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Design, synthesis and evaluation of small molecule CD4-mimics as entry inhibitors possessing broad spectrum anti-HIV-1 activity

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Abstract

Since our first discovery of a CD4-mimic, NBD-556, which targets the Phe43 cavity of HIV-1 gp120, we and other groups made considerable progress in designing new CD4-mimics with viral entry-antagonist property. In our continued effort to make further progress we have synthesized twenty five new analogs based on our earlier reported viral entry antagonist, NBD-11021. These compounds were tested first in HIV-1 Env-pseudovirus based single-cycle infection assay as well as in a multi-cycle infection assay. Four of these new compounds showed much improved antiviral potency as well as cytotoxicity. We selected two of the best compounds **45A (NBD-14009)** and **46A (NBD-14010)** to test against a panel of 51 Env-pseudotyped HIV-1 representing diverse subtypes of clinical isolates. These compounds showed noticeable breadth of antiviral potency with IC₅₀ of as low as 150 nM. These compounds also inhibited cell-to-cell fusion and cell-to-cell HIV-1 transmission. The study is expected to pave the way of designing more potent and selective HIV-1 entry inhibitors targeted to the Phe43 cavity of HIV-1 gp120.

Keywords

HIV-1; ENV-pseudovirus; virus entry antagonist; broad spectrum; structure-activity relationship

1. Introduction

Introduction of highly active anti-retroviral therapy (HAART), which uses a combination of drugs, made a remarkable impact in the AIDS epidemic by converting it to a manageable illness from once considered as a fatal disease. Despite this success more than 36 million people are living with AIDS and nearly 2 million people get newly infected each year (UNAIDS Global AIDS Update 2016). The major stumbling block in making any

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appreciable dent in such high HIV infection rate is drug resistance, side effects of the drugs and patient compliance. Moreover, none of the currently marketed drugs are curative of HIV. Therefore, there is an urgent need to develop new drugs, especially against targets critically important for HIV-1 life cycle. HIV-1 envelope glycoprotein is such a critical target. Soluble CD4 (sCD4) was first used to target gp120 and shown to inhibit a diverse strain of HIV and SIV¹. The clinical trial with sCD4 indicated that even at high dose level it failed to reduce the virus titer in a significant level². The only fusion inhibitor, Fuzeon (Enfuvirtide), a 36-residue based peptide drug that targets envelope glycoprotein gp41, was approved in 2003. Another entry inhibitor, Maraviroc, that targets the host coreceptor CCR5, was approved in 2007. Since then no entry/fusion inhibitors got approval for human use.

We reported the discovery of two small molecule HIV-1 inhibitors in 2005 by targeted screening of commercial libraries to the Phe43 cavity of HIV-1 envelope glycoprotein gp120³. Since then other groups also made considerable progress in designing inhibitors targeting the Phe43 cavity based on our earlier discovery⁴⁻¹⁵. In 2012, we reported the co-crystal structure of NBD-556 bound to gp120 and confirmed that indeed this small molecule of 337 Da molecular weight binds to the Phe43 cavity¹⁶. However, later it was discovered that despite their anti-HIV-1 activity, this molecule also mimicked CD4⁸ and induced conformational changes in gp120 which may make it conducive to CCR5 coreceptor binding. In other words, this molecule behaves as viral entry agonist, an undesirable property of gp120 targeted drugs. We exploited the x-ray structural information of NBD-556 and other next generation NBD compounds bound to HIV-1 gp120¹⁷ to design next generation CD4-mimics with the goal of achieving viral entry antagonist property. We recently reported the structure-based design of NBD-11021 which showed not only much potent broadly neutralizing activity but also this molecule was experimentally confirmed to be a viral entry antagonist, a much desired property of this class of molecules¹⁸.

The x-ray structure of NBD-11021 bound to HIV-1 gp120 confirmed that this molecule binds to the Phe43 cavity¹⁸ but in a slightly different orientation than we observed with NBD-556 and other partial antagonists such as NBD-09027 so that the piperidine “N” forms a H-bond with Asp368, a critical interaction that was missing in all our compounds so far. The wide breadth of anti-HIV-1 activity profile of NBD-11021 against a large panel of HIV-1 pseudoviruses representing diverse subtypes of clinical isolates and availability of its structural information in complex with gp120 motivated us to continue the design of more robust and active analogs of this molecule.

Here we report the design, synthesis, biological evaluation and structure-activity relationships (SAR) of several new analogs of NBD-11021, some of which showed substantial improvement in antiviral activity and cytotoxicity profile. The study is expected to pave the way in designing more potent entry inhibitors with improved selectivity index for further preclinical studies.

2. Results and Discussion

2.1. Chemistry

The basic synthesis routes to make this series of NBD-compounds have been previously reported¹⁸. First, we prepared imine **2** from racemic pipecolic acid and racemic *tert*-butanesulfinamide (**Scheme 1**). Then two protected thiazoles **4** and **9** were prepared from 5-(2-hydroxyethyl)-4-methylthiazole and thiazole **10** was prepared as reported¹⁸. 1,2-addition of thiazoles **10** and **4** to imine **2** yielded a separable mixture of isomeric products **11A/B** and **12A/B** and addition of thiazole **9** gave a single stereoisomer **13B**. After chromatographic purification the stereoisomers were separately used in the following steps. The synthetic intermediates derived from **11A** or **12A** have been labeled with an “A” letter in their numbering system (**14A**, **15A**, etc.). The synthetic intermediates derived from **11B**, **12B** or **13B** have been labeled with a “B” letter in the numbering system (**14B**, **15B**, etc.). After acidic deprotection the amines were acylated with one of three commercially available 5-aryl-pyrrole-2-carboxylic acids (**17-19**). Finally Pd-catalyzed deprotection provided the target compounds **21-28**. Compounds **21A** and **22A** and **21B** and **22B** were obtained from amine **14A** and **14B**, respectively, configurations of which were unambiguously determined in our previous work¹⁸. For the rest of the compounds stereochemistry was assigned based on analogy i.e., isomer with *R,R* or *S,S* configuration should be dominant product in the 1,2-addition to imine **2**.

For the preparation of imidazole-based NBD compounds, we relied on the same strategy (**Scheme 2**). However, the acidic N-H group of imidazole demanded an additional protecting group. After small experimentation we chose *N,N*-dimethylsulfamoyl group, because it can coordinate with Li, thus facilitating C-metalation of imidazole and it can be cleaved in one pot with TBS and SO₂ tBu groups.

To this end imidazole **30** was prepared from (5-methyl-1H-imidazol-4-yl)methanol **29** as described^{19;20} and treated with *N,N*-dimethylsulfamoyl chloride to yield a mixture of isomers **31A** and **31B**. The mixture of **31A** and **31B** was deprotonated with *n*-BuLi and imine **2** was added to give a mixture of eight enantiomeric pairs. This mixture was treated with MeOH-HCl to cleave three of four protecting groups. Fortunately, after deprotection stereoisomers can be separated, the isomer with higher *R_f* was marked as **33A** and the isomer with lower *R_f* was marked as **33B**. Unlike in the case of compound **14-16** no stereochemical assignment was made at this point. Amines **33A** and **33B** were separately converted into compounds **35-37A/B**.

NBD-compounds without piperidine ring were prepared by a slightly modified route. First, amino acetaldehyde dimethyl acetal was protected with alloc and allyl groups and aldehyde functionality was unmasked by treatment with aqueous formic acid (**Scheme 3**). Then, enantiopure *tert*-butanesulfinamide was used to prepare both *R*- and *S*-imine **41**. Separately, enantiomers of **41** were treated with metalated thiazole **10** to give a single addition product **42**.

Compounds derived from (*S*)-**41** and (*R*)-**41** were marked as *S*-**42** and *R*-**42**, respectively (**Scheme 4**). According to the Felkin-Ahn model the newly formed stereo center has

opposite configuration than sulfur-stereo center. In other words compounds *fS*-43, *fS*-44, **45B**, **46A**, **47A** have *R*-configuration, and compounds *fR*-43, *fR*-44, **45A**, **46B**, **47B** have *S*-configuration. Since this was not confirmed unambiguously (by X-Ray analysis or other method) we prefer to use *fS*/*fR* markers for the sake of better reproducibility of this work.

As described in **Scheme 3**, three-step synthesis yielded compounds **45A** - **47B**. The enantiopurity of compounds **45A** and **45B** was measured using chiral column (77% and 88% respectively). We assume that stereochemical integrity is preserved during last three steps of the sequence and compounds **45A**, **46A**, **47A** have the same enantiomeric excess (*ee*) value as the parent amine *fS*-43 (77%). The same implies for compounds **45B**, **46B**, **47B** (*ee*=88%).

2.2. Biological Screening

2.2.1. Antiviral Screening of the new analogs by single-cycle and multi-cycle infectivity assays—We recently reported the successful conversion of a viral entry agonist NBD-556 to a viral entry antagonist NBD-11021¹⁸ by modifying regions II and III of the NBD-556 structure. Here we report the design of a new generation of analogs of NBD-11021 and evaluation of their HIV-1 inhibitory activity in a single-cycle and a multi-cycle infectivity assays (**Table 1**). We observed that compounds **21A** to **28B** did not show any improvement in antiviral activity or toxicity compared to NBD-11021A2 in both assays. It is noteworthy that introduction of a meta fluoro substituent in NBD-11021A2 resulted in very similar results in a single-cycle assay but yielded ~2-3-fold lower activity in the multi-cycle assay (IC₅₀ 0.85 μ M for NBD-11021A2 compared to 1.6 and 2.6 μ M for **21A** and **21B**, respectively). This is contrary to the report that introduction of a fluoro substituent in the same position of the phenyl ring of NBD-556 improved the antiviral activity⁸. Earlier we have reported that introduction of a fluoro had mixed results²¹. However, it is noteworthy to mention that the introduction of a fluorine atom replacing hydrogen is known to improve potency or modulate physicochemical properties such as metabolic stability and p*K*_a^{22,23}. The extension of the primary alcohol substituent (CH₂OH) to longer chains (CH₂CH₂OH and CH₂CH₂CH₂OH) resulted in substantial drop in antiviral activity. In order to understand the role of thiazole ring on the antiviral potency we replaced the thiazole ring with an imidazole ring but maintained other substituents. The imidazole ring containing compounds, **35A-37B**, (**Table 1**) showed improvement in cytotoxicity levels but antiviral activity of all these compounds became worse in both assays. No specific structure-activity relationship (SAR) could be derived from this series. We also explored to alter piperidine ring to a simple amine because the x-ray structure of NBD-11021 bound to HIV-1 gp120 revealed that the hydrophobic part of the piperidine ring is located in the solvent exposed area, which is not desirable¹⁸. We used stereo selective synthesis to obtain the new series of NBD compounds. Compound **45A**, where the Phenyl ring has only 4-Cl group, we observed that the antiviral activity is very similar to piperidine containing compound NBD-11021A2 [IC₅₀ Of 2.2 μ M vs 2.1 μ M for **45A**] in a single-cycle assay; however, cytotoxicity improved by about 2-fold, similar to the imidazole analogs. But in multi-cycle assay although the cytotoxicity improved, the antiviral activity decreased by about 3-fold. **45B**, an isomer of **45A**, showed poor activity. When we introduced a fluorine substitution in the ortho position at the phenyl ring of **45A** the resultant compound **46A** showed improvement in both antiviral and

cytotoxic activities in both single-cycle and multi-cycle assays compared to its chloro analogs, **45A**, **46B**, the other isomer of **46A**, showed very similar antiviral activity and cytotoxicity profiles to **46A** in both single-cycle and multi-cycle antiviral assay. In this series we have observed that the introduction of fluorine in the phenyl ring helped in improving potency as discussed earlier. Replacing chloro in **46A** by methyl yielded **47A** which showed slightly less antiviral potency in a single-cycle assay. The isomer, **47B** showed similar activity.

2.2.2. 45A and 46A are viral entry antagonists.—NBD-11021 represents our first example of a viral entry antagonist¹⁸. Since we made further chemical modifications to derive new analogs it was imperative to investigate whether the two best second generation compounds, **45A** and **46A** are also maintaining the viral entry antagonist trait. In order to verify we infected CD4-negative Cf2Th-CCR5+ cells with recombinant CD4-dependent HIV-1_{ADA} virus in the presence of varied concentrations of **45A** and **46A**. The viral entry agonist NBD-556 and the viral entry antagonist NBD-11021 were used as control (**Figure 1**). As expected, both compounds worked as viral entry antagonists. In other words, they did not enhance HIV-1 infectivity in the CD4 negative cells suggesting that the viral entry antagonist property was preserved in these compounds.

2.2.3. Antiviral activity of 45A and 46A against a panel of HIV-1 Env pseudotyped reference viruses—We reported that NBD-11021 showed a broad range of antiviral activity against a large panel of Env-pseudotyped HIV-1 representing diverse subtypes of clinical isolates of different subtype. Here we present the results of these second generation compounds, **45A** and **46A**, against a panel of 51 Env-pseudotyped viruses including 13 recombinant HIV-1 clones, and we compared their antiviral activity with NBD-11021A2 (**Table 2**). Both, **45A** and **46A** exhibited improved anti-HIV-1 activity with respect to the first viral entry antagonist NBD-11021A2 (IC₅₀ in the range 0.32-4.4 μ M, with the overall mean of 1.65 ± 0.13 μ M). In fact, **45A** was consistently active against all the pseudoviruses tested displaying an IC₅₀ in the range of 0.32-2.3 μ M and the overall mean of 1.05 ± 0.07 μ M. Furthermore, **46A** showed a substantially improved anti-HIV-1 activity. The calculated IC₅₀s of this inhibitor were in the range of 0.15-0.87 μ M and the overall mean was 0.46 ± 0.03 μ M, with a nearly 4-fold improvement of the IC₅₀ with respect to NBD-11021A2. Finally, both **45A** and **46A** exhibited poor activity against the control pseudovirus VSV-G indicating that the inhibition of these compounds is more specific to HIV-1.

2.2.4. 45A and 46A prevent Cell-Cell fusion—CD4-gp120 interaction is critical in initiating the fusion of membranes of infected cells with neighboring cells inducing the formation of syncytia. Since these compounds target HIV-1 entry pathway, we investigated whether **45A** and **46A** could prevent HIV-1 Env-mediated cell-cell fusion. We cocultured MAGI-CCR5 cells with Env-expressing HL2/3 cells in the presence of different concentrations of NBD-compounds. NBD-11021A2 was used as control because we previously reported it to inhibit the cell-cell fusion process. As we expected, both, **45A** and **46A** inhibited the HIV-1 Env-mediated cell-to-cell fusion (**Table 3**).

2.2.5. 45A and 46A inhibit cell-to-cell HIV-1 transmission—The cell-to-cell HIV-1 infection has been shown to be more efficient than the HIV-1 infection by cell-free virus, most likely because viruses are relatively protected from the environment and for the very high concentration of viral particles at the cellular contact sites ²⁴. We previously reported that NBD-11021 inhibits cell-to-cell HIV-1 transmission while NBD-556 failed to do so. Therefore, we tested the activity of **45A** and **46A** in cell-to-cell HIV-1 transmission assay. Ghost X4/R5 cells were used as target and H9/HIV-1_{IIIB} and MOLT-4/HIV-1_{ADA} as effector cells. Our results (**Table 3**) indicate that both **45A** and **46A** has similar activity to NBD-11021A2 against the cell-to-cell transmission of the CXCR4-tropic HIV-1 virus. Moreover, we detected similar but poor activity for **45A**, **46A** and NBD-11021A2 in the CCR5-tropic HIV-1 cell-to-cell transmission assay.

3. Conclusion

In this study we have reported the design, synthesis, antiviral activity and SAR of twenty five new analogs of NBD-11021A2, one of the best structure-based designed lead compounds that we reported earlier¹⁸. The antiviral assays in TZM-bl and MT-2 cells in **Table 1** showed a good correlation ($R^2 = 0.78$; with $p < 0.0001$). It was not possible to derive a correlation on the data in **Table 2** because a diverse set of viruses were used. We also did not attempt to correlate these assays with the cell-cell fusion and virus transmission assays due to completely different nature of the assays. The SAR revealed that the thiazole ring in the series of compounds (**21A – 28B** and **45A – 47B**), showed the best potent compounds when only CH₂OH substituent is present in the thiazole ring. However, when the thiazole ring was replaced by an imidazole ring, the activity dropped substantially although their cytotoxicity improved somewhat. The most dramatic improvement in antiviral activity and cytotoxicity were observed when we replaced the entire piperidine ring with a simple primary amine (CH₂NH₂). The best compound **46A** also showed noticeable improvement in activity compared to NBD-11021A2 when tested against a large panel of pseudoviruses from a diverse set of Env from clinical isolates representing a wide variety of HIV-1 subtypes. **46A** also inhibited cell-to-cell HIV-1 transmission and represents a substantially improved lead compound. It is important to mention that we are also aware that there is potential risk of development of resistance against these compounds since certain sections of gp120 are highly variable. Therefore, we are planning in depth studies on the resistance profile, if any, of these series of compounds. We anticipate that these studies and identification of substantially improved leads are expected to pave the way for designing more potent HIV-1 entry inhibitors.

4. Experimental

4.1. General

Commercial reagents and solvents were used without further purification. All reactions were performed in the air atmosphere unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) carried out on Merck TLC Silica gel plates (60 F254). We used UV light for visualization and basic aqueous potassium permanganate or iodine fumes as a developing agent. NMR (¹H and ¹³C) spectra were recorded on Bruker Avance 400

instrument with operating frequency of 400 and 100 MHz respectively and calibrated using residual undeuterated chloroform ($\delta\text{H} = 7.28$ ppm) and CDCl_3 ($\delta\text{C} = 77.16$ ppm), or undeuterated DMSO ($\delta\text{H} = 2.50$ ppm) and $\text{DMSO}-d_6$ ($\delta\text{C} = 39.51$ ppm) as internal references. The following abbreviations are used to set multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br. = broad.

4.1.1. 5-(2-((tert-butyldimethylsilyl)oxy)ethyl)-4-methylthiazole (4)—4-Methyl-5-thiazoleethanol (10.0 g, 69.8 mmol) was dissolved in DMF (70 mL), imidazole (5.70 g, 83.8 mmol, 1.20 equiv) was added in one portion followed by portion wise addition of TBSCl (11.6 g, 77.0 mmol, 1.10 equiv). The reaction mixture was stirred overnight at 50-60 °C, cooled to r.t., diluted with water (0.5 L) and extracted with hexane (3×100 mL). The combined organic phases were dried over Na_2SO_4 , and evaporated to give an oil which was purified by distillation at reduced pressure. bp: 115 - 120 °C (1-2 torr). Yield: 82% (14.71 g).

^1H NMR (CDCl_3 , 400 MHz): $\delta = 0.03$ (s, 6H), 0.90 (s, 9H), 2.41 (s, 3H), 2.97 (t, $J = 6.4$ Hz, 2H), 3.79 (t, $J = 6.4$ Hz, 2H), 8.57 (s, 1H).

^{13}C NMR (CDCl_3 , 100 MHz): $\delta = -5.3$ (2C), 15.1, 18.4, 26.0 (3C), 30.0, 63.4, 128.2, 149.3, 149.7.

4.1.2. 5-(2-chloroethyl)-4-methylthiazole (5)—4-Methyl-5-thiazoleethanol (39.0 g, 272 mmol) was dissolved in CHCl_3 (270 mL) and SOCl_2 (40 mL, 0.55 mol, ~2 equiv) was added dropwise with cooling on a water bath. The mixture was refluxed for 4 hours, cooled to r.t. and evaporated. The residue was suspended in CH_2Cl_2 and aqueous K_2CO_3 (38 g, 0.28 mol, ~1 equiv in 200 mL H_2O). After stirring for 10 minutes the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2×100 mL). The combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by distillation at reduced pressure. bp: 77 - 78 °C (1-2 torr.); Yield: 84% (36.97 g).

^1H NMR (CDCl_3 , 400 MHz): $\delta = 2.37$ (s, 3H), 3.17 (t, $J = 7.0$ Hz, 2H), 3.62 (t, $J = 7.0$ Hz, 2H), 8.56 (s, 1H).

^{13}C NMR (CDCl_3 , 100 MHz): $\delta = 14.9$, 29.6, 44.2, 127.0, 149.9, 150.2.

4.1.3. 3-(4-methylthiazol-5-yl)propanenitrile (6)—NaCN (11.06, 0.226 mol) was suspended in DMF and 5-(2-chloroethyl)-4-methylthiazole (36.50 g, 0.226 mole) was added and the mixture was stirred for 8 hours at r.t. and then at 60 - 80 °C. The reaction mixture was cooled to r.t., diluted with (0.5 L) and extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were dried over Na_2SO_4 and evaporated. The residue was pure enough for the next step. Yield: 98% (33.55 g).

^1H NMR (CDCl_3 , 400 MHz): $\delta = 2.40$ (s, 3H), 2.60 (t, $J = 7.2$ Hz, 2H), 3.11 (t, $J = 7.2$ Hz, 2H), 8.59 (s, 1H).

^{13}C NMR (CDCl_3 , 100 MHz): $\delta = 14.9$, 19.4, 22.5, 118.3, 127.1, 150.2, 150.3.

4.1.4. 3-(4-Methyl-thiazol-5-yl)-propionic acid methyl ester (7)—3-(4-

methylthiazol-5-yl)propanenitrile (33.52 g, 0.22 mol) was dissolved in MeOH (1 L), H₂SO₄ (90 mL, 1.69 mol, 7.7 equiv) was added dropwise and the reaction mixture was refluxed for two weeks (~8 hours a day). The reaction mixture was evaporated to 1/3 of a volume and poured into aqueous K₂CO₃ (276 g, 2 mol, in 0.5 L H₂O) solution and CH₂Cl₂ (500 mL). After stirring for 10 minutes the organic layer was separated and the aqueous layer was extracted with CH₂Cl₂ (2×100 mL). The combined organic layers were dried over Na₂SO₄ and evaporated. The residue was purified by means of liquid chromatography (eluent: hexanes/EtOAc, 10:1, 5:1). The upper spot is the ester the second spot is a starting material (M_{RCN}=3.50 g).

Yield: 81% (29.51 g; brsm).

¹H NMR (CDCl₃, 400 MHz): δ = 2.42 (s, 3H), 2.64 (t, *J* = 7.5 Hz, 2H), 3.11 (t, *J* = 7.5 Hz, 2H), 3.70 (s, 3H), 8.58 (s, 1H).

¹³C NMR (CDCl₃, 100 MHz): δ = 14.8, 21.6, 35.5, 51.8, 129.6, 149.2, 149.4, 172.3.

4.1.5. 3-(4-methylthiazol-5-yl)propan-1-ol (8)—A solution of 3-(4-methyl-thiazol-5-yl)-propionic acid methyl ester (29.51 g, 0.160 mol) in THF (160 mL) was added dropwise to a suspension of LiAlH₄ (6.10 g, 0.160 mmol) in THF (160 mL). The reaction mixture was stirred for 1 hour, and quenched by successive addition of water (6 mL), 10 % NaOH (6 mL) solution and water (12 mL). The precipitate was filtered and washed several times with THF. The filtrate was evaporated to give title compound, which was used without purification.

Yield: 87% (22.02 g).

¹H NMR (CDCl₃, 400 MHz): δ = 1.79 - 1.92 (m, 2H), 2.36 (s, 3H), 2.85 (t, *J* = 7.6 Hz, 2H), 3.06 (br. s, 1H), 3.65 (t, *J* = 6.2 Hz, 2H), 8.52 (s, 1H).

¹³C NMR (CDCl₃, 100 MHz): δ = 14.7, 22.6, 34.3, 61.1, 131.6, 148.5, 149.1.

4.1.6. 5-(3-((tert-butyldimethylsilyl)oxy)propyl)-4-methylthiazole (9)—3-(4-methylthiazol-5-yl)propan-1-ol (22.02 g, 140 mmol) was dissolved in DMF (140 mL), imidazole (12.40 g, 182 mmol, 1.20 equiv) was added in one portion followed by portion wise addition of TBSCl (25.3 g, 168 mmol, 1.10 equiv). The reaction mixture was stirred overnight at 50-60 °C, cooled to r.t., diluted with water (0.5 L) and extracted with hexane (3×100 mL). The combined organic phases were dried over Na₂SO₄, and evaporated to give an oil which was purified by distillation at reduced pressure. bp:bp: 120 °C (2 torr). Yield: 77% (29.16 g).

¹H NMR (CDCl₃, 400 MHz): δ = 0.06 (s, 6H), 0.91 (s, 9H), 1.75 - 1.86 (m, 2H), 2.39 (s, 3H), 2.85 (t, *J* = 7.5 Hz, 2H), 3.64 (t, *J* = 6.0 Hz, 2H), 8.55 (s, 1H).

¹³C NMR (CDCl₃, 100 MHz): δ = -5.2 (2C), 14.9, 18.4, 22.5, 26.0 (3C), 34.6, 61.7, 131.6, 148.7, 148.9.

4.1.7. General procedure for 1,2-addition reaction.—Appropriate thiazole (**4**, **9** or **10**; 1.3 equiv) was dissolved in THF (1 M) and cooled to -78°C . At this temperature *n*-BuLi (2.5 M, 22 mL, 55 mmol, 1.4 equiv) was added dropwise under the nitrogen atmosphere. The reaction mixture was stirred for 30 minutes at -78°C , and **2** (1 equiv) was added dropwise as a solution in THF (1 M). The reaction mixture was slowly (~1 hour) warmed to 0°C , and poured into saturated NH_4Cl aqueous solution (volume equals to that of the reaction mixture). The biphasic mixture was extracted with CH_2Cl_2 ($3\times 100\text{ mL}$). The combined organic phases were dried over Na_2SO_4 , and evaporated to give a brown oil which was purified by means of column chromatography.

The upper spot on TLC (EtOAc) is thiazole, then imine (if present) then two reaction products. Compounds **11A** and **11B** were synthesized as per the method reported earlier¹⁸.

4.1.8. allyl 2-((5-(2-((tert-butyldimethylsilyl)oxy)ethyl)-4-methylthiazol-2-yl)(1,1-dimethylethylsulfinamido)methyl)piperidine-1-carboxylate (12)—Eluent: hexanes/EtOAc, 3:1, then 1:1, then pure EtOAc.

First fraction: (mixture): 10.19 g. Second fraction: **12A**: 2.13 g; Third fraction: **12B**: 11.04 g.

Second fraction (**12A**): Yield: 15% (2.13 g). $R_f=0.53$ (hexanes/EtOAc, 1:1)

^1H NMR: (CDCl_3 , 400 MHz) δ = 0.03 (s, 6H), 0.89 (s, 9H), 1.19 (s, 6H), 1.24 (s, 3H), 1.40 - 1.88 (m, 9H), 2.34 (s, 3H), 2.93 (t, J = 6.1 Hz, 2H), 3.78 (t, J = 6.1 Hz, 2H), 4.42 - 4.53 (m, 1H), 4.58 - 4.80 (m, 2H), 5.08 (d, J = 9.8 Hz, 1H), 5.24 (d, J = 9.8 Hz, 1H), 5.35 (d, J = 7.1 Hz, 1H), 5.90 - 6.06 (m, 1H).

Third fraction (**12B**): Yield: 42% (11.04 g). $R_f=0.33$ (hexanes/EtOAc, 1:1);

^1H NMR: (CDCl_3 , 400 MHz) δ = 0.03 (s, 6H), 0.89 (s, 9H), 1.24 (s, 6H), 1.28 (s, 3H), 1.34 - 1.96 (m, 7H), 2.31 (s, 3H), 2.89 (t, J = 6.4 Hz, 2H), 3.75 (t, J = 6.4 Hz, 2H), 3.87 - 4.07 (m, 1H), 4.24 - 4.54 (m, 3H), 4.58 - 4.91 (m, 1H), 4.97 (dd, J = 7.8, 5.6 Hz, 1H), 5.16 (d, J = 10.5 Hz, 1H), 5.20 (d, J = 17.5 Hz, 1H), 5.63 - 6.05 (m, 1H).

4.1.9. allyl 2-((5-(3-((tert-butyldimethylsilyl)oxy)propyl)-4-methylthiazol-2-yl)(1,1-dimethylethylsulfinamido)methyl)piperidine-1-carboxylate (13B)—Only one diastereomer was formed in this reaction.

Eluent: hexanes/EtOAc, 3:1, then 1:1, then pure EtOAc; $R_f=0.52$ (EtOAc). Yield: 56% (5.35 g).

^1H NMR: (CDCl_3 , 400 MHz) δ = 0.05 (s, 6H), 0.90 (s, 9H), 1.22 (s, 6H), 1.27 (s, 3H), 1.39 - 1.83 (m, 7H), 1.85 - 2.01 (m, 1H), 2.10 - 2.38 (m, 1H), 2.18 (s, 3H), 2.28 (s, 2H), 2.76 (t, J = 7.3 Hz, 1H), 3.62 (t, J = 6.1 Hz, 2H), 3.85 - 4.08 (m, 1H), 4.16 - 4.57 (m, 3H), 4.95 (dd, J = 8.2, 5.5 Hz, 1H), 5.08 - 5.23 (m, 2H), 5.62 - 5.88 (m, 1H).

Instead a second isomer admixture 1-(5-(3-((*tert*-butyldimethylsilyl)oxy)propyl)-4-methylthiazol-2-yl)hexahydroimidazo[1,5-a]pyridin-3(2H)-one was isolated from the reaction mixture ($R_f=0.40$ (EtOAc), Yield = 32% (2.20 g).

¹H NMR: (CDCl₃, 400 MHz) δ = 0.06 (s, 6 H), 0.91 (s, 9 H), 1.19 - 1.34 (m, 2 H), 1.58 - 1.74 (m, 2 H), 1.74 - 1.85 (m, 2 H), 1.87 - 1.95 (m, 1 H), 1.98 - 2.04 (m, 1 H), 2.31 (s, 3 H), 2.69 (td, J =12.5, 3.2 Hz, 1 H), 2.79 (t, J =7.6 Hz, 2 H), 3.42 - 3.49 (m, 1 H), 3.64 (t, J =5.9 Hz, 2 H), 3.88 - 3.95 (m, 1 H), 4.56 (dd, J =6.5, 1.8 Hz, 1 H), 5.21 (br. s, 1 H).

4.1.10. General procedure for amine deprotection.—1M HCl-MeOH (5 - 20 equiv) solution was prepared by dropwise addition of AcCl to MeOH under a water bath cooling system. The resulting solution was cooled to an ambient temperature and added to a flask containing fully protected compound (**11**, **12** or **13**; **A** or **B** isomers). After dissolution the reaction mixture was stirred for 1 hour, evaporated *in vacuo* (no heating). The residue was dissolved in CH₂Cl₂ and washed with 10% aqueous K₂CO₃ solution. The organic layer was separated and the aqueous layer was extracted with CH₂Cl₂ (2×100 mL). The combined organic layers were dried over Na₂SO₄ and evaporated. The residue was purified by means of chromatography. Eluent: CH₂Cl₂/MeOH (50:1, 10:1).

Compounds **14A** and **14B** were prepared as reported previously¹⁸.

4.1.11. allyl 2-(amino(5-(2-hydroxyethyl)-4-methylthiazol-2-yl)methyl)piperidine-1-carboxylate—First fraction (**15A**). Yield: 70% (909 mg).

¹H NMR: (CDCl₃, 400 MHz) δ = 1.39 - 1.77 (m, 7H), 2.18 (br. s, 3H), 2.34 (s, 3H), 2.98 (t, J = 6.4 Hz, 2H), 2.79 - 3.05 (br. s, 1H), 3.82 (t, J = 6.4 Hz, 1H), 4.00 - 4.33 (m, 1H), 4.33 - 4.51 (m, 1H), 4.52 - 4.58 (m, 1H), 4.65 (d, J = 4.2 Hz, 2H), 5.23 (dd, J = 10.5, 1.3 Hz, 1H), 5.35 (d, J = 17.6 Hz, 1H), 5.92 - 6.05 (m, 1H).

¹³C NMR: (CDCl₃, 100 MHz) δ = 15.1, 19.4, 23.3, 24.6, 30.0, 30.2, 40.9, 59.0, 62.7, 62.8, 117.5, 129.0, 133.1, 149.1, 159.9, 168.4.

Second fraction (**15B**). Yield: 41% (1.65 g).

¹H NMR: (CDCl₃, 400 MHz) δ = 1.38 - 1.57 (m, 1H), 1.56 - 1.74 (m, 4H), 2.05 (br. s, 3H), 2.16 - 2.22 (m, 1H), 2.30 (s, 3H), 2.92 (t, J = 6.1 Hz, 2H), 3.06 (t, J = 13.1 Hz, 1H), 3.77 (t, J = 6.2 Hz, 2H), 3.99 - 4.10 (m, 1H), 4.21 - 4.34 (m, 2H), 4.34 - 4.42 (m, 1H), 4.51 (d, J = 10.0 Hz, 1H), 5.14 (dd, J = 10.5, 1.2 Hz, 1H), 5.19 (dd, J = 17.2, 1.5 Hz, 1H), 5.73 - 5.86 (m, 1H).

¹³C NMR: (CDCl₃, 100 MHz) δ = 15.0, 19.1, 25.2, 25.4, 30.0, 40.5, 52.4, 57.1, 63.1, 66.0, 117.1, 127.8, 133.2, 148.1, 155.4, 163.9.

4.1.12. allyl 2-(amino(5-(3-hydroxypropyl)-4-methylthiazol-2-yl)methyl)piperidine-1-carboxylate (16B)—Yield: 68% (1.84 g). R_f =0.36 (CHCl₃/MeOH, 7:1).

¹H NMR: (CDCl₃, 400 MHz) δ = 1.36 - 1.53 (m, 1H), 1.54 - 1.72 (m, 4H), 1.76 - 1.85 (m, 2H), 1.94 - 2.22 (m, 4H), 2.26 (s, 3H), 2.77 (t, J = 7.5 Hz, 2H), 3.03 (t, J = 12.4 Hz, 1H), 3.62 (t, J = 6.3 Hz, 2H), 4.04 (d, J = 12.0 Hz, 1H), 4.23 - 4.33 (m, 2H), 4.39 (dd, J = 13.3,

5.4 Hz, 1H), 4.49 (d, J = 10.0 Hz, 1H), 5.11 (dq, J = 10.5, 1.4 Hz, 1H), 5.16 (dq, J = 17.2, 1.6 Hz, 1H), 5.69 - 5.84 (m, 1H).

^{13}C NMR: (CDCl_3 , 100 MHz) δ = 14.8, 19.0, 22.7, 25.1, 25.4, 34.2, 40.3, 52.3 (br.), 56.7, 61.2, 65.9, 117.0, 131.3, 133.1, 146.8, 155.2, 169.5.

4.1.13. General procedure for amide coupling.—To a suspension of appropriate aryl-1H-pyrrole-2-carboxylic acid (**17**, **18** or **19**; 1 equiv) in DMF (10 mL per 1 g of acid) DIPEA (1 equiv) was added followed by HBTU (1 equiv). The resulting solution was stirred for 10 minutes followed by the addition of the appropriate amine (1 equiv) in DMF (10 mL per 1 g of amine). The reaction mixture was stirred overnight and most of the DMF was evaporated. The residue was dissolved in CH_2Cl_2 and washed with 5 % aqueous NaOH solution. The organic layer was separated and the aqueous layers were extracted with CH_2Cl_2 (2x50 mL). The combined organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by means of chromatography. Eluent: EtOAc or $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (50:1). The collected material was used in the next step without analysis.

4.1.14. General procedure for deprotection.—To a solution containing alloc-protected compound (1 mmol) and *N,N*-dimethylbarbituric acid (3 mmol) in methanol (10 mL), PPh_3 (10 mol. %) was added under a nitrogen atmosphere followed by $\text{Pd}(\text{dba})_2$ (5 mol. %). The mixture was stirred for 2-3 hours under reflux. After cooling, 100 mL CH_2Cl_2 was added and the organic phase was extracted with 10 % aqueous K_2CO_3 to remove the unreacted NDMBA. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2x100 mL). The combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by means of chromatography. Eluent: $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (50:1, 20:1, 10:1).

For some compounds, preparative HPLC followed by lyophilization was essential to obtain sufficient level of purity.

Purity of the final compounds was checked by LCMS (Column: Onyx C18 50x4.6mm | Solvent A : 0.1% TFA in $\text{AcN}/\text{H}_2\text{O}$ (2.5:97.5), Solvent B : 0.1% TFA in AcN) 3.75mL/min; Gradient: "A" -> 2.2min -> "B" (Hold 0.4min) -> 0.2min -> "A" -> Post Run).

4.1.15. 5-(4-chloro-3-fluorophenyl)-N-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)-1H-pyrrole-2-carboxamide—First fraction (**21A**):

Yield: 40% (103 mg; over two steps). rt = 1.484 min. Purity = 96%. LC-MS: m/z [M^+ +H] = 463 Da.

^1H NMR: (CDCl_3 , 400 MHz) δ = 1.19 - 1.33 (m, 2 H), 1.36 - 1.78 (m, 6 H), 1.82 - 1.92 (m, 1 H), 2.30 (s, 3 H), 2.75 (t, J = 11.7 Hz, 1 H), 3.15 - 3.29 (m, 1 H), 4.70 (s, 2 H), 5.35 - 5.46 (m, 1 H), 6.50 (d, J = 3.7 Hz, 1 H), 6.95 (s, 1 H), 7.31 - 7.40 (m, 2 H), 7.43 (d, J = 10.5 Hz, 1 H), 8.13 (br. s, 1 H), 10.67 (br. s, 1 H).

¹³C NMR (CDCl₃, 100 MHz): δ = 15.1, 24.0, 25.5, 29.8, 46.7, 54.2 (br.), 56.4, 59.1, 108.5, 112.9 (d, J = 22.0 Hz), 114.2, 119.5 (d, J = 18.3 Hz), 121.1, 126.9, 131.1, 132.1 (d, J = 8.8 Hz), 132.4 (d, J = 6.6 Hz), 134.1, 149.3, 158.5 (d, J = 248.8 Hz), 161.3, 168.6.

Second fraction (**21B**):

Yield: 49% (99 mg; over two steps). rt = 1.496 min. Purity = 93%. LC–MS: m/z [M^+ + H] = 463 Da.

¹H NMR: (CDCl₃, 400 MHz) δ = 1.19 - 1.35 (m, 2H), 1.35 - 1.52 (m, 3H), 1.65 - 1.73 (m, 1 H), 1.74 - 1.92 (m, 2 H), 2.31 (s, 3 H), 2.72 (t, J = 10.6 Hz, 1 H), 3.15 - 3.32 (m, 2 H), 4.71 (s, 2 H), 5.29 - 5.43 (m, 1 H), 6.48 (d, J = 3.8 Hz, 1 H), 6.87 (d, J = 3.1 Hz, 1 H), 7.31 - 7.38 (m, 2 H), 7.41 (d, J = 10.5 Hz, 1 H), 8.04 (br. s, 1 H), 10.50 (br. s, 1 H).

4.1.16. 5-(3-fluoro-4-methylphenyl)-N-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)-1H-pyrrole-2-carboxamide—First fraction (22A):

Yield: 58% (183 mg; over two steps). rt = 1.468 min. Purity = 100%. LC–MS: m/z [M^+ + H] = 443 Da.

¹H NMR: (CDCl₃, 400 MHz) δ = 1.27 (s, 1H), 1.32 - 1.74 (m, 6 H), 1.82 (d, J = 11.4 Hz, 1 H), 2.29 (s, 3 H), 2.32 (s, 3 H), 2.73 (t, J = 11.1 Hz, 1 H), 3.12 - 3.23 (m, 1 H), 3.44 - 3.51 (m, 1 H), 4.72 (s, 2 H), 5.41 (d, J = 5.9 Hz, 1 H), 6.49 (d, J = 3.5 Hz, 1 H), 6.94 (d, J = 3.3 Hz, 1 H), 7.18 (t, J = 7.9 Hz, 1 H), 7.20 - 7.32 (m, 2 H), 8.06 (br. s, 1 H), 10.56 (br. s, 1 H).

¹³C NMR (CDCl₃, 100 MHz): δ = 14.5 (d, J = 2.9 Hz), 15.0, 23.9, 24.8, 28.3, 46.6, 54.0, 56.1, 59.2, 107.6, 111.4 (d, J = 24.9 Hz), 113.7, 120.2 (d, J = 4.4 Hz), 123.8 (d, J = 17.6 Hz), 126.0, 131.3 (d, J = 7.3 Hz), 132.0 (d, J = 5.9 Hz), 132.4, 135.4, 149.2, 161.4, 161.7 (d, J = 244.4 Hz), 168.5.

Second fraction (**22B**):

Yield: 34% (122 mg; over two steps). rt = 1.573 min. Purity = 100%. LC–MS: m/z [M^+ + H] = 443 Da.

¹H NMR: (CDCl₃, 400 MHz) δ = 1.23 - 1.44 (m, 4 H), 1.51 - 1.59 (m, 1 H), 1.66 - 1.81 (m, 2 H), 2.23 (d, J = 0.6 Hz, 3 H), 2.26 (s, 3 H), 2.57 (t, J = 11.3 Hz, 1 H), 3.07 (d, J = 11.6 Hz, 1 H), 3.65 (br. s., 3 H), 4.65 (s, 2 H), 5.25 - 5.34 (m, 1 H), 6.42 (d, J = 3.8 Hz, 1 H), 6.89 (d, J = 3.8 Hz, 1 H), 7.11 (t, J = 8.1 Hz, 1 H), 7.18 - 7.27 (m, 1 H), 7.91 (br. s, 1 H), 10.60 (br. s, 1 H).

¹³C NMR (CDCl₃, 100 MHz): δ = 14.3 (d, J = 2.9 Hz), 14.9, 24.0, 25.8, 28.8, 46.4, 53.9, 56.0, 60.2, 107.6, 111.2 (d, J = 24.2 Hz), 113.7, 120.0 (d, J = 2.9 Hz), 123.7 (d, J = 17.6 Hz), 126.0, 131.3 (d, J = 8.8 Hz), 131.9 (d, J = 5.9 Hz), 132.5, 135.3 (d, J = 2.2 Hz), 148.8, 161.0, 161.6 (d, J = 244.4 Hz), 166.6.

4.1.17. 5-(3-fluoro-4-methylphenyl)-N-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)-1H-pyrrole-2-carboxamide—First fraction (23A):

Yield: 17% (53 mg; over two steps). rt = 1.492 min. Purity = 100%. LC–MS: m/z [M⁺ +H] = 457 Da.

¹H NMR: (CDCl₃+CD₃OD, ~5:1, 400 MHz) δ = 1.17 (s, 1 H), 1.39 - 1.55 (m, 1 H), 1.63 - 1.91 (m, 5 H), 2.17 (s, 3 H), 2.24 (s, 3 H), 2.75 - 2.91 (m, 2 H), 3.37 (d, J=12.1 Hz, 1 H), 3.62 (t, J=6.2 Hz, 2 H), 3.66 - 3.72 (m, 1 H), 3.84 (br. s., 4 H), 5.54 (d, J=4.2 Hz, 1 H), 6.41 (d, J=3.7 Hz, 1 H), 7.03 - 7.13 (m, 2 H), 7.29 (dd, J=15.0, 8.5 Hz, 2 H).

¹³C NMR: (CDCl₃+CD₃OD, ~5:1, 100 MHz) δ = 14.1 (d, J= 3.7 Hz), 14.6, 22.5, 22.8, 26.5, 29.5, 45.8, 52.0, 59.3, 62.0, 107.5, 111.2 (d, J= 24.2 Hz), 115.7, 120.1 (d, J= 2.9 Hz), 123.7 (d, J= 17.6 Hz), 125.2, 130.2, 131.1 (d, J= 8.1 Hz), 131.8 (d, J= 5.9 Hz), 135.7 (d, J=2.2 Hz), 148.6, 161.4, 161.5 (d, J= 244.4 Hz), 165.0.

Second fraction (**23B**):

Yield: 9% (72 mg; over two steps). rt = 1.436 min. Purity = 98%. LC–MS: m/z [M⁺ +H] = 457 Da.

¹H NMR: (DMSO-*d*₆, 400 MHz) δ = 1.08 - 1.20 (m, 1 H), 1.28 (t, J=8.7 Hz, 2 H), 1.41 - 1.51 (m, 1 H), 1.73 (d, J=8.7 Hz, 2 H), 2.21 (s, 3 H), 2.25 (s, 3 H), 2.80 (t, J=6.4 Hz, 2 H), 2.91 - 3.03 (m, 2 H), 3.49 - 3.56 (m, 2 H), 4.81 (br. s., 1 H), 5.14 (t, J=8.6 Hz, 1 H), 6.61 (d, J=3.2 Hz, 1 H), 6.96 (d, J=3.3 Hz, 1 H), 7.24 (t, J=8.1 Hz, 1 H), 7.52 (dd, J=7.9, 1.2 Hz, 1 H), 7.62 (d, J=11.6 Hz, 1 H), 8.48 (d, J=8.9 Hz, 1 H), 11.75 (br. s, 1 H). Two exchangeable protons are missed.

¹³C NMR (DMSO-*d*₆, 100 MHz): δ = 13.9 (d, J= 3.2 Hz), 14.9, 24.1, 25.8, 28.8, 29.5, 46.1, 54.4, 59.0, 61.3, 107.4, 110.9 (d, J= 24.1 Hz), 113.1, 120.4 (d, J= 3.2 Hz), 122.3 (d, J= 16.9 Hz), 127.1, 128.5, 131.6 (d, J= 8.8 Hz), 131.8 (d, J= 5.6 Hz), 133.9 (d, J= 2.4 Hz), 147.3, 159.8, 161.0 (d, J= 241.7 Hz), 167.4.

HRMS (ESI): m/z calcd for C₂₄H₃₀FN₄O₂S [M+H]⁺ 457.2068, found 457.2071.

4.1.18. 5-(4-chloro-3-fluorophenyl)-N-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)-1H-pyrrole-2-carboxamide—First fraction (24A**):**

Yield: 26% (58 mg; over two steps). rt = 1.458 min. Purity = 97%. LC–MS: m/z [M⁺ +H] = 477 Da.

¹H NMR: (CDCl₃+CD₃OD, ~1:1, 400 MHz) δ = 1.09 (s, 1 H), 1.33 - 1.48 (m, 1 H), 1.55 - 1.84 (m, 5 H), 2.17 (s, 3 H), 2.74 (t, J=5.7 Hz, 2H), 2.77 - 2.88 (m, 1 H), 3.14 - 3.23 (m, 1 H), 3.31 (d, J=12.1 Hz, 1 H), 3.54 (t, J=6.2 Hz, 2 H), 3.59 - 3.67 (m, 1 H), 4.22 (br. s., 3 H), 5.45 (d, J=5.1 Hz, 1 H), 6.37 (d, J=3.9 Hz, 1 H), 6.96 (d, J=3.9 Hz, 1 H), 7.22 (t, J=7.9 Hz, 1 H), 7.27 - 7.34 (m, 1 H), 7.37 (d, J=10.4 Hz, 1 H).

¹³C NMR: (CDCl₃+CD₃OD, ~1:1, 100 MHz) δ = 14.3, 22.2, 22.5, 29.3, 45.6, 52.0, 59.0, 61.7, 108.1, 112.4, 112.6, 115.5, 119.0 (d, J= 18.3 Hz), 121.0 (d, J= 2.9 Hz), 125.8, 130.1, 130.7, 132.1 (d, J= 7.3 Hz), 134.4 (d, J= 2.2 Hz), 148.5, 158.1 (d, J= 247.4Hz), 161.2, 164.4.

Second fraction (**24B**):

Yield: 11% (109 mg; over two steps). rt = 1.468 min. Purity = 97%. LC–MS: m/z [M⁺ +H] = 477 Da.

¹H NMR: (CDCl₃, 400 MHz) δ = 1.18 - 1.52 (m, 5 H), 1.64 (d, *J*=9.8 Hz, 1 H), 1.70 - 1.89 (m, 2 H), 2.29 (s, 3 H), 2.65 (t, *J*=10.6 Hz, 1 H), 2.89 (t, *J*=5.3 Hz, 2 H), 3.16 (d, *J*=3.8 Hz, 2 H), 3.77 (t, *J*=5.6 Hz, 2 H), 5.22 - 5.47 (m, 1 H), 6.47 (d, *J*=3.4 Hz, 1 H), 6.83 (s, 1 H), 7.22 - 7.38 (m, 2 H), 7.41 (d, *J*=10.4 Hz, 1 H), 7.95 (br. s., 1 H), 10.76 (br. s., 1 H).

¹³C NMR: (CDCl₃, 100 MHz) δ = 15.1, 24.0, 25.7, 28.7, 29.9, 46.4, 53.4, 60.6, 62.5, 108.5, 112.8 (d, *J*= 22.7 Hz), 113.4, 119.4 (d, *J*= 17.6 Hz), 121.1 (d, *J*= 2.9 Hz), 127.0, 129.1, 131.1, 132.4 (d, *J*= 8.1 Hz), 134.1 (d, *J*= 2.2 Hz), 148.8, 158.5 (d, *J*= 248.1 Hz), 160.7, 164.9.

HRMS (ESI): m/z calcd for C₂₃H₂₇ClFN₄O₂S [M+H]⁺ 477.1522, found 477.1522.

4.1.19. 5-(4-chlorophenyl)-N-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)-1H-pyrrole-2-carboxamide—First fraction (25A**):**

Yield: 24% (295 mg; over two steps). rt = 1.423 min. Purity = 95%. LC–MS: m/z [M⁺ +H] = 459 Da.

¹H NMR: (DMSO-*d*₆, 400 MHz) δ = 1.15 - 1.39 (m, 4 H), 1.47 - 1.57 (m, 2 H), 1.65 - 1.74 (m, 1 H), 2.24 (s, 3 H), 2.57 (t, *J*=9.9 Hz, 1 H), 2.80 (t, *J*=6.2 Hz, 2 H), 2.95 - 3.08 (m, 1 H), 3.13 - 3.22 (m, 1 H), 3.52 (t, *J*=6.0 Hz, 2 H), 4.81 (br. s., 1 H), 5.20 (t, *J*=7.5 Hz, 1 H), 6.62 (d, *J*=3.1 Hz, 1 H), 6.96 (d, *J*=3.1 Hz, 1 H), 7.41 (d, *J*=8.3 Hz, 2 H), 7.82 (d, *J*=8.3 Hz, 2 H), 8.51 (d, *J*=8.1 Hz, 1 H), 11.88 (br. s, 1 H)

¹³C NMR (DMSO-*d*₆, 100 MHz): δ = 14.9, 23.6, 25.1, 28.5, 29.5, 45.9, 54.5, 58.4, 61.3, 107.6, 113.5, 126.4 (2C), 127.3, 128.7 (3C), 130.7, 131.1, 133.8, 147.4, 160.1, 166.4.

HRMS (ESI): m/z calcd for C₂₃H₂₈ClN₄O₂S [M+H]⁺ 459.1616, found 459.1619.

Second fraction (**25B**):

Yield: 21% (141 mg; over two steps). rt = 1.500 min. Purity = 100%. LC–MS: m/z [M⁺ +H] = 459 Da.

¹H NMR: (DMSO-*d*₆, 400 MHz) δ = 1.13 - 1.39 (m, 4 H), 1.47 - 1.55 (m, 1 H), 1.70 - 1.80 (m, 2 H), 2.25 (s, 3 H), 2.56 (t, *J*=10.3 Hz, 1 H), 2.80 (t, *J*=6.2 Hz, 2 H), 3.00 (d, *J*=11.1 Hz, 1 H), 3.05 - 3.16 (m, 1 H), 3.48 - 3.56 (m, 2 H), 4.78 - 4.84 (m, 1 H), 5.21 (t, *J*=8.3 Hz, 1 H), 6.62 (d, *J*=2.8 Hz, 1 H), 6.98 (d, *J*=3.1 Hz, 1 H), 7.41 (d, *J*=7.8 Hz, 2 H), 7.82 (d, *J*=7.8 Hz, 2 H), 8.58 (d, *J*=8.9 Hz, 1 H), 11.86 (br. s, 1 H).

¹³C NMR (DMSO-*d*₆, 100 MHz): δ = 14.9, 23.8, 25.2, 28.2, 29.5, 45.9, 54.0, 58.9, 61.3, 107.6, 113.3, 126.4 (2C), 127.3, 128.6 (2C), 128.7, 130.7, 131.1, 133.8, 147.4, 159.8, 167.1.

HRMS (ESI): m/z calcd for C₂₃H₂₈ClN₄O₂S [M+H]⁺ 459.1616, found 459.1624.

4.1.20. 5-(3-fluoro-4-methylphenyl)-N-((5-(3-hydroxypropyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)-1H-pyrrole-2-carboxamide (26B)—Yield: 61% (790 mg; over two steps). rt = 1.491 min. Purity = 93%. LC–MS: m/z [M⁺ +H] = 471 Da.

¹H NMR: (CDCl₃, 400 MHz) δ = 1.17 - 1.41 (m, 4 H), 1.51 (d, *J*=10.5 Hz, 1 H), 1.61 - 1.81 (m, 4 H), 2.15 (s, 3 H), 2.21 (s, 3 H), 2.52 (t, *J*=11.3 Hz, 1 H), 2.67 (t, *J*=7.3 Hz, 2 H), 2.95 - 3.06 (m, 2 H), 3.60 (t, *J*=6.1 Hz, 2 H), 4.71 (br. s., 1 H), 5.29 (dd, *J*=7.9, 5.4 Hz, 1 H), 6.44 (d, *J*=3.5 Hz, 1 H), 6.86 (d, *J*=3.5 Hz, 1 H), 7.08 (t, *J*=8.0 Hz, 1 H), 7.21 - 7.26 (m, 2 H), 7.83 (d, *J*=7.1 Hz, 1 H), 10.86 (br. s., 1 H).

¹³C NMR: (CDCl₃, 100 MHz) δ = 14.3 (d, *J* = 3.2 Hz), 14.7, 22.7, 24.4, 26.4, 29.4, 34.2, 46.5, 53.8, 60.5, 61.0, 107.5, 111.3 (d, *J* = 23.3 Hz), 112.8, 120.1 (d, *J* = 3.2 Hz), 123.4 (d, *J* = 17.7 Hz), 126.4, 131.5 (d, *J* = 8.0 Hz), 131.8 (d, *J* = 5.6 Hz), 131.9, 135.1 (d, *J* = 2.4 Hz), 147.7, 160.7, 161.5 (d, *J* = 244.1 Hz), 164.1.

HRMS (ESI): m/z calcd for C₂₅H₃₂FN₄O₂S [M+H]⁺ 471.2225, found 471.2225.

4.1.21. 5-(4-chloro-3-fluorophenyl)-N-((5-(3-hydroxypropyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)-1H-pyrrole-2-carboxamide (27B)—Yield: 47% (640 mg; over two steps). rt = 1.518 min. Purity = 95%. LC–MS: m/z [M⁺ +H] = 491 Da.

¹H NMR: (CDCl₃, 400 MHz) δ = 0.84 - 0.96 (m, 1 H), 1.23 - 1.55 (m, 5 H), 1.59 - 1.72 (m, 1 H), 1.78 (ddd+m, *J*=14.0, 6.9, 6.7 Hz, 2+1 H), 1.84 - 1.92 (m, 1 H), 2.24 (s, 3 H), 2.73 (t, *J*=7.6 Hz, 2 H), 3.15 - 3.29 (m, 2 H), 3.63 (t, *J*=6.3 Hz, 1 H), 2.50 - 4.00 (br. s, 2H), 5.31 - 5.38 (m, 1 H), 6.51 (d, *J*=3.9 Hz, 1 H), 6.88 (d, *J*=3.8 Hz, 1 H), 7.31 - 7.42 (m, 1 H), 7.45 (d, *J*=10.8 Hz, 1 H), 8.03 (br. s., 1 H), 10.72 (br. s, 1 H).

¹³C NMR: (CDCl₃, 100 MHz) δ = 14.9, 22.7, 24.1, 25.9, 28.9, 34.2, 46.4, 53.3, 60.7, 61.4, 108.3, 112.9 (d, *J* = 22.4 Hz), 113.4, 119.3 (d, *J* = 18.1 Hz), 121.1 (d, *J* = 3.5 Hz), 127.1, 131.1, 132.4, 132.5 (d, *J* = 7.8 Hz), 134.0 (d, *J* = 1.7 Hz), 147.7, 158.5 (d, *J* = 248.3 Hz), 160.7, 164.0.

HRMS (ESI): m/z calcd for C₂₄H₂₉ClFN₄O₂S [M+H]⁺ 491.1678, found 491.1680.

4.1.22. 5-(4-chlorophenyl)-N-((5-(3-hydroxypropyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)-1H-pyrrole-2-carboxamide (28B)—Yield: 24% (105 mg; over two steps). rt = 1.473 min. Purity = 98%. LC–MS: m/z [M⁺ +H] = 473 Da.

¹H NMR: (CDCl₃, 400 MHz) δ = 1.43 - 1.75 (m, 2 H), 1.80 (quin, *J*= 6.9 Hz, 2 H), 1.87 - 2.05 (m, 4 H), 2.30 (s, 3 H), 2.77 (t, *J* = 7.5 Hz, 2 H), 2.95 (td, *J* = 12.9, 2.3 Hz, 1 H), 3.63 (t + br. s, *J* = 6.2 Hz, 2+2H), 5.73 (dd, *J* = 8.1, 2.8 Hz, 1 H), 6.50 (dd, *J* = 3.5, 2.3 Hz, 1 H), 7.04 (dd, *J* = 3.6, 2.3 Hz, 1 H), 7.32 (d, *J* = 8.6 Hz, 2 H), 7.29 - 7.35 (m, 2 H), 7.60 (d, *J* = 8.6 Hz, 2 H), 9.64 (d, *J* = 8.1 Hz, 1 H), 11.34 (br. s, 1 H).

¹³C NMR: (CDCl₃, 100 MHz) δ = 14.9, 22.59, 22.64, 22.9, 26.4, 34.0, 45.2, 51.1, 61.0, 61.4, 107.7, 116.2, 126.0 (2C), 126.4, 129.1 (2C), 130.6, 132.8, 133.6, 135.4, 147.2, 161.0, 169.0.

HRMS (ESI): m/z calcd for $C_{24}H_{30}ClN_4O_2S$ $[M+H]^+$ 473.1773, found 473.1770.

4.1.23. (4-methyl-1H-imidazol-5-yl)methanol hydrochloride (29)—A mixture containing 4-methylimidazole (30.0 g, 365 mmol), paraformaldehyde (12.0 g, 402 mmol), K_2CO_3 (55.0 g, 402 mmol) and isopropanol (150 mL) was heated at 60 °C for two days (~16h). The reaction mixture was cooled to RT and filtered. The inorganic material was washed with isopropanol (50 mL). Concentrated aqueous HCl (79 mL) was added to the filtrate and the solvent was removed *in vacuo*. The residue was recrystallized from ethanol (~50 mL), filtered and washed two times with acetone: ethanol (1:1) mixture.

Yield: 27% (14.88 g).

1H NMR: (DMSO- d_6 , 400 MHz) δ = 2.26 (s, 3H), 4.46 (s, 2H), 8.94 (s, 1H), 14.58 (br. s., 2H).

^{13}C NMR (DMSO- d_6 , 100 MHz): δ = 8.6, 52.0, 125.2, 128.6, 132.1.

4.1.24. 5-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-1H-imidazole (30)—4-Hydroxymethyl-5-methylimidazole hydrochloride (14.88 g, 100.5 mmol) was dissolved in DMF (150 mL). TBSCl (22.70 g, 150.6 mmol, 1.5 equiv) and imidazole (18.97 g, 279 mmol, 2.8 equiv) were added and the reaction mixture stirred at r.t. for 24 hours. The reaction mixture was heated until the point where a homogeneous solution was formed (60-80 °C) and kept at this temperature for two hours. The reaction mixture was cooled to r.t. poured into water and extracted with EtOAc (3×100 mL). The organic layer was washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by chromatography (eluent: 10:1, CH_2Cl_2 /MeOH, R_f =0.35 in the same system). Yield: 74% (6.83 g; Yellow solid).

1H NMR: ($CDCl_3$, 400 MHz) δ = 0.06 (s, 6H), 0.89 (s, 9H), 2.24 (s, 3H), 4.67 (s, 2H), 7.49 (s, 1H), 10.28 (br. s, 1H).

^{13}C NMR: ($CDCl_3$, 100 MHz) δ = -5.2 (2C), 10.9, 18.5, 26.1 (3C), 57.4, 128.0, 130.6, 133.3.

4.1.25. 5-(((tert-butyldimethylsilyl)oxy)methyl)-N,N,4-trimethyl-1H-imidazole-1-sulfonamide (31A) and 4-(((tert-butyldimethylsilyl)oxy)methyl)-N,N,5-trimethyl-1H-imidazole-1-sulfonamide (31B)—To a stirred solution of 5-(((tert-butyldimethylsilyl)oxy)methyl)-4-methyl-1H-imidazole (15.40 g, 68.1 mmol) in CH_2Cl_2 (140 mL) was added Et_3N (9.5 mL, 68.1 mmol) followed by Me_2NSO_2Cl (7.33 mL, 68.1 mmol). The reaction mixture was heated under reflux for 2 hours cooled and washed with water (100 mL). The organic layer was dried over Na_2SO_4 and evaporated.

Chromatographic purification (eluent 5:1→3:1→0:1, hexanes/EtOAc). First fraction = 4.76 g (R_f =0.7, 1:1, hexanes/EtOAc).

Second fraction = 6.50 g

Third fraction: Yield: 75% (5.72 g; $R_f=0.55$, 1:1, hexanes/EtOAc). Typically, a mixture of two spots was collected and used as such. First fraction (**31A**):

^1H NMR: (CDCl_3 , 400 MHz) δ = 0.10 (s, 6H), 0.91 (s, 9H), 2.42 (s, 3H), 2.90 (s, 6H), 4.64 (s, 2H), 7.82 (s, 1H).

^{13}C NMR: (CDCl_3 , 100 MHz) δ = -5.1 (2C), 9.9, 18.5, 26.0 (3C), 38.1, 59.1, 124.8, 136.7, 139.6.

Third fraction (**31B**):

^1H NMR: (CDCl_3 , 400 MHz) δ = 0.13 (s, 6H), 0.92 (s, 9H), 2.25 (s, 3H), 2.93 (s, 6H), 4.78 (s, 2H), 7.85 (s, 1H).

^{13}C NMR: (CDCl_3 , 100 MHz) δ = -5.2 (2C), 13.1, 18.6, 26.0 (3C), 38.2, 53.5, 126.1, 137.7, 139.5.

4.1.26. allyl 2-(amino(5-(hydroxymethyl)-4-methyl-1H-imidazol-2-yl)methyl)piperidine-1-carboxylate (33**)**—A mixture of protected imidazoles **31A** and **31B** (6.97 g, 20.9 mmol, 2.0 equiv) was dissolved in THF (21 mL) and cooled to -78 °C. At this temperature *n*-BuLi (2.5 M, 8.4 mL, 21 mmol, 2.0 equiv) was added dropwise under nitrogen. The reaction mixture was stirred for 10 minutes at -78 °C, and imine **2** (3.14 g, 10.5 mmol) was added dropwise as a solution in THF (21 mL). The reaction mixture was slowly (~1 hour) warmed to 0 °C, and poured into saturated NH_4Cl (0.1 L). The biphasic mixture was extracted with CH_2Cl_2 (3×100 mL). The combined organic phases were dried over Na_2SO_4 , and evaporated. The residue was dissolved in 1M HCl-MeOH solution (200 mL). After dissolution the reaction mixture was stirred for 1 hour, evaporated (no heating), dissolved in CH_2Cl_2 and washed with 10 % aqueous K_2CO_3 . The organic layer was dried over Na_2SO_4 , evaporated and loaded on silica. Chromatographic purification (eluent: 10:1, 4:1 $\text{CH}_2\text{Cl}_2/\text{MeOH}$; TLC in 1:1 $\text{CH}_2\text{Cl}_2/\text{MeOH}$) yielded two fractions (**33A** and **33B**).

First fraction; Yield: 31% (1.013 g; over two steps).

Second fraction: Yield: 29% (0.930 g; over two steps). First fraction (**33A**):

^1H NMR: (CDCl_3 , 400 MHz) δ = 1.13 - 1.32 (m, 2 H), 1.32 - 1.53 (m, 3 H), 1.54 - 1.70 (m, 2 H), 2.10 (s, 3 H), 2.82 - 2.97 (m, 1 H), 4.01 - 4.13 (m, 1 H), 4.47 (br. s + m, 2 + 1H), 4.52 - 4.63 (m, 2 H), 5.18 (d, $J=10.4$ Hz, 1 H), 5.27 (dd, $J=17.0$, 1.1 Hz, 1 H), 5.50 - 6.82 (br. s, 4H), 5.84 - 5.97 (m, 1 H).

^{13}C NMR: (CDCl_3 , 100 MHz) δ = 10.6, 19.2, 25.3, 25.7, 39.9, 48.0, 54.8, 55.6, 66.4, 117.6, 128.3, 130.9, 132.9, 146.2, 156.4.

Second fraction (**33B**):

^1H NMR: (CDCl_3 , 400 MHz) δ = 1.32 - 1.49 (m, 2 H), 1.50 - 1.71 (m, 4 H), 2.14 (s, 3 H), 2.97 (t, $J=12.8$ Hz, 1 H), 3.92 (d, $J=12.5$ Hz, 1 H), 4.25 - 4.65 (m, 2H), 4.45 (br. s, 4 H), 5.10 - 5.18 (m, 2 H), 5.40 - 6.50 (br. s, 4H), 5.75 - 5.86 (m, 1 H).

¹³C NMR: (CDCl₃, 100 MHz) δ = 10.1, 18.8, 25.2 (2C), 29.8, 40.4, 48.4, 55.0, 66.3, 117.3, 126.5, 131.4, 132.9, 147.3, 155.7.

Compounds with general structure labeled as **34** were obtained following general procedure for amide coupling using amines **33A** and **33B** and acids **17-19** as substrates. Eluent: 10/1, CH₂Cl₂/MeOH (R_f =0.4 10:1, CH₂Cl₂/MeOH).

4.1.27. General procedure for deprotection (imidazoles).—To solution containing alloc-protected compound (1 mmol) and *N,N*-dimethylbarbituric acid (3 mmol) in MeOH (10 mL), PPh₃ (10 mol. %) was added under a nitrogen atmosphere followed by Pd(dba)₂ (5 mol. %). The mixture was stirred for 2-3 hours under reflux. After cooling, the reaction mixture was evaporated and the residue was dissolved in CH₂Cl₂ (50 - 100 ml). The organic phase was extracted twice with 5 % aqueous HCl (~10 mL). The combined aqueous layers were evaporated and purified by preparative HPLC.

4.1.28. 5-(4-chlorophenyl)-N-((5-(hydroxymethyl)-4-methyl-1H-imidazol-2-yl)(piperidin-2-yl)methyl)-1H-pyrrole-2-carboxamide (35)—First fraction (**35A**):

Yield: 5% (40.1 mg; over two steps). *rt* = 1.271 min. Purity = 97%. LC-MS: *m/z* [*M*⁺ + *H*] = 428 Da.

¹H NMR: (DMSO-*d*₆, 400 MHz) δ = 1.06 - 1.44 (m, 4 H), 1.45 - 1.60 (m, 1 H), 1.61 - 1.81 (m, 1 H), 2.09 (s, 3 H), 2.82 - 3.16 (m, 4 H), 3.41 (br. s, 3 H), 4.31 (s, 2 H), 4.56 - 4.96 (m, 1 H), 5.05 (br. s., 1 H), 6.63 (br. s., 1 H), 6.90 (br. s., 1 H), 7.44 (br. s., 1 H), 7.81 (br. s., 1 H), 8.16 (br. s., 1 H), 11.82 (br. s., 1 H).

Second fraction (**35B**):

Yield: 7% (51.1 mg; over two steps). *rt* = 1.121 min. Purity = 96%. LC-MS: *m/z* [*M*⁺ + *H*] = 428 Da.

¹H NMR: (DMSO-*d*₆, 400 MHz) δ = 1.36 (d, *J*=9.5 Hz, 4 H), 1.56 - 1.65 (m, 1 H), 1.67 - 1.83 (m, 2 H), 2.10 (s, 3 H), 2.71 - 2.82 (m, 1 H), 3.16 (d, *J*=11.9 Hz, 1 H), 3.28 (t, *J*=7.1 Hz, 1 H), 4.30 (s, 2 H), 5.28 (t, *J*=8.0 Hz, 1 H), 6.63 (d, *J*=2.2 Hz, 1 H), 6.92 (d, *J*=2.8 Hz, 1 H), 7.42 (d, *J*=8.6 Hz, 2 H), 7.82 (d, *J*=8.6 Hz, 2 H), 8.32 (s, 2 H), 8.66 (d, *J*=9.2 Hz, 1 H), 11.97 (br. s, 1 H).

¹³C NMR (DMSO-*d*₆, 100 MHz): δ = 10.5, 22.6, 23.6, 26.2, 45.1, 48.8, 54.4, 58.8, 107.5, 113.6, 126.2 (2C), 127.3, 127.6, 128.7 (2C), 130.7, 131.1, 131.4, 133.5, 143.5, 159.9, 164.9.

HRMS (ESI): *m/z* calcd for C₂₂H₂₇ClN₅O₂ [*M*+*H*]⁺ 428.1848, found 428.1847.

4.1.29. 5-(4-chloro-3-fluorophenyl)-N-((5-(hydroxymethyl)-4-methyl-1H-imidazol-2-yl)(piperidin-2-yl)methyl)-1H-pyrrole-2-carboxamide—First fraction (**36A**):

Yield: 6% (41.6 mg; over two steps).

¹H NMR: (DMSO-*d*₆, 400 MHz) δ = 1.14 - 1.42 (m, 3 H), 1.47 - 1.59 (m, 2 H), 1.67 - 1.79 (m, 1 H), 2.25 (s, 3 H), 2.53 - 2.62 (m, 1 H), 2.97 - 3.07 (m, 1 H), 3.15 - 3.23 (m, 1 H), 4.53 (s, 2 H), 5.21 (t, *J*=7.8 Hz, 1 H), 6.73 (d, *J*=3.8 Hz, 1 H), 6.98 (d, *J*=3.8 Hz, 1 H), 7.56 (t, *J*=8.2 Hz, 1 H), 7.67 (dd, *J*=8.5, 1.5 Hz, 1 H), 7.91 (dd, *J*=11.2, 1.7 Hz, 1 H), 8.24 (s, 1 H), 8.64 (d, *J*=8.4 Hz, 1 H), 11.94 (br. s., 1 H). Two exchangeable protons are missed.

¹³C NMR (DMSO-*d*₆, 100 MHz): δ = 14.9, 23.5, 24.9, 28.4, 45.8, 54.6, 55.1, 58.2, 108.5, 112.5 (d, *J* = 22.5 Hz), 113.3, 117.1 (d, *J* = 17.7 Hz), 121.7 (d, *J* = 3.2 Hz), 127.8, 130.8, 132.7, 132.9 (d, *J* = 8.0 Hz), 133.0, 146.8, 157.5 (d, *J* = 244.1 Hz), 160.1, 164.1, 167.4.

Second fraction (**36B**):

Yield: 6% (53.2 mg; over two steps). *rt* = 1.160 min. Purity = 95%. LC-MS: *m/z* [*M*⁺ + *H*] = 446 Da.

¹H NMR: (DMSO-*d*₆, 400 MHz) δ = 1.16 - 1.43 (m, 4 H), 1.44 - 1.59 (m, 2 H), 1.70 - 1.79 (m, 2 H), 2.26 (s, 3 H), 2.61 (t, *J*=10.6 Hz, 1 H), 3.03 (d, *J*=11.6 Hz, 1 H), 3.13 - 3.22 (m, 1 H), 4.54 (s, 2 H), 5.26 (t, *J*=8.2 Hz, 1 H), 6.73 (d, *J*=3.7 Hz, 1 H), 7.00 (d, *J*=3.8 Hz, 1 H), 7.55 (t, *J*=8.2 Hz, 1 H), 7.67 (dd, *J*=8.6, 1.3 Hz, 1 H), 7.92 (dd, *J*=11.2, 1.7 Hz, 1 H), 8.22 (s, 1 H), 8.68 (d, *J*=8.9 Hz, 1 H), 11.99 (br. s., 1 H).

¹³C NMR (DMSO-*d*₆, 100 MHz): δ = 14.9, 23.5, 24.8, 27.7, 45.8, 53.9, 55.0, 58.7, 108.6, 112.6 (d, *J* = 22.7 Hz), 113.1, 117.1 (d, *J* = 22.7 Hz), 121.8 (d, *J* = 2.9 Hz), 127.8, 130.8, 132.8, 132.9 (d, *J* = 7.3 Hz), 133.2, 146.8, 157.5 (d, *J* = 245.2 Hz), 159.9, 163.9, 167.8.

HRMS (ESI): *m/z* calcd for C₂₂H₂₆ClFN₅O₂ [*M*+*H*]⁺ 446.1754, found 446.1755.

4.1.30. 5-(3-fluoro-4-methylphenyl)-N-((5-(hydroxymethyl)-4-methyl-1H-imidazol-2-yl)(piperidin-2-yl)methyl)-1H-pyrrole-2-carboxamide—First fraction (**37A**):

Yield: 6% (51.5 mg; over two steps). *rt* = 1.102 min. Purity = 100%. LC-MS: *m/z* [*M*⁺ + *H*] = 426 Da.

¹H NMR: (CD₃OD, 400 MHz) δ = 1.54 - 1.62 (m, 2 H), 1.66 - 1.84 (m, 3 H), 1.88 - 1.95 (m, 2 H), 2.23 (s, 3 H), 2.27 (d, *J*=1.2 Hz, 3 H), 3.05 (td, *J*=12.9, 3.0 Hz, 1 H), 3.43 - 3.50 (m, 1 H), 3.66 (s, 1 H), 4.50 (s, 2 H), 5.39 (d, *J*=7.7 Hz, 1 H), 6.56 (d, *J*=3.9 Hz, 1 H), 6.96 (d, *J*=4.0 Hz, 1 H), 7.25 (t, *J*=8.2 Hz, 1 H), 7.34 - 7.41 (m, 2 H), 8.38 (s, 2 H). Two exchangeable protons are missed.

¹³C NMR (CD₃OD, 100 MHz): δ = 10.1, 14.4 (d, *J* = 3.7 Hz), 23.1, 23.5, 27.4, 46.8, 50.1, 56.3, 60.7, 108.8, 112.2 (d, *J* = 24.2 Hz), 115.4, 121.6 (d, *J* = 2.9 Hz), 125.0 (d, *J* = 17.6 Hz), 127.2, 129.3, 133.1 (d, *J* = 8.1 Hz), 133.2 (d, *J* = 5.9 Hz), 134.0, 137.1 (d, *J* = 2.9 Hz), 143.7, 163.1 (d, *J* = 243.0 Hz), 163.5, 168.3.

δ = 49.15 ppm was used as a reference for methanol.

HRMS (ESI): *m/z* calcd for C₂₃H₂₉FN₅O₂ [*M*+*H*]⁺ 426.2300, found 426.2301.

Second fraction (**37B**):

Yield: 6% (54.5 mg; over two steps). *rt* = 1.115 min. Purity = 92%. LC–MS: *m/z* [*M*⁺ + *H*] = 426 Da.

¹H NMR (CD₃OD, 400 MHz): δ = 1.52 - 1.75 (m, 3 H), 1.87 - 1.99 (m, 2 H), 2.03 - 2.13 (m, 1 H), 2.23 (s, 3 H), 2.27 (s, 3 H), 3.10 (td, *J*=12.4, 2.6 Hz, 1 H), 3.44 - 3.51 (m, 1 H), 3.69 - 3.76 (m, 1 H), 4.50 (d, *J*=2.0 Hz, 2 H), 5.48 (d, *J*=6.8 Hz, 1 H), 6.57 (d, *J*=4.0 Hz, 1 H), 6.98 (d, *J*=3.9 Hz, 1 H), 7.26 (t, *J*=8.0 Hz, 1 H), 7.35 - 7.42 (m, 2 H), 8.34 (s, 2 H).

Three exchangeable protons are missed.

¹³C NMR (CD₃OD, 100 MHz): δ = 9.9, 14.4 (d, *J* = 4.74 Hz), 23.0, 23.6, 26.4, 46.5, 49.2, 56.4, 60.6, 108.8, 112.2 (d, *J* = 23.4 Hz), 115.3, 121.6 (d, *J* = 2.9 Hz), 125.0 (d, *J* = 17.6 Hz), 127.2, 129.0, 133.0 (d, *J* = 8.8 Hz), 133.2 (d, *J* = 4.4 Hz), 134.4, 144.0, 163.1 (d, *J* = 3.7 Hz), 163.4, 168.0.

HRMS (ESI): *m/z* calcd for C₂₃H₂₉FN₅O₂ [*M*+*H*]⁺ 426.2300, found 426.2303.

4.1.31. allyl 2,2-dimethoxyethylcarbamate (38)—Amino acetaldehyde dimethyl acetal (5.64 mL, 51.8 mmol, 1.10 equiv) and Et₃N (6.56 mL, 47.0 mmol) were dissolved in CH₂Cl₂ (94 mL) and alloc-Cl (5.00 mL, 47.0 mmol) was added dropwise with cooling of the flask on an ice bath. The reaction was stirred for 1 hour at r.t. and washed with 5-10 % aqueous citric acid. The aqueous layer was extracted with CH₂Cl₂ (100 mL). The combined organic layers were dried over Na₂SO₄ and evaporated.

Yield: 100% (8.86 g; Clear oil); *R*_f=0.6 (hexanes/EtOAc, 1:1).

¹H NMR (CDCl₃, 400 MHz): δ = 3.30 (t, *J* = 5.7 Hz, 2H), 3.37 (s, 6H), 4.36 (t, *J* = 5.3 Hz, 1H), 4.54 (d, *J* = 5.3 Hz, 2H), 5.07 (br. s, 1H), 5.19 (dd, *J* = 10.4, 1.2 Hz, 1H), 5.28 (dd, *J* = 17.2, 1.5 Hz, 1H), 5.90 (dddd, *J* = 16.8, 10.9, 5.6, 5.4 Hz, 1H).

¹³C NMR (CDCl₃, 100 MHz): δ = 42.5, 54.3 (2C), 65.7, 102.9, 117.7, 132.9, 156.4.

4.1.32. allyl allyl(2,2-dimethoxyethyl)carbamate (39)—Allyl 2,2-dimethoxyethylcarbamate (8.86 g, 46.9 mmol) was dissolved in DMF (47 mL) and NaH (60% dispersion in mineral oil, 2.10 g, 52.5 mmol, 1.10 equiv) was added in several portions. When the hydrogen evolution stops allyl bromide (4.47 mL, 51.7 mmol, 1.10 equiv) was added dropwise. After 10 minutes, the TLC shows disappearance of the starting material. The reaction mixture was diluted with water (100 mL) and extracted with EtOAc (3×100 mL). The combined organic layers were dried over Na₂SO₄ and evaporated. Yield: 100% (12.10 g; Clear oil); *R*_f=0.7 (hexanes/EtOAc, 3:1). Mixture of rotamers:

¹H NMR (CDCl₃, 400 MHz): δ = 3.25 - 3.33 (m, 2H), 3.36 (br. s, 6H), 3.89 - 4.00 (m, 2H), 4.46 (d, *J* = 18.8 Hz, 1H), 4.58 (d, *J* = 3.5 Hz, 2H), 5.04 - 5.15 (m, 2H), 5.17 (d, *J* = 10.4 Hz, 1H), 5.27 (d, *J* = 17.1 Hz, 1H), 5.67 - 5.81 (m, 1H), 5.84 - 5.97 (m, 1H).

¹³C NMR (CDCl₃, 100 MHz): δ = 48.1, 48.8, (50.6, 50.7), (54.53, 54.59), 66.1, 103.5, 103.9, 116.4, 116.9, 117.2, 117.4, 133.0, 133.5, 133.6, (155.8, 156.2).

4.1.33. allyl allyl(2-oxoethyl)carbamate (40)—Allyl allyl(2,2-dimethoxyethyl)carbamate was dissolved in formic acid (120 mL) and water (24 mL) was added to the solution. After 30 minutes the TLC shows disappearance of the starting material. After 1 day most of HCOOH was evaporated, the residue was dissolved in EtOAc (250 mL) and was washed with brine until neutral pH (10×100 mL). The organic layer was dried over Na₂SO₄ and evaporated to give pure enough aldehyde. Yield: 98% (8.37 g; over two steps). Yellow oil.

¹H NMR (CDCl₃, 400 MHz): δ = 3.97 (br. s, 2H), 3.98 - 4.05 (m, 2H), 4.61 (dd, *J* = 15.5, 5.0 Hz, 2H), 5.10 - 5.25 (m, 3H), 5.28 (dd, *J* = 26.9, 17.2 Hz, 1H), 5.77 (ddt, *J* = 16.8, 10.5, 6.0 Hz, 1H), 5.91 (dddd, *J* = 28.2, 22.6, 10.8, 5.5 Hz, 1H), 9.58 (s, 1H).

¹³C NMR (CDCl₃, 100 MHz): δ = 50.9, 51.3, 56.1, 56.6, 66.6, 117.8, 117.94, 117.98, 118.6, 132.5, 132.6, 133.0, 198.3.

4.1.34. (*E*)-allyl allyl(2-(*tert*-butylsulfinylimino)ethyl)carbamate (41)—To a solution of aldehyde (4.00 g, 21.9 mmol) in CH₂Cl₂ (22 mL) was added commercially available (*S*)-*N*-*tert*-butylsulfinylamide (2.91 g, 24.0 mmol, 1.10 equiv), PPTS (0.27 g, 0.050 equiv) and MgSO₄ (13.10 g, 109.2 mmol, 5.000 equiv). To control the progress of the reaction 0.5 mL of the DCM solution from the reaction mixture was evaporated and analyzed by NMR (once a day). The mixture was stirred at rt for 30 h, inorganic material was filtered and washed several times with CH₂Cl₂. The filtrate was concentrated in vacuo to give pure enough title compound (*S*)-**41**. Chromatographic purification: Hexanes/EtOAc 3:1. The product always contains inseparable mixture of aldehyde.

Yield: 91% (5.67 g). Clear oil.

The reaction with (*R*)-*N*-*tert*-butylsulfinylamide was conducted in the same manner affording the corresponding *R* enantiomer (*R*)-**41**. Yield: 76% (5.38 g).

¹H NMR (CDCl₃, 400 MHz): δ = 1.20 (s, 9H), 3.87 - 4.10 (m, 2H), 4.18 - 4.37 (m, 2H), 4.51 - 4.68 (m, 2H), 5.12 - 5.24 (m, 3H), 5.24 - 5.36 (m, 1H), 5.74 - 6.00 (m, 2H), 7.97 (t, *J* = 2.7 Hz, 1H).

¹³C NMR (CDCl₃, 100 MHz): δ = 22.3 (3C), 50.3, (50.6, 50.9), (57.0, 57.1), (66.3, 66.5), 117.5, (117.9, 118.1), (132.5, 132.7), 133.1, (155.6, 155.9), 165.1.

4.1.35. allyl allyl(2-(5-(((*tert*-butyldimethylsilyl)oxy)methyl)-4-methylthiazol-2-yl)-2-(1,1-dimethylethylsulfinamido)ethyl)carbamate (42)—5-(((*tert*-butyldimethylsilyl)oxy)methyl)-4-methylthiazole (6.25 g, 25.7 mmol, 1.3 equiv) was dissolved in THF (25 mL) and cooled to -78 °C. At this temperature *n*-BuLi (2.5 M, 11 mL, 27.5 mmol, 1.4 equiv) was added dropwise under a nitrogen atmosphere. The reaction mixture was stirred for 20 minutes at -78 °C, and (*S*)-**41** (5.67 g, 19.8 mmol) was added dropwise as a solution in THF (20 mL). The reaction mixture was slowly (~1 hour) warmed to 0 °C, and poured into water (0.1 L). The biphasic mixture was extracted with CH₂Cl₂ (3×100 mL). The combined organic phases were dried over Na₂SO₄, and evaporated to give

brown oil which was purified by means of column chromatography. Eluent: hexanes/EtOAc (10:1, 5:1, then 1:1).

Yellow oil (*/S*-**42**): *R*_f = 0.4 (hexanes/EtOAc, 1:1). Yield: 66% (6.93 g).

The reaction with (*R*)-**41** was conducted in the same manner to give *R*-**42**.

Yield: 74% (7.40 g).

¹H NMR (CDCl₃, 400 MHz): δ = 0.09 (s, 6H), 0.90 (s, 9H), 1.27 (s, 9H), 2.32 (s, 3H), 3.55 (dd, *J* = 14.7, 2.5 Hz, 1H), 3.83 (dd, *J* = 16.4, 5.1 Hz, 1H), 3.88 - 4.03 (m, 2H), 4.54 - 4.69 (m, 2H), 4.76 (s, 2H), 4.89 (dt, *J* = 9.5, 3.8 Hz, 1H), 5.16 (dt, *J* = 15.9, 1.5 Hz, 3H), 5.19 (d, *J* = 17.1 Hz, 2H), 5.30 (dd, *J* = 17.2, 1.0 Hz, 1H), 5.56 (d, *J* = 2.4 Hz, 1H), 5.81 (ddd, *J* = 22.8, 10.6, 5.5, 1H), 5.93 (ddd, *J* = 22.5, 10.7, 5.4, 2H).

¹³C NMR (CDCl₃, 100 MHz): δ = -5.21 (2C), 15.3, 18.4, 22.9 (3C), 25.9 (3C), 29.8, 50.5, 52.3, 56.3, 58.0, 66.8, 117.0, 117.5, 132.8, 133.1, 133.2, 147.9, 158.6, 170.4.

4.1.36. allyl allyl(2-amino-2-(5-(hydroxymethyl)-4-methylthiazol-2-yl)ethyl)carbamate (43)—The 1M HCl-MeOH solution was prepared by dropwise addition of AcCl to a MeOH (100 mmol). The resulting solution was cooled to an ambient temperature and added to a flask containing **42** (10 mmol). After dissolution the reaction mixture was stirred for 1 hour, evaporated (no heating), dissolved in CH₂Cl₂ and washed with 10 % aqueous K₂CO₃. The organic layer was dried over Na₂SO₄, evaporated and loaded on silica. Eluting with CH₂Cl₂/MeOH (50:1) provided pure amine. *R*_f = 0.3 (CHCl₃/MeOH, 7:1). Yellow oil.

For *R*-**43**: Yield: 75% (3.28 g).

For *S*-**43**: Yield: 64% (2.62 g).

¹H NMR (CDCl₃, 400 MHz): δ = 2.30 (s, 3H), 2.62 (br. s., 3H), 3.45 - 3.68 (m, 2H), 3.74 - 3.94 (m, 2H), 4.36 (t, *J* = 6.7 Hz, 1H), 4.56 (d, *J* = 4.4 Hz, 2H), 4.69 (s, 2H), 5.12 (dd, *J* = 17.7, 9.5 Hz, 2H), 5.18 (d, *J* = 10.4 Hz, 1H), 5.28 (d, *J* = 17.2 Hz, 1H), 5.74 (ddt, *J* = 16.4, 10.7, 5.3 Hz, 1H), 5.89 (ddt, *J* = 16.7, 11.0, 5.3, 1H)

¹³C NMR (CDCl₃, 100 MHz): δ = 15.0, (50.6, 50.8), 53.1, (53.4, 54.1), 56.3, 66.4, 117.0, 117.5, 117.9, 131.7, 132.8, 133.2, 148.8, (156.2, 157.0), (171.7, 172.2).

4.1.37. allyl allyl(2-(5-(4-chlorophenyl)-1H-pyrrole-2-carboxamido)-2-(5-(hydroxymethyl)-4-methylthiazol-2-yl)ethyl)carbamate (44)—To a suspension/solution of 5-(4-chlorophenyl)-1H-pyrrole-2-carboxylic acid (878 mg, 3.96 mmol) in DMF (8.8 mL) DIPEA (0.690 mL, 3.97 mmol) was added followed by HBTU (1.506 g, 3.97 mmol). The resulting solution was stirred for 10 minutes and added to a solution of amine *S*-**43** (1.237 g, 3.97 mmol) in DMF (12 mL). The reaction mixture was stirred for 12 hours, evaporated, diluted with CH₂Cl₂ (100 mL) and successively washed with 5% aqueous NaOH and 10 % citric acid solutions. The organic layer was dried over Na₂SO₄, evaporated

and loaded on silica. Eluting with hexanes: EtOAc (1:1, than pure EtOAc) gave the target compounds.

First enantiomer (*S*-**44**): Yield: 74% (1.52 g).

The second enantiomer (*R*-**44**) was prepared from amine *R*-**43** using the same procedure: Yield: 58% (1.11 g). Light brown oil.

¹H NMR (CDCl₃, 400 MHz): δ = 2.28 (s, 3H), 3.53 (dd, J = 14.5, 3.1 Hz, 1H), 3.72 (dd, J = 16.4, 5.6 Hz, 1H), 3.85 (dd, J = 16.2, 4.8 Hz, 1H), 4.07 (dd, J = 14.8, 10.8 Hz, 1H), 4.51 (d, J = 5.0 Hz, 2H), 4.18 - 4.95 (br. s, 1H), 4.65 (s, 2H), 5.05 - 5.22 (m, 4H), 5.38 (ddd, J = 9.8, 6.8, 3.4 Hz, 1H), 5.62 - 5.83 (m, 2H), 6.44 (t, J = 3.1 Hz, 1H), 6.80 (t, J = 2.3 Hz, 1H), 7.21 (d, J = 8.6 Hz, 2H), 7.48 (d, J = 8.6 Hz, 2H), 8.21 (d, J = 6.7 Hz, 1H), 10.76 (br. s, 1H).

¹³C NMR (CDCl₃, 100 MHz): δ = 14.8, 50.3, 50.6, 52.4, 56.0, 107.9, 113.0, 117.2, 117.7, 126.2 (2C), 126.4, 128.9 (2C), 130.4, 131.9, 132.2, 132.7, 132.8, 135.3, 149.1, 158.1, 161.6, 168.8.

4.1.38. N-(2-amino-1-(5-(hydroxymethyl)-4-methylthiazol-2-yl)ethyl)-5-(4-chlorophenyl)-1H-pyrrole-2-carboxamide (45)—To a solution containing *S*-**44** (R_1 =H, R_2 =Cl) (1.52 g, 2.95 mmol) and *N,N*-dimethylbarbituric acid (2.30 g, 14.7 mmol, 5 equiv) in MeOH (30 mL), PPh₃ (77 mg, 20 mol. %) was added under a nitrogen atmosphere followed by Pd(dba)₂ (85 mg, 10 mol. %). The mixture was stirred for 6 hours at reflux. After cooling, 200 mL CH₂Cl₂ was added and the organic phase was extracted twice with 5 % aqueous K₂CO₃ to remove the unreacted NDMBA. The organic phase was dried over Na₂SO₄ and concentrated. Purification by flash chromatography (CH₂Cl₂: MeOH, 20:1, 5:1, 1:1) afforded amine **45A** as a slightly brown or yellowish solids. R_f =0.5 (DCM/MeOH, 1:1).

Yield: 39% (450 mg); rt = 1.430 min. Purity = 100%. LC-MS: m/z [M^+ +H] = 391 Da.

HRMS (ESI): m/z calcd for C₁₈H₂₀ClN₄O₂S [$M+H$]⁺ 391.0990, found 391.0992.

The second enantiomer **45B** was prepared from *R*-**44** using the same procedure: Yield: 65% (550 mg); rt = 1.342 min. Purity = 100%. LC-MS: m/z [M^+ +H] = 391 Da. **HRMS (ESI):** m/z calcd for C₁₈H₂₀ClN₄O₂S [$M+H$]⁺ 391.0990, found 391.0992.

¹H NMR (DMSO-*d*₆, 400 MHz): δ = 2.27 (s, 3H), 2.99 - 3.08 (m, J = 13.3, 7.9, 1H), 3.15 (dd, J = 13.1, 5.3 Hz, 1H), 4.54 (s, 2H), 5.19 (td, J = 7.8, 5.8 Hz, 1H), 6.65 (d, J = 3.9 Hz, 1H), 7.00 (d, J = 3.9 Hz, 1H), 7.43 (d, J = 8.7 Hz, 2H), 7.85 (d, J = 8.7 Hz, 2H), 8.57 (d, J = 8.1 Hz, 1H), 11.90 (br. s, 1H).

¹³C NMR (DMSO-*d*₆, 100 MHz): δ = 14.9, 45.4, 53.9, 55.1, 107.6, 113.1, 126.4 (2C), 127.5, 128.6 (2C), 130.7, 131.1, 132.6, 133.8, 146.9, 160.4, 169.2.

The enantiopurity of compounds **45A** and **45B** was checked on a chiral column ODRH (a mechanical mixture of enantiomers was used as a reference).

ee (**45A**) = 77% and ee (**45B**) = 88%.

Compounds **46A** - **47B** were prepared using the same two-step procedures as for compound **45A**. Protected compounds were used (*fR-44* and *fS-44*) in the next step after chromatographic purification without analysis.

4.1.39. N-(2-amino-1-(5-(hydroxymethyl)-4-methylthiazol-2-yl)ethyl)-5-(4-chloro-3-fluorophenyl)-1H-pyrrole-2-carboxamide [Two isomers, 46A and 46B

—**46A**: Yield: 50% (755 mg; over two steps). rt = 1.275 min. Purity = 98%. LC–MS: m/z [M⁺ +H] =409 Da.

HRMS (ESI): m/z calcd for C₁₈H₁₉ClFN₄O₂S [M+H]⁺ 409.0896, found 409.0903.

46B: Yield: 54% (710 mg; over two steps). rt = 1.375 min. Purity = 97%. LC–MS: m/z [M⁺ +H] =409 Da.

HRMS (ESI): m/z calcd for C₁₈H₁₉ClFN₄O₂S [M+H]⁺ 409.0896, found 409.0900.

¹H NMR: (DMSO-*d*₆, 400 MHz) δ = 2.27 (s, 3H), 3.18 (dd, *J* = 13.0, 8.7 Hz, 1H), 3.30 (dd, *J* = 13.0, 5.1 Hz, 1H), 4.54 (s, 2H), 5.34 (td, *J* = 8.3, 5.1 Hz, 1H), 4.0 - 6.0 (br. s, 2H), 6.76 (d, *J* = 3.8 Hz, 1H), 6.99 (d, *J* = 3.9 Hz, 1H), 7.57 (t, *J* = 8.3 Hz, 1H), 7.70 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.95 (dd, *J* = 11.2, 2.0 Hz, 1H), 8.97 (d, *J* = 8.1 Hz, 1H), 11.93 (br. s, 1H).

¹³C NMR (DMSO-*d*₆, 100 MHz): δ = 14.8, 43.4, 51.7, 55.0, 108.6, 112.6 (d, *J* = 22.7 Hz), 113.1, 117.0 (d, *J* = 17.6 Hz), 121.7 (d, *J* = 3.7 Hz), 127.9, 130.8, 133.7, 132.9 (d, *J* = 7.3 Hz), 133.1, 146.9, 157.5 (d, *J* = 245.2 Hz), 160.3, 168.0.

4.1.40. N-(2-amino-1-(5-(hydroxymethyl)-4-methylthiazol-2-yl)ethyl)-5-(3-fluoro-4-methylphenyl)-1H-pyrrole-2-carboxamide (Two isomers, 47A and 47B)

—**47A (NBD-14012)**: Yield: 22% (313 mg; over two steps). rt = 1.316 min. Purity = 100%.

HRMS (ESI): m/z calcd for C₁₉H₂₂FN₄O₂S [M+H]⁺ 389.1442, found 389.1448.

47B: Yield: 37% (490 mg; over two steps). rt = 1.339 min. Purity = 100%.

HRMS (ESI): m/z calcd for C₁₉H₂₂FN₄O₂S [M+H]⁺ 389.1442, found 389.1445.

¹H NMR: (DMSO-*d*₆, 400 MHz) δ = 2.23 (s, 3H), 2.27 (s, 3H), 3.18 (dd, *J* = 13.1, 8.8 Hz, 1H), 3.31 (dd, *J* = 13.1, 5.0 Hz, 1H), 4.54 (s, 2H), 5.35 (td, *J* = 8.3, 5.1 Hz, 1H), 4.0-6.0 (br. s, 2H), 6.65 (d, *J* = 3.8 Hz, 1H), 6.96 (d, *J* = 3.8 Hz, 1H), 7.27 (t, *J* = 8.2 Hz, 1H), 7.56 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.66 (dd, *J* = 11.6, 1.7 Hz, 1H), 8.93 (d, *J* = 7.9 Hz, 1H), 11.93 (br. s, 1H).

¹³C NMR (DMSO-*d*₆, 100 MHz): δ = 13.9 (d, *J* = 2.9 Hz), 14.8, 45.1, 53.4, 55.0, 107.4, 111.4 (d, *J* = 24.2 Hz), 112.9, 120.5, 122.3 (d, *J* = 17.6 Hz), 127.2, 131.6 (d, *J* = 8.1 Hz), 131.8 (d, *J* = 5.9 Hz), 132.7, 133.9 (d, *J* = 2.9 Hz), 146.9, 160.4, 161.0 (d, *J* = 242.2 Hz), 168.1.

4.2. Biology

4.2.1. Cells and viruses—MT-2 cells (Human T-cell leukemia cells) were obtained through the NIH AIDS Research and Reference Reagent Program (ARP) from Dr. D. Richman²⁵. TZM-bl cells (a HeLa cell line that expresses CD4, CXCR4 and CCR5 and expresses luciferase and β -galactosidase under control of the HIV-1 promoter) were obtained from Dr. J. C. Kappes, Dr. X. Wu and Tranzyme Inc. through the NIH ARP²⁶. H9/HTLV-/HIV-1_{IIIB} cells were obtained through the NIH ARP from Dr. R. Gallo²⁷. HEK 293T cells were purchased from ATCC. MOLT-4/CCR5 cells were obtained through the NIH ARP from Dr. M. Baba, Dr. H. Miyake and Dr. Y. Iizawa. GHOST X4/R5 cells were obtained through the NIH ARP from Drs. V. N. Kewalramani and D. R. Littman²⁸. MAGI-CCR5 cells were obtained through the NIH ARP from Dr. J. Overbaugh²⁹. HL2/3 cells were obtained through the NIH ARP from Drs. B. K. Felber and G. N. Pavlakis³⁰. CD4-negative Cf2Th-CCR5+ cells and Env expression vector pSVIIIenv-ADA were kindly provided by Dr. J. G. Sodroski³¹. HIV-1 Env molecular clone expression vector pHXB2-env (X4) DNA was obtained through the ARP from Dr. K. Page and Dr. D. Littman³². HIV-1 Env molecular clones of gp160 genes for HIV-1 Env pseudovirus production were obtained as follows: clones representing the standard panels A, A/D, A2/D, D and panel C (QB099.391M.ENV.B1 and QB099.391M.ENV.C8) were obtained through the NIH ARP from Dr. J. Overbaugh^{33;34}. The HIV-1 Env molecular clones panel of subtype A/G and A/E (CRF01_AE clone 269) Env clones were obtained through the NIH ARP from Drs. D. Ellenberger, B. Li, M. Callahan and S. Butera³⁵. The AE clone AA058 was kindly provided by Drs. R. J. McLinden and A. L. Chenine from US Military HIV Program, Henry M. Jackson Foundation (Silver Spring, MD). The HIV-1 Env panel of standard reference subtype B Env clones were obtained through the NIH ARP from Drs. D. Montefiori, F. Gao and M. Li (PVO, clone 4 (SVPB11); TRO, Clone 11 (SVPB12); AC10.0, clone 29 (SVPB13); QH0692, clone 42 (SVPB6); SC422661, clone B (SVPB8)); from Drs. B. H. Hahn and J. F. Salazar-Gonzalez (pREJO4541, clone 67 (SVPB16); pRHPA4259, clone 7 (SVPB14); pWITO4160 clone 33 (SVPB18)); from Drs. B. H. Hahn and D. L. Kothe (pTHRO4156 clone 18 (SVPB15), pCAAN5342 clone A2 (SVPB19))^{26;36;37}. The subtype B pWEAUd15.410.5017 and p1058_11.B11.1550 were obtained through the NIH ARP from Drs. B. H. Hahn, B. F. Keele and G. M. Shaw³⁸. The subtype C HIV-1 reference panel of Env clones were also obtained through the NIH ARP from Drs. D. Montefiori, F. Gao, S. A. Karim and G. Ramjee (Du 156.12; Du172.17); from Drs. D. Montefiori, F. Gao, C. Williamson and S. A. Karim (Du422.1), from Drs. B. H. Hahn, Y. Li and J. F. Salazar-Gonzalez (ZM197M.PB7; ZM233M.PB6; ZM214M.PL15); from Drs. E. Hunter and C. Derdeyn (ZM53M.PB12; ZM135M.PL10a; ZM109F.PB4); from Drs. L. Morris, K. Mlisana and D. Montefiori, (CAP45.2.00.G3; CAP210.2.00.E8)³⁹⁻⁴¹. The HIV-1 Subtype C Panel of Indian gp160 Env Clone HIV-16055-2, clone 3 was obtained through the NIH ARP from Drs. R. Paranjape, S. Kulkarni and D. Montefiori³⁵. HIV-1 Env molecular clones MF535.W0M.ENV.C1 and BF535.W6M.ENV.A1 of subtype D/A, were obtained through the NIH ARP from Dr. J. Overbaugh⁴². The ENV pseudotyped genes of BG505.T332N, KNH1144 and B41 were kindly provided by Dr. J. P. Moore of the Weil Cornell Medical College, NY.

The Env-deleted pro-viral backbone plasmids, pNL4-3.Luc.R-E-DNA (Dr. N. Landau)^{43;44} and pSG3^{env} DNA (Drs. J. C. Kappes and X. Wu)^{26;37} were obtained through the NIH ARP. MLV gag-pol-expressing vector pVPack-GP, Env-expressing vector pVPack-VSV-G and a pFB-luc vector were obtained from Stratagene (La Jolla, CA). HIV-1_{IIIB} laboratory adapted strain was obtained through the NIH ARP.

4.2.2. Pseudovirus preparation—Pseudoviruses capable of single cycle infection were prepared as previously described¹⁸. Briefly, HEK 293T cells were transfected with a mixture of 10 µg of an env-deleted pro-viral backbone plasmid pNL4-3.Luc.R-E-DNA or pSG3^{env} DNA and 10 µg of an HIV-1 Env expression vector using FuGENE 6 (Roche). VSV-G pseudovirus was prepared by transfecting 293T cell with a mixture of 10 µg each of the Env-expressing vector pVPack-VSV-G, the MLV gag-pol-expressing vector pVPack-GP and the pFB-luc vector using FuGENE 6. Pseudovirus-containing supernatants were collected two days after transfection, filtered, and stored in aliquots at –80 °C. Pseudoviruses were tittered to calculate the 50% tissue culture infectious dose (TCID₅₀) by infecting different cell types.

4.2.3. Measurement of antiviral activity

Single-cycle infection assay in TZM-bl cells: The inhibitory activity of the new generation of NBD molecules was tested against the pseudovirus HIV-1_{HXB-2} (X4) in a single cycle infection assay. Additionally, selected small molecules **45A** and **46A** were also tested against a large group of HIV-1 pseudotyped viruses expressing the Env from the panel of standard reference as previously described¹⁸. Briefly, TZM-bl cells were plated at 10⁴ cells / well in a 96-well tissue culture plate and cultured at 37 °C overnight. Graded concentrations of the small molecules were incubated with HIV-1 pseudovirus for 30 minutes. The mixture was added to the cells and cultured for 3 days. Cells were washed with PBS and lysed with 50 µl of cell culture lysis reagent (Promega). 20 µl of lysates were transferred to a white plate and mixed with 100 µl of luciferase assay reagent (Promega). The luciferase activity was immediately measured with a Tecan infinite M1000 reader and the percent inhibition by the compounds and IC₅₀ values were calculated using the GraphPad Prism software (GraphPad).

Multi-cycle infection assay: We evaluated the inhibitory activity of the small molecules on infection by the laboratory-adapted HIV-1_{IIIB} as previously described⁴⁵. Briefly, 10⁴ MT-2 cells / well were infected with HIV-1_{IIIB} at 100 TCID₅₀ (0.0069 MOI) pre-treated for 30 minutes with graded concentrations of small molecules. Following overnight incubation at 37 °C, the culture supernatants were replaced with fresh media. Four days post-infection, the culture supernatants were collected and mixed with an equal volume of 5 % Triton X-100 and tested for p24 antigen by sandwich-ELISA. The percent inhibition of p24 production and IC₅₀ (the half maximal inhibitory concentration) values were calculated by the GraphPad Prism software.

4.2.4. Evaluation of cytotoxicity

TZM-bl cells: The cytotoxicity of the small molecules in TZM-bl cells was measured by the XTT method. Briefly, 100 µl of a compound at graded concentrations was added to equal volume of cells (10⁵/ml) in wells of 96-well plates followed by incubation for 3 days and

addition of XTT as described above. The percent of cytotoxicity and the CC₅₀ values were calculated as above.

MT-2 cells: The cytotoxicity of the small molecules in MT-2 cells which ran parallel to the antiviral assay was measured with a colorimetric method using XTT (PolySciences) as previously described⁴⁵. Briefly, 100 µl of a small molecule at graded concentrations was added to an equal volume of cells (10⁵ cells / ml) in 96-well plates followed by incubation for 4 days. Four hours after the addition of XTT the soluble intracellular formazan was quantitated at 450 nm. The percent of cytotoxicity and the CC₅₀ (the concentration for 50 % cytotoxicity) values were calculated as above.

4.2.5. Assay in Cf2Th-CCR5 cells—CD4-negative Cf2Th-CCR5 cells were plated at 6×10^3 cells / well in a 96-well tissue culture plate and incubated overnight. The cells were infected with the luciferase expressing recombinant CD4-dependent pseudovirus HIV-1_{ADA} as previously described¹⁰. Briefly, 50 µl of a test compound at graded concentrations was mixed with equal volume of the recombinant virus and incubated for 30 minutes. The mixtures were added to the cells and cultured for 48 hours. Cells were washed with PBS and lysed with 40 µl of cell lysis reagent. Lysates were transferred to a white 96-well plate and mixed with 100 µl of luciferase assay reagent. The luciferase activity was immediately measured to obtain the relative infection with respect to the untreated control.

4.2.6. Cell-Cell Fusion—Cell-to-cell fusion assay was performed as previously described^{18;46;47} with some minor modifications. MAGI-CCR5 cells, a HeLa cell line expressing CD4, CXCR4 and CCR5, and HIV-LTR-β-gal under control of HIV-1 Tat were used as target cells and HL 2/3 cells, a HeLa cell line which expresses HIV-1_{HXB2} Env on the surface and Tat, Gag, Rev and Nef proteins in the cytoplasm but does not produce detectable mature virions⁴⁸ were used as effector cells.

Following fusion of the two cell types, Tat produced by the HL2/3 cells activates β-gal expression in Magi cells. Briefly, MAGI-CCR5 cells were plated in a black 96-well plate with clear bottom at 1.5×10^4 /well and cultured for 4 hours at 37 °C. Next, the cells were incubated for 1 hour with escalating concentrations of NBD-compounds. HL 2/3 cells were then added to the culture at 10⁴ cells / well and incubated for 24 hours at 37 °C. β-gal expression was quantified with the Beta-Glo assay system (Promega) following the manufacturer's instructions. The percent inhibition and the IC₅₀ values were calculated using the GraphPad Prism software.

4.2.7. CD4-Dependent cell-to-cell HIV-1 transmission inhibition assay—The CD4-dependent cell-to-cell HIV-1 transmission inhibition assay was performed as previously described with some modifications¹⁸. Briefly, target cells, GHOST (3) X4/R5 which are adherent cells, were plated at 10⁴ / well in a 96-well plate and cultured overnight. As transmitting cells we used 4×10^3 /well chronically infected H9/HIV-1_{IIIIB} for the CXCR4-tropic assay and 2×10^4 /well MOLT-4/CCR5 cells chronically infected with HIV-1_{ADA} for the CCR5-tropic assay. The transmitting cells, which are suspension cells, were treated with 200 µg/mL mitomycin C for 1 hour at 37 °C, washed with complete GHOST-medium and incubated with the target cells and drugs for 4 hours. The target cells were then washed three

times with PBS and incubated with fresh medium for 20 hours. Cells were washed with PBS and lysed with 1% Triton X-100. Intracellular p24 contents were determined by ELISA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

HIV-1	Human Immunodeficiency Virus Type 1
Env	Envelope
AIDS	acquire immunodeficiency syndrome
VSV-G	Vesicular stomatitis virus-G
HBTU	(2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate)
TBSCl	tert-Butyldimethylsilyl chloride
DIPEA	N,N-Diisopropylethylamine
Alloc	allyloxycarbonyl
M_{RCN}	mass of recovered nitrile
brsm	based on recovered starting material

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Figure 1. Infectivity of CD4-negative and CCR5-positive Cf2Th-CCR5 cells by CD4-dependent HIV-1_{ADA}

Cf2Th-CCR5 cells were infected with CD4-dependent HIV-1_{ADA} in the presence of escalating concentrations of NBD-compounds. The relative virus infectivity indicates the amount of infection detected in the presence of the compounds divided by the amount of infection detected in the absence of the compounds. Three independent experiments were performed in triplicate, and the graph is representative of one experiment; the values represent the mean $\pm$ standard deviation.

Scheme-1.

Outline of the synthesis of intermediates and compounds 21A – 28B

Scheme-2.

Outline of the synthesis of intermediates and compounds 29 – 37B

Scheme 3.
Outline of the synthesis of intermediates and compounds 45A (NBD-14009) – 47B

Scheme-4.

Nomenclature *fS* was used to designate compounds obtained from (S)-isomers. Similarly *fR* was used for compounds synthesized from (R)-isomers.

Table 1

Anti-HIV-1 activity (IC₅₀) and cytotoxicity (CC₅₀) of NBD-compounds in single-cycle (TZM-bl cells) and multi-cycle (MT-2 cells) assays.

Compound ID	R1	R2	R3	TZM-bl Cells (Mean ± SD)		MT-2 Cells (Mean ± SD)	
				IC ₅₀ (μM)	CC ₅₀ (μM)	IC ₅₀ (μM)	cc ₅₀ (μm)
<div> </div>							
NBD-11021A2	H	Cl	CH ₂ OH	2.2 ± 0.2	23.6±0.2	0.85 ± 0.06	24.8±2.3
21A	F	Cl	CH ₂ OH	3±0.4	15.2±0.2	1.6±0.2	17.2±0.4
21B	F	Cl	CH ₂ OH	3.2±0.5	17.4±1.6	2.6±0.6	26±0.3
22A	F	CH ₃	CH ₂ OH	2.7±0.2	18±0.6	3±0.6	16.1 ±0.4
22B	F	CH ₃	CH ₂ OH	3.8±0.3	19.2±1.6	1.6±0.3	28.2±0.9
23A	F	CH ₃	CH ₃ CH ₂ OH	10.6±0.1	28.8±1	6.1 ±0.7	29.7±2
23B	F	CH ₃	CH ₃ CH ₂ OH	6.3±0.3	15.3±0.2	3±0.4	21.2±1.1
24A	F	Cl	CH ₃ CH ₂ OH	7.5±0.2	16.1 ±0.2	3.4±0.4	16.9±0.2
24B	F	Cl	CH ₃ CH ₂ OH	8±0.1	15.1 ±0.2	2.9±0.4	16.8±0.6
25A	H	Cl	CH ₃ CH ₂ OH	8.9±0.2	17.3±0.1	2±0.3	26.8±1.3
25B	H	Cl	CH ₃ CH ₂ OH	5.7±0.7	15.4±0.3	4.6±0.4	21 ±0.3
26B	F	CH ₃	CH ₃ CH ₂ CH ₂ OH	7.9±1	17±0.7	7.4±0.4	20.6±1
27B	F	Cl	CH ₃ CH ₂ CH ₂ OH	5.1 ±0.7	8.2±0.3	4.5±0.2	12.6±0.1
28B	H	Cl	CH ₃ CH ₂ CH ₂ OH	7.6±0.8	13.7±1	5.8±1.3	19±0.2
<div> </div>							
35A	H	Cl	CH ₂ OH	14.1 ±0.2	30±0.8	10.2±1.3	36.6±0.8
35B	H	Cl	CH ₂ OH	15.8±0.6	33.8±0.8	18±3.6	45.2±1.4
36A	F	Cl	CH ₂ OH	8.8±0.6	26.7±0.8	10.6±3.2	31.1±0.8
36B	F	Cl	CH ₂ OH	16±0.3	30.7±1.3	22.8±2.8	36.4±2.5
37A	F	CH ₃	CH ₂ OH	9.4±0.2	33.3±0.7	12.9±4.2	28.4±0.5

Compound ID	R ₁	R ₂	R ₃	TZM-bl Cells (Mean ± SD)		MT-2 Cells (Mean ± SD)	
				IC ₅₀ (μM)	CC ₅₀ (μM)	IC ₅₀ (μM)	CC ₅₀ (μM)
37B	F	CH ₃	CH ₂ OH	16.9±0.5	34.5±0.7	22.3±4.7	37.1 ±2

45A (NBD-14009)	H	Cl	CH ₂ OH	2.1 ±0.2	33.6±0.8	2.7±0.33	25.8±0.8
45B	H	Cl	CH ₂ OH	6.5±1.6	34.1 ±3.9	2.1 ±0.04	28.2±0.5
46A (NBD-14010)	F	Cl	CH ₂ OH	0.59±0.06	40.5±1.3	0.52±0.05	30.5±0.6
46B (NBD-14011)	F	Cl	CH ₂ OH	0.68±0.03	26±2.5	0.77±0.01	22.7±0.6
47A (NBD-14012)	F	CH ₃	CH ₂ OH	1.7±0.04	35±1.3	0.91±0.1	31.6±1.4
47B (NBD-14013)	F	CH ₃	CH ₂ OH	1.1±0.1	26.2±1.3	0.65±0.06	24.3±1.5

^aThe reported IC₅₀ and CC₅₀ values represent the means ± standard deviations (SD) of three replicates

Table 2

Antiviral activity of NBD-compounds against a panel of HIV-1 Env Pseudoviruses

Subtype	NIH #	Env	IC ₅₀ (μM) Mean ± S.D. ^a		
			NBD-11021A2	45A (NBD-14009)	46A (NBD-14010)
A	11887	Q259env.w6	1.6±0.1 ^c	1.3±0.02	0.74±0.06
	11888	QB726.70M.ENV.C4	1.3±0.3 ^c	0.8±0.3	0.87±0.2
	11890	QF495.23M.ENV.A1	1.3±0.4 ^c	1.2±0.3	0.62±0.04
	11891	QF495.23M.ENV.A3	0.88±0.4 ^c	0.5±0.08	0.31±0.01
	11892	QF495.23M.ENV.B2	0.6±0.4 ^c	0.74±0.1	0.52±0.07
		BG505-T332N	1±0.1 ^c	0.78±0.2	0.33±0.04
		KNH1144	2.1±0.4 ^c	0.67±0.02	0.43±0.2
A/D	11901	QA790.204I.ENV.A4	1.4±0.05 ^c	2.1±0.3	0.62±0.02
	11903	QA790.204I.ENV.C8	1.5±0.02	0.74±0.06	0.21 ±0.003
	11904	QA790.204I.ENV.E2	0.71 ±0.09	0.47±0.09	0.4±0.05
A2/D	11905	QG393.60M.ENV.A1	0.41±0.2 ^c	1.3±0.1	0.24±0.02
	11906	QG393.60M.ENV.B7	0.8±0.3 ^c	0.41±0.04	0.35±0.01
	11907	QG393.60M.ENV.B8	0.48±0.2 ^c	0.36±0.02	0.37±0.03
A/E	11603 (AE potential)	CRF01_AE clone 269	1.5±0.3 ^c	2±0.2	0.7±0.02
		AA058	4.4±0.1	1±0.2	0.21 ±0.008
A/G	11601	CRF02_AG clone 263	1.4±0.3 ^c	1.5±0.2	0.59±0.09
	11602	CRF02_AG clone 266	1±0.2 ^c	1.6±0.2	0.68±0.02
	11605	CRF02_AG clone 278	1.3±0.5 ^c	1.1±0.2	0.68±0.02
B		B41	0.68±0.1 ^c	0.32±0.02	0.28±0.03
	11563	p1058_11.B11.1550 ^b	1.1±0.8 ^c	0.9±0.07	0.36±0.05
	11578	pWEAUd15.410.5017 ^b	3±0.1 ^c	0.67±0.1	0.28±0.04
	11018	QH0692, clone 42	0.52±0.1 ^c	1.6±0.2	0.49±0.05
	11022	PVO, clone 4	1.9±0.1 ^c	1.3±0.3	0.74±0.07
	11023	TRO, clone 11	1.2±0.05 ^c	1.4±0.04	0.51 ±0.09
	11024	AC10.0, clone 29	0.32±0.01 ^c	0.54±0.1	0.29±0.03
	11033	pWITO4160 clone 33	1.7±0.1 ^c	1.1±0.2	0.5±0.06
	11035	pREJO4541 clone 67	1.6±0.3 ^c	1±0.2	0.43±0.03

			IC ₅₀ (μM) Mean ± S.D. ^a		
Subtype	NIH #	Env	NBD-11021A2	45A (NBD-14009)	46A (NBD-14010)
	11036	pRHPA4259 clone 7	1.4±0.2	0.86±0.3	0.32±0.01
	11037	pTHRO4156 clone 18	1±0.2 ^c	0.82±0.08	0.69±0.08
	11038	pCAAN5342 clone A2	0.6±0.07 ^c	0.39±0.02	0.22±0.02
	11058	SC422661.8	0.33±0.01 ^c	0.54±0.02	0.2±0.01
C	11306	Du156, clone 12	2.2±0.5 ^c	1.3±0.08	0.43±0.08
	11307	Du172, clone 17	1.9±0.5 ^c	0.78±0.1	0.59±0.06
	11308	Du422, clone 1	3.3±0.4 ^c	0.81±0.2	0.62±0.06
	11309	ZM197M.PB7	2±0.2	0.43±0.05	0.3±0.04
	11310	ZM214M.PL15	2.7±0.2 ^c	1.1±0.1	0.42±0.03
	11311	ZM233M.PB6	1.7±0.1	1.1 ±0.1	0.21±0.02
	11313	ZM53M.PB12	1.5±0.2 ^c	1.6±0.2	0.6±0.04
	11314	ZM109F.PB4	2.5±0.06	1.4±0.2	0.75±0.02
	11315	ZM135M.PL10a	2.7±1.2 ^c	0.74±0.09	0.38±0.03
	11316	CAP45.2.00.G3	1.2±0.2	0.63±0.05	0.15±0.003
	11317	CAP210.2.00.E8	2.8±0.7 ^c	0.76±0.05	0.43±0.05
	11502	HIV-16055-2, clone 3	2.3±0.1	1.2±0.2	0.86±0.08
	11908	QB099.391M.ENV.B1	1.7±0.4 ^c	0.89±0.3	0.47±0.03
	11909	QB099.391M.ENV.C8	1.8±0.5 ^c	1.3±0.2	0.49±0.05
D	11911	QA013.70I.ENV.H1	2.6±0.2 ^c	2.2±1.6	0.66±0.04
	11912	QA013.70I.ENV.M12	2.2±0.3 ^c	0.54±0.07	0.3±0.01
	11916	QD435.100M.ENV.B5	1.6±0.4	1.4±0.3	0.26±0.04
	11918	QD435.100M.ENV.E1	3.7±0.1 ^c	0.52±0.3	0.46±0.09
D/A	11526 (mother)	MF535.W0M.ENV.C1	1.8±0.5 ^c	1.5±0.3	0.33±0.02
	11517 (infant)	BF535.W6M.ENV.A1	2.8±0.6 ^c	2.3±0.7	0.66±0.02
Control		VSV-G	7.1±0.7	6.3±0.6	8.7±1.1

^aThe reported IC₅₀ values represent the mean ± standard deviation (SD) of three replicates.

^bR5X4-tropic viruses; all the rest were CCR5-tropic viruses.

^cData previously published (Ref.18).

Table 3

Activity of NBD-compounds in cell-cell fusion assay and cell-to-cell HIV-1 transmission assay.

	NBD-11021A2 IC₅₀ (μM) Mean ± S.D.	45A (NBD-14009) IC₅₀ (μM) Mean ± S.D.	46A (NBD-14010) IC₅₀ (μM) Mean ± S.D.
MAGI-CCR5/ HL2/3	8.9±0.4	11.9±2.4	15.2±2.4
GHOST (3) X4/R5 H9/HIV-1 _{IIIb}	8.8±0.6	8.6±0.3	6.4±0.9
GHOST (3) X4/R5 MOLT-4/CCR5/HIV- 1 _{ADA}	23±3	30±2	24±2.5