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Design, Synthesis, and antiviral activity of a series of CD4-mimetic small-molecule HIV-1 entry inhibitors

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Abstract

We presented our continuing stride to optimize the second-generation NBD entry antagonist targeted to the Phe43 cavity of HIV-1 gp120. We have synthesized thirty-eight new and novel analogs of NBD-14136, earlier designed based on a CH₂OH “positional switch” hypothesis, and derived a comprehensive SAR. The antiviral data confirmed that the linear alcohol towards the “N” (C4) of the thiazole ring yielded more active inhibitors than those towards the “S” (C5) of the thiazole ring. The best inhibitor, NBD-14273 (compound **13**), showed both improved antiviral activity and selectivity index (SI) against HIV-1_{HXB2} compared to NBD-14136. We also tested NBD-14273 against a large panel of 50 HIV-1 Env-pseudotyped viruses representing clinical isolates of diverse subtypes. The overall mean data indicate that antiviral potency against these isolates improved by ~3-fold, and SI also improved ~3-fold compared to NBD-14136. This new and novel inhibitor is expected to pave the way for further optimization to a more potent and clinically relevant inhibitor against HIV-1.

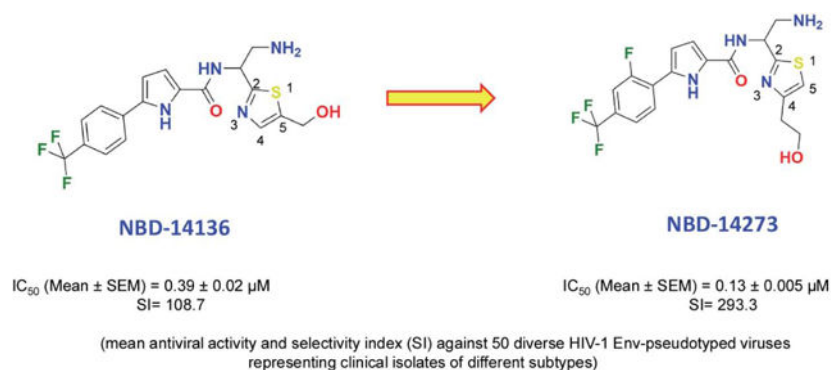
Graphical Abstract

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.



Keywords

HIV-1; ENV-pseudovirus; gp120 entry-antagonist; cytotoxicity; broad-spectrum; structure-activity relationship (SAR); selectivity index (SI)

1. Introduction

Human immunodeficiency virus type-1 (HIV-1) is the causative of the acquired immunodeficiency syndrome (AIDS). Based on the 2020 Global HIV Statistics from UNAIDS, 38 million people are living with HIV globally, with about 1.7 million new infections in 2019. Since the start of the epidemic, about 76 million people have become infected with this deadly disease, and more than 32 million have lost their lives due to AIDS-related illnesses.

However, advances in available therapeutics, particularly combination antiretroviral therapy (cART), significantly improved the treatment of HIV infection and facilitated the shift from high morbidity and mortality to a manageable chronic disease. Despite this remarkable success, current treatments suffer from several limitations, including (1) reliance on daily adherence, (2) long-term use resulting in long-term toxicity, (3) limited treatment options due to the development of drug resistance, (4) high cost, and finally, (5) the non-curative nature of current treatments. Further, despite the tremendous effort and investment for over a decade, the availability of an effective vaccine or microbicide is not on the near horizon, and significant hurdles must be overcome to achieve an effective cure. Thus, the continued development of small-molecule drugs with high potency against novel targets, but minimal side effects is imperative. The development of novel therapeutics will aid in increasing the number of new drugs available and will extend the scope of combination therapy.

Viral attachment and fusion to the host cell membrane are critical to the ability of HIV-1 to enter host cells and initiate its life-cycle by utilizing host cell machinery¹. Therefore, viral attachment and fusion (often collectively termed “viral entry”) have been targets of new drug discovery for years¹⁻⁴. Only two drugs currently on the market that target the HIV-1 entry pathway. FUZEON® (enfuvirtide) targets the envelope glycoprotein gp41^{5, 6}, and SELZENTRY® (maraviroc) targets the host cell receptor CCR5 to prevent viral entry⁷⁻⁹.

Even though HIV-1 gp120 is critical for viral entry and has been the target of drug discovery efforts, no drugs that target gp120 have been approved by the US FDA. BMS-663068, the most advanced inhibitor in this class, is a prodrug of BMS-626529. The safety and efficacy of BMS-663068 were recently demonstrated in a Phase 2b clinical trial, and the compound is currently undergoing Phase III clinical trials.

Our laboratory had focused on developing novel inhibitors targeted to the Phe43 cavity of gp120 since 2005 when we first reported the discovery of NBD-556, the NBD-series CD4-mimic¹⁰. Unfortunately, NBD-556 was shown to enhance HIV-1 infection in CD4⁻-CCR5⁺ cells and thus behaved as an HIV-1 entry agonist^{11, 12}. Since this finding, we and others have endeavored to design CD4 mimics with not only higher potency and lower toxicity but also that are devoid of this unwanted agonist property and serve as HIV-1 entry antagonists¹²⁻²².

Since the discovery of the small molecule CD4-mimic, NBD-556, we systematically showed by x-ray crystallography that it binds to the Phe43 cavity of the HIV-1 gp120²³. The major transformation of CD4-mimic came through the design of NBD-11021. Based on the x-ray structure of NBD-11021¹², we further modified the molecules by replacing the piperazine ring with a simple amine, such as NBD-14010. The x-ray structure of NBD-14010 provided critical information on modifying the thiazole ring substituents to achieve robust interactions with the target protein²⁴. One such essential details lead us to postulate the “Positional switch” hypothesis that resulted in more potent antivirals, such as NBD-14136 (IC₅₀=0.27 μM), NBD-14168 (IC₅₀=0.28 μM), and NBD-14189 (IC₅₀=0.089 μM) that we reported earlier (Figure 1)²⁵. Based on this success, we wanted to expand further the structure-activity relationship (SAR) of these series of compounds with significant emphasis on compounds with the p-CF₃ substituents, which yielded the most active CD4-mimics as antivirals. In this report, we presented our effort to expand the SAR studies of an extensive series of small molecule CD4-mimics to optimize further this class of HIV-1 entry inhibitors that target the Phe43 cavity of envelope glycoprotein gp120 to improve its antiviral activity and selectivity index (SI).

2. Results and Discussions

2.1. Design, anti-HIV-1 screening, and structure-activity relationships (SARs)

In this study, we focused on one of the best lead NBD-14136 containing a trifluoromethyl (CF₃) group at the para position, which showed an IC₅₀ of 0.27 μM and CC₅₀ of 42.4 μM resulting in an SI (where SI = CC₅₀/IC₅₀) = 157. We synthesized a large set of compounds to improve the antiviral activity and SI and derived a comprehensive SAR based on the lead NBD-14136. In most cases, we kept the CF₃ substituent at the para position in the phenyl ring. We also decided to minimally alter the substituents in the phenyl ring and make changes in the thiazole moiety that showed improvement in antiviral activity and selectivity before. Due to the presence of one chiral center, all compounds have two stereoisomers (defined as R and S in Table 1). We observed in our earlier studies that in the majority of the cases, the S isomer had better antiviral potency. Therefore, in the SAR, we will mostly focus on comparing the S isomers. We have initially tested all the synthesized compounds against HIV-1_{HXB2} in TZM-bl cells (Table 1) to identify the best active molecule with higher SI to

further test it against a large panel of HIV-1 ENV-pseudotyped reference viruses from clinical isolates.

Compound **2** with a CF₃ at R₂ and a fluoro (F) at R₄ position with CH₂OH at R₆ positions yielded one of the most active compounds in this series (IC₅₀=0.19 μM and SI=137). However, when we kept all groups the same except changing CH₂OH to CH₂CH₂OH, the cytotoxicity improved by about 1.7-fold, providing compound **13** with similar anti-HIV-1 activity but much improved SI of 220. Moving the F to R₁ in compound **12** had no apparent effect on both antiviral activity and cytotoxicity when compared to compound **2**. We have explored a large number of compounds keeping the substituents the same as in compound **12** by increasing the complexity of the primary alcohol substituent at R₆. In general, the cytotoxicity of the majority of the compounds improved, but the activity varied considerably. When the chain length was increased in compound **17**, the activity and cytotoxicity remained similar to **12**. The antiviral activity dropped considerably when R₆ had branched primary alcohols (compounds **15** and **19**). When we replaced the primary alcohol with an acid, the compounds became insoluble; therefore, we could not perform the antiviral or cytotoxicity assays. We also observed a considerable drop in antiviral activity when R₆ had an amide or ester substituents. Interestingly, when we moved the branched alcohol substituent to the R₇ position, antiviral activity dropped substantially (compound **19**). It appears that bulkier alcohol substituent in either R₆ or R₇ position was not well tolerated. Interestingly, we also observed that when we substituted the R₂ position with a hydrophilic substituent (CN), we virtually lost the antiviral potency (Compound **4**). It was, however, well-established that the Phe43 pocket in that region is hydrophobic. Therefore, the result was not surprising. A chloro (Cl) substituent at R₂ and F in both R₁ and R₃ with CH₂CH₂OH at R₆ (Compound **9**) yielded one of the most active inhibitors with an IC₅₀ of 0.15 μM and SI of 218. Interestingly, when we added a methyl group at position R₁₀ on the pyrrole ring, antiviral activity improved remarkably (compounds **27–30**). Unfortunately, the cytotoxicity also was higher, making the SI values low. We also observed that when the primary amine had two methyl substituents at R₈ and R₉ (compounds **31–34**) and the CH₂OH group was at position R₆, the antiviral activities were also improved substantially, but again these additional methyl groups also made those molecules more cytotoxic (CC₅₀). However, when the CH₂OH group was moved to R₇ (Compound **36**), the antiviral activity dropped by about 2-fold, but cytotoxicity improved to make SI >100. In the same series, when we substituted R₇ with branched alcohol (compounds **37** and **38**), the antiviral potency dropped substantially, but the cytotoxicity improved, although SI values did not improve. Overall, the SAR analyses confirmed that the antiviral activity considerably improved by moving the primary alcohol groups to R₆ from R₇ (as in NBD-14136). Both *p*-Cl and *p*-CF₃ containing compounds (compounds **9** and **13**) showed high potency with high SI values. The data in Table 1 demonstrated that NBD-14242 and NBD-14273 showed very similar antiviral activity and SI (IC₅₀ of 0.15 and 0.18 μM; and 218 and 220, respectively). We selected NBD-14273 for further study.

2.2. NBD-14273 showed entry antagonist features

NBD-556¹⁰, our first-generation gp120-targeted compound, mimic CD4 by inducing a conformational change in gp120 and facilitating HIV infection into CD4-negative cells that

expressed the coreceptor CCR5^{13, 26}. Since then, we validated our new generation inhibitors by confirming that they do not possess this undesirable trait and work as entry antagonists^{27–30}. We selected NBD-14273, which exhibited the best anti-HIV-1 activity and a higher Selectivity Index value (SI = 220). CD4-negative Cf2TH-CCR5 cells were infected with the recombinant CD4-dependant HIV-1_{ADA} virus in the presence of escalating concentrations of NBD-14273 and NBD-556, which was used as a control. While NBD-556 significantly supported the infection of the Cf2Th-CCR5 cells, NBD-14273 did not, indicating that this compound is an HIV-1 entry antagonist (Figure 2). This data assured us of selecting this inhibitor for further evaluation against a large and diverse panel of HIV-1 ENV-pseudotyped clinical isolates.

2.3. Antiviral activities of NBD-14273 against a large and diverse panel of HIV-1 Env-pseudotyped clinical isolates

Since we reported the identification of NBD-556¹⁰ as the first example of HIV-1 entry-inhibitor, we have shown consistent improvement in the anti-HIV-1 activity of our new generations of gp120 entry-antagonists against the control lab-adapted virus HIV-1_{HXB2} and a large panel of diverse HIV-1 Env-pseudotyped clinical isolates^{12, 15, 25, 28, 31}. In this study, the anti-HIV-1 activity of NBD-14273 was evaluated against a collection of 50 HIV-1 clinical isolates of diverse subtypes, including primary, transmitted, and early founder HIV-1 isolates and a selection of 12 recombinant HIV-1 clones (Table 2). All HIV-1 clones tested use the CCR5 coreceptor for entry except for two dual-tropic (CCR5/CXCR4) clones (NIH # 11563 and 11578). We compared the antiviral activity of this compound with that of the previously described inhibitor NBD-14136^{28, 32}. NBD-14273 exhibited a broad-spectrum antiviral activity, as shown by its activity against all the clinical isolates tested, regardless of the viral subtype or coreceptor usage. The anti-HIV-1 activity of NBD-14273 was about 2.4–3.3-fold higher than the anti-viral activity detected for NBD-14136, as demonstrated by their overall mean IC₅₀ values and their antiviral activities against the different HIV subtypes (Table 2). The overall mean IC₅₀ value for NBD-14136 was 0.39 ± 0.02 μM (with the SI of 108.7 and the IC₅₀ values ranged from 0.19–0.79 μM), whereas the overall mean IC₅₀ value determined for NBD-14273 was 0.135 ± 0.005 μM (with the SI of 293.3 and the IC₅₀ values ranged from 0.11–0.22 μM). It is noteworthy to mention that our earlier reported inhibitor NBD-14189, which showed the best activity profile (as low as 63 nM compared to 94 nM for NBD-14273), but NBD-14273 demonstrated better SI (199.1 for NBD-14189 vs. 293 for NBD-14273)²⁵. In this study, NBD-14273 showed better antiviral activity against all the viruses of different subtypes tested in this work. In contrast, this compound exhibited a slightly better cytotoxicity profile (CC₅₀ of 39.6 ± 2 μM) than that for NBD-14136 (CC₅₀ of 42.4 ± 1.0 μM), which resulted in an improvement of the NBD-14273 SI values of 2.2–3-fold with respect to NBD-14136. Moreover, NBD-14273 was poorly active against the pseudovirus VSV-G, which was used here as a control, suggesting that the inhibitory activities of these compounds are specific to HIV-1. Additionally, the toxicity in the U87-CD4-CXCR4 cell line was similar to what we detected in the other cell lines.

Furthermore, we evaluated the anti-HIV-1 activity of NBD-14273 against a panel of HIV-1 clones, which included a group of paired infant and maternal clones. These HIV clones were isolated from chronically infected mothers and their respective infected infants and belonged

to subtypes A and D/A³³. It has been shown that the vertically transmitted HIV-1 infant variants were more challenging to neutralize using combinations of broadly neutralizing antibodies (bNAbs) 2G12, biz, 2F5, and 4E10³³. In this study, we found that NBD14273, as previously shown for NBD-14136³², equally neutralized both the infant and maternal HIV-1 variants (Table 3), as shown by their overall mean of the IC₅₀ values and the IC₅₀ means for infant and maternal viruses. Of note, NBD-14273 was about 4-fold more effective than NBD-14136 against both viral clones of the infant and maternal panel. These findings suggest that NBD-14273 can neutralize both infant and maternal HIV-1 variants.

2.4. Inhibitory activity of NBD-14273 against a large panel of FDA-approved-drug-resistant viruses

We further evaluated the anti-viral activity of NBD-14273 against a panel of drug-resistant viruses, consisting of 5 HIV-1 clones resistant to the entry-inhibitor Enfuvirtide (T-20); 7 HIV-1 multi-drug-resistant clones specifically, these are non-nucleoside reverse transcriptase inhibitor (NNRTI)-resistant viruses, which carry mutations that confer resistance to both NNRTIs and nucleoside reverse transcriptase inhibitors (NRTIs); 3 HIV-1 clones resistant to the integrase inhibitor Raltegravir; and 9 HIV-1 clones resistant to multiple protease inhibitors (Table 4). As a reference, the activity of NBD-14273 was evaluated against the wild type (WT) HIV-1_{NL4-3} clone, which was used to obtain the drug-resistant viruses. For this assay, the TZM-bl cells were infected with the WT HIV-1_{NL4-3} control virus or the drug-resistant viruses pretreated for 30 min with escalating concentrations of NBD-14273. NBD-14273 inhibited the WT HIV-1_{NL4-3}, with an IC₅₀ value of 0.21 μM. All the T-20-resistant viruses were sensitive to NBD-14273; the IC₅₀ values we detected were similar to those determined for the control virus, WT HIV-1_{NL4-3}. Regarding the NNRTI-resistant viruses, also all the viral clones were sensitive to NBD-14273. In some cases, we observed that the drug-resistant viruses appeared to be more sensitive to the gp120 entry-antagonist than the WT virus. The only exceptions were the HIV-1 clone NIH # 12243, which showed a 6.7-fold increase of the IC₅₀ with respect to the IC₅₀ determined for the WT HIV-1_{NL4-3} control virus. NIH #12233 and NIH #12239 clones showed a 3.38- and 3.85-fold increase of the IC₅₀, respectively. The Raltegravir-resistant viruses and the protease inhibitor-resistant viruses were also sensitive to NBD-14273, as demonstrated by their low IC₅₀ values. In conclusion, NBD-14273 was effective against all the drug-resistant viruses tested in this work, indicating that this compound could potentially be used in combination with other antiviral agents. To explain the low sensitivity of the HIV-1 clones, NIH # 12243, NIH #12233, and NIH #12239 to NBD-14273 will require further investigation.

2.5. gp120 entry-antagonists inhibited cell-to-cell HIV-1 transmission

Our earlier generation of gp120 entry-antagonists displays another critical feature: the ability to inhibit cell-to-cell HIV-1 transmission^{25, 32}. Cell-to-cell HIV-1 infection has been reported to be more efficient than HIV-1 cell-free infection^{34, 35} as multiple viral particles can be transmitted at the same time. Additionally, this type of infection is resistant to some potent broadly neutralizing antibodies (bNAbs), including CD4 binding site (CD4bs) antibodies^{36–38} and NRTIs, but not to other antiretrovirals, including entry inhibitors, NNRTIs, and protease inhibitors³⁹. Here, we evaluated the activity of NBD-14273 in the cell-to-cell HIV-1 transmission setting and compared its activity to that of NBD-14136,

which has previously been reported to inhibit cell-to-cell HIV-1 transmission^{25, 32}. We used TZM-bl cells as acceptor cells and H9 cells chronically infected with HIV-1_{IIIB} (CXCR4-tropic), and MOLT-4 cells chronically infected with HIV-1_{ADA} (CCR5-tropic) as donor cells. BMS-626529 was used as a control treatment drug. Our results (Table 5) indicated that all the compounds tested in this assay, including BMS-626529, showed better activity against the CXCR4-tropic virus HIV-1_{IIIB} than against the CCR5-tropic virus HIV-1_{ADA}. Furthermore, NBD-14273 had slightly improved activity with respect to that we detected for NBD-14136. Of note, the IC₅₀ value for NBD-14273 calculated against HIV-1_{ADA} in the CCR5-tropic assay was 1-fold lower than the IC₅₀ values calculated for NBD-14136. When we compared the cell-cell HIV-1 transmission inhibition of NBD-14273 with our earlier reported most active inhibitor NBD-14189, it showed somewhat lower activity²⁵.

3. Conclusion

In summary, we have expanded the SAR of NBD-14136 generated through the “CH2OH” positional switch hypothesis reported earlier. A systematic medicinal chemistry optimization and SAR study yielded several new compounds with improved antiviral potency and SI. We evaluated one of the best inhibitors, NBD-14273, against a large panel of 50 HIV-1 Env-pseudotyped viruses representing clinical isolates of diverse subtypes. The data against this set of clinical isolates indicated an improvement of antiviral potency and selectivity index by ~3-fold. NBD-14273 also showed antiviral activity against a large panel of FDA-approved-drug-resistant HIV viruses. The detailed data is expected to pave the way to optimize this series further to a clinically relevant candidate.

4. Experiment

4.1. Cells and viruses

TZM-bl cells⁴¹, U87CD4+CXCR4+ cells⁴², HIV-1 IIIB, infected H9 Cells⁴³, and MOLT-4 CCR5+ Cells⁴⁴ were obtained through the NIH ARP. HEK 293T cells were purchased from ATCC. CD4-negative Cf2Th-CCR5+ cells and Env expression vector pSVIIEnv-ADA were kindly provided by Dr. J. G. Sodroski⁴⁵. HIV-1 Env molecular clone expression vector pHXB2-env (X4) DNA was also obtained through the NIH ARP⁴⁶. HIV-1 Env molecular clones of gp160 genes for HIV-1 Env pseudovirus production were obtained as follows: clones representing the standard panels A (Q259ENV.W6, QB726.70M.ENV.C4, QF495.23M.ENV.A1, QF495.23M.ENV.A3), A/D, A2/D, D, and C (QB099.391M.Env.B1) were obtained through the NIH ARP from Dr. J. Overbaugh^{47, 48}. The HIV-1 Env molecular clones panel of subtype A/G, A/E, and G Env clones were obtained through the NIH ARP from Drs. D. Ellenberger, B. Li, M. Callahan, and S. Butera⁴⁹. The HIV-1 Env panel of standard reference subtype B Env clones was obtained through the NIH ARP from Drs. D. Montefiori, F. Gao and M. Li (PVO, clone 4 (SVPB11) TRO, Clone 11 (SVPB12), QH0692, clone 42 (SVPB6), SC422661, clone B (SVPB8)); from Drs. B. H. Hahn and J. F. Salazar-Gonzalez (pRHPA4259, clone 7 (SVPB14)); from Drs. B. H. Hahn and D. L. Kothe (pTHRO4156 clone 18 (SVPB15), pCAAN5342 clone A2 (SVPB19))^{50, 51}. The subtype B clones pWEAUd15.410.5017, p1058_11.B11.1550, p1054.TC4.1499, p1006_11.C3.1601, p1056.TA11.1826 and p9021_14.B2.4571 were obtained through the NIH ARP from Drs. B.

H. Hahn, B. F. Keele, and G. M. Shaw⁵². 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 (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; ZM214M.PL15, ZM249M.PL1); from Drs. E. Hunter and C. Derdeyn (ZM53M.PB12; ZM109F.PB4); from Drs. L. Morris, K. Mlisana and D. Montefiori, (CAP210.2.00.E8)^{53–55}. The HIV-1 Subtype C Panel of Indian gp160 Env Clones HIV-16055–2 clone 3, HIV-16936–2 clone 21, HIV-25711–2 clone 4, and HIV-225925–2 clone 22 were obtained through the NIH ARP from Drs. R. Paranjape, S. Kulkarni and D. Montefiori⁴⁹. The panel of paired Infant and maternal HIV-1 Env Molecular Clones were obtained through the NIH ARP from Dr. J. Overbaugh⁵⁶. 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 proviral backbone plasmids pNL4–3.Luc.R-E-DNA (from Dr. N. Landau)^{57, 58}, the pSG3^{env} DNA (from Drs. J. C. Kappes and X. Wu)^{41, 51}, and the pNL4–3 (from Dr. Malcolm Martin)⁵⁹ were obtained through the NIH ARP Division of AIDS, NIAID, NIH. The following molecular clones were also obtained through the NIH ARP, Division of AIDS, NIAID, NIH: the panel of Enfuvirtide (T-20) resistant viruses from Trimeris, Inc.^{59, 60}; the panel Multi-Drug Resistant NNRTI Infectious Clones from Dr. R. Shafer⁶¹; the Raltegravir-resistant infectious molecular clones from Dr. R. Shafer and E. Reuman, M.S⁶² and the multi-drug Protease inhibitor-resistant infectious clones from Dr. R. Shafer⁶³.

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).

4.2. Pseudovirus preparation

Pseudoviruses capable of single-cycle infection were prepared as previously described^{13, 15}. Briefly, 5×10^6 HEK293T cells were transfected with an HIV-1 Env-expression plasmid and either the HIV-1 Env-deleted pro-viral backbone plasmid pSG3^{env} or the pNL4–3.Luc.R-E-DNA, by using FuGENE6 (Promega). The control VSV-G pseudovirus was prepared by transfecting the HEK293T cells with a combination of the Env-expressing plasmid pVPack-VSV-G, the MLV gag-pol-expressing plasmid pVPack-GP, the pFB-Luc plasmid by using FuGENE6. Pseudovirus-containing supernatants were collected two days after transfection, filtered, tittered, and stored in aliquots at $-80\text{ }^{\circ}\text{C}$.

4.3. Measurement of antiviral activity

4.3.1. Single-cycle infection assay in TZM-bl cells—The antiviral activity of the new generation of gp120 entry-antagonists was evaluated in a single-cycle infection assay by infecting TZM-bl cells with HIV-1 pseudotyped with the Env from the lab-adapted CXCR4-tropic HIV-1_{HXB-2}. Additionally, NBD-14136 and NBD-14273 were evaluated against a large group of HIV-1 pseudotyped with the Env from the panel of clinical isolates as previously described^{13, 15}. Briefly, TZM-bl cells were plated at 1×10^4 / well in a 96-well tissue culture plate. Following overnight incubation, the cells were infected with aliquots of

HIV-1 pseudovirus pre-treated with graded concentrations of the small molecules for 30 min. On the third day post-infection, the cells were washed and lysed with 50 μ l of lysis buffer (Promega). 20 μ l of the lysates were transferred to a white plate and mixed with the luciferase assay reagent (Promega). The luciferase activity was measured immediately with a Tecan Spark reader, and the percent inhibition by the compounds and the IC₅₀ (the half-maximal inhibitory concentration) values were calculated using the GraphPad Prism software.

4.3.2. Single-cycle infection assay in U87-CD4-CXCR4 cells—The antiviral activity of NBD-14136 and NBD-14273 was tested against the control pseudovirus VSV-G in U87-CD4-CXCR4 cells. Briefly, U87-CD4-CXCR4 cells were plated in a 96-well tissue culture plate at 1×10^4 /well and cultured at 37 °C. Following overnight incubation, the cells were infected with aliquots of pseudovirus pre-treated with graded concentrations of the small molecules for 30 min. On the third day, post-infection, the cells were washed and lysed with 40 μ l of lysis buffer. The lysates were then transferred to a white plate and mixed with the luciferase assay reagent. The luciferase activity was immediately measured to calculate the percent of inhibition and IC₅₀ values by using the GraphPad Prism software.

4.3.3. 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 at 37 °C. The following day, the cells were infected with the recombinant CD4-dependent pseudovirus HIV-1_{ADA} as previously described¹¹. Briefly, aliquots of HIV-1_{ADA} pseudovirus pre-treated with graded concentrations of NBD-14273 and NBD-556 for 30 min were added to the cells and cultured for 48 h. The cells were then washed with PBS and lysed with 40 μ l of cell lysis reagent. The lysates were mixed with the luciferase assay reagent, and the luciferase activity was measured. For each compound, we calculated the relative infection compared to the untreated control. The Relative virus infectivity indicates the ratio of the amount of infection detected in the presence of the compounds and the amount of infection detected in the absence of the compounds.

4.3.4. Measurement of antiviral activity against drug-resistant viruses in TZM-bl cells—We evaluated the antiviral activity of NBD-14273 against a panel of drug-resistant viruses by infecting TZM-bl cells. Briefly, TZM-bl cells were plated at 10^4 /well in a 96 well plate and cultured overnight. On the following day, the HIV-1 drug-resistant viruses were pre-treated with graded concentrations of the small molecules for 30 min and added to the cells. Following 48 h incubation, the cells were washed and lysed. The cellular lysates were mixed with the luciferase substrate, and the luciferase activity was measured to calculate the percent inhibition by the compounds and the IC₅₀ values using the GraphPad Prism software, as reported above.

4.4. Evaluation of cytotoxicity

4.4.1. TZM-bl cells and U87-CD4-CXCR4 cells—The cytotoxicity of the gp120 entry-inhibitors in TZM-bl and U87-CD4-CXCR4 cells was measured by using the colorimetric CellTiter 96@ Aqueous One Solution Cell Proliferation Assay (MTS) (Promega) following the manufacturer's instructions. Briefly, the cells were seeded in a 96-

well plate and cultured overnight at 37 °C. The following day the cells were cultured with 100 µl of the compounds at graded concentrations and incubated for three days. The MTS reagent was added to the cells and incubated for 4 h at 37 °C. The absorbance was recorded at 490 nm. The percent of cytotoxicity and the CC₅₀ (the concentration for 50 % cytotoxicity) values were calculated as above.

4.4.2. Cf2Th-CCR5 cells—The cytotoxicity of the small molecules in CD4-negative Cf2Th-CCR5 cells was also measured with the colorimetric assay, as above. Briefly, Cf2Th-CCR5 cells were plated in a 96-well plate and cultured at 37 °C. Following overnight incubation, the cells were cultured with 100 µl of the compounds at graded concentrations and cultured for 48 h. The MTS reagent was added to the cells, and 4 h later, the absorbance was recorded at 490 nm. The percent of cytotoxicity and the CC₅₀ values were calculated as above.

4.5. Cell-to-Cell HIV-1 Transmission

The cell-to-cell HIV-1 transmission inhibition assay was performed as previously described^{64, 65}, with minor modifications. Briefly, the indicator TZM-bl cells (used as ‘acceptor’ cells) were plated at 10⁴/well in a 96 well plate 24 h before the assay. For the CXCR4-tropic assay, as ‘transmitting’ cells, we used H9 cells chronically infected with HIV-1_{IIIB} at 2 × 10³ cells/well. For the CCR5-tropic assay, we used MOLT-4/CCR5 cells chronically infected with HIV-1_{ADA} at 2 × 10³ cells/well. The transmitting cells were pre-treated with 200 µg/mL mitomycin C (Sigma) for 1 h at 37 °C, washed and incubated with the TZM-bl cells in the presence of escalating concentrations of compounds for 24 h. Then, the cells were washed and lysed. The lysates were mixed with the luciferase substrate, and the luciferase activity was measured to calculate the percent of inhibition and IC₅₀ values by using the GraphPad Prism software.

4.6. Chemistry

For this study, all commercial reagents and solvents were used without further purification. Unless otherwise stated, all the described reactions were performed in an air atmosphere. Reactions were monitored by thin-layer chromatography (TLC) carried out on Merck TLC Silica gel plates (60 F254), using a UV light for visualization and basic aqueous potassium permanganate or iodine fumes as a developing agent. ¹H and ¹³C NMR spectra were recorded on a Bruker Avance 400 instrument with an operating frequency of 400 and 100 MHz, respectively, and calibrated using residual not-deuterated chloroform (δH = 7.28 ppm) and CDCl₃ (δC = 77.16 ppm) or not-deuterated DMSO (δH = 2.50 ppm) and DMSO-d₆ (δC = 39.51 ppm) as internal references. NMR data spectra abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. The purity of compounds was determined via LCMS (Shimadzu LCMS-2010A) using three types of detection systems such as EDAD, ELSD, and UV with purity cut-off at 95%.

General procedure A: for Suzuki coupling—To a solution containing appropriate bromide (50 mmol, 1 equiv.), (1-(tert-butoxycarbonyl)-1H-pyrrol-2-yl)boronic acid (50 mmol, 1 equiv.) in THF-H₂O (1:1, 100 mL), Na₂CO₃ (100 mmol, 2 equiv.) and Pd(Ph₃P)Cl₂ (1 mol. %) were added under a nitrogen atmosphere. The mixture was stirred at reflux for 8–

15 h (TLC-control). After cooling to room temperature, water (50 mL) and CH₂Cl₂ (50 mL) were added. The organic layer was separated; the aqueous layer was extracted with CH₂Cl₂ (3×50 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Purification by flash chromatography using hexane-EtOAc mixture as eluent afforded the desired compound. Compound **S1a–c** were obtained following the general procedure A (Scheme 1).

Tert-butyl 2-(4-cyanophenyl)-1H-pyrrole-1-carboxylate (S1a)—Eluent: Hexane-EtOAc (from 20:1 to 10:1), R_f=0.3 (10:1, Hexane-EtOAc). Yield = 53%.

¹H NMR (CDCl₃, 400 MHz): δ = 1.43 (s, 9 H), 6.25 – 6.30 (m, 2 H), 7.40 (dd, *J*=3.2, 1.9 Hz, 1 H), 7.45 – 7.48 (m, 2 H), 7.62 – 7.66 (m, 2 H).

¹³C NMR (CDCl₃, 100 MHz): δ = 27.6 (3 C), 84.3, 110.4, 111.0, 116.1, 118.9, 123.9, 129.5 (2 C), 131.3 (2 C), 133.0, 138.7, 148.9.

Tert-butyl 2-(2,4-bis(trifluoromethyl)phenyl)-1H-pyrrole-1-carboxylate (S1b)—Eluent: Hexane-EtOAc (from 1:0 to 20:1), R_f=0.3–0.4 (20:1, Hexane-EtOAc). Yield = 56%.

¹H NMR (CDCl₃, 400 MHz): δ = 1.25 (s, 9 H), 6.20 – 6.24 (m, 1 H), 6.28 (t, *J*=3.3 Hz, 1 H), 7.43 (dd, *J*=3.4, 1.8 Hz, 1 H), 7.53 (d, *J*=7.9 Hz, 1 H), 7.81 (d, *J*=7.4 Hz, 1 H), 7.97 (s, 1 H).

¹³C NMR (CDCl₃, 100 MHz): δ = 27.4 (3 C), 84.0, 110.7, 115.9, 122.4, 123.3 (q, *J*=274.0 Hz), 123.1 (m), 123.6 (q, *J*=272.2 Hz), 127.8 (q, *J*=3.7 Hz), 127.8, 130.6 (q, *J*=33.5 Hz), 130.8 (q, *J*=30.6 Hz), 133.6, 138.0, 148.8.

Tert-butyl 2-(2-fluoro-4-(trifluoromethyl)phenyl)-1H-pyrrole-1-carboxylate (S1c)—Eluent: Hexane-EtOAc (from 30:1 to 20:1), R_f=0.5 (20:1, Hexane-EtOAc). Yield = 70%.

¹H NMR (CDCl₃, 400 MHz): δ = 1.41 (s, 9 H), 6.28 – 6.33 (m, 2 H), 7.30 – 7.38 (m, 1 H), 7.42 – 7.47 (m, 2 H), 7.46 – 7.52 (m, 1 H).

¹³C NMR (CDCl₃, 100 MHz): δ = 27.6, 84.3, 111.0, 112.5 (dq, *J*=25.5, 3.7 Hz), 116.2, 120.8 (dq, *J*=7.6, 3.7 Hz), 123.5 (dq, *J*=272.2, 2.6 Hz), 123.6, 126.8 (d, *J*=14.9 Hz), 126.8, 131.3 (d, *J*=3.3 Hz), 131.4 (qd, *J*=33.2, 7.9 Hz), 149.0, 160.0 (d, *J*=249.2 Hz).

4-(1H-Pyrrol-2-yl)benzotrile (S2a)—To a solution containing **S1a** (10 g, 1 equiv.) in MeOH (20 mL), 1M HCl solution in MeOH (100 mL) was added in one portion. The mixture was stirred at room temperature for 7–8 h, and then the solvent was evaporated. Aqueous K₂CO₃ (10% solution, 100 mL) was added carefully (CO₂ evolution), and the mixture was extracted with CH₂Cl₂ (3×50 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated. The crude product was used in the next step without purification. M = 6.01 g. Yield = 96%.

¹H NMR (CDCl₃, 400 MHz): δ = 6.36 (dt, *J*=3.5, 2.6 Hz, 1 H), 6.69 (ddd, *J*=3.7, 2.6, 1.4 Hz, 1 H), 6.97 (td, *J*=2.7, 1.5 Hz, 1 H), 7.53 – 7.57 (m, 2 H), 7.61 – 7.65 (m, 2 H), 8.71 (br s., 1 H).

^{13}C NMR (CDCl_3 , 100 MHz): δ = 108.7, 108.9, 111.1, 119.4, 121.1, 123.8 (2 C), 130.2, 132.9 (2 C), 136.9.

2-(2,4-Bis(trifluoromethyl)phenyl)-1H-pyrrole (S2b)—To a solution containing **S1b** (10 g, 1 equiv.) in CH_2Cl_2 (130 mL), TFA (10.1 mL, 15.03 g, 132 mmol, 5 equiv.) was added dropwise on the water bath. The mixture was stirred at room temperature overnight, and then the solvent was evaporated. Aqueous K_2CO_3 (10% solution, 100 mL) was added carefully (CO_2 evolution), and the mixture was extracted with CH_2Cl_2 (3 \times 50 mL). The combined organic layers were dried over Na_2SO_4 , filtered, and concentrated. Purification by flash chromatography using hexane-EtOAc mixture (from 30:1 to 10:1) as eluent (R_f = 0.2 in hexane-EtOAc 10:1). M = 6.34 g. Yield = 86%

^1H NMR (CDCl_3 , 400 MHz): δ = 6.38 – 6.42 (m, 1 H), 6.57 – 6.61 (m, 1 H), 6.99 – 7.04 (m, 1 H), 7.73 (d, J =8.2 Hz, 1 H), 7.83 (d, J =8.3 Hz, 1 H), 8.04 (s, 1 H), 8.62 (br. s., 1 H).

^{13}C NMR (CDCl_3 , 100 MHz): δ = 110.1, 111.7, 121.0, 123.7 (q, J =272.0 Hz), 123.9 (q, J =273.3 Hz), 124.1 (m), 126.7 (q, J =31.1 Hz), 127.7, 128.8, (q, J =4.0 Hz) 128.9 (q, J =33.5 Hz), 131.9, 136.0.

2-(2-Fluoro-4-(trifluoromethyl)phenyl)-1H-pyrrole (S2c)—**S2c** was synthesized by the same experimental procedures of **S2b**.

Eluent: Hexane-EtOAc (from 30:1 to 20:1), R_f =0.4 (20:1, Hexane-EtOAc). Yield = 93%.

^1H NMR (DMSO-d_6 , 400 MHz): δ = 6.18 – 6.27 (m, 1 H), 6.72 (dd, J =2.29, 1.25 Hz, 1 H), 6.96 – 7.06 (m, 1 H), 7.56 (d, J =8.25 Hz, 1 H), 7.63 (d, J =11.86 Hz, 1 H), 7.95 (t, J =8.04 Hz, 1 H), 11.54 (br. s., 1 H).

^{13}C NMR (DMSO-d_6 , 100 MHz): δ = 109.7 (d, J =1.7 Hz), 111.4 (d, J =10.0 Hz), 113.6 (dq, J =26.2, 3.9 Hz), 121.4 (d, J =0.9 Hz), 121.6 (dq, J =7.4, 3.7 Hz), 123.5 (d, J =2.8 Hz), 123.6 (qd, J =271.6, 2.4 Hz), 124.7 (d, J =12.0 Hz), 126.5 (qd, J =33.0, 8.3 Hz), 126.6 (d, J =4.4 Hz), 157.3 (d, J =249.0 Hz).

General procedure B: for acylation—Crude pyrrole from the previous step (1 equiv.) was dissolved in CH_2Cl_2 (0.5 M solution), and pyridine (1.2 equiv.) was added, followed by the dropwise addition of TFAA (1.2 equiv.). After completion of the addition, the mixture was stirred for 2 h, and the solvent was evaporated. The product was triturated in water, and the precipitate was filtered, washed with water twice, and dried on a filter. Compound **S3a–c** were obtained following the general procedure B (Scheme 1).

4-(5-(2,2,2-Trifluoroacetyl)-1H-pyrrol-2-yl)benzotrile (S3a)—Yield = 79%.

^1H NMR (DMSO-d_6 , 400 MHz): δ = 7.04 (dd, J =4.2, 2.4 Hz, 1 H), 7.18 – 7.34 (m, 1 H), 7.87 (d, J =8.4 Hz, 2 H), 8.14 (d, J =8.4 Hz, 2 H), 13.06 (br. s., 1 H).

^{13}C NMR (DMSO-d_6 , 100 MHz): δ = 111.0, 112.3, 117.0 (q, J =289.9 Hz), 118.7, 123.0 (q, J =3.7 Hz), 126.8 (2 C), 126.9, 132.8 (2 C), 134.0, 141.1, 168.3 (q, J =35.2 Hz).

1-(5-(2,4-Bis(trifluoromethyl)phenyl)-1H-pyrrol-2-yl)-2,2,2-trifluoroethanone (S3b)—Yield = 85%.

¹H NMR (DMSO-d₆, 400 MHz): δ = 6.56 – 6.62 (m, 1 H), 7.31 (dt, *J*=4.1, 2.1 Hz, 1 H), 7.91 (d, *J*=8.6 Hz, 1 H), 8.14 – 8.26 (m, 2 H), 13.17 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 114.1 (q, *J*=2.4 Hz), 116.9 (q, *J*=289.9 Hz), 121.7 (q, *J*=3.3 Hz), 123.0 (q, *J*=273.9 Hz), 123.3 (qq, *J*=5.9, 4.0 Hz), 123.3 (q, *J*=272.6 Hz), 126.1, 128.5 (q, *J*=31.1 Hz), 129.2 (q, *J*=3.0 Hz), 130.0 (q, *J*=33.2 Hz), 134.0, 134.1, 138.4, 168.8 (q, *J*=35.2 Hz).

2,2,2-Trifluoro-1-(5-(2-fluoro-4-(trifluoromethyl)phenyl)-1H-pyrrol-2-yl)ethanone (S3c)—Yield = 92%.

¹H NMR (DMSO-d₆, 400 MHz): δ = 6.82 (t, *J*=3.66 Hz, 1 H), 7.13 – 7.36 (m, 1 H), 7.64 (d, *J*=8.27 Hz, 1 H), 7.73 (d, *J*=11.13 Hz, 1 H), 8.21 (t, *J*=7.95 Hz, 1 H), 13.05 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 113.6 (dq, *J*=26.0, 3.8 Hz), 114.4 (d, *J*=10.7 Hz), 117.0 (q, *J*=289.9 Hz), 121.5 (dq, *J*=7.2, 3.5 Hz), 122.0 (d, *J*=11.6 Hz), 122.3 (q, *J*=3.0 Hz), 123.3 (qd, *J*=277.2, 2.4 Hz), 126.5, 130.1 (d, *J*=2.6 Hz), 130.7 (qd, *J*=33.4, 8.5 Hz), 135.4 (d, *J*=1.8 Hz), 159.0 (d, *J*=253.0 Hz), 168.7 (q, *J*=35.2 Hz).

General procedure C: for haloform reaction—Appropriate trifluoroethanone (1 equiv.) was added to a solution of NaOH (5 equiv.) in dioxane-H₂O mixture (1:1, 0.5M solution). The resulting reaction mixture was refluxed for 20 h and cooled to room temperature. A concentrated aqueous HCl solution (~12 M, 5 equiv.) was added dropwise. The resulting precipitate was filtered, washed with H₂O, and dried on a filter. Compound **S4a-c** were obtained following the general procedure D (Scheme 1).

5-(4-Cyanophenyl)-1H-pyrrole-2-carboxylic acid (S4a)—Yield = 69%.

¹H NMR (DMSO-d₆, 400 MHz): δ = 6.75 (dd, *J*=3.7, 2.5 Hz, 1 H), 6.84 (dd, *J*=3.8, 2.2 Hz, 1 H), 7.92 (d, *J*=8.6 Hz, 2 H), 7.97 (d, *J*=8.6 Hz, 2 H), 12.18 (br. s., 1 H), 12.72 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 109.3, 116.6, 124.9 (2 C), 125.4, 128.9, 129.9 (2 C), 135.4, 135.6, 161.9, 167.2.

5-(2,4-Bis(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxylic acid (S4b)—Yield = 71%.

¹H NMR (DMSO-d₆, 400 MHz): δ = 6.32 – 6.40 (m, 1 H), 6.83 (dd, *J*=3.7, 2.4 Hz, 1 H), 7.83 (d, *J*=8.6 Hz, 1 H), 8.04 – 8.17 (m, 2 H), 12.22 (br. s., 1 H), 12.55 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 111.9 (q, *J*=3.0 Hz), 115.5, 123.2 (m), 123.4 (q, *J*=273.9 Hz), 123.6 (q, *J*=272.4 Hz), 125.3, 127.8 (q, *J*=30.8 Hz), 128.7 (q, *J*=33.0 Hz), 128.9, 131.4, 134.0, 135.7, 162.0.

5-(2-Fluoro-4-(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxylic acid (S4c)—

Yield = 83%.

¹H NMR (DMSO-d₆, 400 MHz): δ = 6.65 – 6.72 (m, 1 H), 6.80 – 6.92 (m, 1 H), 7.61 (d, *J*=8.27 Hz, 1 H), 7.73 (d, *J*=11.44 Hz, 1 H), 8.20 (t, *J*=7.95 Hz, 1 H), 12.19 (br. s., 1 H), 12.64 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 112.6 (d, *J*=10.6 Hz), 113.6 (dq, *J*=26.2, 3.7 Hz), 116.2, 121.5 (dq, *J*=6.9, 3.2 Hz), 123.5 (qd, *J*=272.2, 2.8 Hz), 123.6 (d, *J*=11.5 Hz), 125.7, 128.7 (d, *J*=2.3 Hz), 128.7 (qd, *J*=33.1, 8.7 Hz), 129.1 (d, *J*=3.2 Hz), 158.4 (d, *J*=250.9 Hz), 161.9.

((3-Fluoro-4-(trifluoromethyl)phenyl)ethynyl)trimethylsilane (S5)—We

synthesized **S5–S8** based on Scheme 2. In a pressurized vessel equipped with magnetic stirring bar containing solution of 4-bromo-2-fluoro-1-(trifluoromethyl)benzene (30 g; 123 mmol, 1 equiv.) in Et₃N (250 mL), TMS-acetylene (24.25 g, 34.2 mL, 247 mmol, 2 equiv.), Pd(Ph₃P)Cl₂ (0.87 g; 1 mol. %), CuI (0.48 g; 2 mol. %) were added under argon atmosphere. It was heated to 50–60 °C, and the mixture was stirred at this temperature for 6–8 h (TLC-control). Water (500 mL) was added and extracted with hexane (3×150 mL). Combined extracts were washed with water and dried with anhydrous sodium sulfate. The solvent was removed by rotary evaporation, and the residue was purified using flash chromatography (eluent: from pure hexane to hexane-EtOAc, 30:1, R_f = 0.6 in hexane). M = 30.1 g. Yield = 94%.

¹H NMR (CDCl₃, 400 MHz): δ = 0.27 (s, 9 H), 7.28 (d, *J*=10.8 Hz, 1 H), 7.32 (d, *J*=8.4 Hz, 1 H), 7.53 (t, *J*=7.7 Hz, 1 H).

¹³C NMR (CDCl₃, 100 MHz): δ = -0.2, 99.0, 102.1 (d, *J*=2.6 Hz), 118.5 (qd, *J*=33.2, 12.5 Hz), 120.1 (d, *J*=22.1 Hz), 122.5 (q, *J*=272.7 Hz), 127.2 (qd, *J*=4.6, 2.2 Hz), 127.8 (d, *J*=3.7 Hz), 129.4 (d, *J*=9.8 Hz), 159.5 (dq, *J*=256.7, 2.0 Hz).

1-tert-Butyl 2-methyl 5-(3-fluoro-4-(trifluoromethyl)phenyl)-3-methyl-1H-

pyrrole-1,2-dicarboxylate (S6)—To a solution of **S5** (20 g, 77 mmol, 1 equiv.) in DMF (400 mL), triethylamine trihydrofluoride (3.72 g, 3.76 mL, 23 mmol, 0.3 equiv.) was added, and the mixture was stirred for 1 h under argon atmosphere. Then methyl (E)-2-(tert-butoxycarbonylamino)-3-iodo-but-2-enoate (26.2 g, 77 mmol, 1 equiv.), Pd(Ph₃P)Cl₂ (2.7 g; 5 mol. %), CuI (1.46 g; 10 mol. %) and Cs₂CO₃ (50.06 g; 154 mmol, 2 equiv.) were added under argon atmosphere. It was heated to 70–80 °C, and the mixture was stirred at this temperature for 10–15 h (TLC-control). Water (800 mL) was added and extracted with Et₂O (3×200 mL). Combined extracts were washed with water and brine, dried over anhydrous Na₂SO₄, filtered, and concentrated. Purification by flash chromatography (eluent: hexane-EtOAc from 20:1 to 5:1, R_f = 0.6 in hexane-EtOAc 5:1). M = 12.73 g, Yield = 41%.

¹H NMR (CDCl₃, 400 MHz): δ = 1.46 (s, 9 H), 2.30 (s, 3 H), 3.89 (s, 3 H), 6.14 (s, 1 H), 7.25 – 7.33 (m, 2 H), 7.61 (t, *J*=7.7 Hz, 1 H).

^{13}C NMR (CDCl_3 , 100 MHz): δ = 12.7, 27.4 (3 C), 51.8, 85.8, 115.2, 117.3 (d, J =21.9 Hz), 117.9 (qd, J =33.3, 12.5 Hz), 122.6 (q, J =272.2 Hz), 123.1, 124.6 (d, J =3.7 Hz), 126.9 (qd, J =4.6, 2.0 Hz), 130.0, 135.2 (d, J =1.8 Hz), 138.1 (d, J =8.9 Hz), 149.3, 159.3 (dq, J =256.3, 2.0 Hz), 161.7.

Methyl 5-(3-fluoro-4-(trifluoromethyl)phenyl)-3-methyl-1H-pyrrole-2-carboxylate (S7)—To a solution of **S6** (12.58 g, 31 mmol, 1 equiv.) in CH_2Cl_2 (150 mL), TFA (17.87 g, 12 mL, 157 mmol, 5 equiv.) was added in one portion, and the mixture was stirred overnight. Then the solvent was evaporated, and 10% aqueous K_2CO_3 was added, and the mixture was extracted with CH_2Cl_2 (3 \times 100 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated. The product was used without further purification. M = 9.28 g. Yield = 98 %.

^1H NMR (DMSO-d_6 , 400 MHz): δ = 2.27 (s, 3 H), 3.81 (s, 3 H), 6.72 (d, J =2.6 Hz, 1 H), 7.70 (t, J =8.1 Hz, 1 H), 7.82 (d, J =8.3 Hz, 1 H), 8.02 (d, J =12.9 Hz, 1 H), 11.99 (br. s., 1 H).

^{13}C NMR (DMSO-d_6 , 100 MHz): δ = 12.8, 51.1, 112.5, 112.8 (d, J =22.7 Hz), 114.2 (qd, J =32.4, 12.4 Hz), 121.0, 121.3, 122.8 (q, J =271.5 Hz), 127.5 (q, J =3.7 Hz), 128.4, 132.3, 137.9 (d, J =9.0 Hz), 159.4 (d, J =251.4 Hz), 161.2.

5-(3-Fluoro-4-(trifluoromethyl)phenyl)-3-methyl-1H-pyrrole-2-carboxylic acid (S8)—To a solution of **S7** (9.19 g, 31 mmol, 1 equiv.) in a mixture of $\text{EtOH-H}_2\text{O}$ (1:1, 120 mL), NaOH (2.44 g, 61 mmol, 2 equiv.) was added in one portion, and the reaction mixture was stirred at reflux for 10–12 h (TLC-control). Then the sodium-salt solution was acidified by the addition of an equivalent amount of HCl (12M, 5.08 mL, 2 equiv.), water (100 mL) was added, and the precipitate was filtered. M = 8.4 g. Yield = 96 %.

^1H NMR (DMSO-d_6 , 400 MHz): δ = 2.28 (s, 3 H), 6.72 (s, 1 H), 7.70 (t, J =8.0 Hz, 1 H), 7.83 (d, J =8.1 Hz, 1 H), 8.03 (d, J =12.9 Hz, 1 H), 11.91 (br. s., 1 H), 12.53 (br. s., 1 H).

^{13}C NMR (DMSO-d_6 , 100 MHz): δ = 12.8, 112.5, 112.6 (d, J =22.7 Hz), 114.0 (qd, J =32.1, 12.7 Hz), 120.9 (d, J =3.0 Hz), 122.4, 122.9 (q, J =270.1 Hz), 127.6 (q, J =3.5 Hz), 127.8, 131.6 (d, J =1.8 Hz), 138.2 (d, J =9.4 Hz), 159.4 (dq, J =249.2, 2.0 Hz), 162.5.

Compounds **S9–S14** were synthesized as per Scheme 3.

5-((Allyloxy)methyl)thiazole (S9)—To a solution of appropriate alcohol (26.1 g, 227 mmol, 1 equiv.) in DMF (226 mL), NaH (40% in mineral oil, 9.97 g, 249 mmol, 1.1 equiv.) was added in several portions at 0 °C. The mixture was stirred until gas evolution stopped. Then allyl bromide (23.5 mL, 32.91 g, 272 mmol, 1.2 equiv.) was added dropwise. The reaction mixture was stirred overnight at room temperature. Water (500 mL) was added and extracted with EtOAc (3 \times 150 mL). Combined extracts were washed with water and brine, dried over Na_2SO_4 , filtered, and concentrated. Purification by distillation under reduced pressure (bp=110–120 °C, 10 torr). M = 28.34 g. Yield = 81%.

^1H NMR (CDCl_3 , 400 MHz): δ = 4.01 (d, J =5.7 Hz, 2 H), 4.71 (s, 2 H), 5.21 (d, J =10.5 Hz, 1 H), 5.29 (dd, J =17.2, 1.6 Hz, 1 H), 5.83 – 5.96 (m, 1 H), 7.77 (s, 1 H), 8.78 (s, 1 H).

^{13}C NMR (CDCl_3 , 100 MHz): $\delta = 63.7, 71.0, 117.9, 134.0, 135.6, 142.0, 153.9$.

3-(Thiazol-5-ylmethoxy)propane-1,2-diol (S10)—To a solution of alkene **S9** (28.34 g, 183 mmol, 1 equiv.) in acetone-water (10:1, 360 mL), NMO monohydrate (37.02 g, 274 mmol, 1.5 equiv.) and potassium osmate (VI) dihydrate (0.67 g, 1 mol. %) were added in that sequence. The mixture was stirred at room temperature for 2–3 h (TLC-control), and then the solvent was evaporated. Crude product was purified by flash chromatography (eluent: from pure EtOAc to CH_2Cl_2 -MeOH 10:1, $R_f = 0.3$ in EtOAc). $M = 32.2$ g, Yield = 93%.

^1H NMR (CDCl_3 , 400 MHz): $\delta = 3.40 - 3.53$ (m, 3 H), 3.58 (dd, $J = 11.4, 3.8$ Hz, 1 H), 3.76 – 3.84 (m, 1 H), 4.09 (br. s., 2 H), 4.67 (s, 2 H), 7.70 (s, 1 H), 8.74 (s, 1 H).

^{13}C NMR (CDCl_3 , 100 MHz): $\delta = 63.6, 65.0, 70.7, 71.5, 135.4, 141.8, 154.3$.

5-((2,3-Bis((tert-butyl)dimethylsilyloxy)propoxy)methyl)thiazole (S11)—To a solution of appropriate diol **S10** (32.2 g, 170 mmol, 1 equiv.) DMF (340 mL), imidazole (46.34 g, 681 mmol, 4 equiv.) was added in one portion, followed by portionwise addition of TBSCl (102.57 g, 681 mmol, 4 equiv.). The reaction mixture was stirred overnight at 60–80 °C, cooled to the room temperature, diluted with water (680 mL), and extracted with EtOAc (3 × 200 mL). The combined organic phases were washed with water (200 mL), brine (200 mL), and dried over anhydrous Na_2SO_4 , filtered and evaporated to give an oil, which was purified by flash chromatography (eluent: hexane-EtOAc from 20:1 to 10:1, $R_f = 0.4$ in hexane-EtOAc 20:1). $M = 59.95$ g. Yield = 84%.

^1H NMR (CDCl_3 , 400 MHz): $\delta = 0.03 - 0.08$ (m, 12 H), 0.88 (s, 18 H), 3.46 (dd, $J = 9.9, 5.7$ Hz, 1 H), 3.53 – 3.61 (m, 3 H), 3.79 – 3.86 (m, 1 H), 4.77 (s, 2 H), 7.81 (s, 1 H), 8.91 (s, 1 H).

^{13}C NMR (CDCl_3 , 100 MHz): $\delta = -5.3, -5.3, -4.6, -4.5, 18.3, 18.4, 25.9$ (3 C), 26.0 (3 C), 64.9, 65.4, 72.3, 72.7, 135.9, 141.9, 153.8.

Allyl allyl(2-amino-2-(5-((3-((tert-butyl)diphenylsilyloxy)-2-hydroxypropoxy)methyl)thiazol-2-yl)ethyl)carbamate (S14)—Experimental procedures are given for the synthesis of R-enantiomer (R):

The thiazole **S11** (10.9 g, 26 mmol, 1 equiv.) was dissolved in THF (26 mL) and cooled to –78 °C. At this temperature, n-BuLi (2.5 M in hexane, 10.96 mL, 1.05 equiv.) was added dropwise under a nitrogen atmosphere. The reaction mixture was stirred for 20 min at –78 °C, and appropriate R-imine (**S11a**: 7.47 g, 26 mmol, 1 equiv.) was added dropwise as a solution in THF (1M, 26 mL). The reaction mixture was slowly (~1 h) warmed to 0 °C and poured into water (150 mL). The organic layer was separated, and water was extracted with CH_2Cl_2 (3 × 100 mL). Combined organic phases were dried over anhydrous Na_2SO_4 , filtered, and evaporated to give a brown oil which was purified by column chromatography (eluent: hexane-EtOAc, gradient from 5:1 to 0:1, $R_f = 0.4$ in hexane-EtOAc 1:1). This product (**S12**) was used without further purification and analysis.

To a solution of protected thiazole from the previous step in MeOH (50 ml), 1M solution of HCl in MeOH (100 ml) was added in one portion. The mixture was stirred for 2–3 h, and the solvent was evaporated. The residue was dissolved in water (50 mL) and extracted with CH₂Cl₂ (2 × 50 mL). After that, solid K₂CO₃ was added carefully (CO₂ evolution!) to the water phase at pH 10–12. Product was extracted from water with CH₂Cl₂ (4 × 50 mL), combined organic layers were dried over anhydrous Na₂SO₄, filtered, and evaporated to give diol **S13**. M = 3.02 g.

¹H NMR (CDCl₃, 400 MHz): δ = 2.26 (br. s., 4 H), 3.49 – 3.72 (m, 6 H), 3.77 – 3.98 (m, 3 H), 4.46 (t, *J*=6.5 Hz, 1 H), 4.60 (d, *J*=5.3 Hz, 2 H), 4.70 (s, 2 H), 5.08 – 5.24 (m, 3 H), 5.30 (d, *J*=17.2 Hz, 1 H), 5.70 – 5.97 (m, 2 H), 7.58 (s, 1 H).

Diol **S13** (3.02 g, 8 mmol, 1 equiv.) was dissolved in CH₂Cl₂ (81 mL), and imidazole (0.66 g, 10 mmol, 1.2 equiv.) was added in one portion, followed by dropwise addition of TBDPSCI (2.33 mL, 2.46 g, 9 mmol, 1.1 equiv.). The reaction mixture was stirred overnight, diluted with water (50 mL), and extracted with CH₂Cl₂ (3 × 50 mL). The combined organic phases were washed with brine and dried over anhydrous Na₂SO₄. It was then filtered and evaporated to give an oil, which was purified by flash chromatography (eluent: hexane-EtOAc, from 1:1 to 0:1 and then CH₂Cl₂-MeOH 10:1, R_f = 0.3 in EtOAc). M = 3.75 g. Yield (over three steps) = 24%.

S-enantiomer (S) was synthesized by the same experimental procedures. Yield (over three steps) = 17%.

¹H NMR (CDCl₃, 400 MHz): δ = 1.06 (s, 9 H), 1.84 (br. s., 2 H), 2.52 (br. s., 1 H), 3.51 – 3.72 (m, 6 H), 3.77 – 4.01 (m, 3 H), 4.45 (t, *J*=6.4 Hz, 1 H), 4.61 (d, *J*=5.3 Hz, 2 H), 4.67 (s, 2 H), 5.08 – 5.19 (m, 2 H), 5.21 (dd, *J*=10.4, 1.2 Hz, 1 H), 5.31 (dd, *J*=17.2, 1.3 Hz, 1 H), 5.71 – 5.98 (m, 2 H), 7.36 – 7.47 (m, 6 H), 7.57 (s, 1 H), 7.65 (d, *J*=6.6 Hz, 4 H).

¹³C NMR (CDCl₃, 100 MHz): δ = 19.1, 26.7 (3 C), (50.4, 50.6), (53.1, 53.2), 64.6, 65.3, 66.1, 70.6, 70.9, (116.8, 117.2), (117.3, 117.7), 127.7 (4 C), 129.7 (2 C), 132.7, 133.0 (2 C), 133.1, 135.1, 135.4 (4 C), 141.4, (155.8, 156.7), (175.5, 176.3).

Compounds **S15**, **S16**, and **S21** were synthesized as per Scheme 4.

Methyl 2-(2-(allyl((allyloxy)carbonyl)amino)-1-aminoethyl)thiazole-4-carboxylate (S15)—A solution of *tert*-butyl 2-bromothiazole-4-carboxylate (10.0 g, 37.9 mmol, 1 equiv.) and **S-imine (S11a)** (21.7 g, 75.6 mmol, 2 equiv.) in THF (38 mL) was cooled to 0°C and *i*-PrMgCl*LiCl solution (1M in tetrahydrofuran, 78 mL, 78 mmol, 2 equiv.) was added maintaining the temperature between 0 and +5 °C. The reaction mixture was then stirred at 0°C for 1h and allowed to warm to room temperature. The reaction was quenched by the addition of aqueous ammonium chloride solution (20% w/w, 0.5 L) and extracted with Et₂O (3 × 100 mL). Combined organic layers were washed with brine (3 × 50 mL) dried over Na₂SO₄ and evaporated to dryness. The residue was dissolved in excess of 1M HCl in methanol (400 mL) and left for 2 days. Methanol was evaporated, and saturated NaHCO₃ (100 mL) was added to the residue. The product was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic layer was dried over anhydrous Na₂SO₄ and evaporated to

dryness. The residue was purified using column chromatography (eluent: hexane-EtOAc from 1:1 to 0:1, and then CH₂Cl₂-MeOH 10:1, R_f = 0.3–0.4 in EtOAc). M = 7.74 g. Yield = 63%.

Reaction with **R**-imine (**S11a**) was performed in the same way. M = 6.22 g. Yield = 51%.

¹H NMR (DMSO-d₆, 400 MHz): δ = 2.48 (br. s., 2 H), 3.36–3.41 (m, 1 H), 3.55–3.68 (m, 1 H), 3.81 (s, 3 H), 3.87 (d, *J*=5.2 Hz, 2 H), 4.32–4.54 (m, 3 H), 5.04–5.18 (m, 3 H), 5.25 (d, *J*=17.3 Hz, 1 H), 5.70–5.95 (m, 2 H), 8.43 (s, 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 50.0, 51.9, 52.4, (52.7, 53.2), 65.3, (116.0, 116.7), (116.5, 116.9), 129.2, 133.3, (133.8, 134.0), 145.7, (155.1, 155.8), 161.4, (177.9, 178.2).

Allyl allyl(2-amino-2-(4-carbamoylthiazol-2-yl)ethyl)carbamate (S16)—Thiazole **S15** (1.5 g, 4.6 mmol) was dissolved in MeOH saturated with NH₃ (~7M, 10 mL). It was heated to 70–80 °C in a pressure vessel on an oil bath, and the mixture was stirred at this temperature for 25–30 h (TLC-control). Then it was cooled to the room temperature and evaporated. The product was used without further purification.

¹H NMR (CDCl₃, 400 MHz): δ = 1.94 (br. s., 2 H), 3.49–3.71 (m, 2 H), 3.75–3.98 (m, 2 H), 4.42–4.63 (m, 3 H), 5.05–5.33 (m, 4 H), 5.66–5.97 (m, 2 H), 6.28 (br. s., 1 H), 7.15 (br. s., 1 H), 8.07 (s, 1 H).

¹³C NMR (CDCl₃, 100 MHz): δ = 50.3, (52.3, 52.5), (53.4, 52.6), 65.9, (116.6, 117.0), (117.1, 117.4), 123.9, 132.2, 132.7, 149.3, (155.6, 156.4), 163.1, (174.9, 175.3).

We prepared **S17–S20** and **S22–S28** were prepared by following the methods reported previously^{25, 27, 32, 67}.

Allyl (1-amino-1-(4-(hydroxymethyl)thiazol-2-yl)-2-methylpropan-2-yl)carbamate (S21)—Experimental procedures for the synthesis of **R**-enantiomer (**R**):

The 4-(((tert-Butyldimethylsilyl)oxy)methyl)thiazole (20.02 g, 87 mmol, 2.5 equiv.) was dissolved in THF (87 mL) and cooled to –78 °C. At this temperature, *n*-BuLi (2.5 M in hexane, 34.92 mL, 2.5 equiv.) was added dropwise under a nitrogen atmosphere. The reaction mixture was stirred for 20 min at –78 °C, and appropriate **R**-imine (**S21a**) (10.0 g, 35 mmol, 1 equiv.) was added dropwise as a solution in THF (1M, 35 mL). The reaction mixture was slowly (~1 h) warmed to 0 °C and poured into water (200 mL). The organic layer was separated, and water was extracted with CH₂Cl₂ (4 × 100 mL). Combined organic phases were dried over anhydrous Na₂SO₄, filtered, and evaporated to give a brown oil which was purified by column chromatography (eluent: hexane-EtOAc, gradient from 5:1 to 0:1, R_f = 0.4 in hexane-EtOAc 1:1). This product was used without analysis.

To a solution of protected thiazole from the previous step in MeOH (50 ml), 1M solution of HCl in MeOH (100 ml) was added in one portion. The mixture was stirred for 2–3 h, and the solvent was evaporated. The residue was dissolved in water (50 mL) and extracted with CH₂Cl₂ (2 × 50 mL). After that, solid K₂CO₃ was added carefully (CO₂ evolution) to the

water phase at pH 10–12. Product was extracted from water with CH₂Cl₂ (4 × 50 mL), combined organic layers were dried over anhydrous Na₂SO₄, filtered, and evaporated to give pure amine. M = 3.2 g. Yield = 32%

S-enantiomer (S) was synthesized by the same experimental procedures. Yield = 29%.

¹H NMR (CDCl₃, 400 MHz): δ = 1.32 (s, 3 H), 1.34 (s, 3 H), 2.49 (br. s., 3 H), 4.45 – 4.56 (m, 3 H), 4.68 (s, 2 H), 5.14 – 5.32 (m, 2 H), 5.59 (s, 1 H), 5.89 (dddd, *J*=16.8, 11.0, 5.7, 5.4 Hz, 1 H), 7.10 (s, 1 H).

¹³C NMR (CDCl₃, 100 MHz): δ = 23.5, 23.7, 56.0, 59.6, 60.5, 65.1, 114.5, 117.5, 132.9, 155.0, 156.3, 172.6.

General Procedure D: for Amide Coupling—DIPEA (1 equiv.) was added to an appropriate acid (1 equiv.) followed by DMF (10 mL per 1 g of acid) and then HBTU (1 equiv.). The resulting solution was stirred for 10 min and added to a solution of appropriate amine (1 equiv.) in DMF (10 mL per 1 g of amine) in several portions. The reaction mixture was stirred overnight; DMF was evaporated, and the residue was dissolved in CH₂Cl₂ (50 mL per 1 g of crude product) and successively washed with 5% aqueous NaOH and 10% tartaric acid solutions (25 mL per 1 g of crude product). The organic layer was dried over anhydrous Na₂SO₄, filtered, evaporated, and dry loaded on silica. Eluting with hexane-EtOAc (1:1, then pure EtOAc) gave the target compounds. Products were used in the next step without analysis.

General Procedure E: for Allyl- and Alloc- Deprotection—To a solution containing protected compound (1 equiv.) and N,N-dimethyl barbituric acid (NDMBA, 3 equiv.) in MeOH (0.1M solution), PPh₃ (10 mol %) was added under a nitrogen atmosphere followed by Pd(dba)₂ (5 mol %). The mixture was stirred for 1 day under reflux. After cooling, 50 mL of CH₂Cl₂ was added, and the organic phase was shaken with 10% aqueous K₂CO₃ (50 mL) to remove the unreacted NDMBA. The organic layer was separated, and the aqueous layer was extracted with CH₂Cl₂-EtOH (~4:1, (3–5) × 50 mL). Combined organic layers were dried over anhydrous Na₂SO₄, filtered, and concentrated. Purification by flash chromatography (twice) (eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1 and 5:1, eluent 2: CH₂Cl₂-MeOH, 4:1, 2:1, 1:1) afforded amine as a slightly yellow or white solid.

General procedure F: for TBDPS- deprotection—TBDPS cleavage: To a solution of TBDPS-protected compound (1 equiv.) in THF (0.1M), solution of TBAF trihydrate (1.1 equiv.) in THF (0.1M) was added in a one portion. The mixture was stirred for 1–2 h at room temperature (TLC-control) and concentrated. Purification by flash chromatography (twice) (eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1, 5:1, 3:1, eluent 2: CH₂Cl₂-MeOH, 4:1, 3:1, 2:1, 1:1) afforded amine as a slightly yellow or white solid.

Compounds 1–38 were synthesized as per Scheme 5.

N-(2-amino-1-(4-(hydroxymethyl)thiazol-2-yl)ethyl)-5-(2-fluoro-4-(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxamide—Compounds **1** (NBD-14250) and **2** (NBD-14251) were obtained following the general procedure D, and E from amine

S17 and acid **S4c** Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1 and 5:1, Eluent 2: CH₂Cl₂-MeOH, 4:1 and 2:1.

1 (NBD-14250); (R): M = 501 mg. Yield = 29% (over two steps). rt = 1.317 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 429 Da.

2 (NBD-14251); (S): M = 432 mg. Yield = 25% (over two steps). rt = 1.325 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 429 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 1.77 (br. s., 2 H), 3.01 (dd, *J*=13.0, 7.9 Hz, 1 H), 3.14 (dd, *J*=13.0, 5.1 Hz, 1 H), 4.54 (s, 2 H), 5.18 – 5.27 (m, 1 H), 5.31 (br. s., 1 H), 6.73 (t, *J*=3.7 Hz, 1 H), 7.09 (d, *J*=3.8 Hz, 1 H), 7.29 (s, 1 H), 7.62 (d, *J*=7.6 Hz, 1 H), 7.74 (d, *J*=11.4 Hz, 1 H), 8.17 (t, *J*=7.9 Hz, 1 H), 8.73 (d, *J*=7.6 Hz, 1 H), 11.95 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 45.7, 54.4, 59.8, 112.2 (d, *J*=10.1 Hz), 112.9, 113.7 (dq, *J*=26.7, 3.2 Hz), 114.1, 121.53 (qd, *J*=7.4, 3.7 Hz), 123.5 (qd, *J*=272.1, 2.8 Hz), 123.7 (d, *J*=11.5 Hz), 127.1 (d, *J*=2.8 Hz), 128.1 (qd, *J*=33.1, 8.3 Hz), 128.4, 128.6 (d, *J*=3.7 Hz), 157.6, 158.1 (d, *J*=250.5 Hz), 160.3, 172.1.

HRMS (ESI) calcd for C₁₈H₁₅F₄N₄O₂S [M - H]⁻ 427.0857, found 427.0858.

N-(2-Amino-1-(4-(hydroxymethyl)thiazol-2-yl)ethyl)-5-(4-cyanophenyl)-1H-pyrrole-2-carboxamide—Compounds **3** (NBD-14260) and **4** (NBD-14261) were obtained following the general procedure D and E from amine **S17** and acid **S4a**.

Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1 and 5:1, Eluent 2: CH₂Cl₂-MeOH, 4:1 and 2:1.

3 (NBD-14260); (R): M = 207 mg. Yield = 17% (over two steps). rt = 1.077 min. Purity = 91%. LC-MS: m/z [M+H]⁺ = 368 Da.

4 (NBD-14261); (S): M = 288 mg. Yield = 21% (over two steps). rt = 1.217 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 368 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 1.75 (br. s., 2 H), 3.01 (dd, *J*=13.1, 7.8 Hz, 1 H), 3.14 (dd, *J*=13.2, 5.3 Hz, 1 H), 4.54 (s, 2 H), 5.17 – 5.26 (m, 1 H), 5.31 (br. s., 1 H), 6.84 (d, *J*=3.9 Hz, 1 H), 7.05 (d, *J*=3.9 Hz, 1 H), 7.29 (d, *J*=0.9 Hz, 1 H), 7.81 (d, *J*=8.0 Hz, 2 H), 8.01 (d, *J*=8.3 Hz, 2 H), 8.66 (d, *J*=7.8 Hz, 1 H), 12.04 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 45.7, 54.4, 59.8, 108.5, 109.6, 113.2, 114.1, 119.1, 125.1 (2 C), 128.8, 132.7 (2 C), 133.1, 136.1, 157.6, 160.4, 172.2.

HRMS (ESI) calcd for C₁₈H₁₆N₅O₂S [M - H]⁻ 366.1030, found 366.1033.

N-(2-Amino-1-(4-(hydroxymethyl)thiazol-2-yl)ethyl)-5-(2,4-bis(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxamide—Compounds **5** (NBD-14313) and **6** (NBD-14314) were obtained following the general procedure D and E

from amine **S17** and acid **S4b**. Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1 and 5:1, Eluent 2: CH₂Cl₂-MeOH, 4:1 and 2:1.

5 (NBD-14313); (S): M = 614 mg. Yield = 35% (over two steps). rt = 1.378 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 479 Da.

6 (NBD-14314); (R): M = 662 mg. Yield = 37% (over two steps). rt = 1.365 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 479 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 1.89 (br. s., 2 H), 3.00 (dd, *J*=13.1, 7.9 Hz, 1 H), 3.14 (dd, *J*=13.2, 5.3 Hz, 1 H), 4.54 (s, 2 H), 5.18 – 5.26 (m, 1 H), 5.31 (br. s., 1 H), 6.39 (d, *J*=3.2 Hz, 1 H), 7.06 (d, *J*=3.9 Hz, 1 H), 7.29 (s, 1 H), 7.84 (d, *J*=8.7 Hz, 1 H), 8.07 – 8.13 (m, 2 H), 8.67 (d, *J*=8.0 Hz, 1 H), 12.04 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 45.4, 54.0, 59.8, 111.6 (q, *J*=3.3 Hz), 112.2, 114.2, 123.3 (br.), 123.4 (q, *J*=273.9 Hz), 123.5 (q, *J*=272.4 Hz), 127.3 (q, *J*=30.8 Hz), 128.1, 128.2 (q, *J*=33.0 Hz), 128.9 (q, *J*=3.0 Hz), 129.9, 133.7, 135.8, 157.7, 160.5, 172.1.

HRMS (ESI) calcd for C₁₉H₁₇F₆N₄O₂S [M +H]⁺ 479.0971, found 479.0971.

N-(1-amino-2-(4-(hydroxymethyl)thiazol-2-yl)propan-2-yl)-5-(3-fluoro-4-(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxamide—Compound **7** (NBD-14278) was obtained following the general procedure D and Boc- and TBS-deprotection from amine racemic amine **S25** and acid **S27**.

Deprotection: To a solution of protected thiazole from the previous step in MeOH (10 ml), 1M solution of HCl in MeOH (50 ml) was added in one portion. The mixture was stirred for 3–4 h, and the solvent was evaporated. After that, 10% aqueous K₂CO₃ (50 mL) was added, and it was extracted with CH₂Cl₂-EtOH (~4:1, 4 × 50 mL). Combined organic layers were dried over Na₂SO₄, filtered, and concentrated. The compound was purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1 and 5:1, Eluent 2: CH₂Cl₂-MeOH, 5:1 and 2:1.

7 (NBD-14278): M = 451 mg. Yield = 32% (over two steps). rt = 1.350 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 443 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 1.77 (s, 3 H), 1.82 (br. s., 2 H), 2.93 (d, *J*=13.2 Hz, 1 H), 3.12 (d, *J*=13.1 Hz, 1 H), 4.51 (d, *J*=2.4 Hz, 2 H), 5.28 (br. s., 1 H), 6.88 (d, *J*=3.9 Hz, 1 H), 7.01 (d, *J*=3.9 Hz, 1 H), 7.23 (s, 1 H), 7.73 (t, *J*=8.1 Hz, 1 H), 7.84 (d, *J*=8.4 Hz, 1 H), 8.00 (d, *J*=13.0 Hz, 1 H), 8.15 (s, 1 H), 12.04 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 23.5, 51.8, 59.9, 60.4, 109.9, 112.3 (d, *J*=22.5 Hz), 112.7, 113.7 (qd, *J*=32.4, 12.5 Hz), 113.8, 120.6 (d, *J*=3.0 Hz), 122.9 (q, *J*=271.5 Hz), 127.6 (q, *J*=4.4 Hz), 129.6, 131.9 (d, *J*=2.2 Hz), 138.5 (d, *J*=9.0 Hz), 156.9, 159.4 (dq, *J*=253.4, 2.2 Hz), 160.1, 176.4.

HRMS (ESI) calcd for C₁₉H₁₇F₄N₄O₂S [M - H]⁻ 441.1014, found 441.1014.

N-(1-Amino-2-(4-(hydroxymethyl)thiazol-2-yl)propan-2-yl)-5-(4-chloro-3,5-difluorophenyl)-1H-pyrrole-2-carboxamide—Compound **8** (NBD-14280) was obtained following the general procedure D and Boc- and TBS-deprotection from amine racemic amine **S25** and acid **S28**.

Deprotection: To a solution of protected thiazole from the previous step in MeOH (10 ml), 1M solution of HCl in MeOH (50 mL) was added in one portion. The mixture was stirred for 3–4 h, and the solvent was evaporated. After that, 10% aqueous K₂CO₃ (50 mL) was added, and it was extracted with CH₂Cl₂-EtOH (~4:1, 4 × 50 mL). Combined organic layers were dried over Na₂SO₄, filtered, and concentrated. The compound was purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1 and 5:1, Eluent 2: CH₂Cl₂-MeOH, 5:1 and 2:1.

8 (NBD-14280): M = 471 mg. Yield = 35% (over two steps). rt = 1.318 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 427 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 1.77 (s, 3 H), 1.86 (br. s., 2 H), 2.93 (d, *J*=13.2 Hz, 1 H), 3.12 (d, *J*=13.2 Hz, 1 H), 4.52 (s, 2 H), 5.29 (br. s., 1 H), 6.84 (d, *J*=3.9 Hz, 1 H), 6.99 (d, *J*=3.9 Hz, 1 H), 7.23 (s, 1 H), 7.84 (d, *J*=9.1 Hz, 2 H), 8.12 (s, 1 H), 11.93 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 23.5, 51.7, 59.9, 60.3, 105.3 (t, *J*=21.3 Hz), 108.3 (d, *J*=23.2 Hz, 2 C), 109.4, 112.5, 113.8, 129.1, 131.6 (t, *J*=2.8 Hz), 132.8 (t, *J*=10.3 Hz), 156.9, 158.3 (dd, *J*=245.8, 4.4 Hz, 2 C), 160.1, 176.4.

HRMS (ESI) calcd for C₁₈H₁₈ClF₂N₄O₂S [M + H]⁺ 427.0802, found 427.0811.

N-(2-Amino-1-(4-(2-hydroxyethyl)thiazol-2-yl)ethyl)-5-(4-chloro-3,5-difluorophenyl)-1H-pyrrole-2-carboxamide—Compounds **9** (NBD-14242) and **10** (NBD-14243) were obtained following the general procedure D and E from amine **S19** and acid **S28**. Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1 and 5:1, Eluent 2: CH₂Cl₂-MeOH, 4:1 and 2:1.

9 (NBD-14242); (S): M = 292 mg. Yield = 22% (over two steps). rt = 1.284 min. Purity = 95%. LC-MS: m/z [M+H]⁺ = 427 Da.

10 (NBD-14243); (R): M = 321 mg. Yield = 24% (over two steps). rt = 1.268 min. Purity = 96%. LC-MS: m/z [M+H]⁺ = 427 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 1.86 (br. s., 2 H), 2.84 (t, *J*=6.8 Hz, 2 H), 3.00 (dd, *J*=13.1, 7.9 Hz, 1 H), 3.15 (dd, *J*=13.1, 5.3 Hz, 1 H), 3.70 (t, *J*=6.9 Hz, 2 H), 4.68 (br. s., 1 H), 5.17 – 5.26 (m, 1 H), 6.85 (d, *J*=3.9 Hz, 1 H), 7.04 (d, *J*=3.9 Hz, 1 H), 7.17 (s, 1 H), 7.86 (d, *J*=9.0 Hz, 2 H), 8.64 (d, *J*=7.9 Hz, 1 H), 11.96 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 34.9, 45.8, 54.5, 60.2, 105.4 (t, *J*=21.5 Hz), 108.4 (d, *J*=25.7 Hz, 2 C), 109.5, 112.7, 114.1, 128.6, 131.9 (t, *J*=2.8 Hz), 132.8 (t, *J*=10.1 Hz), 154.0, 158.4 (dd, *J*=245.9, 4.2 Hz, 2 C), 160.4, 171.5.

HRMS (ESI) calcd for C₁₈H₁₆ClF₂N₄O₂S [M - H]⁻ 425.0656, found 425.0659.

N-(2-Amino-1-(4-(2-hydroxyethyl)thiazol-2-yl)ethyl)-5-(3-fluoro-4-(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxamide—Compounds **11** (NBD-14258) and **12** (NBD-14259) were obtained following the general procedure D and E from amine **S19** and acid **S27**. Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1 and 5:1, Eluent 2: CH₂Cl₂-MeOH, 4:1 and 2:1.

11 (NBD-14258); (R): M = 582 mg. Yield = 31% (over two steps). rt = 1.333 min. Purity = 98%. LC-MS: m/z [M+H]⁺ = 443 Da.

12 (NBD-14259); (S): M = 506 mg. Yield = 27% (over two steps). rt = 1.338 min. Purity = 96%. LC-MS: m/z [M+H]⁺ = 443 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 1.79 (br. s., 2 H), 2.84 (t, *J*=6.8 Hz, 2 H), 3.00 (dd, *J*=13.2, 7.9 Hz, 1 H), 3.15 (dd, *J*=13.2, 5.3 Hz, 1 H), 3.70 (t, *J*=6.9 Hz, 2 H), 4.67 (br. s., 1 H), 5.18 – 5.26 (m, 1 H), 6.89 (d, *J*=3.9 Hz, 1 H), 7.06 (d, *J*=3.9 Hz, 1 H), 7.18 (s, 1 H), 7.73 (t, *J*=8.1 Hz, 1 H), 7.85 (d, *J*=8.4 Hz, 1 H), 8.03 (d, *J*=13.0 Hz, 1 H), 8.67 (d, *J*=7.9 Hz, 1 H), 12.06 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 34.9, 45.8, 54.5, 60.3, 110.0, 112.4 (d, *J*=22.5 Hz), 113.0, 113.3 (qd, *J*=32.6, 12.7 Hz), 114.1, 120.6 (d, *J*=2.6 Hz), 122.9 (q, *J*=271.3 Hz), 127.6 (q, *J*=3.1 Hz), 129.0, 132.1 (d, *J*=1.8 Hz), 138.5 (d, *J*=9.2 Hz), 154.1, 159.5 (dq, *J*=251.3, 2.2 Hz), 160.4, 171.5.

HRMS (ESI) calcd for C₁₉H₁₇F₄N₄O₂S [M - H]⁻ 441.1015, found 441.1015.

N-(2-Amino-1-(4-(2-hydroxyethyl)thiazol-2-yl)ethyl)-5-(2-fluoro-4-(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxamide—Compounds **13** (NBD-14273) and **14** (NBD-14274) were obtained following the general procedure D and E from amine **S19** and acid **S4c**. Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1 and 5:1, Eluent 2: CH₂Cl₂-MeOH, 4:1 and 2:1.

13 (NBD-14273); (S): M = 581 mg. Yield = 37% (over two steps). rt = 1.313 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 443 Da.

14 (NBD-14274); (R): M = 548 mg. Yield = 35% (over two steps). rt = 1.308 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 443 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 1.80 (br. s., 2 H), 2.84 (t, *J*=6.9 Hz, 2 H), 3.00 (dd, *J*=13.2, 7.9 Hz, 1 H), 3.14 (dd, *J*=13.1, 5.1 Hz, 1 H), 3.70 (t, *J*=6.9 Hz, 2 H), 4.68 (br. s., 1 H), 5.18 – 5.26 (m, 1 H), 6.73 (t, *J*=3.7 Hz, 1 H), 7.09 (d, *J*=3.9 Hz, 1 H), 7.18 (s, 1 H), 7.62 (d, *J*=8.2 Hz, 1 H), 7.74 (d, *J*=11.5 Hz, 1 H), 8.17 (t, *J*=8.0 Hz, 1 H), 8.72 (d, *J*=7.9 Hz, 1 H), 12.01 (br. s., 1 H).

^{13}C NMR (DMSO- d_6 , 100 MHz): δ = 34.8, 45.8, 54.5, 60.2, 112.2 (d, $J=10.3$ Hz), 112.9, 113.7 (dq, $J=26.4$, 3.7 Hz), 114.1, 121.5 (qd, $J=7.2$, 3.5 Hz), 123.5 (qd, $J=271.8$, 2.2 Hz), 123.7 (d, $J=11.8$ Hz), 127.1 (d, $J=2.2$ Hz), 128.1 (qd, $J=33.2$, 8.3 Hz), 128.4, 128.6 (d, $J=3.7$ Hz), 154.0, 158.1 (d, $J=250.3$ Hz), 160.3, 171.5.

HRMS (ESI) calcd for $\text{C}_{19}\text{H}_{17}\text{F}_4\text{N}_4\text{O}_2\text{S}$ [M - H] $^-$ 441.1015, found 441.1014.

N-(2-Amino-1-(4-(1,2-dihydroxyethyl)thiazol-2-yl)ethyl)-5-(3-fluoro-4-(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxamide—Compounds **15** (NBD-14287) and **16** (NBD-14288) were obtained following the general procedure D and E and F from amine **S24** and acid **S27**. Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl_3 -MeOH (saturated with $\text{NH}_3 \sim 7\text{M}$), 10:1, 5:1 and 3:1, Eluent 2: CH_2Cl_2 -MeOH, 2:1 and 1:1.

Note that compounds **15** & **16** were obtained as a diastereomeric mixture of 2 single compounds having the absolute configuration of the chiral carbon as R for **16** and S for **15**.

15 (NBD-14287); (S): M = 365 mg. Yield = 19% (over two steps). rt = 1.239 min. Purity = 96%. LC-MS: m/z [M+H] $^+$ = 459 Da.

16 (NBD-14288); (R): M = 402 mg. Yield = 21% (over two steps). rt = 1.304 min. Purity = 95%. LC-MS: m/z [M+H] $^+$ = 459 Da.

^1H NMR (DMSO- d_6 , 400 MHz): δ = 1.80 (br. s., 2 H), 3.01 (ddd, $J=13.2$, 7.7, 1.7 Hz, 1 H), 3.14 (dt, $J=13.2$, 4.7 Hz, 1 H), 3.44 – 3.52 (m, 1 H), 3.71 (dt, $J=10.9$, 3.9 Hz, 1 H), 4.65 (dd, $J=6.7$, 4.2 Hz, 1 H), 4.71 (br. s., 1 H), 5.17 – 5.26 (m, 1 H), 5.35 (br. s., 1 H), 6.89 (d, $J=3.9$ Hz, 1 H), 7.06 (d, $J=3.9$ Hz, 1 H), 7.30 (s, 1 H), 7.74 (t, $J=8.1$ Hz, 1 H), 7.85 (d, $J=8.4$ Hz, 1 H), 8.03 (d, $J=12.9$ Hz, 1 H), 8.68 (d, $J=7.8$ Hz, 1 H), 12.14 (br. s., 1 H).

^{13}C NMR (DMSO- d_6 , 100 MHz): δ = 45.7, (54.5, 54.5), (65.8, 65.8), (71.3, 71.4), 110.0, 112.4 (d, $J=22.5$ Hz), 112.9, 113.7 (qd, $J=32.6$, 12.5 Hz), (114.3, 114.4), 120.6 (d, $J=3.0$ Hz), 122.9 (q, $J=271.5$ Hz), 127.6 (q, $J=3.0$ Hz), 128.9, 132.0 (d, $J=1.8$ Hz), 138.5 (d, $J=9.4$ Hz), (158.3, 158.4), 159.4 (dq, $J=251.2$, 2.2 Hz), (160.3, 160.3), (171.7, 171.8).

HRMS (ESI) calcd for $\text{C}_{19}\text{H}_{17}\text{F}_4\text{N}_4\text{O}_3\text{S}$ [M - H] $^-$ 457.0963, found 457.0956.

N-(2-Amino-1-(4-(3-hydroxypropyl)thiazol-2-yl)ethyl)-5-(3-fluoro-4-(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxamide—Compounds **17** (NBD-14289) and **18** (NBD-142990) were obtained following the general procedure D and E from amine **S20** and acid **S27**. Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl_3 -MeOH (saturated with $\text{NH}_3 \sim 7\text{M}$), 10:1 and 5:1, Eluent 2: CH_2Cl_2 -MeOH, 4:1 and 2:1.

17 (NBD-14289); (S): M = 519 mg. Yield = 27% (over two steps). rt = 1.348 min. Purity = 95%. LC-MS: m/z [M+H] $^+$ = 457 Da.

18 (NBD-14290); (R): M = 392 mg. Yield = 26% (over two steps). rt = 1.370 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 457 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 1.66 (br. s., 2 H), 1.74 – 1.83 (m, 2 H), 2.71 (t, *J*=7.6 Hz, 2 H), 3.00 (dd, *J*=13.2, 7.9 Hz, 1 H), 3.15 (dd, *J*=13.1, 5.3 Hz, 1 H), 3.44 (t, *J*=6.4 Hz, 2 H), 4.49 (br. s., 1 H), 5.17 – 5.25 (m, 1 H), 6.89 (d, *J*=3.9 Hz, 1 H), 7.06 (d, *J*=3.9 Hz, 1 H), 7.13 (s, 1 H), 7.74 (t, *J*=8.1 Hz, 1 H), 7.85 (d, *J*=8.4 Hz, 1 H), 8.03 (d, *J*=12.9 Hz, 1 H), 8.67 (d, *J*=7.9 Hz, 1 H), 12.13 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 27.6, 32.1, 45.8, 54.5, 60.2, 110.0, 112.4 (d, *J*=22.5 Hz), 112.9, 113.1, 113.7 (qd, *J*=32.4, 12.5 Hz), 120.6 (d, *J*=3.0 Hz), 122.9 (q, *J*=271.3 Hz), 127.6 (q, *J*=3.3 Hz), 129.0, 132.1 (d, *J*=1.8 Hz), 138.5 (d, *J*=9.4 Hz), 156.6, 159.4 (dq, *J*=251.2, 2.0 Hz), 160.4, 171.6.

HRMS (ESI) calcd for C₂₀H₁₉F₄N₄O₂S [M - H]⁻ 455.1170, found 455.1170.

N-(2-Amino-1-(5-(1,2-dihydroxyethyl)thiazol-2-yl)ethyl)-5-(3-fluoro-4-(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxamide—Compounds **19** (NBD-14303) and **20** (NBD-14304) were obtained following the general procedure D and E and F from amine **S23** and acid **S27**. Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1, 5:1 and 3:1, Eluent 2: CH₂Cl₂-MeOH, 2:1 and 1:1.

Note that compounds **19** & **20** were obtained as a diastereomeric mixture of 2 single compounds having the absolute configuration of the chiral carbon as R for **20** and S for **19**.

19 (NBD-14303); (S): M = 382 mg. Yield = 31% (over three steps). rt = 1.316 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 459 Da.

20 (NBD-14304); (R): M = 216 mg. Yield = 27% (over three steps). rt = 1.230 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 459 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 1.68 (br. s., 2 H), 3.00 (dd, *J*=13.2, 7.9 Hz, 1 H), 3.14 (dd, *J*=13.2, 5.3 Hz, 1 H), 3.40 – 3.47 (m, 1 H), 3.51 (dd, *J*=10.8, 6.2 Hz, 1 H), 4.75 (t, *J*=5.7 Hz, 1 H), 4.94 (br. s., 1 H), 5.15 – 5.25 (m, 1 H), 5.68 (br. s., 1 H), 6.89 (d, *J*=3.9 Hz, 1 H), 7.06 (d, *J*=3.9 Hz, 1 H), 7.56 (s, 1 H), 7.74 (t, *J*=8.1 Hz, 1 H), 7.85 (d, *J*=8.3 Hz, 1 H), 8.03 (d, *J*=13.0 Hz, 1 H), 8.66 (d, *J*=7.9 Hz, 1 H), 12.08 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = (45.6, 45.7), 54.5, 66.7, 68.1, 110.0, 112.4 (d, *J*=22.6 Hz), 112.9, 113.7 (qd, *J*=32.4, 12.5 Hz), 120.6 (d, *J*=2.9 Hz), 122.9 (q, *J*=271.3 Hz), 127.6 (q, *J*=3.5 Hz), 129.0, 132.0 (d, *J*=2.1 Hz), 138.5 (q, *J*=2.3 Hz), 141.3, 141.4, 159.4 (dq, *J*=251.3, 1.9 Hz), 160.3, (171.2, 171.2).

HRMS (ESI) calcd for C₁₉H₁₉F₄N₄O₃S [M + H]⁺ 459.1109, found 459.1109.

2-[2-Amino-1-[[5-[3-fluoro-4-(trifluoromethyl)phenyl]-1H-pyrrole-2-carbonyl]amino]ethyl]thiazole-4-carboxylic acid—Compounds **21** (NBD-14271) and

22 (NBD-14272) were obtained from direct NaOH hydrolysis of 100mg of the corresponding methyl esters **23** (NBD-14275) and **24** (NBD-14276).

21 (NBD-14271); (S): M = 51 mg. LC-MS: m/z [M+H]⁺ = 443 Da.

22 (NBD-14272); (R): M = 60 mg. LC-MS: m/z [M+H]⁺ = 443 Da.

Due to broad NMR signals and since the corresponding crudes were not active, no further characterization and purification were performed.

Methyl 2-(2-amino-1-(5-(3-fluoro-4-(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxamido)ethyl)thiazole-4-carboxylate—Compounds **23** (NBD-14275) and **24** (NBD-14276) were obtained following the general procedure D and E from amine **S15** and acid **S27**. Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1 and 5:1, Eluent 2: CH₂Cl₂-MeOH, 4:1 and 2:1.

23 (NBD-14275); (S): M = 344 mg. Yield = 21% (over two steps). rt = 1.377 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 457 Da.

24 (NBD-14276); (R): M = 421 mg. Yield = 26% (over two steps). rt = 1.394 min. Purity = 85%. LC-MS: m/z [M+H]⁺ = 457 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 2.04 (br. s., 2 H), 3.06 (dd, *J*=13.2, 7.7 Hz, 1 H), 3.18 (dd, *J*=13.2, 5.4 Hz, 1 H), 3.82 (s, 3 H), 5.22 – 5.29 (m, 1 H), 6.90 (d, *J*=3.9 Hz, 1 H), 7.07 (d, *J*=3.9 Hz, 1 H), 7.74 (t, *J*=8.1 Hz, 1 H), 7.86 (d, *J*=8.4 Hz, 1 H), 8.04 (d, *J*=12.9 Hz, 1 H), 8.44 (s, 1 H), 8.82 (d, *J*=6.4 Hz, 1 H), 12.12 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 45.4, 52.0, 54.5, 110.0, 112.4 (d, *J*=22.5 Hz), 113.1, 113.8 (qd, *J*=32.4, 12.5 Hz), 120.7 (d, *J*=2.8 Hz), 122.9 (q, *J*=271.3 Hz), 127.6 (q, *J*=3.3 Hz), 128.8, 129.1, 132.3 (d, *J*=1.8 Hz), 138.5 (d, *J*=9.2 Hz), 145.5, 159.5 (dq, *J*=251.2, 2.2 Hz), 160.5, 161.3, 173.4.

HRMS (ESI) calcd for C₁₉H₁₅F₄N₄O₃S [M - H]⁻ 455.0806, found 455.0808.

2-(2-Amino-1-(5-(3-fluoro-4-(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxamido)ethyl)thiazole-4-carboxamide—Compounds **25** (NBD-14267) and **26** (NBD-14268) were obtained following the general procedure D and E from amine **S16** and acid **S27**. Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1 and 5:1, Eluent 2: CH₂Cl₂-MeOH, 3:1 and 1:1.

25 (NBD-14267); (R): M = 471 mg. Yield = 35% (over two steps). rt = 1.289 min. Purity = 97%. LC-MS: m/z [M+H]⁺ = 442 Da.

26 (NBD-14268); (S): M = 493 mg. Yield = 36% (over two steps). rt = 1.285 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 442 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 1.99 (br. s., 2 H), 3.06 (dd, *J*=13.3, 7.5 Hz, 1 H), 3.22 (dd, *J*=13.2, 5.4 Hz, 1 H), 5.20 – 5.30 (m, 1 H), 6.90 (d, *J*=3.9 Hz, 1 H), 7.08 (d, *J*=3.9 Hz, 1 H), 7.59 (br. s., 1 H), 7.70 – 7.78 (m, 2 H), 7.86 (d, *J*=8.4 Hz, 1 H), 8.03 (d, *J*=13.0 Hz, 1 H), 8.14 (s, 1 H), 8.75 (d, *J*=6.0 Hz, 1 H), 11.95 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 45.5, 54.5, 110.1, 112.5 (d, *J*=22.5 Hz), 113.2, 113.9 (qd, *J*=32.4, 12.5 Hz), 120.7 (d, *J*=2.8 Hz), 122.9 (q, *J*=271.5 Hz), 124.0, 127.6 (q, *J*=3.3 Hz), 128.8, 132.3 (d, *J*=2.0 Hz), 138.5 (d, *J*=9.2 Hz), 150.1, 159.5 (dq, *J*=251.4, 2.0 Hz), 160.6, 162.5, 173.0.

HRMS (ESI) calcd for C₁₈H₁₄F₄N₅O₂S [M - H]⁻ 440.0810, found 440.0811.

N-(2-Amino-1-(4-(hydroxymethyl)thiazol-2-yl)ethyl)-5-(3-fluoro-4-(trifluoromethyl)phenyl)-3-methyl-1H-pyrrole-2-carboxamide—Compounds 27

(NBD-14325) and **28** (NBD-14324) were obtained following the general procedure D and E from amine **S17** and acid **S8**. Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1 and 5:1, Eluent 2: CH₂Cl₂-MeOH, 3:1 and 2:1.

27 (NBD-14325); (S): M = 454 mg. Yield = 27% (over two steps). rt = 1.327 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 443 Da.

28 (NBD-14324); (R): M = 571 mg. Yield = 34% (over two steps). rt = 1.336 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 443 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 1.81 (br. s., 2 H), 2.32 (s, 3 H), 3.04 (dd, *J*=13.1, 7.2 Hz, 1 H), 3.12 (dd, *J*=13.1, 5.3 Hz, 1 H), 4.55 (s, 2 H), 5.20 – 5.27 (m, 1 H), 5.31 (br. s., 1 H), 6.74 (s, 1 H), 7.31 (s, 1 H), 7.72 – 7.82 (m, 2 H), 7.87 (d, *J*=12.7 Hz, 1 H), 8.28 (d, *J*=7.0 Hz, 1 H), 11.60 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 12.9, 45.9, 54.0, 59.8, 112.0 (d, *J*=22.1 Hz), 112.3, 113.8 (qd, *J*=32.4, 12.4 Hz), 114.2, 120.3 (d, *J*=2.8 Hz), 122.9 (q, *J*=271.5 Hz), 124.7, 125.8, 127.8 (q, *J*=4.1 Hz), 129.9 (d, *J*=1.8 Hz), 138.4 (d, *J*=9.2 Hz), 157.7, 159.6 (dq, *J*=251.6, 2.2 Hz), 160.8, 171.9.

HRMS (ESI) calcd for C₁₉H₁₇F₄N₄O₂S [M-H]⁻ 441.1014, found 441.1013.

N-(2-Amino-1-(5-(hydroxymethyl)thiazol-2-yl)ethyl)-5-(3-fluoro-4-(trifluoromethyl)phenyl)-3-methyl-1H-pyrrole-2-carboxamide—Compounds 29

(NBD-14329) and **30** (NBD-14328) were obtained following the general procedure D and E from amine **S18** and acid **S8**. Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1 and 5:1, Eluent 2: CH₂Cl₂-MeOH, 3:1 and 2:1.

29 (NBD-14329); (S): M = 420 mg. Yield = 24% (over two steps). rt = 1.326 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 443 Da.

30 (NBD-14328); (R): M = 620 mg. Yield = 35% (over two steps). rt = 1.318 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 443 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 2.08 (br. s., 2 H), 2.33 (s, 3 H), 3.05 (dd, *J*=13.1, 7.3 Hz, 1 H), 3.14 (dd, *J*=13.1, 5.1 Hz, 1 H), 4.62 (s, 2 H), 5.18 – 5.25 (m, 1 H), 5.50 (br. s., 1 H), 6.75 (s, 1 H), 7.57 (s, 1 H), 7.73 – 7.82 (m, 2 H), 7.89 (d, *J*=12.5 Hz, 1 H), 8.32 (d, *J*=6.8 Hz, 1 H), 11.64 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 12.9, 45.7, 54.0, 55.8, 112.0 (d, *J*=22.3 Hz), 112.3, 113.7 (qd, *J*=32.6, 12.5 Hz), 120.3 (d, *J*=2.8 Hz), 122.8 (q, *J*=271.3 Hz), 124.6, 125.7, 127.8 (q, *J*=3.5 Hz), 129.9 (d, *J*=2.0 Hz), 138.4 (d, *J*=9.2 Hz), 139.0, 140.2, 159.5 (dq, *J*=251.7, 2.4 Hz), 160.8, 171.5.

HRMS (ESI) calcd for C₁₉H₁₇F₄N₄O₂S [M-H]⁻ 441.1014, found 441.1014.

N-(2-Amino-1-(4-(hydroxymethyl)thiazol-2-yl)-2-methylpropyl)-5-(4-chloro-3,5-difluorophenyl)-1H-pyrrole-2-carboxamide—Compounds **31** (NBD-14246) and **32** (NBD-14247) were obtained following the general procedure D and E from amine **S21** and acid **S28**. Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1 and 5:1, Eluent 2: CH₂Cl₂-MeOH, 4:1 and 2:1.

31 (NBD-14246); (S): M = 364 mg. Yield = 28% (over two steps). rt = 1.362 min. Purity = 95%. LC-MS: m/z [M+H]⁺ = 441 Da.

32 (NBD-14247); (R): M = 315 mg. Yield = 24% (over two steps). rt = 1.377 min. Purity = 96%. LC-MS: m/z [M+H]⁺ = 441 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 1.08 (s, 3 H), 1.13 (s, 3 H), 1.97 (br. s., 2 H), 4.57 (s, 2 H), 5.29 (d, *J*=7.6 Hz, 1 H), 5.33 (br. s., 1 H), 6.84 (d, *J*=3.9 Hz, 1 H), 7.08 (d, *J*=3.9 Hz, 1 H), 7.32 (s, 1 H), 7.83 (d, *J*=8.9 Hz, 2 H), 8.26 (d, *J*=8.4 Hz, 1 H), 12.02 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 27.6, 28.5, 52.7, 59.2, 59.8, 105.4 (t, *J*=21.3 Hz), 108.4 (d, *J*=25.4 Hz, 2 C), 109.5, 113.2, 114.4, 128.4, 132.0 (t, *J*=2.8 Hz), 132.8 (t, *J*=10.1 Hz), 157.3, 158.4 (dd, *J*=246.4, 4.1 Hz, 2 C), 160.0, 170.2.

HRMS (ESI) calcd for C₁₉H₁₈ClF₂N₄O₂S [M - H]⁻ 439.0813, found 439.0813.

N-(2-Amino-1-(4-(hydroxymethyl)thiazol-2-yl)-2-methylpropyl)-5-(4-(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxamide—Compounds **33** (NBD-14248) and **34** (NBD-14249) were obtained following the general procedure D and E from amine **S21** and acid **S26**. Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1 and 5:1, Eluent 2: CH₂Cl₂-MeOH, 4:1 and 2:1.

33 (NBD-14248); (S): M = 435 mg. Yield = 27% (over two steps). rt = 1.354 min. Purity = 97%. LC-MS: m/z [M+H]⁺ = 439 Da.

34 (NBD-14249); (R): M = 501 mg. Yield = 31% (over two steps). rt = 1.360 min. Purity = 99%. LC-MS: m/z [M+H]⁺ = 439 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 1.09 (s, 3 H), 1.14 (s, 3 H), 1.95 (br. s., 2 H), 4.58 (s, 2 H), 5.30 (d, J=8.2 Hz, 1 H), 5.38 (br. s., 1 H), 6.78 (d, J=3.8 Hz, 1 H), 7.08 (d, J=3.8 Hz, 1 H), 7.32 (s, 1 H), 7.71 (d, J=8.4 Hz, 2 H), 8.02 (d, J=8.2 Hz, 2 H), 8.28 (d, J=8.8 Hz, 1 H), 12.05 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 27.6, 28.5, 52.7, 59.2, 59.8, 108.8, 113.5, 114.4, 124.4 (q, J=272.1 Hz), 125.1 (2 C), 125.6 (q, J=4.1 Hz, 2 C), 126.7 (q, J=31.7 Hz), 128.2, 133.4, 135.6, 157.3, 160.0, 170.2.

HRMS (ESI) calcd for C₂₀H₂₀F₃N₄O₂S [M - H]⁻ 437.1265, found 437.1269.

N-(2-Amino-1-(5-(hydroxymethyl)thiazol-2-yl)-2-methylpropyl)-5-(4-(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxamide—Compounds **35** (NBD-14262) and **36** (NBD-14263) were obtained following the general procedure D and E from amine **S22** and acid **S26**. Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1 and 5:1, Eluent 2: CH₂Cl₂-MeOH, 4:1 and 2:1.

35 (NBD-14262); (R): M = 438 mg. Yield = 27% (over two steps). rt = 1.458 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 439 Da.

36 (NBD-14263); (S): M = 77 mg. Yield = 4% (over two steps). rt = 1.306 min. Purity = 94%. LC-MS: m/z [M+H]⁺ = 439 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 1.10 (s, 3 H), 1.14 (s, 3 H), 2.21 (br. s., 2 H), 4.63 (s, 2 H), 5.27 (d, J=8.4 Hz, 1 H), 5.51 (br. s., 1 H), 6.78 (d, J=3.9 Hz, 1 H), 7.06 (d, J=3.9 Hz, 1 H), 7.58 (s, 1 H), 7.72 (d, J=8.4 Hz, 2 H), 8.02 (d, J=8.2 Hz, 2 H), 8.29 (d, J=8.9 Hz, 1 H), 12.07 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 27.5, 28.4, 52.8, 55.8, 59.3, 108.8, 113.6, 124.4 (q, J=271.6 Hz), 125.1 (2 C), 125.6 (q, J=3.7 Hz, 2 C), 126.7 (q, J=31.7 Hz), 128.2, 133.4, 135.6, 138.7, 140.3, 160.0, 170.1.

HRMS (ESI) calcd for C₂₀H₂₀F₃N₄O₂S [M +H]⁺ 437.1265, found 437.1269.

N-(2-amino-1-(5-((2,3-dihydroxypropoxy)methyl)thiazol-2-yl)ethyl)-5-(4-(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxamide—Compounds **37** (NBD-14225) and **38** (NBD-14229) were obtained following the general procedure D and E and F from amine **S14** and acid **S26**. Compounds were purified using column chromatography on silica gel (twice). Eluent 1: CHCl₃-MeOH (saturated with NH₃~7M), 10:1, 5:1 and 3:1, Eluent 2: CH₂Cl₂-MeOH, 2:1 and 1:1.

Note that compounds **37** & **38** were obtained as a diastereomeric mixture of 2 single compounds having the absolute configuration of the chiral carbon as R for **38** and S for **37**.

37 (NBD-14225); (S): M = 342 mg. Yield = 17% (over two steps). rt = 1.342 min. Purity = 100%. LC-MS: m/z [M+H]⁺ = 485 Da.

38 (NBD-14229); (R): M = 381 mg. Yield = 19% (over two steps). rt = 1.456 min. Purity = 95%. LC-MS: m/z [M+H]⁺ = 485 Da.

¹H NMR (DMSO-d₆, 400 MHz): δ = 1.77 (br. s., 2 H), 3.04 (dd, J=13.1, 7.8 Hz, 1 H), 3.17 (dd, J=13.3, 5.4 Hz, 1 H), 3.29 – 3.36 (m, 3 H), 3.45 (dd, J=9.9, 4.6 Hz, 1 H), 3.54 – 3.61 (m, 1 H), 4.65 (s, 2 H), 4.72 (br. s., 2 H), 5.19 – 5.28 (m, 1 H), 6.79 (d, J=3.9 Hz, 1 H), 7.06 (d, J=3.8 Hz, 1 H), 7.66 (s, 1 H), 7.71 (d, J=8.4 Hz, 2 H), 8.04 (d, J=8.2 Hz, 2 H), 8.67 (d, J=7.8 Hz, 1 H), 12.00 (br. s., 1 H).

¹³C NMR (DMSO-d₆, 100 MHz): δ = 45.6, 54.6, 63.0, 64.5, 70.5, 71.8, 108.9, 113.1, 124.4 (q, J=271.6 Hz), 125.1 (2 C), 125.6 (q, J=3.7 Hz, 2 C), 126.6 (q, J=32.2 Hz), 128.3, 133.3, 135.4, 135.6, 141.0, 160.5, 173.1.

HRMS (ESI): Calcd for C₂₁H₂₄F₃N₄O₄S [M +H]⁺ 485.1465, found 485.1466.

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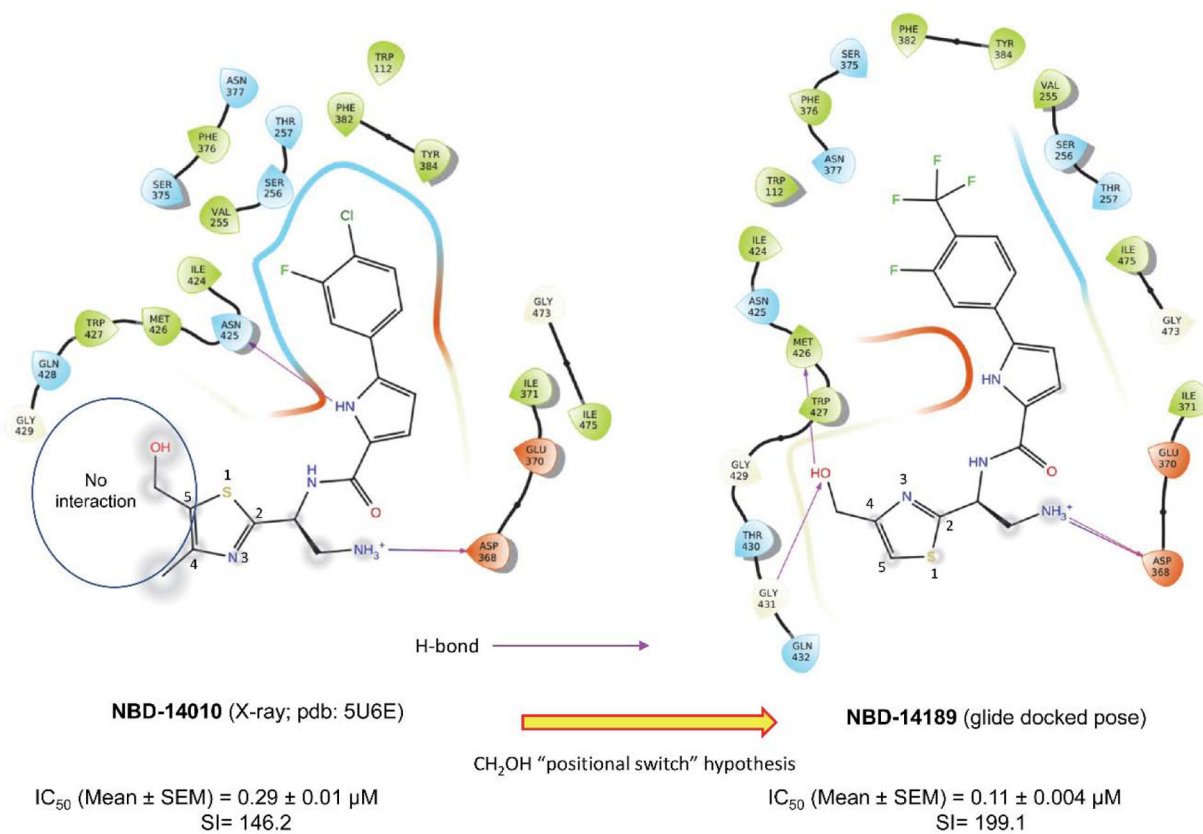
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(against 55 diverse HIV-1 Env-pseudotyped viruses representing clinical isolates of different subtypes)

Figure 1.

"Positional switch" hypothesis indicating no interaction of the CH₂OH group at position 5 in the thiazole ring in the x-ray structure of NBD-14010 bound to HIV-1 gp120. When the CH₂OH was switched to position 4 in the thiazole ring in NBD-14189, it forms H-bond (indicated by violet arrows using a glide docking pose) with Met426 and Gly431²⁵.

Cf2-CCR5 cells infected with HIV-1_{ADA}

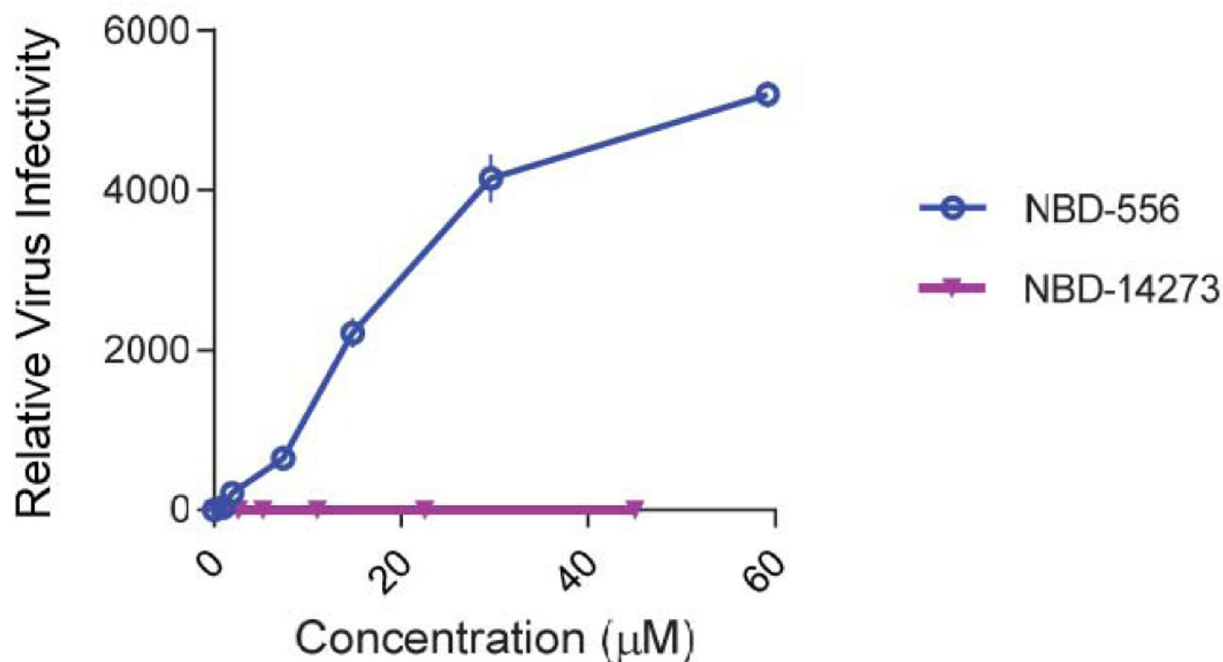
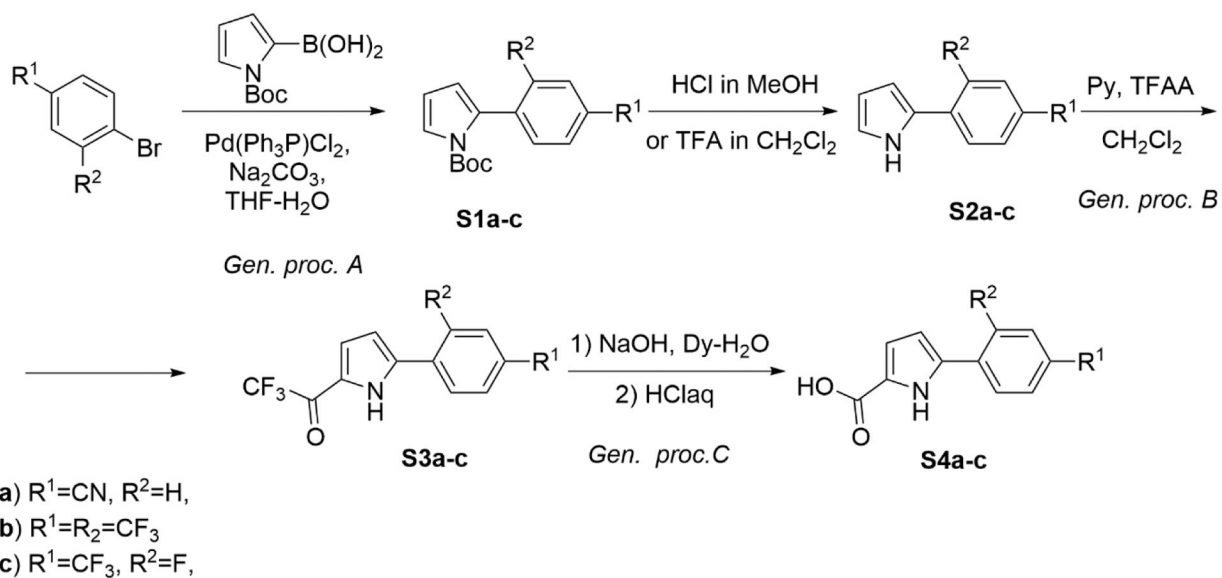
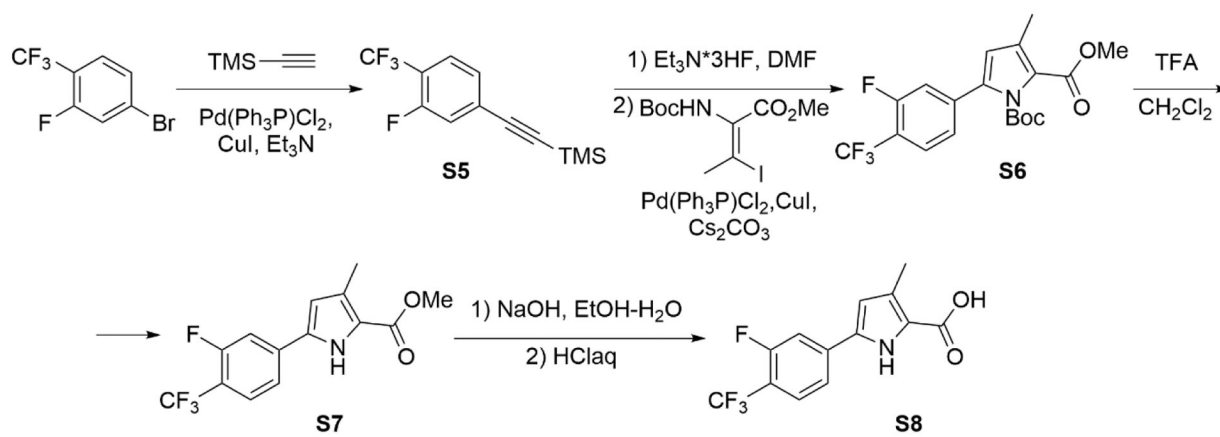


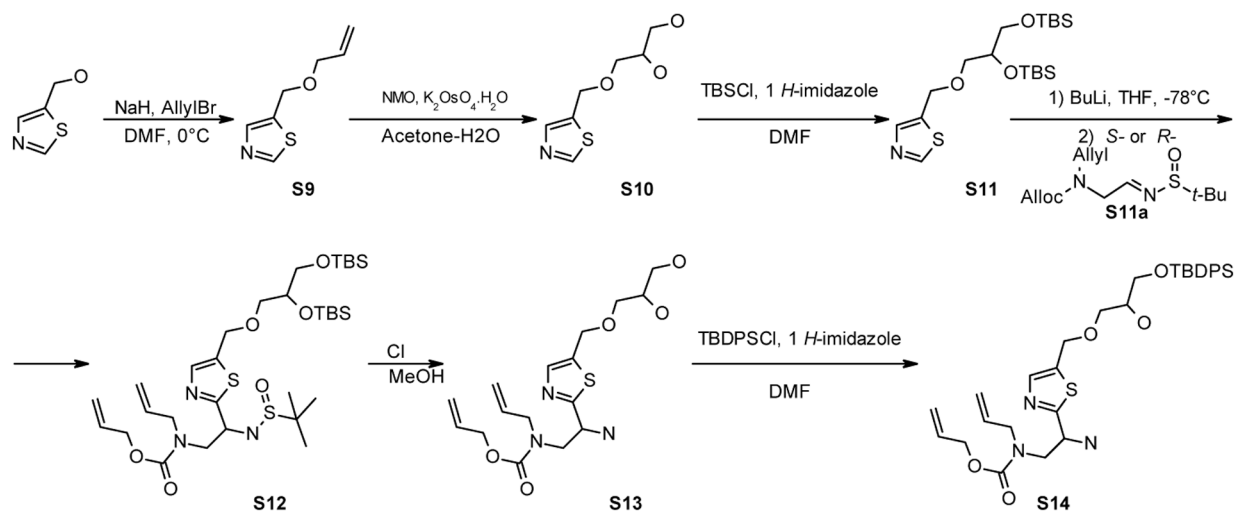
Figure 2. Infectivity of CD4-negative Cf2Th-CCR5 cells by CD4-dependent HIV-1_{ADA}. CCf2Th-CCR5 cells were infected with CD4-dependent HIV-1_{ADA} in the presence of NBD-14273 and NBD-556, which was used as a control. The Relative virus infectivity indicates the ratio of the amount of infection detected in the presence and absence of the compounds. Three independent experiments were performed in triplicate, and the graph is representative of one experiment. The toxicity of the compounds against these cells was evaluated to calculate the CC₅₀ values: for NBD-556, the CC₅₀ was > 60, and for NBD-14273, it was 31.2±1.4. All the values represent the mean ± standard deviation.



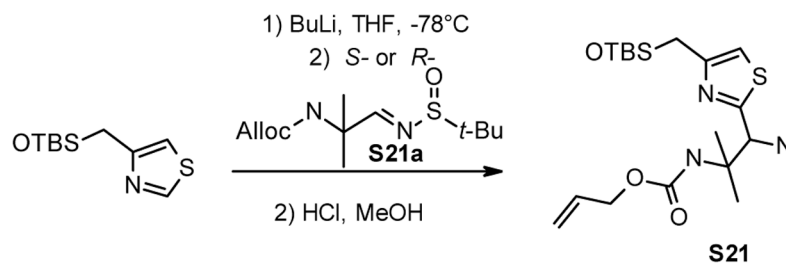
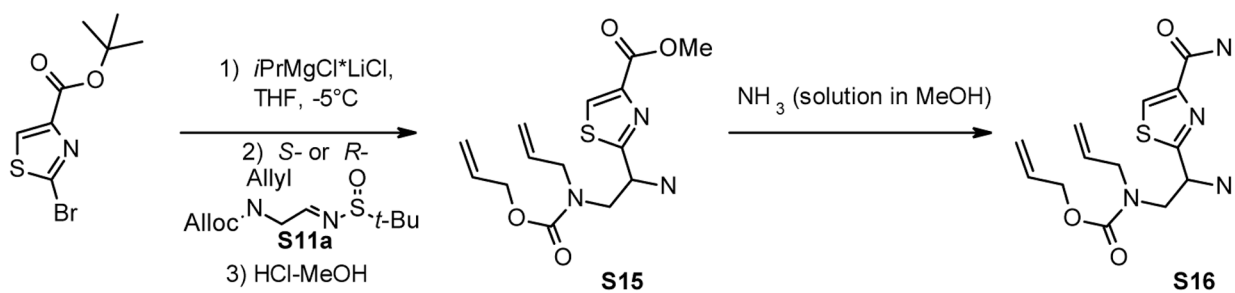
Scheme 1.
Synthesis of **S4a-c**.



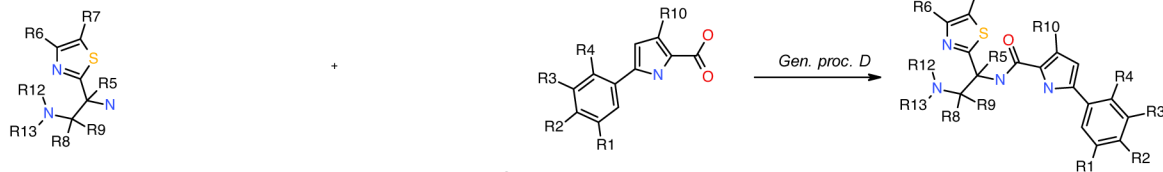
Scheme 2.
Synthesis of **S5**–**S8**.



Scheme 3.
Synthesis of **S9–S14** and **S21**.



Scheme 4.
 Synthesis of **S15**, **S16** and **S21**.



S14, R₆=H, R₅=H, R₇=CH₂OCH₂CH(OH)CH₂OTBDPS, R₈=H, R₉=H, R₁₂=allyl, R₁₃=alloc

S15, R₆=CO₂Me, R₅=H, R₇=H, R₈=H, R₉=H, R₁₂=allyl, R₁₃=alloc

S16, R₆=CONH₂, R₅=H, R₇=H, R₈=H, R₉=H, R₁₂=allyl, R₁₃=alloc

S17, R₆=CH₂OH, R₇=H, R₅=H, R₈=H, R₉=H, R₁₂=allyl, R₁₃=alloc

S18, R₆=H, R₇=CH₂OH, R₅=H, R₈=H, R₉=H, R₁₂=allyl, R₁₃=alloc

S19, R₆=(CH₂)₂OH, R₇=H, R₅=H, R₈=H, R₉=H, R₁₂=allyl, R₁₃=alloc,

S20, R₆=(CH₂)₃OH, R₇=H, R₅=H, R₈=H, R₉=H, R₁₂=allyl, R₁₃=alloc

S21, R₆=CH₂OH, R₇=H, R₅=H, R₈=CH₃, R₉=CH₃, R₁₂=H, R₁₃=alloc

S22, R₆=H, R₇=CH₂OH, R₅=H, R₈=CH₃, R₉=CH₃, R₁₂=H, R₁₃=alloc

S23, R₆=H, R₇=CH(OH)CH₂OTBDPS, R₅=H, R₈=H, R₉=H, R₁₂=allyl, R₁₃=alloc

S24, R₆=CH(OH)CH₂OTBDPS, R₅=H, R₇=H, R₈=H, R₉=H, R₁₂=allyl, R₁₃=alloc

S25, R₆=CH₂OTBS, R₅=Me, R₇=H, R₈=H, R₉=H, R₁₂=H, R₁₃=boc

S4a, R₁₀=H, R₂=CN, R₁=H, R₃=H, R₄=H

S4b, R₁₀=H, R₂=CF₃, R₁=H, R₃=H, R₄=CF₃

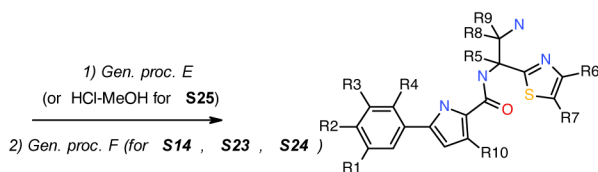
S4c, R₁₀=H, R₂=CF₃, R₁=H, R₃=H, R₄=F

S8, R₁₀=CH₃, R₂=CF₃, R₁=F, R₃=H, R₄=H

S26, R₁₀=H, R₂=CF₃, R₁=H, R₃=H, R₄=H

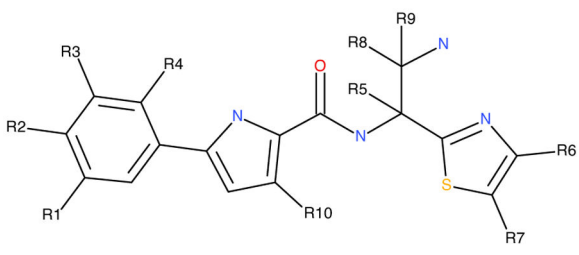
S27, R₁₀=H, R₂=CF₃, R₁=F, R₃=H, R₄=H

S28, R₁₀=H, R₂=Cl, R₁=F, R₃=F, R₄=H

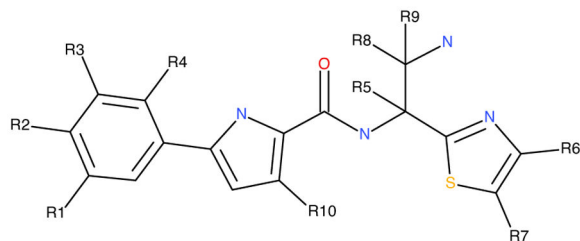


Compounds 1-38

Scheme 5.
Synthesis of compounds 1–38.

Table 1.Anti-HIV-1 activity (IC₅₀) of NBD compounds in a single-cycle assay against HIV-1_{HXB2}


| New No | Code (enantiomer) | R ₁ | R ₂ | R ₃ | R ₄ | R ₅ | R ₆ | R ₇ | R ₉ | R ₉ | R ₁₀ | IC ₅₀ ^b | CC ₅₀ ^b | SI |
|--------|----------------------------|----------------|-----------------|----------------|-----------------|-----------------|--|--------------------|----------------|----------------|-----------------|-------------------------------|-------------------------------|-------|
| | NBD-14136 (R) ^a | H | CF ₃ | H | H | H | H | CH ₂ OH | H | H | H | 0.27±0.2 | 42.4±1 | 157 |
| 1 | NBD-14250 (R) | H | CF ₃ | H | F | H | CH ₂ OH | H | H | H | H | 0.61±15 | 26.8±0.25 | 43.9 |
| 2 | NBD-14251 (S) | H | CF ₃ | H | F | H | CH ₂ OH | H | H | H | H | 0.187±0.01 | 25.7±1.2 | 137 |
| 3 | NBD-14260 (R) | H | CN | H | H | H | CH ₂ OH | H | H | H | H | >16 | >82 | - |
| 4 | NBD-14261 (S) | H | CN | H | H | H | CH ₂ OH | H | H | H | H | >16 | >82 | - |
| 5 | NBD-14313 (S) | H | CF ₃ | H | CF ₃ | H | CH ₂ OH | H | H | H | H | 4.2±0.4 | 33.4±2 | 7.9 |
| 6 | NBD-14314 (R) | H | CF ₃ | H | CF ₃ | H | CH ₂ OH | H | H | H | H | 6.3±1 | 37.6±3.6 | 5.9 |
| 7 | NBD-14278-rac | F | CF ₃ | H | H | CH ₃ | CH ₂ OH | H | H | H | H | 1.6±0.08 | 45.3±1 | 28 |
| 8 | NBD-14280-rac | F | Cl | F | H | CH ₃ | CH ₂ OH | H | H | H | H | 2.6±0.4 | 47.7±1.3 | 18.3 |
| 9 | NBD-14242 (S) | F | Cl | F | H | H | CH ₂ CH ₂ OH | H | H | H | H | 0.15±0.001 | 32.8±1 | 218 |
| 10 | NBD-14243 (R) | F | Cl | F | H | H | CH ₂ CH ₂ OH | H | H | H | H | 0.98±0.01 | 32.8±0.5 | 33.5 |
| 11 | NBD-14258 (R) | F | CF ₃ | H | H | H | CH ₂ CH ₂ OH | H | H | H | H | 0.51±0.05 | 24.2±0.8 | 47.4 |
| 12 | NBD-14259 (S) | F | CF ₃ | H | H | H | CH ₂ CH ₂ OH | H | H | H | H | 0.2±0.02 | 24.4±0.7 | 122 |
| 13 | NBD-14273 (S) | H | CF ₃ | H | F | H | CH ₂ CH ₂ OH | H | H | H | H | 0.18±0.02 | 39.6±2 | 220 |
| 14 | NBD-14274 (R) | H | CF ₃ | H | F | H | CH ₂ CH ₂ OH | H | H | H | H | 0.15±0.01 | 23.4±0.9 | 156 |
| 15 | NBD-14287 (S) | F | CF ₃ | H | H | H | CH(OH)CH ₂ OH | H | H | H | H | 0.55±0.05 | 72.4±3.1 | 131.6 |
| 16 | NBD-14288 (R) | F | CF ₃ | H | H | H | CH(OH)CH ₂ OH | H | H | H | H | 2.4±0.1 | 56.3±8.8 | 23.4 |
| 17 | NBD-14289 (S) | F | CF ₃ | H | H | H | CH ₂ CH ₂ CH ₂ OH | H | H | H | H | 0.25±0.01 | 22.6±2.5 | 90.4 |
| 18 | NBD-14290 (R) | F | CF ₃ | H | H | H | CH ₂ CH ₂ CH ₂ OH | H | H | H | H | 3.5±0.2 | 38±1.3 | 10.8 |



| New No | Code (enantiomer) | R ₁ | R ₂ | R ₃ | R ₄ | R ₅ | R ₆ | R ₇ | R ₉ | R ₉ | R ₁₀ | IC ₅₀ ^b | CC ₅₀ ^b | SI |
|--------|-------------------|----------------|-----------------|----------------|----------------|----------------|--------------------|---|-----------------|-----------------|-----------------|-------------------------------|-------------------------------|-------|
| 19 | NBD-14303 (S) | F | CF ₃ | H | H | H | H | CH(OH)CH ₂ OH | H | H | H | 6.2±0.9 | 98±3.5 | 15.8 |
| 20 | NBD-14304 (R) | F | CF ₃ | H | H | H | H | CH(OH)CH ₂ OH | H | H | H | 0.59±0.1 | 70.5±4.5 | 119.4 |
| 21 | NBD-14271 (S) | F | CF ₃ | H | H | H | COOH | H | H | H | H | Not soluble | - | - |
| 22 | NBD-14272 (R) | F | CF ₃ | H | H | H | COOH | H | H | H | H | Not soluble | - | - |
| 23 | NBD-14275 (S) | F | CF ₃ | H | H | H | COOCH ₃ | H | H | H | H | 2.9±0.4 | 41.6±1.3 | 14.3 |
| 24 | NBD-14276 (R) | F | CF ₃ | H | H | H | COOCH ₃ | H | H | H | H | 4.9±0.9 | 23.4±0.5 | 4.8 |
| 25 | NBD-14267 (R) | F | CF ₃ | H | H | H | CONH ₂ | H | H | H | H | 2.3±0.1 | 25.9±0.3 | 11.3 |
| 26 | NBD-14268 (S) | F | CF ₃ | H | H | H | CONH ₂ | H | H | H | H | >10 | >52 | |
| 27 | NBD-14325 (S) | F | CF ₃ | H | H | H | CH ₂ OH | H | H | H | CH ₃ | 0.54±0.1 | 18.5±0.2 | 34.2 |
| 28 | NBD-14324 (R) | F | CF ₃ | H | H | H | CH ₂ OH | H | H | H | CH ₃ | 0.57±0.1 | 14.1±1.6 | 24.7 |
| 29 | NBD-14329 (S) | F | CF ₃ | H | H | H | H | CH ₂ OH | H | H | CH ₃ | 0.15±0.02 | 10.3±0.5 | 20.6 |
| 30 | NBD-14328 (R) | F | CF ₃ | H | H | H | H | CH ₂ OH | H | H | CH ₃ | 0.21±0.04 | 10.4±0.4 | 49.5 |
| 31 | NBD-14246 (S) | F | Cl | F | H | H | CH ₂ OH | H | CH ₃ | CH ₃ | H | 0.12±0.002 | 16±1 | 133 |
| 32 | NBD-14247 (R) | F | Cl | F | H | H | CH ₂ OH | H | CH ₃ | CH ₃ | H | 0.15±0.004 | 15.8±0.5 | 105 |
| 33 | NBD-14248 (S) | H | CF ₃ | H | H | H | CH ₂ OH | H | CH ₃ | CH ₃ | H | 0.13±0.005 | 17.5±1 | 134.6 |
| 34 | NBD-14249 (R) | H | CF ₃ | H | H | H | CH ₂ OH | H | CH ₃ | CH ₃ | H | 1.3±0.03 | 19.9±0.5 | 15.3 |
| 35 | NBD-14262 (R) | H | CF ₃ | H | H | H | H | CH ₂ OH | CH ₃ | CH ₃ | H | 0.68±0.12 | 23.1±0.5 | 34 |
| 36 | NBD-14263 (S) | H | CF ₃ | H | H | H | H | CH ₂ OH | CH ₃ | CH ₃ | H | 0.23±0.04 | 25.1±1 | 109 |
| 37 | NBD-14225 (S) | H | CF ₃ | H | H | H | H | CH ₂ OCH ₂ CH(OH)CH ₂ OH | H | H | H | >13 | >61.9 | - |
| 38 | NBD-14229 (R) | H | CF ₃ | H | H | H | H | CH ₂ OCH ₂ CH(OH)CH ₂ OH | H | H | H | 2.1±0.2 | ~80 | ~38 |

^aData from Reference²⁵

^bThe reported IC₅₀ and CC₅₀ values represent the mean ± standard deviation (SD), n=3

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Table 2.

Neutralization Activity of gp120 entry-antagonists against a Panel of HIV-1 Env Pseudoviruses

| Subtype | NIH # | ENVs | IC ₅₀ μM ^a | |
|---------|-------|-------------------------------|----------------------------------|-------------|
| | | | NBD-14136 ^c | NBD-14273 |
| A | 11887 | Q259ENV.W6 | 0.46±0.06 | 0.2±0.01 |
| | 11888 | QB726.70M.ENV.C4 | 0.31±0.07 | 0.11±0.005 |
| | 11890 | QF495.23M.ENV.A1 | 0.44±0.05 | 0.21±0.02 |
| | 11891 | QF495.23M.ENV.A3 | 0.26±0.04 | 0.11±0.01 |
| | | BG505-T332N | 0.41±0.03 | 0.118±0.003 |
| | | KNH1144 | 0.62±0.08 | 0.11±0.02 |
| A/D | 11901 | QA790.204I.ENV.A4 | 0.29±0.01 | 0.12±0.004 |
| | 11904 | QA790.204I.ENV.E2 | 0.41±0.03 | 0.11±0.02 |
| A2/D | 11905 | QG393.60M.ENV.A1 | 0.34±0.02 | 0.13±0.003 |
| | 11906 | QG393.60M.ENV.B7 | 0.6±0.005 | 0.127±0.005 |
| A/G | 11591 | CRF02_AG Clone 211 | 0.58±0.05 | 0.22±0.04 |
| | 11594 | CRF02_AG clone 250 | 0.41±0.01 | 0.13±0.005 |
| | 11595 | CRF02_AG clone 251 | 0.36±0.06 | 0.11±0.01 |
| | 11598 | CRF02_AG clone 255 | 0.33±0.01 | 0.13±0.01 |
| | 11599 | CRF02_AG clone 257 | 0.39±0.01 | 0.13±0.006 |
| | 11600 | CRF13_cpx clone 258 | 0.52±0.07 | 0.175±0.03 |
| | 11602 | CRF02_AG clone 266 | 0.51±0.06 | 0.16±0.02 |
| AE | 11603 | CRF01_AE clone 269 | 0.52±0.02 | 0.13±0.01 |
| B | | B41 | 0.36±0.04 | 0.139±0.001 |
| | 11018 | QH0692, clone 42 | 0.27±0.01 | 0.13±0.01 |
| | 11022 | PVO, clone 4 | 0.41±0.06 | 0.094±0.007 |
| | 11023 | TRO, clone 11 | 0.39±0.02 | 0.135±0.001 |
| | 11036 | RHPA4259 clone 7 | 0.37±0.07 | 0.13±0.003 |
| | 11037 | THRO4156 clone 18 | 0.32±0.02 | 0.13±0.005 |
| | 11038 | CAAN5342 clone A2 | 0.22±0.03 | 0.12±0.002 |
| | 11058 | SC422661.8 | 0.32±0.08 | 0.12±0.02 |
| | 11560 | 1006_11.C3.1601 | 0.58±0.06 | 0.135±0.002 |
| | 11561 | 1054.TC4.1499 | 0.25±0.02 | 0.14±0.01 |
| | 11562 | 1056.TA11.1826 | 0.58±0.01 | 0.13±0.01 |
| | 11563 | 1058 11.B11.1550 ^b | 0.29±0.02 | 0.17±0.01 |
| | 11572 | 9021_14.B2.4571 | 0.36±0.08 | 0.13±0.006 |
| | 11578 | WEAUd15.410.5017 ^b | 0.76±0.04 | 0.13±0.004 |
| C | 11307 | Du172, clone 17 | 0.3±0.03 | 0.12±0.001 |
| | 11308 | Du422, clone 1 | 0.63±0.09 | 0.22±0.02 |

| | | | IC ₅₀ μM ^a | |
|-----------------------------|--|------------------------|----------------------------------|-----------------------------|
| Subtype | NIH # | ENVs | NBD-14136 ^c | NBD-14273 |
| | 11309 | ZM197M.PB7, SVPC6 | 0.27±0.01 | 0.12±0.01 |
| | 11310 | ZM214M.PL15, SVPC7 | 0.24±0.007 | 0.12±0.003 |
| | 11312 | ZM249M.PL1, SVPC10 | 0.55±0.07 | 0.17±0.01 |
| | 11313 | ZM53M.PB12, SVPC11 | 0.54±0.03 | 0.136±0.002 |
| | 11314 | ZM109F.PB4 | 0.29±0.01 | 0.12±0.004 |
| | 11317 | CAP210.2.00.E8, SVPC17 | 0.47±0.03 | 0.11±0.003 |
| | 11502 | HIV-16055-2, clone 3 | 0.29±0.06 | 0.123±0.01 |
| | 11504 | HIV-16936-2, clone 21 | 0.47±0.05 | 0.24±0.04 |
| | 11506 | HIV-25711-2, clone 4 | 0.26±0.02 | 0.12±0.01 |
| | 11507 | HIV-225925-2, clone 22 | 0.24±0.02 | 0.11±0.005 |
| | 11908 | QB099.391M.ENV.B1 | 0.42±0.05 | 0.113±0.006 |
| D | 11911 | QA013.70I.ENV.H1 | 0.32±0.01 | 0.12±0.01 |
| | 11912 | QA013.70I.ENV.M12 | 0.22±0.01 | 0.11±0.006 |
| | 11916 | QD435.100M.ENV.B5 | 0.3±0.01 | 0.11±0.01 |
| | 11918 | QD435.100M.ENV.E1 | 0.19±0.04 | 0.113±0.002 |
| G | 11596 | CRF02_G clone 252 | 0.26±0.03 | 0.11±0.01 |
| Mean ± SEM (μM): | Overall (n=50) SI | | 0.39±0.02 108.7 | 0.135±0.005 293.3 |
| | Subtype A (n=6) SI | | 0.42±0.05 101 | 0.143±0.02 277 |
| | Subtype A_{rec} (n=12) SI | | 0.44±0.03 96.4 | 0.139±0.009 285 |
| | Subtype B (n=14) SI | | 0.39±0.04 108.7 | 0.131±0.004 302.3 |
| | Subtype C (n=13) SI | | 0.38±0.04 111.6 | 0.14±0.01 282.9 |
| | Subtype D (n=4) SI | | 0.26±0.03 163.1 | 0.11±0.002 360 |
| Control | VSV-G ^d | IC ₅₀ | >20 | 11.9±0.5 |
| | | CC ₅₀ | 46.3±10.6 | 33.5±0.6 |
| IC50 μM (Color Code) | | 0.2 | >0.2 0.5 | >0.5 |

^aThe reported IC₅₀ values represent the means ± standard deviations (n = 3).

^bR5X4-tropic virus all the rest are CCR5-tropic viruses.

^cData previously published³²

^dVSV-G was tested in U87-CD4-CCR5 cells

Table 3.

Neutralization activity of NBD-14273 against an HIV-1 panel of Paired Infant (B) and Maternal (M) Env Molecular Clones.

| | | | IC_{50} (μM) ^a | |
|-------------------------|----------------|-------------------|------------------------------------|---------------------|
| Subtype | NIH # | ENV | NBD-14136 ^b | NBD-14273 |
| A | 11518-B | BG505.W6M.ENV.C2 | 0.92±0.16 | 0.22±0.02 |
| A | | MG505.W0M.ENV.A2 | 0.93±0.07 | 0.17±0.05 |
| A | 11519-B | B1206.W6P.ENV.A1A | 0.75±0.06 | 0.18±0.06 |
| A | | MI206.W0M.ENV.D1 | 0.7±0.02 | 0.22±0.07 |
| A | 11521-B | BJ613.W6M.ENV.E1 | 0.8±0.07 | 0.13±0.01 |
| A | | MJ613.W0M.ENV.A2 | 0.5±0.05 | 0.136±0.02 |
| D/A | 11524-B | BL035.W6M.ENV.C1 | 0.52±0.16 | 0.24±0.03 |
| D/A | | ML035.W0M.ENV.G2 | 0.59±0.08 | 0.18±0.03 |
| A | 11525-B | BL274.W6M.ENV.A3 | 0.63±0.12 | 0.15±0.020.136±0.01 |
| A | | ML274.W0M.ENV.B1 | 0.66±0.1 | |
| Mean ± SEM (μM): | | Overall (n=10) | 0.7±0.05 | 0.176±0.01 |
| | | Infant (B) (n=5) | 0.72±0.07 | 0.184±0.02 |
| | | Mother (M) (n=5) | 0.68±0.07 | 0.168±0.016 |

^aThe reported IC_{50} values represent the means ± standard deviations (n = 3).

^bData previously published³²

Table 4.

Inhibitory activity of NBD-14273 against a large panel of FDA approved drug-resistant viruses

| | NIH catalog# | Major mutations ^a | NBD-14273 IC ₅₀ (μ M) ^b | Fold increase/Sensitive |
|-----------------------------------|--------------|---|---|-------------------------|
| NL4-3 WT (wild-type) | #114 | - | 0.21 \pm 0.04 | - |
| ENTRY (Enfuvirtide)-resistant | #9498 | V38A, N42T | 0.4 \pm 0.08 | 1.9 |
| | #9490 | V38A | 0.44 \pm 0.02 | 2.09 |
| | #9496 | V38E, N42S | 0.31 \pm 0.03 | 1.48 |
| | #9491 | N42T, N43K | 0.62 \pm 0.07 | 2.95 |
| | #9489 | D36G | 0.34 \pm 0.06 | 1.62 |
| Multi-drug (NNRTI)- resistant | #12227 | K101P, K103N | 0.11 \pm 0.006 | Sensitive |
| | #12229 | L100I, K103N | 0.39 \pm 0.4 | 1.86 |
| | #12231 | K103N, Y181C | 0.12 \pm 0.006 | Sensitive |
| | #12233 | K101E, Y181V | 0.71\pm0.02 | 3.38 |
| | #12237 | Y181C, G190A | 0.3 \pm 0.02 | 1.4 |
| | #12239 | K101E, E138K, Y181C | 0.81\pm0.08 | 3.85 |
| | #12241 | K101E, G190S | 0.26 \pm 0.01 | Sensitive |
| Integrase (Raltegravir)-resistant | #12243 | L100I, M230L | 1.4\pm0.05 | 6.7 |
| | #11847 | G140S, Q148H | 0.17 \pm 0.02 | Sensitive |
| | #11850 | E92Q, N155H | 0.11 \pm 0.1 | Sensitive |
| | #11851 | N155H | 0.12 \pm 0.01 | Sensitive |
| | #11800 | 11I, 32I, 33F, 46I, 47V, 54M, 58E, 73S, 84V, 89V, 90M | 0.1 \pm 0.01 | Sensitive |
| | #11801 | 10F, 33F, 43T, 46L, 54V, 82A, 84V, 90M | 0.12 \pm 0.02 | Sensitive |
| | #11803 | 33F, 43T, 46I, 48V, 50V, 54S, 82A | 0.38 \pm 0.1 | 1.8 |
| | #11804 | 32I, 46I, 47V, 84V | 0.24 \pm 0.02 | Sensitive |
| | #11805 | 48V, 53L, 54V, 82A, 90M | 0.16 \pm 0.05 | Sensitive |
| | #11807 | 32I, 33F, 47A, 82A, 90M | 0.12 \pm 0.03 | Sensitive |
| | #11808 | 10F, 11I, 33F, 43T, 46L, 54V, 73S, 82A, 84V, 89V, 90M | 0.17 \pm 0.03 | Sensitive |
| | #12465 | 46I, 54V, 58E, 74P, 82L, 90M | 0.13 \pm 0.02 | Sensitive |
| | #12466 | 32I, 33F, 43T, 46I, 47V, 54M, 73S, 82A, 89V, 90M | 0.11 \pm 0.02 | Sensitive |
| | #12467 | | 0.15 \pm 0.07 | Sensitive |

^a Mutants were reported here as per the data obtained from <https://www.aidsreagent.org/> and associated references indicated in the Experimental Section.

^b The reported IC₅₀ values represent the means \pm standard deviations (n = 3).

Table 5.

Inhibitory activity against Cell-to-Cell HIV transmission by NBD-14273.

| Compound | IC ₅₀ (μM) ^a | |
|------------------------|------------------------------------|----------------------------------|
| | TZM-bl/H9-HIV-1 _{IIIB} | TZM-bl/Molt-HIV-1 _{ADA} |
| NBD-14136 ^b | 0.46±0.2 | 0.89±0.14 |
| NBD-14273 | 0.39±0.02 | 0.45±0.01 |
| BMS-626529 | 0.02±0.005 | ~0.2 |

^aThe reported IC₅₀ values represent the means ± standard deviation (SD), n=3.^bData previously published³²

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