Published in final edited form as:

J Med Chem. 2012 May 24; 55(10): 4764–4775. doi:10.1021/jm3002247.

Design, synthesis and antiviral activity of entry inhibitors that target the CD4-binding site of HIV-1

Francesca Curreli¹, Spreeha Choudhury¹, Ilya Pyatkin², Victor P. Zagorodnikov², Anna Khulianova Bulay², Andrea Altieri², Young Do Kwon³, Peter D. Kwong³, and Asim K. Debnath^{1,*}

¹Lindsley F Kimball Research Institute, The New York Blood Center, 310 E 67th Street, New York, NY 10065, USA.

²ASINEX Ltd, 20 Geroev Panfilovtzev, building 1, Moscow 125480, Russia.

³Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA.

Abstract

The CD4 binding site on HIV-1 gp120 has been validated as a drug target to prevent HIV-1 entry to cells. Previously, we identified two small molecule inhibitors consisting of a 2,2,6,6-tetramethylpiperidine ring linked by an oxalamide to a *p*-halide-substituted phenyl group, which target this site, specifically, a cavity termed “Phe43 cavity”. Here we use synthetic chemistry, functional assessment and structure-based analysis to explore variants of each region of these inhibitors for improved antiviral properties. Alterations of the phenyl group and of the oxalamide linker indicated that these regions were close to optimal in the original lead compounds. Design of a series of compounds, where the tetramethylpiperidine ring was replaced with new scaffolds, lead to improved antiviral activity. These new scaffolds provide insight into the surface chemistry at the entrance of the cavity and offer additional opportunities by which to optimize further these potential-next-generation therapeutics and microbicides against HIV-1.

Introduction

Human immunodeficiency virus type-1 (HIV-1) is the etiological agent responsible for the AIDS pandemic. According to 2011 UNAIDS report, 34 million people are living with HIV worldwide – a rise of 17% compared to 2001. However, the availability of low cost highly active antiretroviral therapies (HAART) has helped to lower the rate of new infection and death. Despite the success emergence of resistance to the available drugs is a major problem, which justifies continuous search for novel drugs against this virus.

The first step in HIV life cycle is the entry/fusion of the virus to the target cells. The envelope glycoprotein gp120 initiates this process by binding to the cellular receptor CD4 on cell surface. This binding induces substantial structural rearrangement in gp120 which is required for its subsequent binding to cellular chemokine receptor CXCR4 or CCR5^{1,2}. This interaction helps to expose the fusion domain of gp41 that initiates fusion and the entry of the virus to the host cells. Although all these steps are legitimate targets for developing entry inhibitors only drugs targeting gp41^{3,4} and CCR5⁵ have been successfully developed and

*To whom correspondence should be addressed: Tel. (212) 570-3373; adebnath@nybloodcenter.org.

Supporting Information Available: Detail synthesis (Scheme-S1) of succinamide series compounds (**41-51**), their structures and antiviral activity have been reported (Table-S1). Figure-S1 lists 50 top GLIDE XP-scored poses. This material is available free of charge via the Internet at <http://pubs.acs.org>.

clinically available. The x-ray structure of gp120 bound to CD4 and the fragment of an antibody (Fab) 17b was solved in 1998⁶. The structure reveals that phenylalanine at position 43 (Phe43) binds to the vestibule of a cavity of gp120, termed “Phe43 cavity”, and makes major hydrophobic contacts. This hydrophobic contribution accounts for 28% of the contact interactions between CD4 and gp120 residues; however, another residue arginine 59 (Arg59) of CD4 also makes critical interaction by forming a salt-bridge with aspartic acid residue at position 368 (Asp368) of gp120. In addition, gp120 residues in the span of 365-371 and 425-430 contribute to about 58 % of the interactions⁶. Several hydrophobic residues in those two regions are critical and make hydrophobic contacts with the hydrophobic residues in CD4. Insertions into this cavity were found to enhance the affinity of CD4 and of CD4-mimetics⁷, and we were the first to identify small molecule inhibitors, NBD-556 (**1**) and NBD-557 (**2**)⁸ (Figure-1), which appear to insert into the “Phe43 cavity”^{9,10}. These two compounds show low micromolar potency against several laboratory strains and primary isolates. In addition, these molecules induce conformational changes in gp120 similar to CD4¹¹. Recently, we co-crystallized **1** with the clade C strain C1086 version of gp120 core ¹²_e. The structure reveals that **1** binds within the “Phe43 cavity” of gp120 with its 4-chlorophenyl group extended deep inside the cavity surrounded by hydrophobic residues, such as, Trp112, Val255, Trp427 and Met475. The distal NH of the oxalamide group from 4-chlorophenyl ring forms an H-bond with Gly473. However, no apparent interaction of the tetramethylpiperidine ring with any residues in the cavity was observed; rather it was exposed outside of the pocket. The structural insight led us (this manuscript) and others to believe that tetramethylpiperidine moiety can be replaced with other groups that may be able to take advantage of additional interactions, especially with Asp368, near the entrance of the cavity and yield more potent antivirals^{9,13,14}.

In this report, we present the systematic modification of **1** to more potent inhibitors using structure-based design, synthesis, antiviral screening and structure-activity relationship analyses. Our data indicate that structure-guided modifications, especially of Region III with new scaffolds never reported before, improved the activity of several analogs by ~6-fold compared to **1**.

Results and Discussion

Virtual screening to identify new scaffolds to replace 2,2,6,6-tetramethylpiperidine ring

We initiated a strategy for designing next generation entry inhibitors targeted to “Phe43 cavity” based on our earlier identified small molecule CD4-mimetics **1**⁸ (Figure 1) and the x-ray structure of **1** bound to HIV-1 gp120 core. In our initial attempt for a limited structure-activity relationship (SAR) study on the **1** we wanted to extend the hydrophobic portion of the oxalamide chain in Region II (Figure 1) as defined by LaLonde et al¹⁴ and designed several succinamide containing compounds (Supporting Information). However, these compounds failed to show any antiviral activity in both single-cycle and multi-cycle inhibition assay. Recently, LaLonde et al also reported similar observation when testing a succinamide analog of **1**¹⁴. In addition, we (this report) and LaLonde et al observed that variations of the oxalamide chain resulted in either poor activity or no antiviral activity at all¹⁴. Therefore, we turned our attention to modify the 2,2,6,6-tetramethylpiperidine ring. The structural insights described before motivated us to replace the 2,2,6,6-tetramethylpiperidine ring with other scaffolds which may provide a similar interaction as was observed between Arg59 and Asp368 as well as interact with other available binding residues. Since **1** was prepared by simple coupling reaction between 2-(4-chlorophenylamino)-2-oxoacetyl chloride and 4-amino-2,2,6,6-tetramethylpiperidine, we first identified 208 commercially available amines with diverse structures to replace the tetramethylpiperidine part and created a virtual library with 33 anilines consisting of 6864

compounds. Beside 4-chloroaniline we also used a variety of substituted anilines to investigate how they fit in the “Phe43 cavity”. The structure of the compounds in the virtual library was in 2D format; therefore, we used LigPrep module within Maestro (Schrodinger, San Diego, CA) to generate 3D structures. The LigPrep module not only converts 2D structure to 3D but also considers tautomeric, stereochemical and ionization states of the structure and generates conformations and performs energy minimization. The LigPrep application generated 20,688 structures representing all tautomeric, stereochemical and ionization states of the molecules. We then performed a GLIDE-based docking^{15,16} of these structures using the standard precision (SP) mode and selected 500 best scored structures to dock again using the extra precision (XP) scoring mode. We selected 50 top scored structures from this simulation. However, it is worth mentioning that within the top 50 structures same molecule appeared multiple times due to different stereochemical, tautomeric and ionization states. We noticed that the top XP scored molecule (−8.8) and several others within the top 50 molecules contained (2-(amino)piperidine-2-yl)methyl)-4-methylthiazolyl-5-yl)methanol scaffold with variable substitutions in the phenylamine moiety. Another major observation was that the positively charged piperidine ring of the scaffold formed a salt-bridge with Asp368 of gp120 (Figure-2). This interaction was absent in most of the other top scored compounds. Since we wanted to capture this interaction in our newly designed compounds we decided to use this scaffold and its structural variations as the main target for synthesis.

Initially, we restricted our synthesis effort using 4-chlorophenylamine in Region I (Figure-1) due to the fact that we observed earlier that phenyl moiety could tolerate limited substitutions and 4-chloro was the most active so far. We also selected few other top scored compounds for synthesis which did not satisfy the salt-bridge formation to compare their activity; however, we needed to make some minor changes due to the lack of availability of the exact reagents that were used to create the virtual library (Table-2). All these compounds with the variations were docked using the same method and inspected for their fit in the Phe43 cavity. All of them fit very well as the original scaffold with high score (>−7.0).

Chemistry

The oxalamide derivatives were prepared by adopting the general synthetic Scheme-1. This allows probing different haloaryl derivatives in Region I and virtually any kind of available amine in Region III (Figure-1). A series of commercially available aryl amines (Table-1-2 and Table-S1) have been coupled with the ethyl 2-chloro-2-oxoacetate (*a*). The resulting intermediate was hydrolyzed (*b*) and coupled with commercially available amines (*c*) (Table-1-2 and Table-S1). When Boc-protected amines were used an additional step of cleaving was necessary (*d*). The resulting coupling product was HPLC purified and characterized by ¹H-NMR and LCMS. Some of the reported compounds were isolated as diastereoisomeric mixture with variable purity. No further attempt was made to separate individual isomer at this stage.

Structure-Activity Relationship (SAR)

In our initial attempt to understand the SAR we decided to explore few compounds by replacing oxalamide moiety in region II of our earlier reported compounds, **1**, to succinamide (Table-S1 in Supporting Information). No docking-based selection method was used rather those compounds were synthesized based on reagent availability. However, none of the compounds showed any noticeable antiviral activity either in single-cycle or in multi-cycle assay. Based on the docking simulation the best XP-scored compound had the 4-bromo-3-fluorophenyl moiety (Fig-S1). However, we decided initially to explore the 4-chloroaniline based on our earlier reported compound **1**. Therefore, we synthesized the compound, NBD-09027 (**6**) and it showed low μM potency with good selectivity index (SI)

in both single-cycle (in TZM-bl cells) and multi-cycle (in MT-2 cells) assays. Therefore, we concentrated our attention to understand the SAR of the piperidine ring of our initial best hit, **6**. Then we synthesized two additional compounds by moving the nitrogen from position 2 in the initial hit to 3 (**7**) and 4 (**8**) positions. Both compounds showed substantial improvement in antiviral activity in a single-cycle pseudovirus based assay. In addition, the toxicity in TZM-bl cells also decreased remarkably. Unfortunately, both compounds were much less active in MT-2 cell based multi-cycle infectivity assay. A similar study was done with our follow up most active compound NBD-10007 (**9**). Again we observed remarkable improvement in antiviral potency as well as toxicity for both **10** and **11** in TZM-bl based pseudovirus assay. However, both compounds again showed much lower potency in MT-2 based infectivity assay. Since these two assay methods are different and different cells were used, some variations in activity were expected. However, no clear SAR could be discerned from the multi-cycle infectivity based antiviral data. Recently, a multi-center comparison study of HIV neutralization assay found out that pseudovirus assays are generally more sensitive than multi-cycle virus inhibition assay; however, it also depends on the virus and inhibitor used¹⁷. Presence of either hydroxymethanol or hydroxyethanol on the thiazole ring did not make any appreciable differences in antiviral activity. These groups most likely working as solubilizing unit of these molecules than conferring any direct binding. Next, we have substituted H of the NH of the piperidine ring with methyl (**12**) and acetyl (**13**). The antiviral activity of methyl substituted piperidine (**12**) improved compared to its unsubstituted counterpart (**9**) but the toxicity level remained the same. The acetyl substituted piperidine analog (**13**) showed much worse activity but the toxicity improved substantially. In both cases, the antiviral activity in MT-2 cells was poor as was observed with unsubstituted analogs. Complete removal of the piperidine ring with methylaminoethyl ($-\text{CH}_2\text{NHCH}_3$) moiety also showed potent antiviral activity in single-cycle pseudovirus-based assay but showed poor activity in MT-2 cell based multi-cycle infectivity assay. Again the toxicity of this analog was high. We also tested three structurally diverse analogs (**17-19**) but they either had poor activity or no activity at all in both infectivity assay.

Since **6** and **9** showed much better activity profile in both assay, we restricted our synthesis effort in designing analogs based only on these two active inhibitors. This time, we focused our attention mostly to include substitutions to the phenyl ring (Table-2). Substituting 4-Cl with CF_3 (**20**) resulted in somewhat reduced antiviral activity. When acetyl group was introduced at the *para* position of the phenyl ring (**23**) the compound became totally inactive. The introduction of a smaller substituent (F) at the *ortho* position of the phenyl ring was found to be detrimental to the antiviral activity (**21**). However, interestingly, 4-Cl and 4- CH_3 group with 3-F (**27**, **28**, **38** and **39**) in both $-\text{CH}_2\text{OH}$ and $-\text{CH}_2\text{CH}_2\text{OH}$ substituted thiazole showed much improved activity. This is consistent with the observation reported with 2,2,6,6-tetramethylpiperidine containing compounds⁹ and recently with a large number of compounds where 4-amino-2,2,6,6-tetramethylpiperidine was replaced by diverse amines¹⁴. In a more drastic move we have replaced the phenyl ring with a seven membered alicyclic ring and those compounds (**29** and **40**) showed no antiviral activity. This confirms our earlier observation that the phenyl ring can only accommodate limited substitutions due to the narrow hydrophobic pocket in the "Phe43 cavity".

To understand the expected binding mode of two of the most active compounds that contained 4-Cl with 3-F substituents in the phenyl ring NBD-11009 (**27**) and NBD-11018 (**39**) we performed GLIDE-based docking simulations in XP mode as described before. The top scoring conformations of these two inhibitors indicated two possible binding modes (Figure-3 a-d). In both cases, the 4-Cl-3-F-phenyl ring was surrounded by hydrophobic residues similar to that we observed with **6**; however, there was considerable difference between two binding modes of the piperidine-thiazolyl moiety of **27** (Figure-3 a & b). Surprisingly, the positively charged piperidine nitrogen of the top scored (-8.22) **27**

conformation did not form any H-bond/salt-bridge with Asp368 whereas in the next best scored (−7.89) conformation it indeed formed the H-bond/salt-bridge with Asp368. In both cases the −CH₂OH formed H-bond with Trp427. On the contrary, the positively charged piperidine nitrogen in both top scored (−7.96) and the next best scored (−7.81) conformations of **39** formed H-bond/salt-bridge with Asp368 (Figure-3 c and d). This difference in the binding mode may be attributed to the larger size of the ethanol moiety on the thiazole ring. However, the −CH₂CH₂OH group had different binding modes in two different conformations. One noticeable feature was that in all these conformations the oxalamide moiety had the same interaction. One of its NH attached to the phenyl ring formed H-bond with Asn425. This is somewhat different than what we observed with **6** (Figure-2) where in addition to the same H-bond the distal NH of the oxalamide linker also formed an H-bond with Met426. This predicted binding information is expected to guide future optimization of this class of inhibitors.

Conclusions

After our first discovery of two small molecule inhibitors that target the “Phe43 cavity” in 2005, it has been conclusively shown that these compounds bind to the cavity with remarkable ability to alter the conformation of gp120 similar to that observed when CD4 bound to gp120. This extraordinary ability of these small molecules resulted in solving the crystal structures of these molecules with gp120, which provided details of the binding mode of these compounds in the cavity. Despite many attempts by us and others using traditional medicinal chemistry, the 2,2,6,6-tetramethylpiperidine ring could not be replaced successfully by any other chemical scaffold until this report to improve the antiviral activity. In fact, all attempts reported until recently¹⁴ indicate failure to obtain compounds with activity better than **1** and **2**. In this study, we have successfully utilized the structural insights of the x-ray crystal structure of **1** bound to gp120 to design a large number of molecules with new scaffold replacing 2,2,6,6-tetramethylpiperidine ring. Several of these new compounds showed improved potency in single-cycle as well as multi-cycle HIV-1 inhibition assays. The most notable achievement is the identification of new scaffolds which should provide opportunities to optimize these compounds further. Since these compounds prevent HIV-1 from entering to cells they may serve as potential leads to develop as next-generation therapeutics and microbicides against HIV-1.

Experimental section

All reagents were purchased from commercial suppliers or obtained in-house (Asinex) and were used without further purification. The melting points were measured on a Sanyo Gallenkamp melting point apparatus (Sanyo Gallenkamp, UK). The LC/MS analyses for the compounds were done at Surveyor MSQ (Thermo Finnigan, USA) with APCI ionization. The ¹H NMR spectra were recorded on MERCURY plus 400 MHz spectrometer (Varian, Germany). Chemical shift values are given in ppm relative to tetramethylsilane (TMS), with the residual solvent proton resonance as internal standard. High-resolution mass spectra (HRMS) were recorded on a Bruker APEX II ICR-FTMS. Purity of compounds was determined by HPLC. Purity of compounds were 95% except otherwise mentioned. HPLC analyses were performed on a Water XTerra MS C18 analytical column (2.1 × 30 mm, particle size 3.5 μm) using DMSO:Acetonitrile (50:50), at a flow rate of 15 ml/min at 25 °C. The oxalamide compounds were isolated as diastereoisomers with variable isomer ratios.

MT-2 cells (Human T-cell leukemia cells, obtained through the AIDS Research and Reference Reagent Program from Dr. D. Richman.) were grown in RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum (FBS), penicillin and streptomycin (100 U/ l each). TZM-bl cells (a HeLa cell line that expresses CD4, CXCR4 and CCR5 and

expresses luciferase and β -galactosidase under control of the HIV-1 promoter, obtained from Dr. John C. Kappes, Dr. Xiaoyun Wu and Tranzyme Inc) and 293T cells (ATCC) were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco) supplemented with 10% FBS, penicillin and streptomycin. pNL4-3.Luc.R-E DNA was obtained through the AIDS Research and Reference Reagent Program from Dr. N. Landau and env expression vector pHXB2-env (X4) DNA from Dr. K. Page and Dr. D. Littman¹⁸.

Virtual screening using GLIDE-based docking

We used the automated docking software GLIDE 5.7 (Schrödinger, Portland, OR) within Schrödinger Suite 2011 that applies a two-stage scoring process to sort out the best conformations and orientations of the ligand (defined as pose) based on its interactions with the receptor. GLIDE has been used successfully in drug design¹⁹⁻²⁵. We used our recently solved x-ray crystal structure of 1 with the clade C strain C1086 version of gp120 core at 2.7 Å resolutions for docking simulations (pdb ID: 3TGS)¹². Threedimensional coordinates of the ligands, their isomeric, ionization and tautomeric states were generated using the LigPrep (including Ionizer) module within the Schrödinger Suite 2011 programs. The protein was prepared using the "protein preparation tool" and the structures were minimized with MacroModel software within Schrödinger Suite 2011. A grid file encompassing the area in the cavity that contains information on the properties of the associated receptor was created. Conformational flexibility of the ligands was handled via an exhaustive conformational search. Initially, we used Schrödinger's proprietary GlideScore scoring function in standard precision (SP) mode. We selected 500 top-scored compounds to dock again in extra precision (XP) mode to score the optimized poses. 50 top-scored ligands were selected from this simulation for further study.

Synthesis of oxalamide series compounds (6-40)

Since the compounds from oxalamide series were synthesized by general Scheme-1, we presented a representative synthesis (Scheme-2) of one of the most active compounds (**27**) from Table-3 and all other compounds in this series were synthesized by following this method. The deprotection step (d) was only used when an N-Boc protected amines were used as described below.

Ethyl 2-(4-chloro-3-fluorophenylamino)-2-oxoacetate (**2'**)

TEA (2.28g, 0.023 mol) was added at once to a solution of 3g (0.0206 mol) 4-Chloro-3-fluoroaniline in 50 ml of DCM and then ethyl 2-chloro-2-oxoacetate (2.81g, 0.0206 mol) was added dropwise at 0°C. Reaction mixture was stirred at 0°C for 1h and then continued at r.t. for 6h. The mixture was washed with 25% aqueous solution of K₂CO₃ (2×50ml) and water (50ml). The product was dried over Na₂SO₄ and evaporated. The residue was washed with ether and dried on the air to give ethyl 2-(4-chloro-3-fluorophenylamino)-2-oxoacetate (**2'**) (3.97g, 78.6%) as a white powder; LC-MS (APCI⁺) m/z: calcd for C₁₀H₉ClFNO₃: 245.03; found: 245 (M+H⁺).

2-(4-chloro-3-fluorophenylamino)-2-oxoacetic acid (**3'**)

Ethyl 2-(4-chloro-3-fluorophenylamino)-2-oxoacetate (4.18g, 0.017mol) to a solution of NaOH (1.361g, 0.0340 mol) in mixture 50 ml EtOH and 50 ml water was added and the resulting mixture was stirred at r.t. for 6h. The mixture was acidified with 2N HCl to pH 4-5 at 0°C. The precipitate was filtered, washed with water and dried on the air to afford 2-(4-chloro-3-fluorophenylamino)-2-oxoacetic acid (**3'**) (2g, 55.2%) as a white solid. LC-MS (APCI⁺) m/z: calcd for C₈H₅ClFNO₃: 216.99; found: 217 (M+H⁺).

Tert-butyl 2-((2-(4-chloro-3-fluorophenylamino)-2-oxoacetamido)(5-(hydroxymethyl)-4-methylthiazol-2-yl)methyl)piperidine-1-carboxylate (4')

A mixture of 2-(4-chloro-3-fluorophenylamino)-2-oxoacetic acid (**3'**) (0.3g, 1.378 mmol), TBTU (0.530g, 1.65 mmol) and TEA (0.166g, 1.65mmol) in DCM (20ml) was stirred at r.t. for 1 h, then 2-[Amino-(5-hydroxymethyl-4-methyl-thiazol-2-yl)-methyl]-piperidine-1-carboxylic acid tert-butyl ester (0.470g, 1.378mmol) was added and stirring was continued for 6h. Reaction mixture was washed with 25% aqueous solution of K₂CO₃ (2×50ml) and water (50ml). The product was dried over Na₂SO₄ and evaporated. The residue was purified by column chromatography on silica gel (EtOAc/Hexane1/1) to afford tert-butyl 2-((2-(4-chloro-3-fluorophenylamino)-2-oxoacetamido)(5-(hydroxymethyl)-4-methylthiazol-2-yl)methyl)piperidine-1-carboxylate (**4'**) (0.53g, 72%) as a white solid; LC-MS (APCI⁺) m/z: calcd for C₂₄H₃₀ClFN₄O₅S: 540.16; found: 541 (M+H⁺).

N¹-(4-chloro-3-fluorophenyl)-N²-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.2HCl (27)

Tert-butyl 2-((2-(4-chloro-3-fluorophenylamino)-2-oxoacetamido) (5-(hydroxymethyl)-4-methylthiazol-2-yl)methyl)piperidine-1-carboxylate (**4'**) (0.250 g, 0.462 mmol) was dissolved in dioxane (20 ml) and 15% solution HCl in dioxane (20 ml) was added and the resulting mixture was stirred at r.t. for 6h. Solvent was distilled off and residue was triturated with acetone/ether to give N¹-(4-chloro-3-fluorophenyl)-N²-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide hydrochloride (25) (0.114 g, 52%). (Diastereoisomeric mixture 1:5); LC-MS (APCI⁺) m/z: calcd for C₁₉H₂₂ClFN₄O₃S: 440.11; found: 441 (M+H⁺), 443.15 (M⁺+2), 444.15. HPLC: > 92%. ¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H, 1.32-1.75 (m, 6H, CH₂), 2.27 (s, 3H, -CH₃), 2.75-3.00 (m, 1H, CH₂-N), 3.27 (m, 1H, CH₂-N), 3.80 (m, 1H, CH-N), 4.56 (s, 2H, -CH₂-OH), 5.38 (t, 1H for one isomer, CH-), 5.51 (t, 1H for the other, CH-), 6.00-6.30 (H₂O + H⁺ + -OH signals), 7.51 (t, 1H, ArH-), 7.59 (d, 1H, ArH-), 7.87 (d, 1H, ArH-), 8.50-9.50 (m br, 2H, NH₂⁺), 9.53 (br, 1H, CONH), 11.00 (s, 1H for one isomer, CONH), 11.09 (s, 1H for the other, CONH).

N¹-(4-chlorophenyl)-N²-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.HCl (6)

White solid - Yield: 42% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₁₉H₂₃ClN₄O₃S: 422.12; found: 422.98 (M+H⁺). HPLC: 95.8%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H, 1.32-1.90 (m, 6H), 2.27 (s, 3H, -CH₃), 2.75-3.00 (m, 1H), 3.33, (m, 1H), 3.80 (m, 1H), 4.56 (s, 2H, -CH₂-OH), 5.38 (t, 1H for one isomer), 5.51 (t, 1H for the other), 7.40 (d, 2H), 7.84 (d, 2H), 8.50-9.30 (m br, 2H, NH₂⁺), 9.61 (d, 1H for one isomer, NH), 9.68 (d, 1H for the other, NH), 11.80 (s, 1H for one isomer, CONH), 10.91 (s, 1H for the other, CONH).

N¹-(4-chlorophenyl)-N²-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(piperidin-3-yl)methyl)oxalamide.2HCl (7)

White solid - Yield: 50% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₁₉H₂₃ClN₄O₃S: 422.12; found: 423.26 (M+H⁺). HPLC: > 97.5%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H, 1.34 (m, 1H), 1.65 (br, 2H), 1.82 (m, 2H), 2.27 (s, 3H, -CH₃), 2.55 (m, 1H), 2.75 (m, 2H, -CH₂-NH), 3.15 (m, 2H, -CH₂-NH), 4.56 (s, 2H, -CH₂-OH), 5.08 (m, 1H), 7.40 (d, 2H, Ar-H), 7.83 (d, 2H, Ar-H), 8.80 (br, 1H, NH₂⁺), 9.30 (br d & d, 1H, NH₂⁺), 9.50 (d & d, 1H, CONH), 10.75 (s, 1H, CONH).

***N*¹-(4-chlorophenyl)-*N*²-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(piperidin-4-yl)methyl)oxalamide.2HCl (8)**

White solid - Yield: 46% (Single diastereoisomer). LC-MS (APCI⁺) m/z: calcd for C₁₉H₂₃ClN₄O₃S: 422.12; found: 423.26 (M+H⁺). HPLC: 95.0%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.49 (br m, 2H), 1.68 (br d, 1H), 1.93 (br d, 1H), 2.26 (s, 3H, -CH₃), 2.35 (br m, 1H), 2.80 (br m, 2H), 3.25 (br m, 2H), 3.55 (m, 1H), 4.56 (s, 2H, -CH₂-OH), 4.68 (br t, 1H), 4.95 (m, 1H), 7.39 (d, 2H, Ar-H), 7.83 (d, 2H, Ar-H), 8.68 (br, 1H, NH₂⁺), 9.08 (br, 1H, NH₂⁺), 9.41 (d, 1H, CONH), 10.78 (s, 1H, CONH).

***N*¹-(4-chlorophenyl)-*N*²-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.HCOOH (9)**

White solid - Yield: 55% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₂₀H₂₅ClN₄O₃S: 436.13; found: 437.30 (M+H⁺). HPLC: > 95.0%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.10-1.80 (m, 6H), 2.26 (s, 3H, -CH₃), 2.75-3.00 (m, 3H), 3.00-3.80 (12H +NH, + H₂O signal), 5.00 (br, 1H for one isomer), 5.10 (br, 1H for the other), 7.41 (d, 2H), 7.82 (d, 2H), 9.10 (br 1H for one isomer, NH₂⁺), 9.38 (br, 1H for the other, NH₂⁺), 10.85 (s, 1H, NH).

***N*¹-(4-chlorophenyl)-*N*²-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-3-yl)methyl)oxalamide.2HCl (10)**

White solid - Yield: 37% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₂₀H₂₅ClN₄O₃S: 436.13; found: 437.27 (M+H⁺). HPLC: 98.2%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.40 (m, 1H), 1.65 (br, 2H), 1.82 (m, 2H), 2.27 (s, 3H, -CH₃), 2.55 (br, 1H), 2.65-2.88 (m, 4H), 3.13 (m, 2H), 3.55 (m, 2H, -CH₂-CH₂-OH), 5.07 (m, 1H), 7.39 (d, 1H, Ar-H), 7.82 (d, 1H, Ar-H), 8.75 (br, 1H, NH₂⁺), 9.11 (br, 1H for one isomer, NH₂⁺), 9.24 (br, 1H for the other, NH₂⁺), 9.48 (d, 1H for one isomer, CO-NH), 9.51 (d, 1H for the other, CO-NH), 10.75 (s, 1H, CO-NH).

***N*¹-(4-chlorophenyl)-*N*²-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-4-yl)methyl)oxalamide.2HCl (11)**

White solid - Yield: 44% (Single diastereoisomer). LC-MS (APCI⁺) m/z: calcd for C₂₀H₂₅ClN₄O₃S: 436.13; found: 437.21 (M+H⁺). HPLC: 95%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.45 (m, 2H), 1.68 (d, 1H), 1.95 (d, 1H), 2.25 (s, 3H, CH₃), 2.35 (m, 1H), 2.80 (m, 4H), 3.24 (m, 2H), 3.40-3.70 (m, 3H), 4.95 (t, 1H), 7.39 (d, 2H, Ar-H), 7.82 (d, 2H, Ar-H), 8.65 (br, 1H, NH₂⁺), 8.88 (br, 1H, NH₂⁺), 9.35 (d, 1H, CO-NH), 10.75 (s, 1H, CO-NH).

***N*¹-(4-chlorophenyl)-*N*²-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(1-methylpiperidin-2-yl)methyl)oxalamide (12)**

White solid - Yield: 38%. (Single diastereoisomer). LC-MS (APCI⁺) m/z: calcd for C₂₁H₂₇ClN₄O₃S: 450.15; found: 451.12 (M+H⁺). HPLC: 93.0%.

¹H NMR (CDCl₃, 45 °C, 400 MHz) δ_H: 1.20-2.20 (m, 8H), 2.35 (s, 3H, -CH₃), 2.38 (s, 3H, -CH₃), 2.71 (br, 1H, -CH-NMe), 2.95 (m, 4H, -CH₂-CH₂-OH), 3.80 (m, 2H, -CH₂-CH₂-OH), 5.10 (br, 1H, CONH-CH), 7.31 (d, 2H Ar-H), 7.53 (d, 2H Ar-H), 8.40 (br, 1H, CONH-CH), 9.28 (s, 1H, Ar-NHCO-).

***N*¹-((1-acetylpiperidin-2-yl)(5-(2-hydroxyethyl)-4-methylthiazol-2-yl)methyl)-*N*²-(4-chlorophenyl)oxalamide (13)**

White solid - Yield: 36% (Single diastereoisomer). LC-MS (APCI⁺) m/z: calcd for C₂₂H₂₇ClN₄O₄S: 478.14; found: 479.12 (M+H⁺). HPLC: 90.0%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.10-2.20 (m, 9H), 2.27 (s, 3H, -CH₃), 2.80 (m, 2H), 3.56 (m, 2H, -CH₂-OH), 4.75, (m, 1H), 5.12 (m, 1H), 5.48 (m, 1H,), 7.41 (d, 2H), 7.82 (d, 2H), 8.35 (br 1H, CO-NH), 10.75 (s, 1H, CO-NH).

***N*¹-(4-chlorophenyl)-*N*²-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(pyrrolidin-2-yl)methyl)oxalamide.HCOOH (14)**

White solid - Yield: 39% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₁₈H₂₁ClN₄O₃S: 408.10; found: 409.28 (M+H⁺). HPLC: 93.2%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.40-1.90 (m, 4H, -CH₂-), 2.27 (s, 3H, -CH₃), 2.87 (m, 2H), 3.10-3.70 (NH+ H₂O signal), (3.80 (m, 1H), 4.56 (s, 2, -CH₂-OH), 4.98 (br, 1H for one isomer), 5.08 (br, 1H for the other), 7.40 (d, 2H), 7.82 (d, 2H), 9.08 (br 1H for one isomer, NH₂⁺), 9.40 (br, 1H for the other, NH₂⁺), 10.85 (s, 1H, NH).

***N*¹-(4-chlorophenyl)-*N*²-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(pyrrolidin-2-yl)methyl)oxalamide.HCOOH (15)**

White solid - Yield: 53% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₁₉H₂₃ClN₄O₃S: 422.12; found: 423.27 (M+H⁺). HPLC: 95.0%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.52 (m, 1H, -CH₂-), 1.69 (m, 2H, -CH₂-), 1.85 (m, 1H, -CH₂-), 2.27 (s, 3H, -CH₃), 2.80 (t, 2H, -CH₂-CH₂-OH), 2.87 (m, 2H, CH₂-N), 3.53 (m, 2H, -CH₂-CH₂-OH), 3.85 (m, 1H, CH-N), 4.98 (br, 1H for one isomer), 5.08 (br, 1H for the other), 7.40 (d, 2H), 7.82 (d, 2H), 9.08 (br 1H for one isomer, NH₂⁺), 9.46 (br, 1H for the other, NH₂⁺), 10.85 (s, 1H, NH).

***N*¹-(4-chlorophenyl)-*N*²-(1-(5-(2-hydroxyethyl)-4-methylthiazol-2-yl)-2-(methylamino)ethyl)oxalamide.2HCl (16)**

White solid - Yield: 41%. LC-MS (APCI⁺) m/z: calcd for C₁₇H₂₁ClN₄O₃S: 396.10; found: 397.08 (M+H⁺). HPLC: 94.2%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 2.27 (s, 3H, -CH₃), 2.59 (br, 3H, NH-CH₃), 2.82 (m, 2H, -CH₂-CH₂-OH), 3.55 (m, 3H, -NCH₃), 3.60 (m, 2H, -CH₂-OH), 5.55 (m, 1H, CH-HetAr), (m, 1H), 7.41 (d, 2H, ArH), 7.82 (d, 2H, ArH), 9.05 (br 1H, NH), 9.28 (br 1H, NH), 9.75 (br d, 1H, NH), 10.85 (s, 1H, NH).

***N*¹-(4-chlorophenyl)-*N*²-(1-ethyl-1H-pyrazol-4-yl)oxalamide (17)**

White solid - Yield: 35%. Mp: 232-233. LC-MS (APCI⁺) m/z: calcd for C₁₃H₁₃ClN₄O₂: 292.07; found: 293.04 (M+H⁺). HPLC: 98.8%.

¹H NMR (DMSO-d₆, 400 MHz) δ_H: 1.38 (t, 3H, -CH₃), 4.11 (q, 2H, -CH₂-), 7.41 (d, 2H, Ar-H), 7.70 (s, 1H, HetAr-H), 7.86 (d, 2H, Ar-H), 8.10 (s, 1H, HetAr-H), 10.75 (s, 1H, NH), 11.10 (s, 1H, NH).

(2S,4R,5S)-methyl 4-(2-(4-chlorophenylamino)-2-oxoacetamido)-5-phenylpyrrolidine-2-carboxylate (18)

White solid - Yield: 39%. LC-MS (APCI⁺) m/z: calcd for C₂₀H₂₀ClN₃O₄: 401.11; found: 402.14 (M+H⁺). HPLC: 95 %.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 2.18 (m, 2H, -CH₂-), 3.30 (br, 1H, -NH-), 3.69 (s, 3H, O-CH₃), 4.05 (m, 1H, -N-CH-Ph), 4.17 (m, 1H, -CH-), 4.27 (m, 1H, -CH-), 7.20 (t, 1H, Ar-H), 7.28 (t, 2H, Ar-H meta), 7.38 (d, 2H, Ar-H), 7.44 (d, 2H, Ar-H), 7.81 (d, 2H, Ar-H), 9.12 (br 1H, NH), 10.58 (s, 1H, Ar-NH).

N¹-(4-chlorophenyl)-N²-((1S,2R)-1-morpholino-1-phenylpropan-2-yl)oxalamide (19)

White solid - Yield: 30%. LC-MS (APCI⁺) m/z: calcd for C₂₁H₂₄ClN₃O₃: 401.15; found: 402.28 (M+H⁺). HPLC: 95%.

¹H NMR (CDCl₃, 45 °C, 400 MHz) δ_H: 1.10 (d, 3H, -CH₃), 2.45 (br, 2H, -N-CH₂-), 2.56 (br, 2H, -N-CH₂-), 3.31 (d, 1H, Ph-CH-N), 3.79 (m br, 4H, -O-CH₂-), 4.65 (m, 1H, NCH-CH₃), 7.28-7.45 (m, 5H, Ar-H), 7.52 (br d, 2H, Ar-H), 7.57 (br d, 2H, Ar-H).

N¹-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)-N²-(4-(trifluoromethyl)phenyl)oxalamide.2HCl (20)

White solid - Yield: 56% (Diastereoisomeric mixture 1:20). LC-MS (APCI⁺) m/z: calcd for C₂₀H₂₃F₃N₄O₃S: 456.14; found: 457.90 (M+H⁺). HPLC: 97.7%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.30-1.76 (m, 6H), 2.27 (s, 3H, -CH₃), 2.62 (br, 1H, CH), 3.30 (br d, 1H), 3.82 (br, 1H), 4.56 (s, 2H, -CH₂-O), 5.40 (t, 1H for one isomer), 5.51 (t, 1H for the other), 7.65 (d, 2H), 8.20 (d, 2H), 8.61-9.50 (m, 2H, NH₂⁺), 9.65 (br d, 1H, CONH), 11.00 (s, 1H for one isomer, CONH), 11.08 (s, 1H for the other, CONH).

N¹-(2,4-difluorophenyl)-N²-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.HCl (21)

White solid - Yield: 58% (Diastereoisomeric mixture 1:5). LC-MS (APCI⁺) m/z: calcd for C₁₉H₂₂F₂N₄O₃S: 424.14; found: 425.86 (M+H⁺). HPLC: 95.6%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.30-1.80 (m, 6H), 2.27 (s, 3H, -CH₃), 2.80-4.00 (m, 3H + H₂O signal), 4.56 (s, 2H, -CH₂-O), 5.35 (t, 1H for one isomer), 5.41 (t, 1H for the other), 7.11 (t, 1H), 7.34 (t, 3H), 7.61 (m, 3H), 8.38 (br 1H, NH₂⁺), 8.70 (br d, 1H, NH₂⁺), 9.51 (d, 1H for one isomer, NH), 9.70 (d, 1H for the other, NH), 10.25 (s, 1H for one isomer, Ar-CONH), 10.30 (s, 1H for the other, Ar-CONH).

N¹-(3,4-difluorophenyl)-N²-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.HCl (22)

White solid - Yield: 45% (Diastereoisomeric mixture 3:2). LC-MS (APCI⁺) m/z: calcd for C₁₉H₂₂F₂N₄O₃S: 424.14; found: 425.90 (M+H⁺). HPLC: 98.3%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.30-1.80 (m, 6H), 2.27 (s, 3H -CH₃), 2.82 (br, 1H), 3.30 (br, 1H), 3.82 (m, 1H), 4.56 (s, 2H, -CH₂-O), 5.40 (t, 1H for one isomer), 5.55 (t, 1H for the other), 7.40 (m, 1H), 7.67 (d, 1H), 7.91 (m, 1H), 8.60 (br, 1H for one isomer, NH₂⁺), 8.90 (br, 1H for the other, NH₂⁺), 9.20 (br, 1H for one isomer, NH₂⁺), 9.35 (br, 1H for the other, NH₂⁺), 9.68 (br d+d, 1H, CONH), 10.80 (s, 1H, CONH-Ar), 11.00 (s, 1H, CONH-Ar).

***N*¹-(4-acetylphenyl)-*N*²-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.HCl (23)**

White solid - Yield: 42% (Diastereoisomeric mixture 3:2). LC-MS (APCI⁺) m/z: calcd for C₂₁H₂₆N₄O₄S: 430.17; found: 431.19 (M+H⁺). HPLC: 96.5%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.30-1.80 (m, 6H), 2.27 (s, 3H, -CH₃), 2.52 (s, 3H, -CH₃), 2.82 (br, 1H), 3.30, (br, 1H), 3.82 (m, 1H), 4.56 (s, 2H, -CH₂-O), 5.40 (t, 1H for one isomer), 5.55 (t, 1H for the other), 7.90 (s, 4H, ArH), 8.60 (br, 1H for one isomer, NH₂⁺), 8.95 (br, 1H for the other, NH₂⁺), 9.10 (br, 1H for one isomer, NH₂⁺), 9.25 (br, 1H for the other, NH₂⁺), 9.65 (d, 1H for one isomer, CONH), 9.71 (d, 1H for the other, CONH), 10.80 (s, 1H, CONH-Ar), 11.00 (s, 1H, CONH-Ar).

***N*¹-(3-chloro-4-fluorophenyl)-*N*²-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.2HCl (24)**

White solid - Yield: 33% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₁₉H₂₂ClFN₄O₃S: 440.11; found: 441.13 (M+H⁺). HPLC: 92.7%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.30-1.80 (m, 6H), 2.27 (s, 3H, -CH₃), 2.82 (br, 1H), 3.30, (br, 1H), 3.82 (br, 1H), 4.56 (s, 2H, -CH₂-O), 5.40 (t, 1H for one isomer), 5.55 (t, 1H for the other), 7.40 (t, 1H, ArH), 7.82 (m, 1H, ArH), 8.08 (m, 1H, ArH), 8.60 (br, 1H for one isomer, NH₂⁺), 8.95 (br, 1H for the other, NH₂⁺), 9.19 (br, 1H for one isomer, NH₂⁺), 9.40 (br, 1H for the other, NH₂⁺), 9.68 (d+d, 1H, CONH), 10.80 (s, 1H, CONH-Ar), 11.00 (s, 1H, CONH-Ar).

***N*¹-(4-fluorophenyl)-*N*²-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.HCl (25)**

White solid - Yield: 50% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₁₉H₂₃FN₄O₃S: 406.15; found: 407.16 (M+H⁺). HPLC: 94.7%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.30-1.80 (m, 6H), 2.27 (s, 3H, -CH₃), 2.80-3.00 (br, 1H), 3.30 (br, 1H), 3.82 (m, 1H), 4.51(s, 2H, -CH₂-O), 5.40 (t, 1H for one isomer), 5.51 (t, 1H for the other), 7.12 (m, 2H, ArH), 7.75 (m, 2H, ArH), 8.60 (br, 1H for one isomer, NH₂⁺), 8.95 (br, 1H for the other, NH₂⁺), 9.19 (br, 1H for one isomer, NH₂⁺), 9.40 (br, 1H for the other, NH₂⁺), 9.65 (d+d, 1H, CONH), 10.70 (s, 1H for one isomer, CONH-Ar), 10.75 (s, 1H for the other, CONH-Ar).

***N*¹-(2-fluoro-4-methylphenyl)-*N*²-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.HCl (26)**

White solid - Yield: 39% (Diastereoisomeric mixture 1:9). LC-MS (APCI⁺) m/z: calcd for C₂₀H₂₅FN₄O₃S: 420.16; found: 421.19 (M+H⁺). HPLC: 95.1%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.30-1.80 (m, 6H), 2.27 (s, 3H, -HetAr-CH₃), 2.31 (s, 3H, Ar-CH₃), 2.75-3.00 (br, 1H), 3.30 (br, 1H), 3.82 (m, 1H), 4.51(s, 2H, -CH₂-O), 5.40 (t, 1H for one isomer), 5.51 (t, 1H for the other), 7.02 (d, 1H, ArH), 7.10 (d, 1H, ArH), 7.50 (t, 1H, ArH), 8.51 (br, 1H for one isomer, NH₂⁺), 8.61 (br, 1H for the other, NH₂⁺), 9.10 (br d, 1H for one isomer, NH₂⁺), 9.25 (br, 1H for the other, NH₂⁺), 9.60 (d, 1H for one isomer, CONH), 9.67 (d, 1H for the other, CONH), 10.10 (s, 1H for one isomer, CONH-Ar), 10.20 (s, 1H for the other, CONH-Ar).

***N*¹-(3-fluoro-4-methylphenyl)-*N*²-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.HCl (28)**

White solid - Yield: 34% (Diastereoisomeric mixture 2:3). LC-MS (APCI⁺) m/z: calcd for C₂₀H₂₅FN₄O₃S:420.16; found: 421.19 (M+H⁺). HPLC: 95.8%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.30-1.80 (m, 6H), 2.26 (s, 3H, -CH₃), 2.80 (m, 1H), 3.30, (br, 1H), 3.80 (m, 1H), 4.56 (s, 2H, CH₂-OH), 5.40 (t, 1H for one isomer), 5.51 (t, 1H for the other), 7.24 (m, 1H, ArH), 7.55 (d, 1H, ArH), 7.66 (d, 1H, ArH), 8.51 (br, 1H for one isomer, NH₂⁺), 8.65 (br, 1H for the other, NH₂⁺), 9.11 (br, 1H for one isomer, NH₂⁺), 9.24 (br, 1H for the other, NH₂⁺), 9.61 (d, 1H for one isomer, CONH), 9.68 (d, 1H for the other, CONH), 10.75 (s, 1H for one isomer, CONH-Ar), 10.82 (s, 1H for the other, CONH-Ar).

***N*¹-cycloheptyl-*N*²-((5-(hydroxymethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.HCl (29)**

White solid - Yield: 28% (Diastereoisomeric mixture 2:3). LC-MS (APCI⁺) m/z: calcd for C₂₀H₃₂N₄O₃S: 408.22; found: 409.57 (M+H⁺). HPLC: 97.5%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.30-1.80 (m, 18H), 2.26 (s, 3H, -CH₃), 2.75-3.00 (m, 1H), 3.27, (m, 1H), 3.40, (m, 1H), 3.76 (m, 2H), 4.56 (s, 2H, CH₂-OH), 5.33 (t, 1H for one isomer), 5.50 (t, 1H for the other), 8.30-8.55 (br m, 1H, NH), 8.53 (br, 1H for one isomer, NH), 8.77 (br, 1H for the other, NH), 9.11 (br, 1H for one isomer, NH), 9.25 (br, 1H for the other, NH), 9.40 (br, 1H, CO-NH).

***N*¹-(4-chlorophenyl)-*N*²-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.HCOOH (30)**

White solid - Yield: 55% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₂₀H₂₅ClN₄O₃S:436.13; found: 437.30 (M+H⁺). HPLC: > 95.0%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.10-1.80 (m, 6H), 2.26 (s, 3H, -CH₃), 2.75-3.00 (m, 3H), 3.00-3.80 (12H +NH,+ H₂O signal), 5.00 (br, 1H for one isomer), 5.10 (br, 1H for the other), 7.41 (d, 2H), 7.82 (d, 2H), 9.10 (br 1H for one isomer, NH₂⁺) 9.38 (br, 1H for the other, NH₂⁺), 10.85 (s, 1H, NH).

***N*¹-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)-*N*²-(4-(trifluoromethyl)phenyl)oxalamide.2HCl (31)**

White solid - Yield: 39% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₂₁H₂₅F₃N₄O₃S: 470.16; found: 471.21 (M+H⁺). HPLC: 95.5%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.30-1.80 (m, 6H), 2.25 (s, 3H, -CH₃) 2.75-3.00 (m, 3H), 3.30, (br, 1H), 3.55 (m, 2H, -CH₂-CH₂-OH), 3.80 (br, 1H), 5.38 (t, 1H for one isomer), 5.55 (t, 1H for the other), 7.77 (d, 2H, ArH), 8.02 (d, 2H, ArH), 8.60 (br, 1H for one isomer, NH₂⁺), 8.91 (br, 1H for the other, NH₂⁺), 9.12 (br, 1H for one isomer, NH₂⁺), 9.26 (br, 1H for the other, NH₂⁺), 9.63 (d, 1H for one isomer, CONH), 9.70 (d, 1H for the other, CONH), 10.98 (s, 1H for one isomer, CONH-Ar), 11.20 (s, 1H for the other, CONH-Ar).

***N*¹-(2,4-difluorophenyl)-*N*²-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.HCl (32)**

White solid - Yield: 43% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₂₀H₂₄F₂N₄O₃S: 438.15; found 438.50 (M+H⁺). HPLC: 97.5%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.30-1.80 (m, 6H), 2.25 (s, 3H, -CH₃), 2.75-3.00 (m, 3H), 3.30, (br, 1H), 3.55 (m, 2H, -CH₂-CH₂-OH), 3.80 (br, 1H), 5.39 (t, 1H for one isomer), 5.50 (t, 1H for the other), 7.07 (m, 1H, ArH), 7.28 (m, 1H, ArH), 7.60 (m, 1H, ArH), 8.50 (br, 1H for one isomer, NH₂⁺), 8.74 (br, 1H for the other, NH₂⁺), 9.09 (br, 1H for one isomer, NH₂⁺), 9.21 (br, 1H for the other, NH₂⁺), 9.57 (d, 1H for one isomer, CONH), 9.70 (d, 1H for the other, CONH), 11.01 (s, 1H for one isomer, CONH-Ar), 11.32 (s, 1H for the other, CONH-Ar).

***N*¹-(3,4-difluorophenyl)-*N*²-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.2HCl (33)**

White solid - Yield: 32% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₂₀H₂₄F₂N₄O₃S: 438.15; found: 439.19 (M+H⁺). HPLC: 97.9%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.30-1.80 (m, 6H), 2.25 (s, 3H, -CH₃), 2.75-3.00 (m, 3H), 3.30, (br, 1H), 3.55 (m, 2H, -CH₂-CH₂-OH), 3.80 (br, 1H), 5.36 (t, 1H for one isomer), 5.50 (t, 1H for the other), 7.40 (m, 1H, ArH), 7.65 (m, 1H, ArH), 7.91 (m, 1H, ArH), 8.56 (br, 1H for one isomer, NH₂⁺), 8.90 (br, 1H for the other, NH₂⁺), 9.11 (br d, 1H for one isomer, NH₂⁺), 9.29 (br, 1H for the other, NH₂⁺), 9.61 (d, 1H for one isomer, CONH), 9.69 (d, 1H for the other, CONH), 10.85 (s, 1H for one isomer, CONHAr), 11.00 (s, 1H for the other, CONH-Ar).

***N*¹-(4-acetylphenyl)-*N*²-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.2HCl (34)**

White solid - Yield: 44% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₂₂H₂₈N₄O₄S: 444.18; found: 445.20 (M+H⁺). HPLC: 96.6%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.30-1.80 (m, 6H), 2.25 (s, 3H, -CH₃), 2.52 (s, 3H, CO-CH₃), 2.75-3.00 (br, 3H), 3.30, (br, 1H), 3.55 (m, 2H, -CH₂-CH₂-OH), 3.80 (br, 1H), 5.36 (t, 1H for one isomer), 5.50 (t, 1H for the other), 7.86 (s, 4H, ArH), 8.56 (br, 1H for one isomer, NH₂⁺), 8.90 (br, 1H for the other, NH₂⁺), 9.10 (br, 1H for one isomer, NH₂⁺), 9.25 (br, 1H for the other, NH₂⁺), 9.61 (d, 1H for one isomer, CONH), 9.69 (d, 1H for the other, CONH), 10.86 (s, 1H for one isomer, CONH-Ar), 11.00 (s, 1H for the other, CONH-Ar).

***N*¹-(3-chloro-4-fluorophenyl)-*N*²-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.2HCl (35)**

White solid - Yield: 35% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₂₀H₂₄ClFN₄O₃S: 454.12; found: 455.14 (M+H⁺). HPLC: 97.0%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H: 1.30-1.80 (m, 6H), 2.25 (s, 3H, -CH₃), 2.75-3.00 (m, 3H), 3.30, (br, 1H), 3.55 (m, 2H), 3.80 (br, 1H), 5.39 (t, 1H for one isomer), 5.51 (t, 1H for the other), 7.40 (t, 1H, ArH), 7.80 (m, 1H, ArH), 8.15 (m, 1H, ArH), 8.58 (br, 1H for one isomer, NH₂⁺), 8.90 (br, 1H for the other, NH₂⁺), 9.15 (br, 1H for one isomer, NH₂⁺), 9.30 (br, 1H for the other, NH₂⁺), 9.61 (d, 1H for one isomer, CONH), 9.69 (d, 1H for the other, CONH), 10.86 (s, 1H for one isomer, CONH-Ar), 11.00 (s, 1H for the other, CONH-Ar).

***N*¹-(4-fluorophenyl)-*N*²-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.HCl (36)**

White solid - Yield: 47% (Diastereoisomeric mixture 1:1.2). LC-MS (APCI⁺) m/z: calcd for C₂₀H₂₅FN₄O₃S: 420.16; found: 421.18 (M+H⁺). HPLC: 95.3%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H, 1.30-1.80 (m, 6H), 2.26 (s, 3H, -CH₃), 2.75-3.00 (m, 3H), 3.30 (br, 1H), 3.55 (m, 2H), 3.80 (br, 1H), 5.39 (t, 1H for one isomer), 5.50 (t, 1H for the other), 7.17 (m, 2H, ArH), 7.82 (m, 2H, ArH), 8.51 (br, 1H for one isomer, NH₂⁺), 8.90 (br, 1H for the other, NH₂⁺), 9.12 (br, 1H for one isomer, NH₂⁺), 9.28 (br, 1H for the other, NH₂⁺), 9.51 (d, 1H for one isomer, CONH), 9.60 (d, 1H for the other, CONH), 10.70 (s, 1H for one isomer, CONH-Ar), 10.76 (s, 1H for the other, CONH-Ar).

***N*¹-(2-fluoro-4-methylphenyl)-*N*²-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.2HCl (37)**

White solid - Yield: 65% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₂₁H₂₇FN₄O₃S: 434.18; found: 435.21 (M+H⁺). HPLC: 93.4%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H, 1.30-1.80 (m, 6H), 2.26 (s, 3H, -CH₃), 2.75-3.00 (m, 3H), 3.30, (br, 1H), 3.60 (m, 2H), 3.80 (br, 1H), 5.35 (t, 1H for one isomer), 5.50 (t, 1H for the other), 7.01 (d, 1H, ArH), 7.10 (d, 1H, ArH), 7.51 (m, 1H, ArH), 8.51 (br, 1H for one isomer, NH₂⁺), 8.65 (br, 1H for the other, NH₂⁺), 9.11 (br, 1H for one isomer, NH₂⁺), 9.24 (br, 1H for the other, NH₂⁺), 9.53 (d, 1H for one isomer, CONH), 9.70 (d, 1H for the other, CONH), 10.15 (s, 1H for one isomer, CONH-Ar), 10.24 (s, 1H for the other, CONH-Ar).

***N*¹-(3-fluoro-4-methylphenyl)-*N*²-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.HCl (38)**

White solid - Yield: 47% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₂₁H₂₇FN₄O₃S: 434.18; found: 435.24 (M+H⁺). HPLC: 96.1%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H, 1.30-1.80 (m, 6H), 2.20 (s, 3H, Ar-CH₃), 2.26 (s, 3H, HetAr-CH₃), 2.75-3.00 (m, 3H), 3.30 (br, 1H), 3.55 (m, 2H, -CH₂-CH₂-OH), 3.80 (s, 1H), 5.39 (t, 1H for one isomer), 5.57 (t, 1H for the other), 7.24 (t, 1H, ArH), 7.55 (d, 1H, ArH), 7.70 (d, 1H, ArH), 8.55 (br, 1H for one isomer, NH₂⁺), 8.91 (br, 1H for the other, NH₂⁺), 9.12 (br d, 1H for one isomer, NH₂⁺), 9.26 (br, 1H for the other, NH₂⁺), 9.58 (d, 1H for one isomer, CONH), 9.65 (d, 1H for the other, CONH), 10.75 (s, 1H for one isomer, CONH-Ar), 10.80 (s, 1H for the other, CONH-Ar).

***N*¹-(4-chloro-3-fluorophenyl)-*N*²-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.HCl (39)**

White solid - Yield: 50% (Diastereoisomeric mixture 4:5). LC-MS (APCI⁺) m/z: calcd for C₂₀H₂₄ClFN₄O₃S: 454.12; found: 455.18 (M+H). HPLC: 96.2%.

¹H NMR (DMSO-d₆, 50 °C, 400 MHz) δ_H, 1.30-1.80 (m, 6H), 2.26 (s, 3H, -CH₃), 2.75-3.00 (m, 3H), 3.30 (br, 1H), 3.60 (m, 2H, -CH₂-CH₂-OH), 3.80 (m, 1H), 5.35 (t, 1H for one isomer), 5.50 (t, 1H for the other), 7.55 (t, 1H, ArH), 7.65 (d, 1H, ArH), 7.90 (d, 1H, ArH), 8.59 (br, 1H for one isomer, NH₂⁺), 8.90 (br, 1H for the other, NH₂⁺), 9.11 (br, 1H for one isomer, NH₂⁺), 9.24 (br, 1H for the other, NH₂⁺), 9.60 (d, 1H for one isomer, CONH), 9.68 (d, 1H for the other, CONH), 10.92 (s, 1H for one isomer, CONH-Ar), 11.09 (s, 1H for the other, CONH-Ar).

***N*¹-cycloheptyl-*N*²-((5-(2-hydroxyethyl)-4-methylthiazol-2-yl)(piperidin-2-yl)methyl)oxalamide.HCl (40)**

Yellow oil -Yield: 57% (Diastereoisomeric mixture 1:1). LC-MS (APCI⁺) m/z: calcd for C₂₁H₃₄N₄O₃S: 422.24; found: 423.60 (M+H⁺). HPLC: 98.5%.

^1H NMR (DMSO- d_6 , 50 °C, 400 MHz) δ_{H} , 1.30-1.80 (m, 18H), 2.26 (s, 3H, $-\text{CH}_3$), 2.75-3.00 (m, 3H), 3.30 (br, 1H), 3.40 (m, 1H), 3.58 (m, 2H, $-\text{CH}_2-\text{CH}_2-\text{OH}$), 3.80 (m, 1H), 5.35 (t, 1H for one isomer), 5.50 (t, 1H for the other), 8.30-8.55 (br m, 1H σ , NH), 8.53 (br, 1H for one isomer, NH), 8.77 (br, 1H for the other, NH), 9.11 (br, 1H for one isomer, NH), 9.25 (br, 1H for the other, NH), 9.40 (br, 1H, CO-NH).

Measurement of antiviral activity

Pseudoviruses preparation

To prepare the X4-tropic pseudovirus NL4-3-HXB2-Luc 5×10^6 293T cells were seeded in a T75 flask and transfected 24 hrs later in 20ml medium with a mixture of 10 g of pNL4-3.Luc.R-E- DNA and 10 g of env expression vector pHXB2-env (X4) DNA using FuGENE 6 (Roche). Pseudovirus-containing supernatant was collected 2 days after transfection and stored in aliquots at -80°C . Pseudovirus was titered by infecting the TZM-bl cells to calculate the 50% tissue culture infectious dose (TCID₅₀). TZM-bl cells were plated in 96 wells plates at 10^4 cells/well 24hrs before infection. On the day of the infection 100 μl of serial twofold dilutions of pseudovirus were added to the cells. After 3 days incubation the cells were washed 2 times with PBS and lysed with 50 μl of cell culture lysis reagent (Promega). 20 μl of lysates were transferred to a white 96-well plate (Costar) and mixed with 100 μl of luciferase assay reagent (Luciferase Assay System, Promega). The luciferase activity was immediately measured with a Tecan infinite M1000 reader (Tecan). Wells producing relative luminescence units (RLU) 4 times the background were scored as positive and the TCID₅₀ was calculated by the Spearman-Kärber statistical method.

Single-cycle neutralization assay

The inhibitory activity of small molecules was tested on NL4-3-HXB2-Luc pseudotyped virus expressing Env of the HIV-1_{HXB2} (X4). Briefly, 100 l of TZM-bl cells at 1×10^5 cells/ml was added to the wells of a 96-well tissue culture plate and cultured at 37 °C overnight. 50 l of a test compound at graded concentrations was mixed with 50 l of the NL4-3-HXB2-Luc virus at about 100 TCID₅₀. After incubation at 37 °C for 30 min, the mixtures were added to the cells and incubated at 37 °C for 3 days. The cells were then harvested and lysed for measuring luciferase activity as described above.

Multi-cycle neutralization assay

The inhibitory activity of small molecules on infection by laboratory-adapted HIV-1 IIIB strain was determined as previously described²⁶. In brief, 1×10^4 MT-2 cells were infected with HIV-1 at 100 TCID₅₀ (50% tissue culture infective dose) (0.01MOI) in 200 μl medium in the presence or absence of small molecules at graded concentrations and incubated overnight. The culture supernatants were then removed and replaced with fresh media. On the fourth day post-infection, 100 l of culture supernatants were collected from each well, mixed with equal volume of 5% Triton X-100 and tested for p24 antigen by sandwich-ELISA. The percent inhibition of p24 production and IC₅₀ values were calculated by the GraphPad Prism software (GraphPad Software Inc.).

Determination of cytotoxicity

In TZM-bl cells

The cytotoxicity of small molecules in TZM-bl cells was measured by a colorimetric method using XTT (sodium 3'-(1-(phenylamino)-carbonyl)-3,4-tetrazolium-bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate), a light yellowish tetrazolium dye, as previously described²⁷. Briefly, 100 l of a compound at graded concentrations was added to equal volume of cells ($10^5/\text{ml}$) in wells of 96-well plates followed by incubation at 37 °C for 3 days and addition

of XTT (PolySciences, Inc., Warrington, PA). The soluble intracellular formazan was quantitated colorimetrically at 450 nm 4 h later. The percent of cytotoxicity and the CC₅₀ (the concentration for 50% cytotoxicity) values were calculated by the GraphPad Prism software (GraphPad Software Inc.).

In MT-2 cells

Cytotoxicity of small molecules in MT-2 cells was measured as previously described.²⁶ Briefly, 100 µl of a small molecule at graded concentrations was added to an equal volume of MT-2 cells (10⁵ cells/ml) in 96-well plates followed by incubation at 37 °C for 4 days. After addition of XTT, the soluble intracellular formazan was quantitated colorimetrically and the percent of cytotoxicity and the CC₅₀ values were calculated as above.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by an intramural fund from the New York Blood Center (AKD), the Intramural AIDS Targeted Antiviral Program (IATAP) and intramural funding from the National Institute of Allergy and Infectious Diseases.

Abbreviations used

SAR	Structure-activity relationship
LC-MS	Liquid Chromatography-Mass Spectroscopy
NMR	Nuclear Magnetic Resonance
DCM	dichloromethane
TBTU	N,N,N',N'-Tetramethyl-O-(Benzotriazol-1-yl)Uranium tetrafluoroborate

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1 (NBD-556); R = Cl

2 (NBD-557); R = Br

Figure-1.
Structure of **1** and **2** identifying different pharmacophoric regions.

Figure-2.

GLIDE-based docking of compound **6** in the “Phe43 cavity”. (a) Compound **6** is shown as docked inside the cavity. The 4-chlorophenyl moiety is located deep inside the cavity. The protonated “N” of piperidine ring is within salt-bridge (H-bond interaction) distance from Asp368; (b) the interactions of **6** with the residues in the “Phe43 cavity” of HIV-1 gp120 as mapped by the Maestro software in Schrödinger Suit 2011.

Figure-3.

Binding mode of two of the most active compounds, **27** and **39**. (a & b), the docking-based top scored two conformations of **27** showed distinct differences in binding of the piperidine-thiazolyl moiety. The piperidine NH formed an H-bond/salt-bridge with Asp368 in the second best scored conformation but not in the top scored conformation. (c & d) Both top scored two conformations of **39**, on the other hand, formed the H-bond/salt-bridge with Asp368. However, the ethanol moiety in the thiazolyl ring showed two distinct binding modes. The symbol notations are same as in Figure-2.

R¹ = Substituted anilines and aromatic amines as shown in Table-1 and 2; R = Primary amines as shown in Table-1

Scheme-1.

a. ClCOOEt, NEt₃, DCM; b. NaOH, EtOH, H₂O, c. TBTU, NEt₃, amine, d. HCl/
Dioxane (this step is only required to deprotect N-Boc amines)

Scheme-2.

a. ClC(=O)OEt, NEt₃, DCM; b. NaOH, EtOH, H₂O, c. TBTU, NEt₃, amine, d. HCl/Dioxane

Table-1

Structure and antiviral activity of oxalamide series compounds in single-cycle (TZM-bl) and multi-cycle (MT-2) inhibition assays.

No	R ¹	R ²	TZM-bl cells		MT-2 Cells	
			IC ₅₀ (μ M $\pm$ SD)	^a CC ₅₀ (μ M $\pm$ SD)	IC ₅₀ (μ M $\pm$ SD)	^a CC ₅₀ (μ M $\pm$ SD)
6	OH		4.3 $\pm$ 1.0	>22 (10%)	4.7 $\pm$ 0.6	>108 (40%)
7	OH		1.4 $\pm$ 0.4	~60.2	12 $\pm$ 1.1	>81

No	R ¹	R ²	TZM-bl cells		MT-2 Cells	
			IC ₅₀ (μ M $\pm$ SD)	aCC ₅₀ (μ M $\pm$ SD)	IC ₅₀ (μ M $\pm$ SD)	aCC ₅₀ (μ M $\pm$ SD)
8	OH		0.83 $\pm$ 0.14	~81	29.8 $\pm$ 3.5	>81
9	CH ₂ OH		4.6 $\pm$ 0.7	32.8 $\pm$ 0.6	4.2 $\pm$ 0.2	>62 (0%)
10	CH ₂ OH		0.65 $\pm$ 0.1	~59.4	13.1 $\pm$ 2.4	>78.5

No	R ¹	R ²	TZM-bl cells		MT-2 Cells	
			IC ₅₀ (μ M $\pm$ SD)	aCC ₅₀ (μ M $\pm$ SD)	IC ₅₀ (μ M $\pm$ SD)	aCC ₅₀ (μ M $\pm$ SD)
11	CH ₂ OH		1.0 $\pm$ 0.8	~85.4	30.5 $\pm$ 3.4	>78.5
12	CH ₂ OH		1.9 $\pm$ 0.25	36 $\pm$ 3.3	28.6 $\pm$ 1.6	~88
13	CH ₂ OH		19.7 $\pm$ 1.3	>84 (20%)	>52	>84
14	OH		4.8 $\pm$ 0.5	12.4 $\pm$ 0.7	15.4 $\pm$ 2.6	~45

No	R ¹	R ²	TZM-bl cells		MT-2 Cells	
			IC ₅₀ (μ M $\pm$ SD)	aCC ₅₀ (μ M $\pm$ SD)	IC ₅₀ (μ M $\pm$ SD)	aCC ₅₀ (μ M $\pm$ SD)
15	CH ₂ OH		4.2 $\pm$ 0.3	~4.9	16.2 $\pm$ 1.7	~42
16	CH ₂ OH		2.8 $\pm$ 0.3	16.6 $\pm$ 1.0	24.3 $\pm$ 2.2	>43

No	R	TZM-bl cells		MT-2 Cells	
		IC ₅₀ (μM ± SD)	<i>a</i> CC ₅₀ (μM ± SD)	IC ₅₀ (μM ± SD)	<i>a</i> CC ₅₀ (μM ± SD)
17		5.9 ± 0.4	~8	11.9 ± 1.2	10.7 ± 1.6
18		>99.6	>99.6	>24.9	>99.6

	No	R	TZM-bl cells		MT-2 Cells	
			IC ₅₀ ($\mu\text{M} \pm \text{SD}$)	aCC ₅₀ ($\mu\text{M} \pm \text{SD}$)	IC ₅₀ ($\mu\text{M} \pm \text{SD}$)	aCC ₅₀ ($\mu\text{M} \pm \text{SD}$)

	19		>99.6	>99.6	>24.9	>99.6
	1		4.2 $\pm$ 0.5	>60 (10%)	8 $\pm$ 0.2	~150

^aThe number in parenthesis indicates % toxicity at that dose.

Table-2

Structure-activity relationship analysis (SAR) of oxalamide compounds in single-cycle (TZM-bl) and multi-cycle (MT-2) assays.

No	R ¹	R ²	TZM-bl cells		MT-2 Cells	
			IC ₅₀ (μ M $\pm$ SD)	^a CC ₅₀ (μ M $\pm$ SD)	IC ₅₀ (μ M $\pm$ SD)	^a CC ₅₀ (μ M $\pm$ SD)
6		*-CH ₂ OH	4.3 $\pm$ 1.1	>22 (10%)	4.7 $\pm$ 0.6	>108 (40%)
20		*-CH ₂ OH	10.3 $\pm$ 1.6	>75.5 (10%)	4.6 $\pm$ 0.3	>75.5
21		*-CH ₂ OH	>87	>87	~110	>110
22		*-CH ₂ OH	8.4 $\pm$ 2.0	>87 (0%)	14 $\pm$ 3.8	>87
23		*-CH ₂ OH	>86	>86	~107	>107
24		*-OH	6.5 $\pm$ 1.0	>77.8 (0%)	9.5 $\pm$ 1.4	>77.8
25		*-CH ₂ OH	7.4 $\pm$ 0.4	>90 (0%)	47.9 $\pm$ 5.5	>113

N ^o	R ¹	R ²	TZM-bl cells		MT-2 Cells	
			IC ₅₀ ($\mu\text{M} \pm \text{SD}$)	aCC ₅₀ ($\mu\text{M} \pm \text{SD}$)	IC ₅₀ ($\mu\text{M} \pm \text{SD}$)	aCC ₅₀ ($\mu\text{M} \pm \text{SD}$)
26		*-CH ₂ OH	>88	>88	~66	>109
27		*-CH ₂ OH	1.6 $\pm$ 0.07	~58.4	3.8 $\pm$ 0.7	~77.8
28		*-CH ₂ OH	2.7 $\pm$ 0.41	>88 (0%)	5.3 $\pm$ 0.5	>88 (30%)
29		*-CH ₂ OH	>90	>90	39.3 $\pm$ 5	>90
30		*-CH ₂ CH ₂ OH	4.6 $\pm$ 0.7	32.8 $\pm$ 0.6	4.2 $\pm$ 0.3	>62 (0%)
31		*-CH ₂ CH ₂ OH	4.9 $\pm$ 0.5	>74 (35%)	17.4 $\pm$ 5.7	>39
32		*-OH	>84	>84	>105	>105
33		*-CH ₂ CH ₂ OH	8.2 $\pm$ 0.5	>78.2 (10%)	24.1 $\pm$ 4.6	>78.2
34		*-CH ₂ CH ₂ OH	>77.3	>77.3	24.5 $\pm$ 1.5	>48.3

No	R ¹	R ²	TZM-bl cells		MT-2 Cells	
			IC ₅₀ (μ M $\pm$ SD)	aCC ₅₀ (μ M $\pm$ SD)	IC ₅₀ (μ M $\pm$ SD)	aCC ₅₀ (μ M $\pm$ SD)
35		*-CH ₂ CH ₂ OH	9.0 $\pm$ 1.0	>75.8 (20%)	19.5 $\pm$ 1.8	~94.7
36		*-CH ₂ CH ₂ OH	~22	>88	21.6 $\pm$ 3.5	~109
37		*-CH ₂ CH ₂ OH	>78.8	>78.8	45.8 $\pm$ 3.5	>78.8
38		*-CH ₂ CH ₂ OH	1.66 $\pm$ 0.06	>85 (40%)	3.7 $\pm$ 0.7	~85
39		*-OH	1.98 $\pm$ 0.19	~61	3.5 $\pm$ 0.9	>41 (10%)
40		*-CH ₂ CH ₂ OH	>88	>88	19.6 $\pm$ 4	~60

^aThe number in parenthesis indicates % toxicity at that dose.