chlorobenzyl)-4-hydroxypyrrolidin-1-yl)piperidine-1-carboxylate (**72**).* The title compound was prepared according to the General Procedure B starting from compound **63** obtained in the previous step. It was purified by silica-gel column chromatography using hexanes : AcOEt (4 : 1) solvent system yielding 146 mg of **70**

(0.37 mmol, 40%). LC-MS (ES+): m/z 295.4/297.3 $[M - Boc + 1]^+$, 395.4/397.3 $[M + H]^+$. ^1H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.28 – 7.23 (m, 2H), 7.20 – 7.16 (m, 2H), 4.08 – 4.02 (m, 1H), 3.92 – 3.86 (m, 2H), 3.03 – 2.97 (m, 2H), 2.84 (dd, *J* = 13.3, 4.5 Hz, 1H), 2.75 (dd, *J* = 10.1, 6.1 Hz, 1H), 2.71 – 2.60 (m, 4H), 2.55 (dd, *J* = 13.3, 9.1 Hz, 1H), 1.84 – 1.76 (m, 1H), 1.70 – 1.61 (m, 2H), 1.36 (s, 9H), 1.31 – 1.12 (m, 2H).

*Step 7: Synthesis of (3R,5S)-1-(1-(3-amino-1H-1,2,4-triazol-3-yl)piperidin-5-yl)-5-(4-chlorobenzyl)pyrrolidin-3-ol bis-2,2,2-trifluoroacetate (**28**).* The title compound was prepared according to the General Procedure C and purified by preparative HPLC to give **28** as a bis-trifluoroacetate salt (105 mg, 33%). LC-MS (ES+): m/z 377.4/379.4 $[M + H]^+$. ^1H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.39 – 7.36 (m, 2H), 7.33 – 7.30 (m, 2H), 4.44 – 4.39 (m, 1H), 4.02 – 3.95 (m, 1H), 3.91 – 3.83 (m, 2H), 3.56 – 3.46 (m, 1H), 3.43 – 3.37 (m, 1H), 3.37 – 3.28 (m, 1H), 3.25 (dd, *J* = 13.5, 4.4 Hz, 1H), 3.04 (dd, *J* = 13.5, 11.3 Hz, 1H), 2.94 – 2.85 (m, 2H), 2.22 – 2.17 (m, 1H), 2.17 – 2.09 (m, 1H), 2.07 – 2.00 (m, 1H), 1.84 – 1.68 (m, 3H).

5-(4-((2S,4R)-2-(4-chlorobenzyl)-4-methoxypyrrolidin-1-yl)piperidin-1-yl)-1H-1,2,4-triazol-3-amine 2,2,2-trifluoroacetate (29). *Step 1: Synthesis of tert-butyl 4-((2S,4R)-2-(4-chlorobenzyl)-4-methoxypyrrolidin-1-yl)piperidine-1-carboxylate (**75**).* Starting from 50 mg of compound **72** (0.13 mmol), the title compound was prepared in a similar methylation procedure as that used for **50**. Purification by column chromatography (hexanes : AcOEt, 1 : 1) gave 41 mg (0.10 mmol, 77%) of **75** in a form of pale yellow oil. LC-MS (ES+): m/z 409.4/411.4 $[M + H]^+$. ^1H NMR (700 MHz, DMSO-d_6) δ 7.28 – 7.24 (m, 2H), 7.18 – 7.15 (m, 2H), 3.92 – 3.86 (m, 2H), 3.75 – 3.70 (m, 1H), 3.13 (s, 3H), 3.05 – 2.99 (m, 1H), 2.82 (dd, *J* = 13.3, 4.5 Hz, 1H), 2.78 – 2.72 (m, 2H), 2.72 – 2.61 (m, 4H), 2.49 – 2.44 (m, 8H), 1.79 (dt, *J* = 13.2, 7.1 Hz, 1H), 1.70 – 1.63 (m, 2H), 1.43 – 1.38 (m, 1H), 1.36 (s, 10H), 1.32 – 1.23 (m, 1H), 1.24 – 1.17 (m, 1H).

*Step 2: Synthesis of 5-(4-((2S,4R)-2-(4-chlorobenzyl)-4-methoxypyrrolidin-1-yl)piperidin-1-yl)-1H-1,2,4-triazol-3-amine bis-2,2,2-trifluoroacetate (**29**).* The title compound was prepared according to the General Procedure C and purified by preparative HPLC to give 21 mg of **29** as a bis-trifluoroacetate salt (0.034 mmol, 34%). LC-MS (ES+): m/z 391.4/393.4 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.39 – 7.34 (m, 2H), 7.30 – 7.26 (m, 2H), 4.07 – 4.03 (m, 1H), 4.03 – 3.96 (m, 1H), 3.89 – 3.82 (m, 2H), 3.56 (d, *J* = 12.4 Hz, 1H), 3.52 – 3.46 (m, 1H) 3.36 – 3.28 (m, 1H), 3.27 (s, 3H), 3.21 (dd, *J* = 13.5, 4.5 Hz, 1H), 2.92 – 2.80 (m, 3H), 2.18 – 2.08 (m, 2H), 2.03 – 1.98 (m, 1H), 1.83 – 1.77 (m, 1H), 1.79 – 1.65 (m, 2H).

(S)-5-(4-(3-(4-chlorobenzyl)morpholino)piperidin-1-yl)-4H-1,2,4-triazol-3-amine (30). *Step 1: Synthesis of 2-chloro-N-[(1S)-1-(4-chlorobenzyl)-2-hydroxyethyl]acetamide (**65**).* To a solution of amino alcohol **64** (5.0 g, 27.09 mmol) in THF (162 mL) TEA (4.6 mL, 32.50 mmol) was added and the solution was cooled to 0 °C. Chloroacetyl chloride (2.1 mL, 27.09 mmol) was added slowly in such a manner that the internal temperature of the reaction was below 5 ºC. The cooling bath was then removed and the mixture was stirred for further 20 min. Diethyl ether was then added and the whole reaction mixture was sequentially washed with 1M HCl, 1M NaOH, brine, dried over $MgSO_4$, filtered and evaporated to dryness to give the crude product. Crystallization from hot diethyl ether gave compound **65** (6.0 g, 85%). LC-MS (ES+): m/z 262.2/264.2 $[M + H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.05 (d, *J* = 8.5 Hz, 1H), 7.33 – 7.25 (m, 2H), 7.23 – 7.14 (m, 2H), 4.85 (t, *J* = 5.5 Hz, 1H), 3.95 (s, 2H), 3.90 – 3.78 (m, 1H), 3.38 – 3.25 (m, 3H), 2.80 (dd, *J* = 13.7, 5.4 Hz, 1H), 2.60 (dd, *J* = 13.7, 8.6 Hz, 1H).

*Step 2: Synthesis of (5S)-5-(4-chlorobenzyl)morpholin-3-one (**66**).* To a solution of 5.1 g of compound **65** (19.45 mmol) in THF (195 mL) sodium hydride 50% in mineral oil (2.8 g, 58.35 mmol) was added all at once at 0 °C and the reaction mixture was allowed to stir at room

temperature for 2 h. The excess of sodium hydride was then carefully quenched by dropwise addition of brine and then additional volume of brine was added causing phases separation. The organic layer was separated and the aqueous layer was additionally extracted with diethyl ether. Combined organic extracts were then dried over $MgSO_4$, filtered and the solvents were evaporated. Crude product was sufficiently pure to be used in the next step without any additional purification (2.6 g, 59%). LC-MS (ES+): m/z 226.2/228.2 $[M + H]^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 7.33 – 7.28 (m, 2H), 7.14 – 7.07 (m, 2H), 6.10 (brs, 1H), 4.20 – 4.09 (m, 2H), 3.87 (dd, *J* = 11.8, 3.7 Hz, 1H), 3.74 – 3.65 (m, 1H), 3.55 (dd, *J* = 11.8, 6.2 Hz, 1H), 2.90 – 2.80 (m, 1H), 2.71 (dd, *J* = 13.6, 8.5 Hz, 1H).

*Step 3: Synthesis of (5S)-5-(4-chlorobenzyl)morpholine (**67**).* To a solution of morpholin-3-one **66** (2.3 g, 10.36 mmol) in THF (30 mL) BH_3·DMS complex (3.1 mL, 31.08 mmol) was added and the reaction mixture was refluxed for 3 h, after which time the TLC control showed complete consumption of the starting material. Reaction mixture was cooled to room temperature and 2M HCl was cautiously added (30 mL). The resulting reaction mixture was refluxed for 2 h and cooled back to room temperature. The pH of the solution was then made strongly alkaline (~10) by a dropwise addition of 4M NaOH. The organic layer was separated and the aqueous layer was additionally extracted with diethyl ether. Combined organic extracts were then dried over $MgSO_4$, filtered and the solvents were evaporated. The crude morpholine **67** was sufficiently pure to be used in the next step without any additional purification (1.6 g, 7.54 mmol, 73%). LC-MS (ES+): m/z 212.2/214.2 $[M + H]^+$. ^1H NMR (500 MHz, $CDCl_3$) δ 7.28 (d, *J* = 8.3 Hz, 2H), 7.11 (d, *J* = 8.3 Hz, 2H), 3.81 – 3.75 (m, 2H), 3.55 – 3.49 (m, 1H), 3.24 (t, *J* = 10 Hz, 1H), 3.0 – 2.98 (m, 1H), 2.89 – 2.8 (m, 2H), 2.62 (dd, *J* = 4.9, 13.5 Hz, 1H), 2.44 (dd, *J* = 9.2, 13.5 Hz, 1H).

*Step 4: Synthesis of tert-butyl (R)-4-(2-(4-chlorobenzyl)morpholin-1-yl)piperidine-1-carboxylate (**73**)*. The title compound was prepared according to the General Procedure B starting from morpholine **67**. Product was purified by silica-gel chromatography (elution with hexanes : AcOEt; 1 : 1) yielding 1.58 g (4.0 mmol, 53%) of pure compound **73**. LC-MS (ES+): m/z 395.1/397.1 $[M + H]^+$. ^{1}H NMR (700 MHz, DMSO-d_6) δ 7.33 – 7.29 (m, 2H), 7.25 – 7.20 (m, 2H), 3.92 (t, J = 14.8 Hz, 2H), 3.68 – 3.58 (m, 2H), 3.31 (dd, J = 11.6, 5.5 Hz, 1H), 3.11 – 2.97 (m, 2H), 2.91 (d, J = 13.8 Hz, 1H), 2.79 – 2.69 (m, 3H), 2.69 – 2.62 (m, 2H), 1.79 – 1.75 (m, 1H), 1.76 – 1.71 (m, 1H), 1.38 (s, 9H), 1.36 – 1.21 (m, 2H), one proton overlapping with water signal.

*Step 5: Synthesis of (S)-5-(4-(3-(4-chlorobenzyl)morpholino)piperidin-1-yl)-4H-1,2,4-triazol-3-amine (**30**).* The title compound was prepared according to the General Procedure C and purified by crystallization from hot acetonitrile. 694 mg of **30**, (1.84 mmol, 46% over three steps) was obtained. HRMS calculated for $C_{18}H_{26}ClN_6O$ $[M + H]^+$ 377.1851, HRMS found 377.1851. ^{1}H NMR (700 MHz, DMSO-d_6 + D_2O, 348K) δ 7.35 – 7.31 (m, 2H), 7.28 – 7.19 (m, 2H), 5.68 (brs, 2H), 4.89 – 4.65 (m, 1H), 3.87 – 3.69 (m, 3H), 3.63 – 3.48 (m, 3H), 3.37 – 3.32 (m, 2H), 3.23 (dd, J = 11.1, 5.4 Hz, 1H), 2.94 – 2.76 (m, 3H), 2.71 – 2.59 (m, 2H), 1.75 (d, J = 12.4 Hz, 1H), 1.67 (d, J = 12.4 Hz, 1H), 1.47 (qd, J = 12.0, 4.1 Hz, 1H), 1.34 (qd, J = 12.0, 4.2 Hz, 1H).

5-(4-(3-(4-chlorobenzyl)morpholino)piperidin-1-yl)-4H-1,2,4-triazol-3-amine (*rac*-30).

This compound was synthesized for the purpose of assessment of the optical purity of compound **30** (see Supporting Information). Starting from racemic 2-amino-3-(4-chlorphenyl)propan-1-ol, the synthesis was accomplished exactly in the same manner as for compound **30**. 105 mg of ***rac*-30** was obtained.

Biological Methods (*In Vitro*).

Enzymatic Assays: IC_{50} Determination toward Human and Mouse AMCase and Human and Mouse CHIT1. Human and mouse AMCase and human and mouse CHIT1 recombinant proteins were produced in CHO-K1 cells after transient transfection with plasmid coding full-length protein with C-terminal His-tag. The proteins were purified by nickel-affinity chromatography. Chitinolytic activity of recombinant enzymes was measured using standard assay as previously described [21, 27, 28]. For determination of enzymatic activity 103 μM 4-methylumbelliferyl β-D-N,N′diacetylchitobioside hydrate and 5.2 ng per well of hAMCase, 46 μM 4-methylumbelliferyl β-D-N,N′-diacetylchitobioside hydrate and 3 ng per well of mAMCase, 5 μM 4-methylumbelliferyl β-D-N,N′,N″-triacetylchitotrioside and 0.2 ng per well of hCHIT1 or 20 μM 4-methylumbelliferyl β-D-N,N′,N″-triacetylchitotrioside and 2 ng per well of mCHIT1 were used. Appropriate substrate, enzyme, and varying concentrations of compounds in assay buffer (0.1 M citrate, 0.2 M dibasic phosphate, 1 mg/mL BSA) were incubated in a 96-well black microtiter plate with shaking in the dark at 37 °C for 60 min followed by addition of stop solution (0.3 M glycine/NaOH buffer, pH 10.5). Substrate hydrolysis product 4-methlyumbelliferone was measured fluorometrically using Spark M10 (Tecan) microplate reader (excitation 355 nm/emission 460 nm). IC_{50} values of all inhibitors against AMCase and CHIT1 were determined from dose−response sigmoidal curves of the % of inhibition *vs* log(inhibitor concentration) using GraphPad Prism version 6.0. Experiments were performed in duplicate.

hERG Binding Assay. To characterize the affinity of compounds to the hERG channel, the Predictor hERG fluorescence polarization assay kit (Invitrogen) was used according to the manufacturer's protocol [31].

Biological Methods (*In Vivo*). All *in vivo* experiments were performed in accordance with the guidelines of the Institute for Animal Care and Use Committee in Poland, and all protocols were approved by Local Ethic Committee, Warsaw, Poland.

Pharmacokinetic Measurements in Mice. The PK properties of compound **30** were evaluated in female BALB/c mice following single intravenous bolus (3 mg/kg) or oral (10 mg/kg)administration. Compound **30** was prepared in a 30% PEG400/70% water vehicle for intravenous bolus and oral administrations at 5 mL/kg, respectively, and administered to 2 mice/group per time point with samples collected out to 24 h post-dose. Blood collection was performed by cardiac puncture under anesthesia with sampling of blood into K_2EDTA anticoagulant tubes, followed by centrifugation to obtain plasma. Samples were stored frozen at −80 °C or lower prior to compound extraction and LC/MS/MS analysis. Pharmacokinetic parameters of compound **30** in mice were calculated by standard noncompartmental modeling from the systemic plasma concentration−time profile.

Pharmacokinetic Measurements in Rats. The PK properties of compound **30** were evaluated in male Sprague-Dawley (SD) rats following single intravenous bolus (3 mg/kg) or oral (10 mg/kg) administration. Compound **30** was prepared in a 10% Solutol/10% ethanol/5% glucose in saline vehicle for intravenous bolus and oral administrations at 1.5 mL/kg or 5 mL/kg, respectively. Blood collection was performed at 5 min (IV only), 15 min , 30 min, 1h, 2h, 4h, 6 and 12h post compound **30** administration (n = 2) serially from same animal by tail vein puncture without anesthesia. Blood was collected into K_2EDTA anticoagulant tubes and followed by centrifugation to obtain plasma. Plasma samples snap frozen and stored at −80 °C or lower prior to compound extraction and LC/MS/MS analysis. Pharmacokinetic parameters of compound **30** in rats were

calculated by standard noncompartmental modeling from the systemic plasma concentration−time profile.

Measurement of Compound 30 Concentration in Brain. In order to assess the concentration of compound **30** present in the brain at steady state in relation to that in blood, compound **30** was administered to female BALB/c mice following single oral administration at 10 mg/kg dose. Compound **30** was prepared in a 10% ethanol/10% solutol/5% glucose in saline vehicle at 5 mL/kg and administered to 2 mice/group per time point. Blood and brain were collected at 30 min, 2 h and 4 h post administration. Firstly, blood collection was performed by cardiac puncture under anesthesia with sampling of blood into K_2EDTA anticoagulant tubes, followed by centrifugation to obtain plasma. Then, brain was dissected, weighted and homogenized in 10% ethanol in water. The samples were stored frozen at −80°C prior to compound extraction and LC/MS/MS analysis. The relative brain bioavailability was calculated as $F_{rel(brain/plasma)}=AUC_{brain(p.o.)}/AUC_{plasma(p.o.)}\times 100\%$.

House Dust Mite (HDM) Mouse Model of Allergic Pulmonary Inflammation. Determination of the therapeutic efficacy of compound **30** was carried in 3-week-long exposure HDM-induced mouse model of allergic inflammation. Briefly, 2 groups (n=8; HDM with or without compound **30** treatment) of age-matched 8-week-old female C57BL/6 mice (Charles River, Germany) were subjected to intranasal exposure of 40 μg of HDM extract (Greer, Lenoir, NC) in 25 μl PBS solution 5 times per week for 19 days. In addition, naive mice were subjected to PBS intranasal challenges at times of HMD challenges (n=8). Compound **30** was administered orally (20 μL/g) in a dose of 50 mg/kg starting on day 7 onwards representing therapeutic treatment scheme as compared to vehicle treated controls. The mice were allowed to rest 24h post last HDM and compound **30** administration, and on day 19th animals were euthanized by lethal Morbital®

injection (sodium pentobarbital 26.7 mg/mL, pentobarbital 133.3 mg/mL; Biovet, Poland) and material was collected for subsequent assessment of pulmonary inflammation. Briefly, lungs were washed *via* trachea using 1 ml of PBS to collect BALf. The BALf was centrifuged (10 min, 2000 rpm, 4°C) and cells pellet was resuspended in 300 μL of PBS and subsequently used for FACS analysis for CD45-positive leukocyte population identification.

Pulmonary Inflammation Assessment. The inflammatory cells influx was analyzed in BALf. Total BALf cells were counted manually using Burker chamber and were further characterized with flow cytometry (FACSAria, BD) to determine total leukocyte (CD45-positive) total numbers in BALf.

Molecular modeling

The initial conformation of compound **2** was taken from the experimentally solved structure (PDB ID: 5NR8). Three dimensional structure of compound **30** was built using the structure of **2** and OpenBabel [37], and further refined using MOPAC7 with PM3 method [38]. Molecular docking was performed using rDock with desolvation scoring function [39] to AMCase (PDB ID: 3RM4) and CHIT1 (PDB ID: 5NR8). For each ligand, 100 poses were generated, and top scoring 10 models were inspected visually. Poses with a binding mode most similar to the native ligands were accepted for the further analysis.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Computational models of AMCase with compounds **2** and **30** and CHIT1 with compound **30** in PDB format.

Molecular formula strings, IC_{50} (CSV)

^{1}H NMR spectra and HPLC profiles for compounds **4**−**30**, chiral HPLC profiles for compounds **30** and ***rac*-30**, crystallographic information for compound **50**, analysis of Trp99 side-chain orientation in published AMCase crystallographic structures (PDF), comparison of human and murine AMCase sequences and their binding sites (experimentally solved and homology model, respectively), comparison of human and murine CHIT1 sequences and their binding sites (experimentally solved and homology model, respectively)

Accession Codes

Coordinates have been deposited in the CCDC with accession code for compound **50**: CCDC 1901012. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/structures

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): Some of the authors are current employees of OncoArendi Therapeutics S.A. and own company stocks.

ACKNOWLEDGEMENTS

Studies were supported by two projects: (1) “Development of a first-in-class small molecule drug candidate for treatment of idiopathic pulmonary fibrosis through chitotriosidase inhibition” (POIR.01.01.01-00-0551/15), acronym IPF and (2) “Preclinical research and clinical trials of a first-in-class development candidate in therapy of asthma and inflammatory bowel disease” (POIR.01.01.01-00-0168/15), acronym IBD – both cofinanced by the National Centre for Research and Development in the framework of European Funds Smart Growth. We thank Dr. Damian

Trzybiński and Prof. Krzysztof Woźniak for collecting and analyzing the crystallographic data. This research was carried out at the Biological and Chemical Research Centre, University of Warsaw, co-financed from the European Regional Development Fund. The data were collected at the Core Facility for Crystallographic and Biophysical Research to support the development of medicinal products sponsored by the Foundation for Polish Science (FNP).

ABBREVIATIONS USED

COPD, chronic obstructive pulmonary disease; CHIT1, chitotriosidase; hCHIT1, human chitotriosidase; mCHIT1, mouse chitotriosidase; AMCase, acidic mammalian chitinase; hAMCase, human acidic mammalian chitinase; mAMCase, mouse acidic mammalian chitinase; IL-13, interleukin-13; Th2, type 2 helper T cell; HDM, house dust mite; TGFβ, transforming growth factor beta; DAT, dopamine active transporter; CL, clearance; AUC_{0-inf}, area under the curve; C_{max}, peak plasma concentration of a drug after administration; t_{max}, time to reach C_{max}; F, bioavailability; PBS, phosphate buffered saline; BALf, bronchoalveolar lavage fluid; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; FACS, fluorescence-activated cell sorting; CHO-K1, Chinese hamster ovarian cell, K1 type; tR, retention time.

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