Bioorg. Med. Chem. 32 (2021) 116000

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

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

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ARTICLE INFO

Keywords:
HIV-1
ENV-pseudovirus
Gp120 entry-antagonist
Cytotoxicity
Broad-spectrum
Structure–activity relationship (SAR)
selectivity index (SI)

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 CH_2OH "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.

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 machinery.[1] Therefore, viral attachment and fusion (often collectively termed "viral entry") have been targets of new drug discovery for years.[1–4] 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 entry.[7–9]

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

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https://doi.org/10.1016/j.bmc.2021.116000
Received 3 December 2020; Accepted 31 December 2020
Available online 8 January 2021

to the Phe43 cavity of gp120 since 2005 when we first reported the discovery of NBD-556, the NBD-series CD4-mimic.[10] 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 antagonists.[12–22]

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 gp120.[23] The major transformation of CD4-mimic came through the design of NBD-11021. Based on the x-ray structure of NBD-11021,[12] 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 protein.[24] One such essential details lead us to postulate the "Positional switch" hypothesis that resulted in more potent antivirals, such as NBD-14136 ($IC_{50} = 0.27$ μM), NBD-14168 ($IC_{50} = 0.28$ μM), and NBD-14189 ($IC_{50} = 0.089$ 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-CF_3 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 (CF_3) group at the para position, which showed an IC_{50} of 0.27 μM and CC_{50} of 42.4 μM resulting in an SI (where $SI = CC_{50}/IC_{50}$) = 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 CF_3 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 $HIV\text{-}1_{HXB2}$ 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 CF_3 at R_2 and a fluoro (F) at R_4 position with CH_2OH at R_6 positions yielded one of the most active compounds in this series ($IC_{50} = 0.19$ μM and SI = 137). However, when we kept all groups the same except changing CH_2OH to CH_2CH_2OH, 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 R_1 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

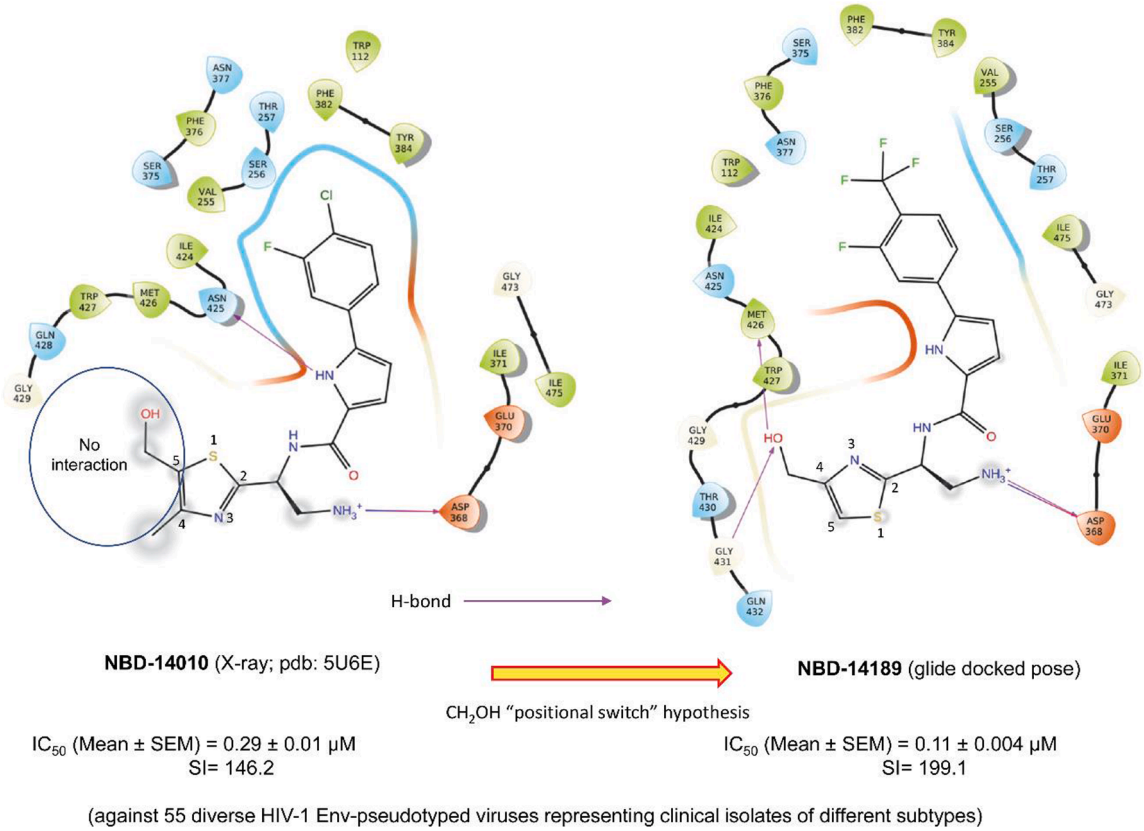
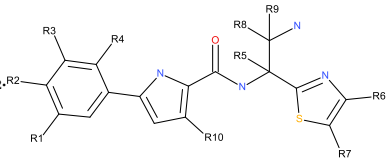


Figure 1. "Positional switch" hypothesis indicating no interaction of the CH_2OH group at position 5 in the thiazole ring in the x-ray structure of NBD-14010 bound to HIV-1 gp120. When the CH_2OH was switched to position 4 in the thiazole ring in NBD-14189, it forms H-bond (indicated by violet arrows using a glide docking pose) with Met426 and Gly431.[25]

Table 1

Anti-HIV-1 activity (IC_{50}) 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_2OH	H	H	H	0.27 ± 02	42.4 ± 1	157
1	NBD-14250 (R)	H	CF_3	H	F	H	CH_2OH	H	H	H	H	0.61 ± 15	26.8 ± 0.25	43.9
2	NBD-14251 (S)	H	CF_3	H	F	H	CH_2OH	H	H	H	H	0.187 ± 0.01	25.7 ± 1.2	137
3	NBD-14260 (R)	H	CN	H	H	H	CH_2OH	H	H	H	H	>16	>82	–
4	NBD-14261 (S)	H	CN	H	H	H	CH_2OH	H	H	H	H	>16	>82	–
5	NBD-14313 (S)	H	CF_3	H	CF_3	H	CH_2OH	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_2OH	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_2OH	H	H	H	H	1.6 ± 0.08	45.3 ± 1	28
8	NBD-14280-rac	F	Cl	F	H	CH_3	CH_2OH	H	H	H	H	2.6 ± 0.4	47.7 ± 1.3	18.3
9	NBD-14242 (S)	F	Cl	F	H	H	CH_2CH_2OH	H	H	H		0.15 ± 0.001	32.8 ± 1	218
10	NBD-14243 (R)	F	Cl	F	H	H	CH_2CH_2OH	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_2CH_2OH	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_2CH_2OH	H	H	H	H	0.2 ± 0.02	24.4 ± 0.7	122
13	NBD-14273 (S)	H	CF_3	H	F	H	CH_2CH_2OH	H	H	H	H	0.18 ± 0.02	39.6 ± 2	220
14	NBD-14274 (R)	H	CF_3	H	F	H	CH_2CH_2OH	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_2OH	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_2OH	H	H	H	H	2.4 ± 0.1	56.3 ± 8.8	23.4
17	NBD-14289 (S)	F	CF_3	H	H	H	$CH_2CH_2CH_2OH$	H	H	H	H	0.25 ± 0.01	22.6 ± 2.5	90.4
18	NBD-14290 (R)	F	CF_3	H	H	H	$CH_2CH_2CH_2OH$	H	H	H	H	3.5 ± 0.2	38 ± 1.3	10.8
19	NBD-14303 (S)	F	CF_3	H	H	H	H	$CH(OH)CH_2OH$	H	H	H	6.2 ± 0.9	98 ± 3.5	15.8
20	NBD-14304 (R)	F	CF_3	H	H	H	H	$CH(OH)CH_2OH$	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	COOCH3	H	H	H	H	2.9 ± 0.4	41.6 ± 1.3	14.3
24	NBD-14276 (R)	F	CF_3	H	H	H	COOCH3	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_2OH	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_2OH	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_2OH	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_2OH	H	H	CH_3	0.21 ± 0.04	10.4 ± 0.4	49.5
31	NBD-14246 (S)	F	Cl	F	H	H	CH_2OH	H	CH_3	CH_3	H	0.12 ± 0.002	16 ± 1	133
32	NBD-14247 (R)	F	Cl	F	H	H	CH_2OH	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_2OH	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_2OH	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_2OH	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_2OH	CH_3	CH_3	H	0.23 ± 0.04	25.1 ± 1	109
37	NBD-14225 (S)	H	CF_3	H	H	H	H	$CH_2OCH_2CH(OH)$ CH_2OH	H	H	H	>13	>61.9	–
38	NBD-14229 (R)	H	CF_3	H	H	H	H	$CH_2OCH_2CH(OH)$ CH_2OH	H	H	H	2.1 ± 0.2	~80	~38

[a] Data from Reference 25.

[b] The reported IC_{50} and CC_{50} values represent the mean ± standard deviation (SD), n = 3.

compound **12** by increasing the complexity of the primary alcohol substituent at R_6. 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 R_6 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 R_6 had an amide or ester substituents. Interestingly, when we moved the branched alcohol substituent to the R_7 position, antiviral activity dropped substantially (compound **19**). It appears that bulkier alcohol substituent in either R_6 or R_7 position was not well tolerated. Interestingly, we also observed that when we substituted the R_2 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 R_2 and F in both R_1 and R_3 with CH_2CH_2OH at R_6 (Compound **9**) yielded one of the most active inhibitors with an IC_{50} of 0.15 μM and SI of 218. Interestingly, when we added a methyl group at position R_{10} 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 R_8 and R_9 (compounds **31**–**34**) and the CH_2OH group was at position R_6, the antiviral activities were also improved substantially, but again these

additional methyl groups also made those molecules more cytotoxic (CC_{50}). However, when the CH_2OH group was moved to R_7 (Compound **36**), the antiviral activity dropped by about 2-fold, but cytotoxicity improved to make SI > 100. In the same series, when we substituted R_7 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 R_6 from R_7 (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 (IC_{50} 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-556,[10] 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.[24,27] 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-556[10] 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.[12,15,24,25] 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 co-receptor 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] 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 antiviral activity detected for NBD-14136, as demonstrated by their overall mean IC_{50} values and their antiviral activities against the different HIV subtypes (Table 2). The overall mean IC_{50} value for NBD-14136 was 0.39 ± 0.02 μM (with the SI of 108.7 and the IC_{50} values ranged from 0.19 to 0.79 μM), whereas the overall mean IC_{50} value determined for NBD-14273 was 0.135 ± 0.005 μM (with the SI of 293.3 and the IC_{50} values ranged from 0.11 to 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 (CC_{50} of 39.6 ± 2 μM) than that for NBD-14136 (CC_{50} 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/A.[29] 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 4E10.[29] In this study, we found that NBD14273, as previously shown for NBD-14136,[28] equally neutralized both the infant and maternal HIV-1 variants (Table 3), as shown by their overall mean of the IC_{50} values and the IC_{50} 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.

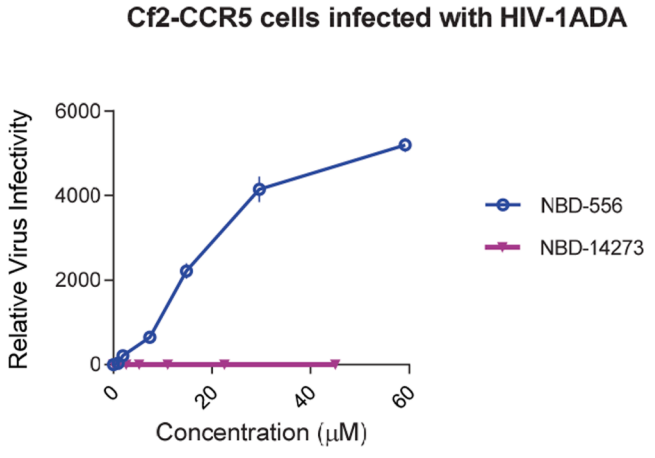


Figure 2. Infectivity of CD4-negative Cf2Th – CCR5 cells by CD4-dependent HIV-1_{ADA}. CCf2Th – CCR5 cells were infected with CD4-dependent HIV-1_{ADA} in the presence of NBD-14273 and NBD-556, which was used as a control. The Relative virus infectivity indicates the ratio of the amount of infection 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 CC_{50} values: for NBD-556, the CC_{50} was > 60, and for NBD-14273, it was 31.2 ± 1.4. All the values represent the mean ± standard deviation.

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-

Table 2
Neutralization Activity of gp120 entry-antagonists against a Panel of HIV-1 Env Pseudoviruses.

			IC_{50} μM[a]	
Subtype	**NIH #**	**ENVs**	**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

(*continued on next page*)

Table 2 (*continued*)

	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
	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)	0.39±0.02	0.135±0.005
		SI	**108.7**	**293.3**
		Subtype A (n=6)	0.42±0.05	0.143±0.02
		SI	**101**	**277**
		Subtype A_{rec} (n=12)	0.44±0.03	0.139±0.009
		SI	**96.4**	**285**
		Subtype B (n=14)	0.39±0.04	0.131±0.004
		SI	**108.7**	**302.3**
		Subtype C (n=13)	0.38±0.04	0.14±0.01
		SI	**111.6**	**282.9**
		Subtype D (n=4)	0.26±0.03	0.11±0.002
		SI	**163.1**	**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**

[a]The reported IC_{50} values represent the means ± standard deviations (n = 3).
[b]R5X4-tropic virus all the rest are CCR5-tropic viruses.
[c]Data previously published.[28]
[d]VSV-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.

Subtype	NIH #	ENV	IC_{50} (μM)[a]	
			NBD-14136[b]	NBD-14273
A	**11518-B**	BG505.W6M.ENV.*C2*	0.92 ± 0.16	0.22 ± 0.02
A	**11528-M**	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	**11531-M**	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	**11535-M**	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	**11538-M**	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	**11540-M**	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

[a] The reported IC_{50} values represent the means ± standard deviations (n = 3).
[b] Data previously published[28]

14273 inhibited the WT HIV-1_{NL4-3}, with an IC_{50} value of 0.21 μM. All the T-20-resistant viruses were sensitive to NBD-14273; the IC_{50} 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 IC_{50} with respect to the IC_{50} 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 IC_{50}, respectively. The Raltegravir-resistant viruses and the protease inhibitor-resistant viruses were also sensitive to NBD-14273, as demonstrated by their low IC_{50} 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.[25,28] Cell-to-cell HIV-1 infection has been reported to be more efficient than HIV-1 cell-free infection[30,31] 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[32-34] and NRTIs, but not to other antiretrovirals, including entry inhibitors, NNRTIs, and protease inhibitors.[35] 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.[25,28] 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 IC_{50} value for NBD-14273 calculated

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} (μM)[b]	Fold increase/ Sensitive
NL4-3 wt (wild-type)	#114	–	0.21 ± 0.04	–
ENTRY (Enfuvirtide)-resistant	#9498	V38A, N42T	0.4 ± 0.08	1.9
	#9490	V38A	0.44 ± 0.02	2.09
	#9496	V38E, N42S	0.31 ± 0.03	1.48
	#9491	N42T, N43K	0.62 ± 0.07	2.95
	#9489	D36G	0.34 ± 0.06	1.62
Multi-drug (NNRTI)-resistant	#12227	K101P, K103N	0.11 ± 0.006	Sensitive
	#12229	L100I, K103N	0.39 ± 0.4	1.86
	#12231	K103N, Y181C	0.12 ± 0.006	Sensitive
	#12233	K101E, Y181V	**0.71 ± 0.02**	**3.38**
	#12237	Y181C, G190A	0.3 ± 0.02	1.4
	#12239	K101E, E138K, Y181C	**0.81 ± 0.08**	**3.85**
	#12241	K101E, G190S	0.26 ± 0.01	Sensitive
	#12243	L100I, M230L	**1.4 ± 0.05**	**6.7**
Integrase (Raltegravir-resistant)	#11847	G140S, Q148H	0.17 ± 0.02	Sensitive
	#11850	E92Q, N155H	0.11 ± 0.1	Sensitive
	#11851	N155H	0.12 ± 0.01	Sensitive
Multiple-PI-resistant	#11800	11I, 32I, 33F, 46I, 47V, 54M, 58E, 73S, 84V, 89V, 90M	0.1 ± 0.01	Sensitive
	#11801	10F, 33F, 43T, 46L, 54V, 82A, 84V, 90M	0.12 ± 0.02	Sensitive
	#11803	33F, 43T, 46I, 48V, 50V, 54S, 82A	0.38 ± 0.1	1.8
	#11804	32I, 46I, 47V, 84V	0.24 ± 0.02	Sensitive
	#11805	48V, 53L, 54V, 82A, 90M	0.16 ± 0.05	Sensitive
	#11807	32I, 33F, 47A, 82A, 90M	0.12 ± 0.03	Sensitive
	#11808	10F, 11I, 33F, 43T, 46L, 54V, 73S, 82A, 84V, 89V, 90M	0.17 ± 0.03	Sensitive
	#12465	46I, 54V, 58E, 74P, 82L, 90M	0.13 ± 0.02	Sensitive
	#12466	32I, 33F, 43T, 46I, 47V, 54M, 73S, 82A, 89V, 90M	0.11 ± 0.02	Sensitive
	#12467		0.15 ± 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 IC_{50} values represent the means ± 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

[a] The reported IC_{50} values represent the means ± standard deviation (SD), n = 3.
[b] Data previously published.[28]

against HIV-1_{ADA} in the CCR5-tropic assay was 1-fold lower than the IC_{50} 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 activity.[25]

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 cells,[36] U87CD4 + CXCR4 + cells,[37] HIV-1 IIIB, infected H9 Cells,[38] and MOLT-4 CCR5 + Cells[39] were obtained through the NIH ARP. HEK 293T cells were purchased from ATCC. CD4-negative Cf2Th-CCR5+ cells and Env expression vector pSVIIIenv-ADA were kindly provided by Dr. J. G. Sodroski.[40] HIV-1 Env molecular clone expression vector pHXB2-env (X4) DNA was also obtained through the NIH ARP.[41] 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, QB726.70 M.ENV.C4, QF495.23 M.ENV.A1, QF495.23 M.ENV.A3), A/D, A2/D, D, and C (QB099.391 M.Env.B1) were obtained through the NIH ARP from Dr. J. Overbaugh.[42,43] 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. Butera.[44] 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)).[45,46] 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. Shaw.[47] 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).[48–50] 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. Montefiori.[44] The panel of paired Infant and maternal HIV-1 Env Molecular Clones were obtained through the NIH ARP from Dr. J. Overbaugh.[29] 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),[51,52] the pSG3Δ^{env} DNA (from Drs. J. C. Kappes and X. Wu),[36,46] and the pNL4-3 (from Dr. Malcolm Martin)[53] 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.[53,54]; the panel Multi-Drug Resistant NNRTI Infectious Clones from Dr. R. Shafer[55]; the Raltegravir-resistant infectious molecular clones from Dr. R. Shafer and E. Reuman, M.S[56] and the multi-drug Protease inhibitor-resistant infectious clones from Dr. R. Shafer.[57]

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$^{\Delta env}$ 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 °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 μl of lysis buffer (Promega). 20 μl 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 IC_{50} (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 μl 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 IC_{50} 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 described.[11] 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 μl 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 IC_{50} 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 CC_{50} (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 CC_{50} 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[58,59], with minor modifications. Briefly, the indicator TZM-bl cells (used as 'acceptor' cells) were plated at 10^4/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 2×10^3 cells/well. For the CCR5-tropic assay, we used MOLT-4/CCR5 cells chronically infected with HIV-1_{ADA} at 2×10^3 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 IC_{50} 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 ^{13}C 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 $CDCl_3$ (δC = 77.16 ppm) or not-deuterated DMSO (δH = 2.50 ppm) and DMSO-d_6 (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%.

4.6.1. 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-H_2O (1:1, 100 mL), Na_2CO_3 (100 mmol, 2 equiv.) and Pd(Ph_3P)Cl_2 (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 CH_2Cl_2 (50 mL) were added. The organic layer was separated; the aqueous layer was extracted with CH_2Cl_2 (3x50 mL). The combined organic layers were dried over Na_2SO_4, 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).

4.6.2. Tert-butyl 2-(4-cyanophenyl)-1H-pyrrole-1-carboxylate (*S1a*)

Eluent: Hexane-EtOAc (from 20:1 to 10:1), Rf = 0.3 (10:1, Hexane-EtOAc). Yield = 53%.

^1H NMR ($CDCl_3$, 400 MHz): δ = 1.43 (s, 9H), 6.25–6.30 (m, 2H), 7.40 (dd, J = 3.2, 1.9 Hz, 1H), 7.45–7.48 (m, 2H), 7.62–7.66 (m, 2H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 27.6 (3C), 84.3, 110.4, 111.0, 116.1, 118.9, 123.9, 129.5 (2C), 131.3 (2C), 133.0, 138.7, 148.9.

4.6.3. Tert-butyl 2-(2,4-bis(trifluoromethyl)phenyl)-1H-pyrrole-1-carboxylate (S1b)

Eluent: Hexane-EtOAc (from 1:0 to 20:1), Rf = 0.3–0.4 (20:1, Hexane-EtOAc). Yield = 56%.

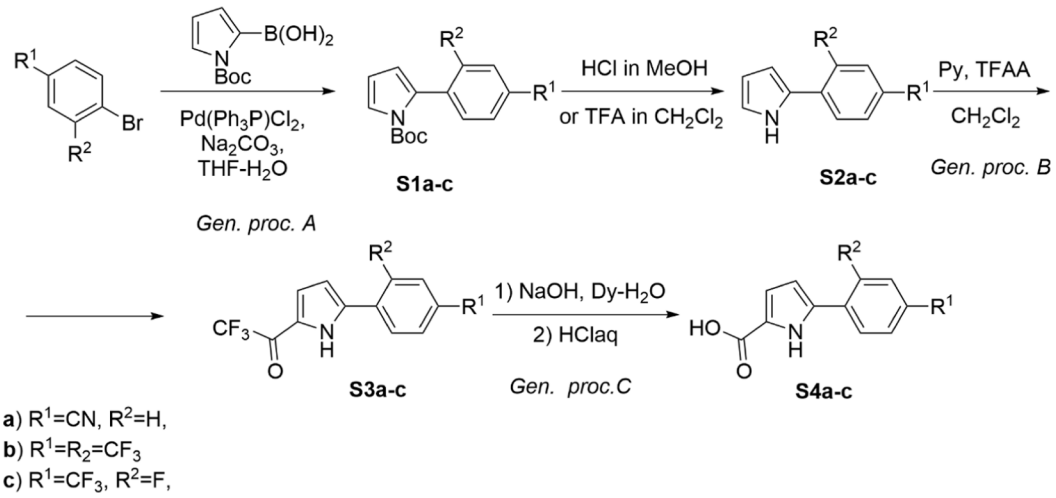
^1H NMR ($CDCl_3$, 400 MHz): δ = 1.25 (s, 9H), 6.20–6.24 (m, 1H), 6.28 (t, J = 3.3 Hz, 1H), 7.43 (dd, J = 3.4, 1.8 Hz, 1H), 7.53 (d, J = 7.9 Hz, 1H), 7.81 (d, J = 7.4 Hz, 1H), 7.97 (s, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 27.4 (3C), 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.

4.6.3.1. Tert-butyl 2-(2-fluoro-4-(trifluoromethyl)phenyl)-1H-pyrrole-1-carboxylate (*S1c*).

Eluent: Hexane-EtOAc (from 30:1 to 20:1), Rf = 0.5 (20:1, Hexane-EtOAc). Yield = 70%.

^1H NMR ($CDCl_3$, 400 MHz): δ = 1.41 (s, 9H), 6.28–6.33 (m, 2H), 7.30–7.38 (m, 1H), 7.42–7.47 (m, 2H), 7.46–7.52 (m, 1H).



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

^{13}C NMR ($CDCl_3$, 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).

*4.6.3.2. 4-(1H-Pyrrol-2-yl)benzonitrile (**S2a**).* To a solution containing **S1a** (10 g, 1 equiv.) in MeOH (20 mL), 1 M 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 K_2CO_3 (10% solution, 100 mL) was added carefully (CO_2 evolution), and the mixture was extracted with CH_2Cl_2 (3x50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude product was used in the next step without purification. M = 6.01 g. Yield = 96%.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.36 (dt, J = 3.5, 2.6 Hz, 1H), 6.69 (ddd, J = J = 3.7, 2.6, 1.4 Hz, 1H), 6.97 (td, J = 2.7, 1.5 Hz, 1H), 7.53–7.57 (m, 2H), 7.61–7.65 (m, 2H), 8.71 (br. s., 1H).

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

*4.6.3.3. 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 CH2Cl2 (3x50 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 (Rf = 0.2 in hexane-EtOAc 10:1). M = 6.34 g. Yield = 86%.

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

^{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.

*4.6.3.4. 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), Rf = 0.4 (20:1, Hexane-EtOAc). Yield = 93%.

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

^{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).

4.7. 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).

*4.7.1. 4-(5-(2,2,2-Trifluoroacetyl)-1H-pyrrol-2-yl)benzonitrile (**S3a**)*

Yield = 79%.

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

^{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 (2C), 126.9, 132.8 (2C), 134.0, 141.1, 168.3 (q, J = 35.2 Hz).

*4.7.2. 1-(5-(2,4-Bis(trifluoromethyl)phenyl)-1H-pyrrol-2-yl)-2,2,2-trifluoroethanone (**S3b**)*

Yield = 85%.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 6.56–6.62 (m, 1H), 7.31 (dt, J = 4.1, 2.1 Hz, 1H), 7.91 (d, J = 8.6 Hz, 1H), 8.14–8.26 (m, 2H), 13.17 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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).

*4.7.3. 2,2,2-Trifluoro-1-(5-(2-fluoro-4-(trifluoromethyl)phenyl)-1H-pyrrol-2-yl)ethanone (**S3c**)*

Yield = 92%.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 6.82 (t, J = 3.66 Hz, 1H), 7.13–7.36 (m, 1H), 7.64 (d, J = 8.27 Hz, 1H), 7.73 (d, J = 11.13 Hz, 1H), 8.21 (t, J = 7.95 Hz, 1H), 13.05 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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).

4.8. General procedure C: For haloform reaction

Appropriate trifluoroethanone (1 equiv.) was added to a solution of NaOH (5 equiv.) in dioxane-H_2O mixture (1:1, 0.5 M 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 H_2O, and dried on a filter. Compound **S4a-c** were obtained following the general procedure D (Scheme 1).

4.8.1. 5-(4-Cyanophenyl)-1H-pyrrole-2-carboxylic acid (S4a)

Yield = 69%.

^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.75$ (dd, $J = 3.7$, 2.5 Hz, 1H), 6.84 (dd, $J = 3.8$, 2.2 Hz, 1H), 7.92 (d, $J = 8.6$ Hz, 2H), 7.97 (d, $J = 8.6$ Hz, 2H), 12.18 (br. s., 1H), 12.72 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 109.3$, 116.6, 124.9 (2C), 125.4, 128.9, 129.9 (2C), 135.4, 135.6, 161.9, 167.2.

4.8.2. 5-(2,4-Bis(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxylic acid (S4b)

Yield = 71%.

^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.32$–6.40 (m, 1H), 6.83 (dd, $J = 3.7$, 2.4 Hz, 1H), 7.83 (d, $J = 8.6$ Hz, 1H), 8.04–8.17 (m, 2H), 12.22 (br. s., 1H), 12.55 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 111.9$ (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.

4.8.3. 5-(2-Fluoro-4-(trifluoromethyl)phenyl)-1H-pyrrole-2-carboxylic acid (S4c)

Yield = 83%.

^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 6.65$–6.72 (m, 1H), 6.80–6.92 (m, 1H), 7.61 (d, $J = 8.27$ Hz, 1H), 7.73 (d, $J = 11.44$ Hz, 1H), 8.20 (t, $J = 7.95$ Hz, 1H), 12.19 (br. s., 1H), 12.64 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 100 MHz): $\delta = 112.6$ (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.

4.8.4. ((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 Et_3N (250 mL), TMS-acetylene (24.25 g, 34.2 mL, 247 mmol, 2 equiv.), Pd$(Ph_3P)Cl_2$ (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, Rf = 0.6 in hexane). M = 30.1 g. Yield = 94%.

^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 0.27$ (s, 9H), 7.28 (d, $J = 10.8$ Hz, 1H), 7.32 (d, $J = 8.4$ Hz, 1H), 7.53 (t, $J = 7.7$ Hz, 1H).

^{13}C NMR ($CDCl_3$, 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).

4.8.5. 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$(Ph_3P)Cl_2$ (2.7 g; 5 mol. %), CuI (1.46 g; 10 mol. %) and Cs_2CO_3 (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 Et_2O (3 × 200 mL). Combined extracts were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash chromatography (eluent: hexane-EtOAc from 20:1 to 5:1, Rf = 0.6 in hexane-EtOAc 5:1). M = 12.73 g, Yield = 41%.

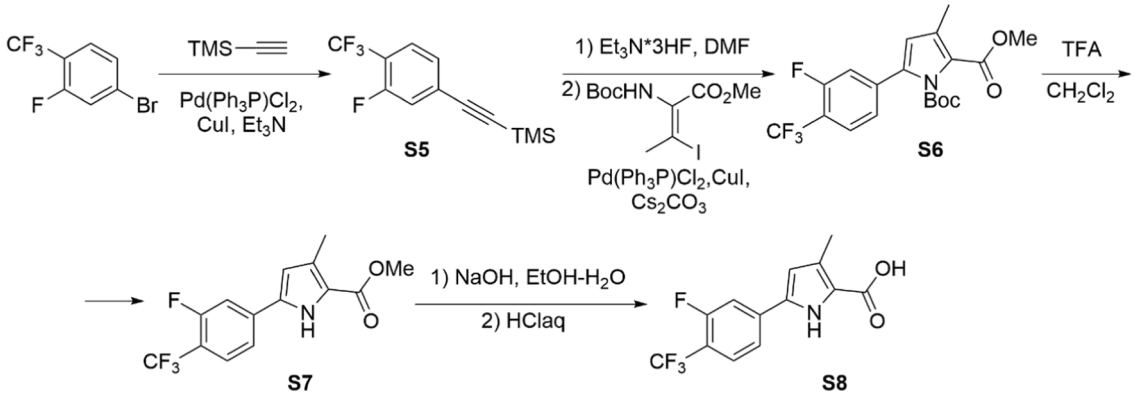
^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.46$ (s, 9H), 2.30 (s, 3H), 3.89 (s, 3H), 6.14 (s, 1H), 7.25–7.33 (m, 2H), 7.61 (t, $J = 7.7$ Hz, 1H).

^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 12.7$, 27.4 (3C), 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.

4.8.6. 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 (3x100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The product was used without further purification. M = 9.28 g. Yield = 98%.

^{1}H NMR (DMSO-d_6, 400 MHz): $\delta = 2.27$ (s, 3H), 3.81 (s, 3H), 6.72 (d, $J = 2.6$ Hz, 1H), 7.70 (t, $J = 8.1$ Hz, 1H), 7.82 (d, $J = 8.3$ Hz, 1H), 8.02



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

(d, J = 12.9 Hz, 1H), 11.99 (br. s., 1H).

^{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.

4.8.7. 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 EtOH-H_2O (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 (12 M, 5.08 mL, 2 equiv.), water (100 mL) was added, and the precipitate was filtered. M = 8.4 g. Yield = 96%.

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

^{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**.**

4.8.8. 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 × 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%.

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

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

4.8.9. 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, Rf = 0.3 in EtOAc). M = 32.2 g, Yield = 93%.

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

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

4.8.10. 5-((2,3-Bis((tert-butyldimethylsilyl)oxy)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 ℃, 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, Rf = 0.4 in hexane-EtOAc 20:1). M = 59.95 g. Yield = 84%.

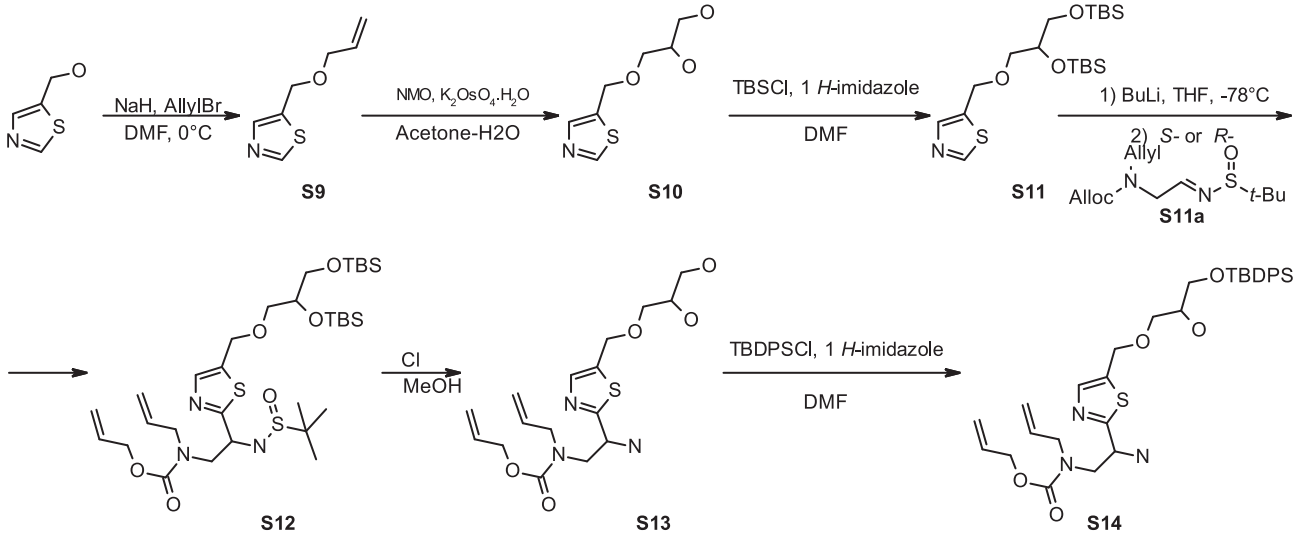
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.03–0.08 (m, 12H), 0.88 (s, 18H), 3.46 (dd, J = 9.9, 5.7 Hz, 1H), 3.53–3.61 (m, 3H), 3.79–3.86 (m, 1H), 4.77 (s, 2 H), 7.81 (s, 1H), 8.91 (s, 1H).

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

4.8.11. Allyl allyl(2-amino-2-(5-((3-((tert-butyldiphenylsilyl)oxy)-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 (1 M, 26 mL). The reaction mixture was slowly (~1h) 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, Rf = 0.4 in hexane-EtOAc 1:1). This product (**S12)** was used without



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

further purification and analysis.

To a solution of protected thiazole from the previous step in MeOH (50 mL), 1 M 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 CH_2Cl_2 (2 × 50 mL). After that, solid K_2CO_3 was added carefully (CO_2 evolution!) to the water phase at pH 10–12. Product was extracted from water with CH_2Cl_2 (4 × 50 mL), combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated to give diol **S13**. M = 3.02 g.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.26 (br. s., 4H), 3.49–3.72 (m, 6H), 3.77–3.98 (m, 3H), 4.46 (t, J = 6.5 Hz, 1H), 4.60 (d, J = 5.3 Hz, 2H), 4.70 (s, 2H), 5.08–5.24 (m, 3H), 5.30 (d, J = 17.2 Hz, 1H), 5.70–5.97 (m, 2H), 7.58 (s, 1H).

Diol **S13** (3.02 g, 8 mmol, 1 equiv.) was dissolved in CH_2Cl_2 (81 mL), and imidazole (0.66 g, 10 mmol, 1.2 equiv.) was added in one portion, followed by dropwise addition of TBDPSCl (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 CH_2Cl_2 (3 × 50 mL). The combined organic phases were washed with brine and dried over anhydrous Na_2SO_4. 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 CH_2Cl_2-MeOH 10:1, Rf = 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%.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.06 (s, 9H), 1.84 (br. s., 2H), 2.52 (br. s., 1H), 3.51–3.72 (m, 6H), 3.77–4.01 (m, 3H), 4.45 (t, J = 6.4 Hz, 1H), 4.61 (d, J = 5.3 Hz, 2H), 4.67 (s, 2H), 5.08–5.19 (m, 2H), 5.21 (dd, J = 10.4, 1.2 Hz, 1H), 5.31 (dd, J = 17.2, 1.3 Hz, 1H), 5.71–5.98 (m, 2H), 7.36–7.47 (m, 6H), 7.57 (s, 1H), 7.65 (d, J = 6.6 Hz, 4H).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 19.1, 26.7 (3C), (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 (4C), 129.7 (2C), 132.7, 133.0 (2C), 133.1, 135.1, 135.4 (4C), 141.4, (155.8, 156.7), (175.5, 176.3).

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

4.8.12. 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 (1 M 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 1 h 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 Et_2O (3 × 100 mL). Combined organic layers were washed with brine (3 × 50 mL) dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in excess of 1 M HCl n methanol (400 mL) and left for 2 days. Methanol was evaporated, and saturated $NaHCO_3$ (100 mL) was added to the residue. The product was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified using column chromatography (eluent: hexane-EtOAc from 1:1 to 0:1, and then CH_2Cl_2-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%.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.48 (br. s., 2H), 3.36–3.41 (m, 1H), 3.55–3.68 (m, 1H), 3.81 (s, 3H), 3.87 (d, J = 5.2 Hz, 2H), 4.32–4.54 (m, 3H), 5.04–5.18 (m, 3H), 5.25 (d, J = 17.3 Hz, 1H), 5.70–5.95 (m, 2H), 8.43 (s, 1H).

^{13}C NMR (DMSO-d_6, 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).

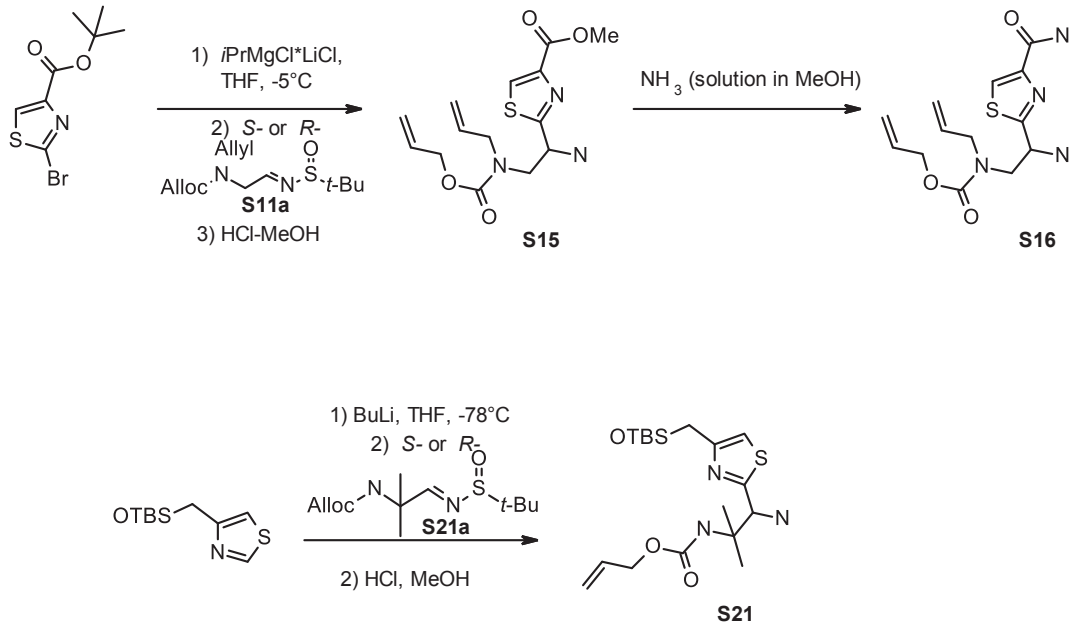
4.8.13. Allyl allyl(2-amino-2-(4-carbamoylthiazol-2-yl)ethyl)carbamate (S16)

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.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.94 (br. s., 2H), 3.49–3.71 (m, 2H), 3.75–3.98 (m, 2H), 4.42–4.63 (m, 3H), 5.05–5.33 (m, 4H), 5.66–5.97 (m, 2H), 6.28 (br. s., 1H), 7.15 (br. s., 1H), 8.07 (s, 1H).

^{13}C NMR ($CDCl_3$, 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



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

methods reported previously.[25,27,28,60]

4.8.14. 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 (1 M, 35 mL). The reaction mixture was slowly (~1h) warmed to 0 °C and poured into water (200 mL). The organic layer was separated, and water was extracted with CH_2Cl_2 (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, Rf = 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), 1 M 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 CH_2Cl_2 (2 × 50 mL). After that, solid K_2CO_3 was added carefully (CO_2 evolution) to the water phase at pH 10–12. Product was extracted from water with CH_2Cl_2 (4 × 50 mL), combined organic layers were dried over anhydrous Na_2SO_4, 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%.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.32 (s, 3H), 1.34 (s, 3H), 2.49 (br. s., 3H), 4.45–4.56 (m, 3H), 4.68 (s, 2H), 5.14–5.32 (m, 2H), 5.59 (s, 1H), 5.89 (dddd, *J* = 16.8, 11.0, 5.7, 5.4 Hz, 1H), 7.10 (s, 1H).

^{13}C NMR ($CDCl_3$, 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.

4.9. 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 CH_2Cl_2 (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 Na_2SO_4, 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.

4.10. 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 (0.1 M solution), PPh_3 (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 CH_2Cl_2 was added, and the organic phase was shaken with 10% aqueous K_2CO_3 (50 mL) to remove the unreacted NDMBA. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2-EtOH (~4:1, (3–5) × 50 mL). Combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash chromatography (twice) (eluent 1: $CHCl_3$-MeOH (saturated with NH_3~7M), 10:1 and 5:1, eluent 2: CH_2Cl_2-MeOH, 4:1, 2:1, 1:1) afforded amine as a slightly yellow or white solid.

4.11. General procedure F: For TBDPS- deprotection

TBDPS cleavage: To a solution of TBDPS-protected compound (1 equiv.) in THF (0.1 M), solution of TBAF trihydrate (1.1 equiv.) in THF (0.1 M) 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: $CHCl_3$-MeOH (saturated with NH3~7M), 10:1, 5:1, 3:1, eluent 2: CH_2Cl_2-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.

4.11.1. 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: $CHCl_3$-MeOH (saturated with NH_3~7M), 10:1 and 5:1, Eluent 2: CH_2Cl_2-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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.77 (br. s., 2H), 3.01 (dd, *J* = 13.0, 7.9 Hz, 1H), 3.14 (dd, *J* = 13.0, 5.1 Hz, 1H), 4.54 (s, 2H), 5.18–5.27 (m, 1H), 5.31 (br. s., 1H), 6.73 (t, *J* = 3.7 Hz, 1H), 7.09 (d, *J* = 3.8 Hz, 1H), 7.29 (s, 1H), 7.62 (d, *J* = 7.6 Hz, 1H), 7.74 (d, *J* = 11.4 Hz, 1H), 8.17 (t, *J* = 7.9 Hz, 1H), 8.73 (d, *J* = 7.6 Hz, 1H), 11.95 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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 $C_{18}H_{15}F_4N_4O_2S$ $[M-H]^-$ 427.0857, found 427.0858.

4.11.2. 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: $CHCl_3$-MeOH (saturated with NH_3~7M), 10:1 and 5:1, Eluent 2: CH_2Cl_2-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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.75 (br. s., 2H), 3.01 (dd, *J* = 13.1, 7.8 Hz, 1H), 3.14 (dd, *J* = 13.2, 5.3 Hz, 1H), 4.54 (s, 2H), 5.17–5.26 (m, 1H), 5.31 (br. s., 1H), 6.84 (d, *J* = 3.9 Hz, 1H), 7.05 (d, *J* = 3.9 Hz, 1H), 7.29 (d, *J* = 0.9 Hz, 1H), 7.81 (d, *J* = 8.0 Hz, 2H), 8.01 (d, *J* = 8.3 Hz, 2H), 8.66 (d, *J* = 7.8 Hz, 1H), 12.04 (br. s., 1H).

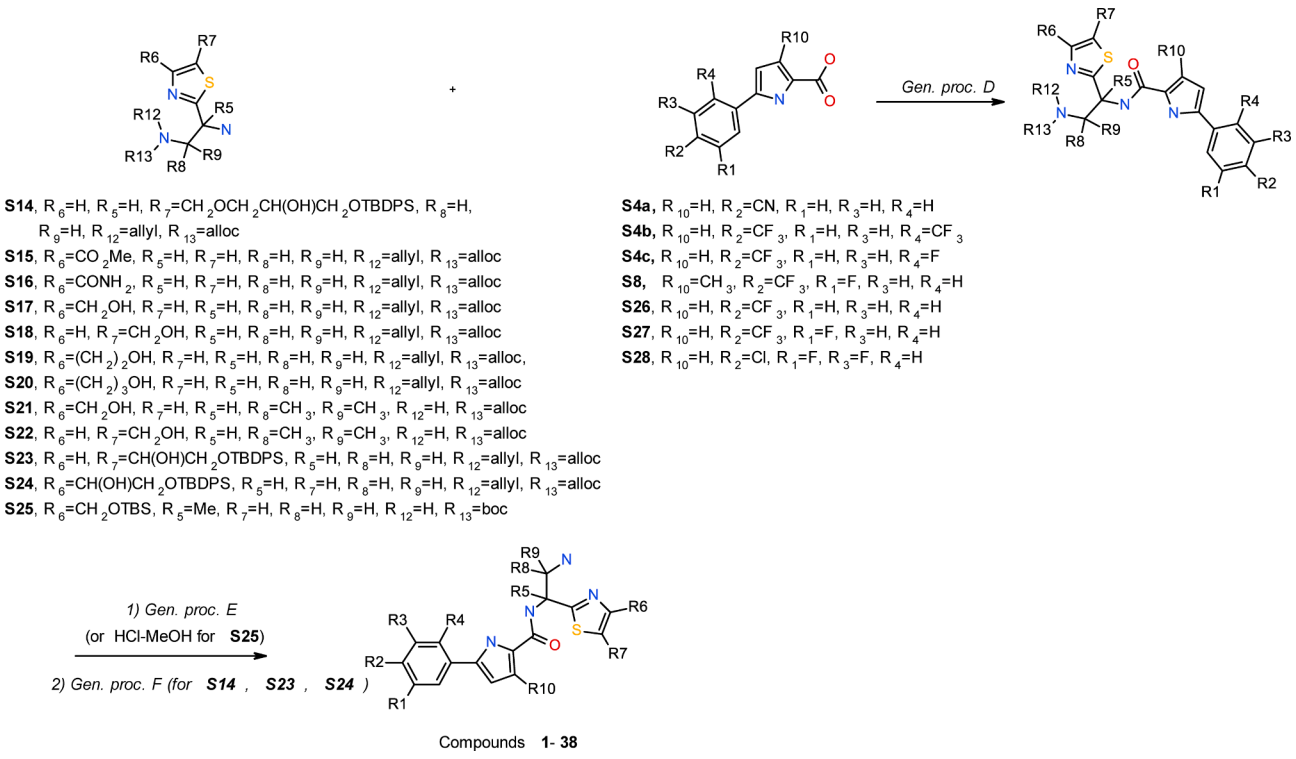
^{13}C NMR (DMSO-d_6, 100 MHz): δ = 45.7, 54.4, 59.8, 108.5, 109.6, 113.2, 114.1, 119.1, 125.1 (2C), 128.8, 132.7 (2C), 133.1, 136.1, 157.6, 160.4, 172.2.

HRMS (ESI) calcd for $C_{18}H_{16}N_5O_2S$ $[M - H]^-$ 366.1030, found 366.1033.

4.11.3. 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: $CHCl_3$-MeOH (saturated with NH_3~7M), 10:1 and 5:1, Eluent 2: CH_2Cl_2-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.



S14, R_6=H, R_5=H, R_7=$CH_2OCH_2CH(OH)CH_2OTBDPS$, R_8=H, R_9=H, R_{12}=allyl, R_{13}=alloc
S15, R_6=CO_2Me, R_5=H, R_7=H, R_8=H, R_9=H, R_{12}=allyl, R_{13}=alloc
S16, R_6=$CONH_2$, R_5=H, R_7=H, R_8=H, R_9=H, R_{12}=allyl, R_{13}=alloc
S17, R_6=CH_2OH, R_7=H, R_5=H, R_8=H, R_9=H, R_{12}=allyl, R_{13}=alloc
S18, R_6=H, R_7=CH_2OH, R_5=H, R_8=H, R_9=H, R_{12}=allyl, R_{13}=alloc
S19, R_6=$(CH_2)_2OH$, R_7=H, R_5=H, R_8=H, R_9=H, R_{12}=allyl, R_{13}=alloc,
S20, R_6=$(CH_2)_3OH$, R_7=H, R_5=H, R_8=H, R_9=H, R_{12}=allyl, R_{13}=alloc
S21, R_6=CH_2OH, R_7=H, R_5=H, R_8=CH_3, R_9=CH_3, R_{12}=H, R_{13}=alloc
S22, R_6=H, R_7=CH_2OH, R_5=H, R_8=CH_3, R_9=CH_3, R_{12}=H, R_{13}=alloc
S23, R_6=H, R_7=$CH(OH)CH_2OTBDPS$, R_5=H, R_8=H, R_9=H, R_{12}=allyl, R_{13}=alloc
S24, R_6=$CH(OH)CH_2OTBDPS$, R_5=H, R_7=H, R_8=H, R_9=H, R_{12}=allyl, R_{13}=alloc
S25, R_6=CH_2OTBS, R_5=Me, R_7=H, R_8=H, R_9=H, R_{12}=H, R_{13}=boc

S4a, R_{10}=H, R_2=CN, R_1=H, R_3=H, R_4=H
S4b, R_{10}=H, R_2=CF_3, R_1=H, R_3=H, R_4=CF_3
S4c, R_{10}=H, R_2=CF_3, R_1=H, R_3=H, R_4=F
S8, R_{10}=CH_3, R_2=CF_3, R_1=F, R_3=H, R_4=H
S26, R_{10}=H, R_2=CF_3, R_1=H, R_3=H, R_4=H
S27, R_{10}=H, R_2=CF_3, R_1=F, R_3=H, R_4=H
S28, R_{10}=H, R_2=Cl, R_1=F, R_3=F, R_4=H

Scheme 5. Synthesis of compounds 1–38.

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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.89 (br. s., 2H), 3.00 (dd, J = 13.1, 7.9 Hz, 1H), 3.14 (dd, J = 13.2, 5.3 Hz, 1H), 4.54 (s, 2H), 5.18–5.26 (m, 1H), 5.31 (br. s., 1H), 6.39 (d, J = 3.2 Hz, 1H), 7.06 (d, J = 3.9 Hz, 1H), 7.29 (s, 1H), 7.84 (d, J = 8.7 Hz, 1H), 8.07–8.13 (m, 2H), 8.67 (d, J = 8.0 Hz, 1H), 12.04 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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 $C_{19}H_{17}F_6N_4O_2S$ $[M + H]^+$ 479.0971, found 479.0971.

4.11.4. 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), 1 M 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 K_2CO_3 (50 mL) was added, and it was extracted with CH_2Cl_2-EtOH (~4:1, 4 × 50 mL). Combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The compound was purified using column chromatography on silica gel (twice). Eluent 1: $CHCl_3$-MeOH (saturated with NH_3~7M), 10:1 and 5:1, Eluent 2: CH_2Cl_2-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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.77 (s, 3H), 1.82 (br. s., 2H), 2.93 (d, J = 13.2 Hz, 1H), 3.12 (d, J = 13.1 Hz, 1H), 4.51 (d, J = 2.4 Hz, 2H), 5.28 (br. s., 1H), 6.88 (d, J = 3.9 Hz, 1H), 7.01 (d, J = 3.9 Hz, 1H), 7.23 (s, 1H), 7.73 (t, J = 8.1 Hz, 1H), 7.84 (d, J = 8.4 Hz, 1H), 8.00 (d, J = 13.0 Hz, 1H), 8.15 (s, 1H), 12.04 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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 $C_{19}H_{17}F_4N_4O_2S$ $[M - H]^-$ 441.1014, found 441.1014.

4.11.5. 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), 1 M 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 K_2CO_3 (50 mL) was added, and it was extracted with CH_2Cl_2-EtOH (~4:1, 4 × 50 mL). Combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The compound was purified using column chromatography on silica gel (twice). Eluent 1: $CHCl_3$-MeOH (saturated with NH3~7M), 10:1 and 5:1, Eluent 2: CH_2Cl_2-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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.77 (s, 3H), 1.86 (br. s., 2H), 2.93 (d, J = 13.2 Hz, 1H), 3.12 (d, J = 13.2 Hz, 1H), 4.52 (s, 2H), 5.29 (br. s., 1H), 6.84 (d, J = 3.9 Hz, 1H), 6.99 (d, J = 3.9 Hz, 1H), 7.23 (s, 1H), 7.84 (d, J = 9.1 Hz, 2H), 8.12 (s, 1H), 11.93 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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. 2C), 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, 2C), 160.1, 176.4.

HRMS (ESI) calcd for $C_{18}H_{18}ClF_2N_4O_2S$ $[M + H]^+$ 427.0802, found 427.0811.

4.11.6. 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: $CHCl_3$-MeOH (saturated with NH_3~7M), 10:1 and 5:1, Eluent 2: CH_2Cl_2-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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.86 (br. s., 2H), 2.84 (t, J = 6.8 Hz, 2H), 3.00 (dd, J = 13.1, 7.9 Hz, 1H), 3.15 (dd, J = 13.1, 5.3 Hz, 1H), 3.70 (t, J = 6.9 Hz, 2H), 4.68 (br. s., 1H), 5.17–5.26 (m, 1H), 6.85 (d, J = 3.9 Hz, 1H), 7.04 (d, J = 3.9 Hz, 1H), 7.17 (s, 1H), 7.86 (d, J = 9.0 Hz, 2H), 8.64 (d, J = 7.9 Hz, 1H), 11.96 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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, 2C), 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, 2C), 160.4, 171.5.

HRMS (ESI) calcd for $C_{18}H_{16}ClF_2N_4O_2S$ [M - H]- 425.0656, found 425.0659.

4.11.7. 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: $CHCl_3$-MeOH (saturated with NH_3~7M), 10:1 and 5:1, Eluent 2: CH_2Cl_2-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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.79 (br. s., 2H), 2.84 (t, J = 6.8 Hz, 2H), 3.00 (dd, J = 13.2, 7.9 Hz, 1H), 3.15 (dd, J = 13.2, 5.3 Hz, 1H), 3.70 (t, J = 6.9 Hz, 2H), 4.67 (br. s., 1H), 5.18–5.26 (m, 1H), 6.89 (d, J = 3.9 Hz, 1H), 7.06 (d, J = 3.9 Hz, 1H), 7.18 (s, 1H), 7.73 (t, J = 8.1 Hz, 1H), 7.85 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 13.0 Hz, 1H), 8.67 (d, J = 7.9 Hz, 1H), 12.06 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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 $C_{19}H_{17}F_4N_4O_2S$ $[M - H]^-$ 441.1015, found 441.1015.

4.11.8. 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: $CHCl_3$-MeOH (saturated with NH_3~7M), 10:1 and 5:1, Eluent 2: CH_2Cl_2-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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.80 (br. s., 2H), 2.84 (t, J = 6.9 Hz, 2H), 3.00 (dd, J = 13.2, 7.9 Hz, 1H), 3.14 (dd, J = 13.1, 5.1 Hz, 1H), 3.70 (t, J = 6.9 Hz, 2H), 4.68 (br. s., 1H), 5.18–5.26 (m, 1H), 6.73 (t, J = 3.7 Hz, 1H), 7.09 (d, J = 3.9 Hz, 1H), 7.18 (s, 1H), 7.62 (d, J = 8.2 Hz, 1H), 7.74 (d, J = 11.5 Hz, 1H), 8.17 (t, J = 8.0 Hz, 1H), 8.72 (d, J = 7.9 Hz, 1H), 12.01 (br. s., 1H).

^{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 $C_{19}H_{17}F_4N_4O_2S$ $[M - H]^-$ 441.1015, found 441.1014.

4.11.9. 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 NH_3~7M), 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 an 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.

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

^{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 $C_{19}H_{17}F_4N_4O_3S$ $[M - H]^-$ 457.0963, found 457.0956.

4.11.10. 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 NH_3~7M), 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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.66 (br. s., 2H), 1.74–1.83 (m, 2H), 2.71 (t, J = 7.6 Hz, 2H), 3.00 (dd, J = 13.2, 7.9 Hz, 1H), 3.15 (dd, J = 13.1, 5.3 Hz, 1H), 3.44 (t, J = 6.4 Hz, 2H), 4.49 (br. s., 1H), 5.17–5.25 (m, 1H), 6.89 (d, J = 3.9 Hz, 1H), 7.06 (d, J = 3.9 Hz, 1H), 7.13 (s, 1H), 7.74 (t, J = 8.1 Hz, 1H), 7.85 (d, J = 8.4 Hz, 1H), 8.03 (d, J = 12.9 Hz, 1H), 8.67 (d, J = 7.9 Hz, 1H), 12.13 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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 $C_{20}H_{19}F_4N_4O_2S$ $[M - H]^-$ 455.1170, found 455.1170.

4.11.11. 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: $CHCl_3$-MeOH (saturated with NH_3~7M), 10:1, 5:1 and 3:1, Eluent 2: CH_2Cl_2-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 an 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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.68 (br. s., 2H), 3.00 (dd, *J* = 13.2, 7.9 Hz, 1H), 3.14 (dd, *J* = 13.2, 5.3 Hz, 1H), 3.40–3.47 (m, 1H), 3.51 (dd, *J* = 10.8, 6.2 Hz, 1H), 4.75 (t, *J* = 5.7 Hz, 1H), 4.94 (br. s., 1H), 5.15–5.25 (m, 1H), 5.68 (br. s., 1H), 6.89 (d, *J* = 3.9 Hz, 1H), 7.06 (d, *J* = 3.9 Hz, 1H), 7.56 (s, 1H), 7.74 (t, *J* = 8.1 Hz, 1H), 7.85 (d, *J* = 8.3 Hz, 1H), 8.03 (d, *J* = 13.0 Hz, 1H), 8.66 (d, *J* = 7.9 Hz, 1H), 12.08 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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 $C_{19}H_{19}F_4N_4O_3S$ $[M + H]^+$ 459.1109, found 459.1109.

4.11.12. 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 100 mg 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.

4.11.13. 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: $CHCl_3$-MeOH (saturated with NH_3~7M), 10:1 and 5:1, Eluent 2: CH_2Cl_2-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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.04 (br. s., 2H), 3.06 (dd, *J* = 13.2, 7.7 Hz, 1H), 3.18 (dd, *J* = 13.2, 5.4 Hz, 1H), 3.82 (s, 3H), 5.22–5.29 (m, 1H), 6.90 (d, *J* = 3.9 Hz, 1H), 7.07 (d, *J* = 3.9 Hz, 1H), 7.74 (t, *J* = 8.1 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 8.04 (d, *J* = 12.9 Hz, 1H), 8.44 (s, 1H), 8.82 (d, *J* = 6.4 Hz, 1H), 12.12 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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 $C_{19}H_{15}F_4N_4O_3S$ $[M - H]^-$ 455.0806, found 455.0808.

4.11.14. 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: $CHCl_3$-MeOH (saturated with NH_3~7M), 10:1 and 5:1, Eluent 2: CH_2Cl_2-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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.99 (br. s., 2H), 3.06 (dd, *J* = 13.3, 7.5 Hz, 1H), 3.22 (dd, *J* = 13.2, 5.4 Hz, 1H), 5.20–5.30 (m, 1H), 6.90 (d, *J* = 3.9 Hz, 1H), 7.08 (d, *J* = 3.9 Hz, 1H), 7.59 (br. s., 1H), 7.70–7.78 (m, 2H), 7.86 (d, *J* = 8.4 Hz, 1H), 8.03 (d, *J* = 13.0 Hz, 1H), 8.14 (s, 1H), 8.75 (d, *J* = 6.0 Hz, 1H), 11.95 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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 $C_{18}H_{14}F_4N_5O_2S$ $[M - H]^-$ 440.0810, found 440.0811.

4.11.15. 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: $CHCl_3$-MeOH (saturated with NH_3~7M), 10:1 and 5:1, Eluent 2: CH_2Cl_2-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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.81 (br. s., 2H), 2.32 (s, 3H), 3.04 (dd, *J* = 13.1, 7.2 Hz, 1H), 3.12 (dd, *J* = 13.1, 5.3 Hz, 1H), 4.55 (s, 2H), 5.20–5.27 (m, 1H), 5.31 (br. s., 1H), 6.74 (s, 1H), 7.31 (s, 1H), 7.72–7.82 (m, 2H), 7.87 (d, *J* = 12.7 Hz, 1H), 8.28 (d, *J* = 7.0 Hz, 1H), 11.60 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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 $C_{19}H_{17}F_4N_4O_2S$ $[M-H]^-$ 441.1014, found 441.1013.

4.11.16. 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: $CHCl_3$-MeOH (saturated with NH_3~7M), 10:1 and 5:1, Eluent 2: CH_2Cl_2-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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.08 (br. s., 2H), 2.33 (s, 3H), 3.05 (dd, *J* = 13.1, 7.3 Hz, 1H), 3.14 (dd, *J* = 13.1, 5.1 Hz, 1H), 4.62 (s, 2H), 5.18–5.25 (m, 1H), 5.50 (br. s., 1H), 6.75 (s, 1H), 7.57 (s, 1H), 7.73–7.82 (m, 2H), 7.89 (d, *J* = 12.5 Hz, 1H), 8.32 (d, *J* = 6.8 Hz, 1H), 11.64 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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 $C_{19}H_{17}F_4N_4O_2S$ $[M-H]^-$ 441.1014, found 441.1014.

4.11.17. 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: $CHCl_3$-MeOH (saturated with NH3~7M), 10:1 and 5:1, Eluent 2: CH_2Cl_2-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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.08 (s, 3H), 1.13 (s, 3H), 1.97 (br. s., 2H), 4.57 (s, 2H), 5.29 (d, J = 7.6 Hz, 1H), 5.33 (br. s., 1H), 6.84 (d, J = 3.9 Hz, 1H), 7.08 (d, J = 3.9 Hz, 1H), 7.32 (s, 1H), 7.83 (d, J = 8.9 Hz, 2H), 8.26 (d, J = 8.4 Hz, 1H), 12.02 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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, 2C), 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, 2C), 160.0, 170.2.

HRMS (ESI) calcd for $C_{19}H_{18}ClF_2N_4O_2S$ $[M - H]^-$ 439.0813, found 439.0813.

4.11.18. 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: $CHCl_3$-MeOH (saturated with NH3~7M), 10:1 and 5:1, Eluent 2: CH_2Cl_2-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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.09 (s, 3H), 1.14 (s, 3H), 1.95 (br. s., 2H), 4.58 (s, 2H), 5.30 (d, J = 8.2 Hz, 1H), 5.38 (br. s., 1H), 6.78 (d, J = 3.8 Hz, 1H), 7.08 (d, J = 3.8 Hz, 1H), 7.32 (s, 1H), 7.71 (d, J = 8.4 Hz, 2H), 8.02 (d, J = 8.2 Hz, 2H), 8.28 (d, J = 8.8 Hz, 1H), 12.05 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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 (2C), 125.6 (q, J = 4.1 Hz, 2C), 126.7 (q, J = 31.7 Hz), 128.2, 133.4, 135.6, 157.3, 160.0, 170.2.

HRMS (ESI) calcd for $C_{20}H_{20}F_3N_4O_2S$ $[M - H]^-$ 437.1265, found 437.1269.

4.11.19. 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: $CHCl_3$-MeOH (saturated with NH3~7M), 10:1 and 5:1, Eluent 2: CH_2Cl_2-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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.10 (s, 3H), 1.14 (s, 3H), 2.21 (br. s., 2H), 4.63 (s, 2H), 5.27 (d, J = 8.4 Hz, 1H), 5.51 (br. s., 1H), 6.78 (d, J = 3.9 Hz, 1H), 7.06 (d, J = 3.9 Hz, 1H), 7.58 (s, 1H), 7.72 (d, J = 8.4 Hz, 2H), 8.02 (d, J = 8.2 Hz, 2H), 8.29 (d, J = 8.9 Hz, 1H), 12.07 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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 (2C), 125.6 (q, J = 3.7 Hz, 2C), 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 $C_{20}H_{20}F_3N_4O_2S$ $[M + H]^+$ 437.1265, found 437.1269.

4.11.20. 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: $CHCl_3$-MeOH (saturated with NH_3~7M), 10:1, 5:1 and 3:1, Eluent 2: CH_2Cl_2-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.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.77 (br. s., 2H), 3.04 (dd, J = 13.1, 7.8 Hz, 1H), 3.17 (dd, J = 13.3, 5.4 Hz, 1H), 3.29–3.36 (m, 3H), 3.45 (dd, J = 9.9, 4.6 Hz, 1H), 3.54–3.61 (m, 1H), 4.65 (s, 2H), 4.72 (br. s., 2H), 5.19–5.28 (m, 1H), 6.79 (d, J = 3.9 Hz, 1H), 7.06 (d, J = 3.8 Hz, 1H), 7.66 (s, 1H), 7.71 (d, J = 8.4 Hz, 2H), 8.04 (d, J = 8.2 Hz, 2H), 8.67 (d, J = 7.8 Hz, 1H), 12.00 (br. s., 1H).

^{13}C NMR (DMSO-d_6, 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 (2C), 125.6 (q, J = 3.7 Hz, 2C), 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 $C_{21}H_{24}F_3N_4O_4S$ $[M + H]^+$ 485.1465, found 485.1466.

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 paper.

Acknowledgments

This study was supported by funds from NIH Grant R01 AI104416 (AKD), and the New York Blood Center (AKD).

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