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Tuning Side Arm Electronics in Unsymmetrical Cyclotriazadisulfonamide (CADA) Endoplasmic Reticulum (ER) Translocation Inhibitors to Improve their Human Cluster of Differentiation 4 (CD4) Receptor Down-Modulating Potencies

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Abstract

Cyclotriazadisulfonamide prevents HIV entry into cells by down-modulating surface CD4 receptor expression through binding to the CD4 signal peptide. According to a two-site binding model, 28 new unsymmetrical analogs bearing a benzyl tail group and 9 bearing a cyclohexylmethyl tail have been designed and synthesized. The most potent new CD4 down-modulator (**40** (CK147): IC₅₀ 63 nM) has a 4-dimethylaminobenzenesulfonyl sidearm. One of the two sidearms was varied with substituents in different positions. This gave a range of CD4 down-modulation potencies that correlated well with anti-HIV-1 activities. The sidearms of 21 of the new benzyl-tailed analogs were modeled by means of quantum mechanical calculations. For CADA analogs with arenesulfonamide sidearms, the pIC₅₀ values for CD4 down-modulation correlated with the component of the electric dipole moment in the aromatic ring, suggesting that an attractive electronic interaction is a major factor determining the stability of the complex between the molecule and its target.

Graphical Abstract

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Supporting Information. Detailed synthetic procedures and characterization data (¹H and ¹³C NMR, IR, MS and combustion microanalysis) for compounds **9**, **10**, **43–48**, **52–57**, **62** and **63**, characterization data only for compounds **12–15**, **17–19**, **21**, **22**, **26–38** and **59–61**, and Table S1 listing antiviral activities of CADA compounds on X4-tropic and R5-tropic HIV-1 strains. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.

INTRODUCTION

Cyclotriazadisulfonamide (CADA) compounds inhibit replication of various strains of human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) by selectively down-modulating expression of cell-surface cluster of differentiation 4 (CD4).^{1–6} This type 1 integral transmembrane glycoprotein is expressed on immune cells and is the primary receptor required by HIV to enter host cells.^{7,8} Thus, CADA compounds are effective HIV entry inhibitors, a class of compounds that are of continuing interest for treatment of HIV infection because of their different resistance profile, compared to other anti-HIV agents.^{5,9–11} They are unique among viral entry inhibitors in that they do not simply bind to and block accessibility of a cell-surface receptor, they reduce CD4 expression levels below the threshold concentration required for HIV entry.² Accordingly, CADA compounds are also of potential interest for chemotherapy of various immunologically based diseases involving CD4⁺ T-cells, including asthma, rheumatoid arthritis, and diabetes, and also for preventing organ transplant rejection.^{12,13} While CD4 down-modulation may at first sight appear to be a therapeutic strategy that could compromise the immune system, the African green monkey avoids progression to acquired immunodeficiency syndrome (AIDS) when infected by SIV through decreased expression of CD4 on T cells, and the resulting CD4[–] cells are immunocompetent.¹⁴

Recent studies have shown that CADA (Figure 1a) down-modulates cell-surface CD4 by a novel mechanism of action.¹⁵ Surface expression of most type 1 transmembrane proteins requires transport of the nascent protein across the membrane of the endoplasmic reticulum (ER) during translation, a process known as co-translational translocation.^{16–19} The N-terminal signal peptide (SP) emerges first from the exit channel of the ribosome and is recognized by the signal recognition particle, which docks the translating ribosome to the mouth of an ER transmembrane channel (a translocon). The SP binds to the wall of this channel, in some cases (as with CD4)¹⁵ initially with the N terminus facing the ER interior (the lumen), then it reorients with the N terminus facing the cytosol,^{18,20–22} allowing SP cleavage, translocation of the protein into the lumen, and subsequent transport to the cell membrane. CADA inhibits requisite inversion of the SP, blocking translocation of the protein into the ER lumen, and diverts CD4 to the cytosol, where it is degraded by proteolysis.¹⁵ CADA directly binds the 25-residue signal peptide of human (and apparently, more generally primate) CD4, but not mouse CD4, which has less homology with the human CD4 SP. Hence, the significance of CADA compounds may go far beyond CD4 down-

modulation in that they might unlock a novel approach to suppressing other cell-surface proteins of therapeutic interest.

In order to deduce the physicochemical properties of the CADA binding site, we have performed several structure-activity relationship (SAR) studies in which the two sidearms (Ar^1 and Ar^2) and the tail group (R) were varied (Figure 1b).^{2,6,23,24} A quantitative structure-activity relationship (QSAR) study showed the importance of a relatively large, hydrophobic tail group (R) for high CD4 down-modulation and anti-HIV potency.²⁴ While CADA was found to interact mainly with the signal peptide of the nascent CD4 protein, the tail group may play an important role in anchoring the molecule to the hydrophobic lateral gate of the translocon. The QSAR study also showed that electron-donating substituents in the para positions of the two benzenesulfonyl sidearms increase potency.²⁴ More recently, this effect was found to be greatest in unsymmetrical CADA compounds with two different sidearms (Ar^1 Ar^2).²³ The most potent unsymmetrical analog for CD4 down-modulation in CHO•CD4-YFP cells was found to be VGD020²³ (**1**, Figure 1c), which was more potent than either symmetrical analog with either two *p*-methylbenzenesulfonyl (tosyl) or two *p*-methoxybenzenesulfonyl sidearms.

We also found previously that the 12-membered ring adopts the same folded conformation in X-ray structures of various CADA compounds.²⁴ There is a consistent twist about the isobutylene head group, placing the two side arms in two different orientations. This led to a “two-site” model in which the two side arm-binding regions are not symmetrically disposed and have different steric and electronic requirements (Figure 1d). We hypothesize that when CADA compounds bind, they stabilize a folded conformation of the CD4 SP, which prevents further translocation of the nascent CD4 protein toward the ER lumen. The folded SP may form the CADA binding site, placing certain amino acid side chains in close proximity with the two side arms. The purpose of this study was to vary substituents (Y) and substitution patterns on the nontosyl side arm to determine the nature of interactions of this sidearm with residues forming its binding site (Figure 1d).

RESULTS AND DISCUSSION

Synthesis.

A series of target CADA analogs was designed, based on the structure of compound **1**, which has a cyclohexylmethyl tail group, one tosyl side arm and one *p*-methoxybenzenesulfonyl sidearm.²³ The objective was to explore the steric and electronic requirements of the binding region for the nontosyl sidearm. Most of the new analogs were prepared by means of the five-step synthesis shown in Scheme 1, which had been developed previously for the synthesis of unsymmetrical CADA analogs.²³ A majority of the target compounds were designed with a benzyl tail group, which generally gives better yields and improves the ease of chromatographic purification during the later synthetic steps. A smaller number of cyclohexylmethyl tail analogs were also prepared, particularly to test the increase in potency relative to some of the more potent benzyl tail analogs. The substituted aromatic sidearm is introduced by sulfonylation in the fourth step of the sequence then palladium-catalyzed macrocyclization produces the target compounds. Many of these macrocycles

were then converted to additional targets by modification of substituents on the nontosyl aromatic ring, as described later.

The starting material, *N*-(3-aminopropyl)-*p*-toluenesulfonamide (**2**), was previously prepared in 48% yield by reaction of tosyl chloride with excess 1,3-diaminopropane in toluene, followed by recrystallization to remove the side product, 1,3-bis[*N*-(*p*-toluenesulfonamido)]propane.²³ We have found that the ratio of monotosyl to ditosyl derivatives in the crude product can be increased by conducting this reaction in dichloromethane (DCM), rather than toluene, resulting in an increased yield of 81%. The benzyl or cyclohexylmethyl tail groups are then installed quantitatively by condensation of **2** with benzaldehyde or cyclohexanecarboxaldehyde, followed by reducing the resulting Schiff base with sodium borohydride (Scheme 1).²³ Three-carbon chain elongation was achieved by reaction of **3** or **4** with commercially available *N*-(3-bromopropyl)phthalimide in acetonitrile (AN) in the presence of sodium carbonate and catalytic lithium iodide. Cyclohexylmethylamine **6** was purified by chromatography, as described previously,²³ while benzyl analog **5** was converted to the HCl salt, which was purified by trituration with diethyl ether. Both phthalimide intermediates were deprotected quantitatively to the corresponding primary amines, **7** and **8**, by hydrazinolysis in ethanol at room temperature.

Reaction of benzylamine intermediate **7** with 14 different sulfonyl chlorides under Schotten-Baumann conditions gave the 14 different open-chain disulfonamides (**9–22**) listed in the table in Scheme 1. Most of these sulfonyl chlorides were commercially available, but 3-(dimethylamino)benzenesulfonyl chloride,²⁵ *p*-(dimethylamino)benzenesulfonyl chloride,^{26,27} benzo[d][1,3]dioxole-5-sulfonyl chloride,²⁸ and *N*-methylpyrrole-3-sulfonyl chloride²⁹ were prepared by literature procedures. Intermediate **19** with a *N,N*-dimethylaminosulfonyl side arm was prepared to test the effect of an electron-donating group lacking an aromatic ring. Two cyclohexylmethyl tail intermediates, **23** and **24** were prepared by sulfonylation of cyclohexylmethylamine **8**. All 16 of the open-chain intermediates were cyclized by palladium catalyzed reaction with 2-methylene-1,3-propanebis(*t*-butylcarbonate), as described previously.²³ The yields for this macrocyclization reaction after rigorous purification of each target compound varied from 15 to 57%. In most cases, byproducts were observed, likely arising from differences in acidity between the two sulfonamide NH groups. The *t*-butoxide anion generated by decomposition of *t*-butylcarbonate in this reaction serves as the base to deprotonate the sulfonamide NH groups. Sodium carbonate is added to scavenge acidic protons that can quench *t*-butoxide. Selective deprotonation of more acidic sulfonamide NH groups can lead to bimolecular substitution reactions competing more effectively with macrocyclization of the intermediate. Finally, Scheme 1 also shows the conversion of *p*-fluorobenzenesulfonamide CK201 (**37**) to methylthio analog CK204 (**41**) via ipso substitution (S_NAr)³⁰ in low, but useful, yield.

Previous SAR studies showed that the CD4 down-modulation potency of CADA analogs is enhanced by electron-donating groups on benzenesulfonamide sidearms, particularly in the para position.^{23,24} Hence, the three nitro compounds with benzyl tail groups listed in Scheme 1 (**27**, **28**, and **34**) and previously prepared VGD023²³ (**42**), the *p*-nitro analog with a cyclohexylmethyl tail, were reduced with sodium borohydride and cupric acetate in ethanol/water³¹ to the corresponding primary amino compounds **43–46** in good to excellent

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yields (Scheme 2). Tertiary heterocyclic amines were also of interest, so the *p*-amino analog with a cyclohexylmethyl tail (**46**) was condensed with 2,5-dimethoxytetrahydrofuran in a modified Paal-Knorr synthesis^{32,33} to produce pyrrole **47** (Scheme 2). Also, **46** was treated with bis(2-chloroethyl) ether³⁴ to give morpholine analog **48** in low yield. Also shown in Scheme 2 is the reaction of *o*-nitro benzyl tail analog **27** with mercaptoethanol and DBU to give one-armed analog **49** by ipso attack and decomposition of the resulting Meisenheimer complex to SO₂ and 2-(*o*-nitrophenylthio)ethanol.³⁵ This compound was mainly of interest for the synthesis of additional unsymmetrical analogs with one benzamide sidearm, as described later.

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Since *p*-methoxy sidearm analog **1** was the most potent CADA compound at the beginning of the current study, variations with *p*-oxygen substituents having different steric and electronic properties were of interest (Scheme 3). In the cyclohexylmethyl tail series, **1** was demethylated with boron tribromide to give phenol **51**, which was alkylated quantitatively to produce *p*-ethoxy analog **52**. To further probe steric tolerance of the binding site in this region and produce analogs that might potentially be conjugated to imaging tags or solid supports, phenol **51** was alkylated with *N*-(3-bromopropyl)phthalimide to give **53**, which was converted to primary amine **54** by hydrazinolysis. In the benzyl tail series, BBr₃ demethylation of **50** (VGD027²³) gave phenol **55**, which was alkylated with propargyl bromide to give *p*-propargyloxy analog **56**. Also shown in Scheme 3 is the conversion of **55** to the *N,N*-dimethylthiocarbamate ester **57**. This was intended as an intermediate in the synthesis of the *p*-thiol analog via a reported rearrangement,^{36,37} which did not succeed in this case.

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The final planned series of analogs has one tosyl side arm and also various benzamide sidearms, as shown in Scheme 4. This series was designed to determine if two arenesulfonamide sidearms are required for high potency and if the trends of substituent effects in arenesulfonamides are similar in benzamides. One-armed analog **49** was accordingly acylated with benzoyl chloride and various para substituted benzoyl chlorides in good yields, as shown in Scheme 4. By the same method used in the arenesulfonamide sidearm series, *p*-nitro analog **60** was also reduced to *p*-amino analog **62**. Finally, **49** was also treated with *N*-morpholinosulfonyl chloride³⁸ to prepare sulfamate **63**, in which the nontosyl sidearm contains an electron-donating tertiary amino group but lacks an aromatic ring (Scheme 4).

Potency.

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A total of 37 new CADA compounds were synthesized, 28 with benzyl tail groups and 9 with cyclohexylmethyl tails. Samples of each of the new compounds were tested for CD4 down-modulation, either in their analytically pure (>95%) free base forms or as hydrochloride salts, as indicated in the Experimental Section or Supporting Information for compositions determined by combustion microanalysis. As for previous CADA compounds,^{23,24} most of the samples tested were stoichiometric hydrates of the monohydrochloride salts (or the dihydrochloride salts when a basic site was present in a sidearm). The CD4 down-modulation potencies are described here as IC₅₀ values (concentration producing a 50% decrease in CD4 expression, measured in Chinese hamster ovary/CD4/yellow fluorescent

protein (CHO•CD4-YFP) cells after 24 h of drug treatment). The IC₅₀ values for all 28 new benzyl-tailed analogs are given in Table 1, along with that of previously reported reference compound **50**, which has a *p*-methoxybenzenesulfonyl sidearm.

The structure-activity relationships for benzyl-tailed compounds displayed in Table 1 show a number of interesting trends. As observed previously for symmetrical and unsymmetrical CADA compounds, electron-donating groups (EDGs) in the para position of the benzenesulfonamide sidearm contribute to high potency. The most potent compounds have the best EDGs in the para position, dimethylamino in **32** (IC₅₀ 0.17 μM), methylthio in **41** (IC₅₀ 0.23 μM), and methoxy in **50** (IC₅₀ 0.29 μM), while **27** with *para*-nitro, a strong electron-withdrawing group (EWG), is only weakly active (IC₅₀ 5.25 μM). Even in the absence of a phenyl ring on the sulfonyl group, dimethylamino and *N*-morpholino groups in **35** and **63** give significant potencies (IC₅₀ 1.58 and 1.08 μM, respectively). The benzamide sidearm compounds **58–62** are decidedly less potent than the corresponding sulfonamides, **50**, CADA²⁴ (IC₅₀ 0.40 μM), **27**, **25**, and **45**, suggesting either that the aromatic rings are oriented differently because of the nearly planar amide group or that the sulfonyl group is important as a double hydrogen-bond acceptor (HBA).

In the sulfonamide series, when an additional methoxy group is placed in the meta position of the *p*-methoxybenzenesulfonyl sidearm of **50** (IC₅₀ 0.29 μM), potency is unchanged (IC₅₀ 0.31 μM for **31**), within experimental error, suggesting that the total electron density of the benzene ring is not important. When the second methoxy group is moved to the ortho position in **30**, potency is reduced (IC₅₀ 1.35 μM), suggesting that an ortho group could disrupt the binding conformation of this sidearm. Replacement of the *p*-methoxy group of **50** with trifluoromethoxy in **29** increases the IC₅₀ value by almost 1 μM, while replacement of methoxy by propargyl in **56** increases it by only about 0.1–0.2 μM, suggesting that electronics are more important than sterics in this position. Finally, replacing OMe or NMe₂ with OH or NH₂, respectively, greatly decreases potency, despite the similar electron donating abilities of OR vs. OH and NR₂ vs. NH₂. The increases in IC₅₀ value for these changes are ca. 3.3 μM (**50** to **55**) and 2.2 μM (**32** to **45**), respectively. This suggests that the introduction of hydrogen-bond donating (HBD) groups on the benzene ring may greatly change the manner in which the molecule binds and is oriented within the binding site.

The IC₅₀ values for the 9 new cyclohexylmethyl-tailed analogs are given in Table 2, along with that of previously reported reference compound **1**, which has a *p*-methoxybenzenesulfonyl side arm. All of the new cyclohexylmethyl tail compounds also have a single substituent in the para position of the nontosyl sidearm. Replacing the benzyl tail with the more hydrophobic cyclohexylmethyl group increases the potency of the reference compound **50** by a factor of 2 for **1** (IC₅₀ 0.15 μM). For the compounds with the best EDG (dimethylamino), the cyclohexylmethyl tail group increases potency by a factor of 3: IC₅₀ 0.17 μM for **32** to 0.063 μM for **40**, which is now the most potent CADA analog synthesized to date. As observed in the benzyl-tailed series, replacing NMe₂ with HBD group NH₂ greatly decreases potency (IC₅₀ 1.0 μM for **46**), and the same effect is observed for replacement of OMe by OH (IC₅₀ 0.15 for **1** vs. 1.14 μM for **51**). Replacing NMe₂ in **40** with heterocyclic substituents *N*-pyrrolyl or *N*-morpholino decreases potency by factors of 3 (IC₅₀ 0.18 μM for **47**) or 2 (IC₅₀ 0.13 μM for **48**), respectively, again suggesting a steric

constraint for nonlinear substituents in the binding site at this ring position. Compounds **52** (*p*-ethoxy, IC₅₀ 0.19 μM) and **53** (*p*-(3-(*N*-phthalimido)propyloxy), IC₅₀ 0.75 μM) further probe sterics in this area and show a very small decrease in potency for a change from OMe to OEt, and a more significant drop with the much larger substituent in **53**. Finally, replacement of the phthalimide group on this chain with NH₂ (compound **54**) makes CD4 down-modulation undetectable, again showing the deleterious effect of hydrogen bond donors on potency.

As a consequence of reduced CD4 cell surface levels caused by treatment with active compounds, HIV is prevented from infecting its target cells. Thus, we tested the activities of the compounds against the infection of the T-cell line MT-4 with the CXCR4-using HIV-1 laboratory strain NL4.3 (Tables 1 and 2). As observed for previously reported analogs,^{2,24} for the new unsymmetrical compounds a good correlation was seen between potencies for CD4 receptor down-modulation and for inhibition of HIV entry into CD4⁺ T-helper lymphocytes (Figure 2a). Only the most potent 27 new compounds were included in this correlation because the CD4 down-modulation assay is more sensitive than the anti-HIV assay, as can be gleaned from Figure 2a, and the 10 least potent compounds did not give exact IC₅₀ values for inhibition of HIV replication *in vitro*. In addition, the antiviral potencies of a representative set of analogs were determined against a CCR5-using and a CXCR4-using HIV-1 strain (BaL and NL4.3, respectively; Table S1 in Supporting Information). Comparison of the antiviral pIC₅₀ values of these compounds between both representative HIV-1 strains revealed a remarkable correlation (Figure 2b), indicating that the new unsymmetrical compounds exert equal anti-HIV activities, irrespective of the co-receptor used by the viral strain.

Molecular modeling.

The SAR study just discussed has revealed three important lessons about the effects of nontosyl side-arm substituents on CD4 down-modulation activity: 1) potency is enhanced by strong EDGs, particularly in the para position of the ring, 2) steric bulk in the para position only weakly decreases potency, and 3) HBD groups, such as OH and NH₂ greatly decrease potency. How can these effects be understood in the context of the hypothesis that CADA compounds inhibit co-translational translocation of CD4 by stabilizing a folded conformation of the CD4 signal peptide in the transmembrane channel (translocon)? The effect of introducing a HBD group is interesting because a new hydrogen bond should, in principle, stabilize a complex between a small molecule and a peptide or protein target. Adding a HBD group should increase water solubility and may affect distribution within the cell, but CADA compounds are otherwise quite hydrophobic and even an analog with an OH or NH₂ group should easily cross the cell membrane. Remembering that the SP is a relatively small target (only 25 amino acids), the binding site is likely to be poorly defined and may be conformationally stabilized by interactions between the SP and the protein subunits of the translocon. While the SP has been identified as the principal target of CADA compounds, part of the molecule, especially the hydrophobic tail, may also interact with the translocon. Introducing a good HBD group might disrupt the folded SP conformation needed to block translocation, either by changing the way the molecule interacts with the SP, or by

weakening its hold on the SP because of a new interaction between the molecule and a hydrogen-bond acceptor on the translocon.

We propose that non-HBD polar substituents on the nontosyl side arm directly affect the strength of the interaction between the small molecule and the CD4 SP. Our initial hypothesis was that the nontosyl side arm might act as a pi-electron donor, so EDGs enhance this interaction with the SP by increasing the electron density of this aromatic ring. Returning to Table 1, the significant activity of *o*-nitro analog **28** (IC₅₀ 0.70 μM) contradicts this hypothesis because NO₂ is well known as a powerful EWG. We then noticed that when the NO₂ group is moved from the ortho to the meta position in **34**, the IC₅₀ increases to 2.02 μM, and then to 5.25 μM for *p*-nitro analog **27**. This strongly suggested that the important parameter is not the electron density of the aromatic ring, but its *dipole moment*. The sulfonyl group itself is a strong EWG and produces an electric dipole in the benzene ring in the absence of other substituents, as shown in Figure 3a. Conjugating EDGs in the para position set up a push-pull situation, increasing the electric dipole component in the plane of the aromatic ring, with its positively-charged end toward the para ring carbon atom (Figure 3b). A strong EWG in the para position competes with the sulfonyl group and would produce a small dipole in the opposite direction if it is a better EWG (e.g. nitro) than sulfonyl (Figure 3c). Vector addition of the dipole produced by the sulfonyl group and the dipole generated by a strong EWG in the ortho position would produce a sizeable dipole moment oriented as shown in Figure 3d.

The activities of the dimethoxy analogs **30** and **31** are also consistent with this dipole moment theory. The 3,4-dimethoxybenzenesulfonyl analog **31** has the same potency (IC₅₀ 0.31 μM) as the *p*-dimethoxy reference compound **50** (IC₅₀ 0.29 μM), within experimental error. The second methoxy group should increase the net dipole, but change its orientation and possibly also produce steric hindrance. The 2,4-dimethoxy analog **30** is significantly less potent (IC₅₀ 1.35 μM). In this case, the two EDGs compete for orientation of the dipole, so its component along the axis between the sulfonyl group and the para position will be much smaller.

The dipole moment of a specific aromatic ring has not often been recognized as a dominant factor in small molecule-protein interactions, though two QSAR studies of sulfonamide carbonic anhydrase inhibitors have found dipole moments to be a dominant factor in modeling inhibition activity, one using the dipole component along the S-N bond³⁹ and the other using the total molecular dipole moment.⁴⁰ A more recent study of antileishmanial benzenesulfonamides also used the molecular dipole moment as a prominent factor in the QSAR model.⁴¹ No specific interaction between the sulfonamide dipole and amino acid residues was proposed in any of these publications, but in these cases in the literature, and also in the binding of CADA compounds to the CD4 SP, the arenesulfonamide may pi-stack with an aromatic side chain or with a planar functional group of the peptide/protein, such as an amide linkage or a polar side chain group.

To test the theory that the arenesulfonamide dipole is a major contributor to the strength of the interaction between CADA compounds and the CD4-SP, we performed quantum mechanical calculations on a series of 23 *N,N*-dimethylarenesulfonamides modeling the

nontosyl sidearms of 21 new benzyl-tailed CADA analogs shown in Schemes 1–4, plus CADA and **50**. The initial study group omitted dimethylaminosulfamide **35**, which lacks an arene ring, but included *N*-morpholinosulfamide **63**, in which the morpholine ring may bind in an orientation that is similar to the benzene rings of other analogs. The geometries were optimized for each molecule and aligned with the coordinate system shown in Figure 4a. Then the dipole moments were calculated, resulting in a total dipole moment (μ_{tot}) and the x, y, and z components of the dipole for each molecule (μ_x , μ_y , and μ_z , respectively), which are listed in Table 3. Chemists conventionally draw dipole vectors pointing from the positive charge to the negative charge, as in Figure 3, while the true mathematical sign of a dipole moment is opposite.⁴² Hence, the x component of the dipole produced by the sulfonamide substituent shown in Figure 3a is positive along the x-axis defined in Figure 4a, rather than negative. The energy-minimized structure of the side-arm model for compound **25** lacking an R substituent on the benzenesulfonamide ring is shown in Figure 4b, along with the orientation of the calculated dipole.

The total dipole moments and the x, y, and z components of the dipole moments for each model compound were plotted separately against the CD4 down-modulation pIC_{50} values of each CADA analog. The use of pIC_{50} values in these plots was based on the assumption that the IC_{50} value for CD4 down-modulation is primarily determined by the affinity of the compound for its target, the CD4 SP, in which case the pIC_{50} value should be roughly proportional to the negative enthalpy of binding ($-\Delta H$). The best correlation in these initial plots (not shown) was observed for the plot of the x component vs. pIC_{50} , but this correlation was poor ($R^2 = 0.173$). Considering that for this correlation to be meaningful, the ring attached to the sulfonyl group of all the compounds has to rest in the same position in the binding site, **36** and **63** lacking carbocyclic aromatic rings were excluded from the study. Also considering that potency-reducing HBD groups may alter the overall binding configuration, phenol **55** and anilines **43–45** were also excluded. Finally, thiocarbamate **57** was removed from the study group because the bulky thiocarbamate substituent would not likely be coplanar with the benzene ring and would have its own dipole outside the xy plane; this could confound the correlation. A plot of the x-component of the dipole moment of the remaining 16 model compounds against the CD4 down-modulation pIC_{50} values of the corresponding CADA compounds (Figure 5) shows a significant correlation ($R^2 = 0.625$).

The roughly linear correlation observed between the CD4 down-modulation pIC_{50} values of 16 CADA compounds and the x-components of the dipole moments calculated for the nontosyl sidearms of each compound suggests that the electron distribution in this sidearm plays a major role in stabilizing the complex with the CD4 SP. The x-component of the sidearm dipole lies in the plane of the aromatic ring and contains the C-S bond of the arenesulfonamide group. The sulfonamide EWG establishes a positive dipole along the x-axis ($\mu_x = 2.81$ D for **25**) and this is increased to 6.09 D by the strong EDG (*p*-NMe₂) of **32**, the most potent compound in the series. Accordingly, the weakest CD4 down-modulator in the correlation displayed in Figure 5 is *p*-nitro analog **27**, and in this case μ_x is actually reversed (−1.24 D). Moving the nitro group to the ortho position in **28** restores the effective orientation of μ_x and increases it relative to the unsubstituted **25**. Accordingly, **28** ($\mu_x = 3.20$ D) is more potent than **25**.

There are many possible reasons why the correlation shown in Figure 5 is significant, but not more perfect. First, compounds with different physiochemical properties may distribute differently between the aqueous medium and the cells, and also within the cells, resulting in different concentrations at the CD4 SP target within the translocon. Second, the positions of substituents on the aromatic ring alter the conformation of the arenesulfonamide group, especially when the substituent is in the ortho position. This could change van der Waals, hydrogen bonding, and polar interactions between the sulfonamide group and the binding site. Additionally, substituent changes do not simply alter molecular dipoles, they also change sterics and can produce new interactions within the binding site. Considering all this, it is remarkable that a significant correlation is observed between potency and a single molecular variable (μ_x). This indicates that the distribution of electrons in the nontosyl sidearm has a strong influence on affinity of the compound for the CD4 SP, and that this aromatic ring likely forms a strong polar interaction with one or more key amino acid residues or other functional groups within the binding site.

CONCLUSIONS

A total of 28 new unsymmetrical analogs bearing a benzyl tail group and 9 new unsymmetrical analogs bearing a cyclohexylmethyl tail have been designed, synthesized and tested for CD4 down-modulation and suppression of HIV-1 replication *in vitro*. This resulted in a wide range of CD4 down-modulation potencies, which correlated well with anti-HIV potencies. The most potent new CD4 down-modulator is compound **40** (IC₅₀ 63 nM), with a cyclohexylmethyl tail, a tosyl sidearm and a second 4-dimethylaminobenzenesulfonyl sidearm. For a series of 16 CADA analogs with arenesulfonamide side arms, a significant correlation was observed between the pIC₅₀ values of the compounds for CD4 down-modulation and the component of the electric dipole moment in the aromatic ring and along the C-S axis. This indicates that one side arm may pi-stack with a key functional group in the CD4 SP, and that the resulting attractive interaction is a major factor determining the stability of the complex between the molecule and the CD4 SP target. The results of this study should enable design of more potent CADA analogs and help determine molecular details of the interaction between CADA compounds and the CD4 SP, which will lead to a more complete understanding of this unique mechanism of action.

EXPERIMENTAL SECTION

Biological Evaluation – CD4 down-modulation.

To study the effect of the CADA analogs on CD4 expression, CHO cells, stably expressing CD4-YFP (human CD4 fused at its COOH-terminus to the yellow fluorescent protein), were treated for 24 h with serial dilutions (1:5) of the compounds at 37 °C. Cells were then washed, fixed in 1% formaldehyde and analyzed immediately. Data were acquired with a FACSCalibur flow cytometer (BD Biosciences) using the 488 nm laser line and CellQuest software (BD Biosciences). YFP was measured with a FL-1 detector and data were analyzed with FLOWJO software (Tree Star, San Carlos, CA). Down-modulation of CD4 was evaluated by the decrease in fluorescence intensity on CADA-treated cells relative to matched, untreated cells. To calculate the efficiency of CD4 down-modulation, the median

fluorescence intensity (MFI) for YFP for each sample was expressed as a percentage of the MFI of control cells (after subtracting the background MFI of the non-transfected control cells).

Viral replication.

MT-4 cells (obtained from the American Type Culture Collection) were seeded in 96-well plates at 3.75×10^5 cells/mL (150 μ L/well) filled with culture medium (Roswell Park Memorial Institute 1640, 10% (v/v) fetal bovine serum, 2 mM L-glutamine) and test compounds. Cells were pre-incubated with the compounds for 15 minutes before addition of the laboratory HIV-1 strain NL4.3 (obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH) at (50 μ L; 30 pg/well) and incubation at 37°C. Four days after infection, cell viability was quantified by the MTS/phenazine ethosulfate (PES) method. Absorbance was measured at 490 and 700 nm and used to calculate the 50% inhibitory concentration (IC_{50}) of each compound for viral replication.

To compare the antiviral effect of CADA analogs on X4-tropic and R5-tropic HIV-1 strains, we used $CD4^+$, $CXCR4^+$, and $CCR5^+$ TZM-bl cells expressing firefly luciferase and Escherichia coli β -galactosidase (a kind gift from Dr. G. Vanham, ITG, Antwerp, Belgium). Cells (50 μ L; 2×10^5 cells/mL) were resuspended in cell culture medium supplemented with 15 μ g/mL diethylaminoethyl-dextran (DEAE-Dextran; Sigma-Aldrich, Diegem, Belgium) and pre-incubated for 30 min at 37 °C in 96-well plates with test compounds diluted in 100 μ L of cell culture medium. Next, HIV-1 X4 strain NL4.3 or HIV-1 R5 strain BaL (also obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID) were added (50 μ L) according to the $TCID_{50}$ of the viral stocks. Two days post-infection, viral replication was measured by luminescence. Steadylite plus reagent (Perkin Elmer, Zaventem, Belgium) was mixed with lyophilized substrate according to manufacturer's guidelines. Supernatant (120 μ L) was removed and 75 μ L of Steadylite plus substrate solution was added to the 96-well plates. Next, the plates were incubated in dark for 10 min in a closed plate shaker (PHMP, Grant, Shepreth, Cambridgeshire, UK). Finally, cell lysis was scored microscopically and 100 μ L was transferred to white lumitrac 96-well plates (Greiner Bio-One, Frickenhausen, Germany) to measure the relative luminescence units (RLUs) using a SpectraMax L microplate reader and Softmax Pro software (Molecular Devices, Sunnyvale, CA, USA) with an integration time of 0.6 sec and a dark adapt of 5 min.

Cytotoxicity.

MT-4 cells were seeded in transparent 96-well plates at 10^4 cells per well in Dulbecco's Modified Eagle Medium (Life Technologies, Waltham, MA, USA) with 10% (v/v) fetal bovine serum and 10 mM HEPES. Subsequently, compounds were added and the cell/compound mixture was incubated at 37 °C for 48 h. The 50% cytotoxic concentration (CC_{50}) of each compound was determined from the reduction of viability of cells exposed to the compound, as measured by the MTS/phenazine ethosulfate (PES) method.

Computational Modeling.

Model *N,N*-dimethylsulfonamide molecules, including *N,N*-dimethylbenzenesulfonamides (Figure 3a), representing the sidearms of 21 new unsymmetrical disulfonamide CADA compounds with a benzyl tail, plus CADA and compound **50**, were built and visualized using the open-source molecular editor Avogadro.^{43,44} Geometry optimizations were then performed using Gaussian 03 Rev C.025⁴⁵ accessed via the web browser-based graphical user interface (GUI) WebMO (version 12.1).⁴⁶ Geometry optimizations of the model molecules were done using a B3LYP^{47–50} functional with a 6–31G(d)^{51–54} standard basis set. The benzene rings of the geometrically optimized molecular structures were then aligned approximately in the xy plane, keeping the S-C1-C4 atoms aligned long the x axis using the program Avogadro.^{43,44} In order to obtain accurate results, single-point calculations of the geometrically optimized and aligned molecular structures of the model molecules were performed at B3LYP^{47–50} with def2-TZVP^{55,56} basis set levels in the program ORCA 2.9.1.⁵⁷

Chemistry – General Methods.

All reactions were performed under an atmosphere of dry nitrogen. Reagents and solvents purchased from Aldrich Chemical Company, Acros Organics, or Fisher Scientific were of ACS reagent grade or better and were used without purification, unless indicated otherwise. Anhydrous AN was distilled from CaH₂. HCl (2 N) in methanol/water was prepared from 42 mL of con. aq. HCl (12.1 N) and 210 mL of methanol. For macrocyclization reactions, the disulfonamide intermediate (previously washed with aq. NaOH, as described for deprotonation of HCl salts) and 2-methylene-1,3-propanebis(*tert*-butylcarbonate) were dried *in vacuo* for at least 16 h then dissolved in anhydrous AN with dppb and Pd₂(dba)₃ added last. All the equipment required for macrocyclization reactions, including magnetic stir bars, spatulas, syringes and needles, were dried overnight at 110 °C. Trituration of HCl salts was done by sonication in anhydrous diethyl ether (5 – 15 mL, unless indicated otherwise) for 5 min and filtration, repeating the process two more times. “Overnight” periods are ca. 16 h. Organic solutions were dried with anhydrous Na₂SO₄, and sample drying *in vacuo* was performed at 0.1 mm and room temperature. Column chromatography was performed with Sorbent Technologies neutral alumina (50–200 μm) or standard grade silica (32–63 μm), unless noted otherwise. Chromatotron chromatography was performed with Sorbent Technologies neutral alumina (UV254 with gypsum). Melting points were measured on a Thomas-Hoover or Mel-Temp apparatus and are uncorrected. ¹H NMR (400 MHz or 500 MHz) and ¹³C NMR (100 MHz or 125 MHz) spectra were acquired on a Varian 400 or a Varian Unity+ 500 spectrometer. All chemical shifts (δ) are reported in ppm units relative to solvent resonances, as follows: ¹H, CDCl₃/TMS = 0.00, DMSO-d₆ = 2.50, CD₃OD = 3.31; ¹³C, CDCl₃ = 77.23, DMSO-d₆ = 39.7, CD₃OD = 49.15 ppm. Infrared spectra (IR) were recorded on a Nicolet 6700 FTIR spectrometer. Low-resolution mass spectra (MS) were acquired on a Waters Micromass ZQ electrospray ionization quadrupole mass spectrometer with positive ion detection (capillary voltage = 3.5 kV). High-resolution mass spectra (HRMS) were acquired on an Agilent 6230 TOF mass spectrometer. Samples for elemental analysis were dried at 78 °C (0.1 mm) for 2 days, unless stated otherwise, and microanalysis

was performed by NuMega Resonance Labs, Inc. Samples for biological testing were greater than 95% pure, as shown by combustion microanalysis.

***N*-(3-Aminopropyl)-*p*-toluenesulfonamide (2).**

A solution of 40 g (0.21 mol) of TsCl in 200 mL of DCM was added dropwise to 156 g (2.10 mol) of 1,3-diaminopropane, which was stirred vigorously at 0 °C. The resulting solution was stirred overnight and allowed to gradually warm to room temperature. The solvent was then removed by rotary evaporation, then a mixture of the residue and 800 mL of 1:1 (v/v) methanol/water was stirred for 30 min at room temperature. Cooling overnight in a refrigerator gave white crystals that were collected by vacuum filtration, washed with cold water and dried *in vacuo* yielding 0.45 g of 1,3-bis(*p*-toluenesulfonamido)propane side product. The filtrate was concentrated to dryness by rotary evaporation. The residue was dried *in vacuo* and recrystallized by dissolving in minimal boiling water and storage in a refrigerator for 2 d. The resulting white solid was collected by vacuum filtration, washed with cold water, and dried *in vacuo*, yielding 39 g (81%) of the desired product, mp 112–115°C (lit.^{58,59,23} 113–115, 112–114, 114–117 °C).

***N*-(3-(*N'*-Phthalimido)propyl)-*N*-(3-(*p*-toluenesulfonamido)propyl)benzylamine (5).**

A mixture of 4.98 g (15.6 mmol) of **3**,²³ 1.58 g (14.9 mmol) of Na₂CO₃, 0.57 g (4.2 mmol) of LiI and 95 mL of AN was stirred at room temperature as a solution of 8.44 g (31.5 mmol) of *N*-(3-bromopropyl)phthalimide in 15 mL of AN was added dropwise over 30 min. The resulting mixture was stirred and boiled under reflux for 24 h, cooled to room temperature, then filtered through a fine porosity sintered glass funnel. The residue washed with 30 mL of AN then the combined filtrates were concentrated by rotary evaporation. The residue was dried *in vacuo* giving 8.1 g of a viscous brown oil, which was stirred at room temperature for 5 h with 95 mL of 2 N HCl in MeOH/H₂O. The mixture was concentrated to dryness by rotary evaporation then dried *in vacuo*. The residue was triturated with ether (3 × 50 mL) then dried *in vacuo*, yielding 8.22 g (97 %) of **5**•HCl as a beige solid, mp 197–201 °C (dec). ¹H NMR (500 MHz, CDCl₃) δ 11.99 (bs, 1 H, NH⁺), 7.83 (m, 2 H, Phth), 7.73 (m, 4 H, *o*-Ts, Phth), 7.56 (m, 2 H, *o*-Bn), 7.28 (m, 5 H, *m*-Ts, *m,p*-Bn), 6.37 (bs, 1 H, TsNH), 4.34 (m, 2 H, CH₂Ph), 3.73 (t, 8 Hz, 2 H, CH₂NPhth), 3.26 (bs, 2 H, CH₂NBn), 3.13 (bs, 2 H, CH₂NBn), 3.06 (t, 8 Hz, 2 H, CH₂NHTs), 2.41 (s, 3 H, CH₃), 2.30 (m, 2 H, CCH₂C), 2.18 (m, 2 H, CCH₂C). ¹³C NMR (125 MHz, CD₃OD) δ 168.3, 143.6, 137.0, 134.1, 131.9, 130.6, 129.8, 129.5, 126.7, 122.9, 56.8, 50.3, 49.6, 39.6, 34.2, 23.8, 22.8, 20.0. IR (neat, cm⁻¹) 3092 (w), 2493 (w), 1767 (w), 1705 (s), 1398 (m), 1329 (m), 1156 (s), 1094 (m), 1025 (m), 811 (m), 759 (m), 717 (s), 661 (m). MS *m/z* 506 (MH⁺). Anal. Calcd. for C₂₈H₃₁N₃O₄S•HCl: C, 62.04; H, 5.95; N, 7.75. Found: C, 61.70; H, 6.33; N, 7.97. A mixture of **5**•HCl, 70 mL of 2 N aq. NaOH, 70 mL of DCM and 70 mL of sat. aq. NaCl was stirred at room temperature for 5 h, then the layers were separated. The aqueous layer was extracted with DCM (2 × 50 mL). The combined organic solutions were washed with 50 mL of sat. aq. NaCl, then dried. Filtration, rotary evaporation and drying *in vacuo* gave 7.35 g (93 %) of pure **5**. ¹H NMR (400 MHz, CDCl₃/TMS) δ 7.85 (d, 8 Hz, 2 H, Phth), 7.71 (m, 4 H, *o*-Ts, Phth), 7.24 (m, 7 H, Bn, *m*-Ts), 5.99 (bs, 1 H, TsNH), 3.59 (t, 6 Hz, 2 H, CH₂NPhth), 3.48 (s, 2 H, CH₂Ph), 3.04 (m, 2 H, CH₂NHTs), 2.46 (t, 6 Hz, 2 H, CH₂NBn), 2.42 (t, 6 Hz, 2 H, CH₂NBn), 2.35 (s, 3 H, CH₃), 1.80 (m, 2 H, CCH₂C), 1.65 (m, 2 H,

CCH₂C). ¹³C NMR (100 MHz, CDCl₃) δ 168.3, 142.9, 137.3, 134.1, 133.9, 132.0, 129.5, 129.1, 128.4, 127.1, 123.3, 123.2, 59.0, 58.5, 51.8, 50.9, 42.3, 36.0, 25.9, 21.4.

***N*-(3-Aminopropyl)-*N*-(3-(*p*-toluenesulfonamido)propyl)benzylamine (7).**

A mixture of 8.32 g (16.5 mmol) of **5**, 12.5 mL of hydrazine monohydrate and 240 mL of EtOH was stirred at room temperature overnight. The reaction mixture was filtered and the residue was washed with 20 mL of EtOH. The combined filtrates were concentrated to minimum volume by rotary evaporation, made basic (pH 10) with aq. NaOH then extracted with DCM (3 × 50 mL). The combined extracts were dried, filtered, and concentrated by rotary evaporation. The residue was dried *in vacuo* to give 6.2 g (100 %) of **7** as a light yellow, viscous oil. ¹H NMR (400 MHz, CDCl₃) δ 7.70 (d, 8 Hz, 2 H, *o*-Ts), 7.23 (m, 7 H, Bn, *m*-Ts), 3.47 (s, 2 H, CH₂Ph), 2.98 (t, 6 Hz, 2 H, CH₂NHTs), 2.67 (t, 6 Hz, 2 H, CH₂NH₂), 2.43 (m, 4 H, CH₂N), 2.42 (s, 3 H, CH₃), 1.61 (m, 4 H, CCH₂C). ¹³C NMR (100 MHz, CDCl₃) δ 142.9, 138.8, 137.4, 129.5, 129.0, 128.4, 127.1, 127.0, 52.7, 51.4, 42.8, 40.3, 30.1, 25.6, 21.5. IR (neat, cm⁻¹) 3384 (w), 2942 (w), 2859 (w), 1696 (w), 1631 (w), 1453 (m), 1319 (m), 1150 (s), 1085 (m), 816 (m), 715 (s), 694 (s). MS *m/z* 376 (MH⁺). Anal. Calcd. for C₂₀H₂₉N₃O₂S•1.25H₂O: C, 60.35; H, 7.98; N, 10.56. Found: C, 60.12; H, 7.88; N, 10.60.

Procedure for synthesis of 11–15, 17–19, 21 and 22.

Benzo[d][1,3]dioxole-5-sulfonyl chloride⁶⁰ and 3-(dimethylamino)benzenesulfonyl chloride⁶¹ were prepared according to literature procedures. Following is a representative example: ***N'*-(*p*-nitrobenzenesulfonyl)-*N''*-(*p*-toluenesulfonyl)-[*N,N*-bis(3-aminopropyl)benzylamine] (11)**. A mixture of 0.62 g (1.7 mmol) of **7**, 0.37 g (1.7 mmol) of *p*-nitrobenzenesulfonyl chloride, 11 mL of DCM, 11 mL of sat. aq. Na₂CO₃ and 11 mL of sat. aq. NaCl was stirred at room temperature for 24 h, then the layers were separated. The aqueous layer was extracted with DCM (2 × 10 mL). The combined organic solutions were dried, filtered and concentrated by rotary evaporation. The residue was dried *in vacuo*, then stirred with 13 mL of 2 N HCl in MeOH/H₂O for 5 h. The solvent was removed by rotary evaporation and the residue was dried *in vacuo*. The resulting brown glassy solid was triturated with ether then dried *in vacuo*, yielding 0.84 g (85 %) of **11**•HCl as a brown solid. A mixture of 0.84 g of **11**•HCl, 20 mL of DCM, 20 mL of 2 N aq. NaOH and 20 mL of sat. aq. NaCl at was stirred room temperature for 4 h, then the layers were separated. The aqueous layer was extracted with DCM (2 × 15 mL). The combined organic solutions were dried, filtered and concentrated by rotary evaporation. The residue was dried *in vacuo* yielding 0.75 g (81 %) of pure **11**. ¹H NMR (500 MHz, CDCl₃) δ 8.24 (d, 9 Hz, 2 H, *m*-ArSO₂), 7.92 (d, 9 Hz, 2 H, *o*-ArSO₂), 7.62 (d, 8 Hz, 2 H, *o*-Ts), 7.24 (d, 8 Hz, 2 H, *m*-Ts), 7.19 (m, 2 H, *m*-Bn), 7.13 (m, 3 H, *o,p*-Bn), 5.58 (bs, 2 H, NH), 3.38 (s, 2 H, CH₂Ph), 2.95 (t, 6 Hz, 2 H, CH₂NHSO₂Ar), 2.87 (t, 6 Hz, 2 H, CH₂NHTs), 2.40 (m, 4 H, CH₂NBn), 2.36 (s, 3 H, CH₃), 1.63 (m, 4 H, CCH₂C). ¹³C NMR (125 MHz, CD₃OD) δ 146.0, 143.5, 138.0, 136.7, 129.7, 129.1, 128.6, 128.3, 127.5, 127.1, 124.3, 58.9, 52.4, 52.0, 42.4, 26.2, 26.0, 21.5. A mixture of 0.1 g of **11** and 5 mL of 2 N HCl in MeOH/H₂O was stirred at room temperature for 5 h. The solvent was removed by rotary evaporation and the residue was dried *in vacuo*. The resulting brown glassy solid was triturated with ether, then dried *in vacuo* yielding 85 mg of pure **11**•HCl as a brown solid, mp 163–170 °C (dec). ¹H NMR (500

MHz, CD₃OD) δ 8.41 (d, 8 Hz, 2 H, *m*-ArSO₂), 8.06 (d, 8 Hz, 2 H, *o*-ArSO₂), 7.73 (d, 8 Hz, 2 H, *o*-Ts), 7.54 (m, 5 H, Bn), 7.40 (d, 8 Hz, 2 H, *m*-Ts), 4.37 (s, 2 H, CH₂Ph), 3.24 (m, 4 H, CH₂NBn), 2.99 (t, 7 Hz, 2 H, CH₂NHSO₂), 2.91 (t, 7 Hz, 2 H, CH₂NHSO₂), 2.42 (s, 3 H, CH₃), 1.98 (m, 4 H, CCH₂C). ¹³C NMR (125 MHz, CD₃OD) δ 150.2, 145.8, 143.6, 136.9, 130.9, 130.0, 129.5, 129.2, 128.0, 126.7, 124.1, 57.1, 50.0, 49.8, 39.7, 39.6, 23.9, 23.8, 20.0. IR (neat, cm⁻¹) 3078 (w), 2940 (w), 2557 (w), 1528 (m), 1455 (w), 1325 (m), 1172 (s), 1088 (m), 1072 (m), 913 (w), 854 (w), 812 (w), 738 (s), 685 (s), 658 (s). MS *m/z* 561 (MH⁺). Anal. Calcd. for C₂₆H₃₂N₄O₆S₂•HCl: C, 52.30; H, 5.57; N, 9.38. Found: C, 52.20; H, 5.74; N, 9.42.

***N'*-(*p*-(Dimethylamino)benzenesulfonyl)-*N''*-(*p*-toluenesulfonyl)-[*N,N*-bis(3-aminopropyl)benzylamine] (16).**

A mixture of 0.28 g (0.75 mmol) of **7**, 0.17 g (0.77 mmol) of *p*-(dimethylamino)benzenesulfonyl chloride,^{62,63} 5 mL of DCM, 5 mL of sat. aq. Na₂CO₃ and 5 mL of sat. aq. NaCl was stirred vigorously at room temperature for 24 h then the layers were separated. The aqueous layer was extracted with DCM (2 × 10 mL). The combined organic solutions were dried, filtered and concentrated by rotary evaporation. The residue was dried *in vacuo* then stirred with 10 mL of 2 N HCl in MeOH/H₂O at room temperature for 5 h. The solvent was removed by rotary evaporation and the residue was dried *in vacuo*, triturated with ether and dried *in vacuo*, yielding 0.43 g (91 %) of pure **16**•2HCl, mp 70–85 °C (dec). ¹H NMR (500 MHz, CD₃OD) δ 7.86 (d, 8 Hz, 2 H, *o*-ArSO₂), 7.71 (d, 8 Hz, 2 H, *o*-Ts), 7.53 (m, 2 H, *o*-Bn), 7.48 (m, 3 H, *m,p*-Bn), 7.37 (m, 4 H, *m*-ArSO₂, *m*-Ts), 4.32 (s, 2 H, CH₂Ph), 3.18 (m, 10 H, (CH₃)₂N, CH₂NBn), 2.90 (m, 4 H, CH₂NHSO₂), 2.40 (s, 3 H, TsCH₃), 1.95 (m, 4 H, CCH₂C). ¹³C NMR (125 MHz, CD₃OD) δ 143.5, 136.9, 130.9, 129.9, 129.5, 129.1, 129.0, 128.8, 126.7, 57.0, 50.0, 39.6, 23.6, 20.0. IR (neat, cm⁻¹) 2955 (w), 2949 (w), 2579 (m), 1708 (m), 1592 (m), 1451 (m), 1335 (s), 1219 (m), 1158 (s), 1109 (m), 1087 (m), 1023 (m), 974 (w), 941 (m), 870 (m), 812 (m), 800 (m), 736 (s), 690 (s), 650 (s), 586 (m). MS *m/z* 559 (MH⁺). Anal. Calcd. for C₂₈H₃₈N₄O₄S₂•2HCl•H₂O: C, 51.76; H, 6.52; N, 8.62. Found: C, 52.12; H, 6.93; N, 8.97. A mixture of 0.43 g of **16**•2HCl, 20 mL of DCM, 20 mL of 2 N aq. NaOH and 20 mL of sat. aq. NaCl was stirred at room temperature for 4 h, then the layers were separated. The aqueous layer was extracted with DCM (2 × 25 mL). The combined organic solutions were dried, filtered and concentrated by rotary evaporation. The residue was dried *in vacuo* giving 0.37 g (88 %) of **16** as a yellowish-brown, viscous residue. ¹H NMR (500 MHz, CDCl₃) δ 7.70 (d, 8 Hz, 2 H, *o*-Ts), 7.65 (d, 8 Hz, 2 H, *o*-ArSO₂), 7.25 (m, 7 H, *m*-Ts, Bn), 6.66 (d, 8 Hz, 2 H, *m*-ArSO₂), 3.32 (s, 2 H, CH₂Ph), 3.03 (s, 6 H, (CH₃)₂N), 2.90 (m, 4 H, CH₂NHSO₂), 2.42 (s, 3 H, TsCH₃), 2.40 (m, 4 H, CH₂NBn), 1.62 (m, 4 H, CCH₂C). ¹³C NMR (125 MHz, CDCl₃) δ 152.7, 143.1, 138.3, 137.0, 129.6, 129.0, 128.9, 128.4, 127.2, 127.0, 125.0, 110.9, 58.7, 52.1, 52.0, 42.3, 42.2, 40.1, 26.04, 26.01, 21.5.

***N'*-(3-*N*-Methylpyrrole-1-sulfonyl)-*N''*-(*p*-toluenesulfonyl)-[*N,N*-bis(3-aminopropyl)benzylamine] (20).**

A mixture of 0.13 g (0.72 mmol) of 3-*N*-methylpyrrole-1-sulfonyl chloride,^{64–66} 0.30 g (0.80 mmol) of **7** and 5 mL of DCM was stirred vigorously at room temperature for 24 h. Concentration by rotary evaporation and drying *in vacuo* gave 0.41 g (91 %) of crude

product, which was partially purified by silica column chromatography and then by chromatotron (70:30 (v/v) ethyl acetate/hexane), yielding 113 mg (27 %) of product. After many unsuccessful purification attempts, this impure material was used in the next step.

***N'*-(*p*-Butoxybenzenesulfonyl)-*N'*-(*p*-toluenesulfonyl)-[*N,N*-bis(3-aminopropyl)cyclohexylmethamphetamine] (23).**

Prepared from **8**²³ and *p*-butoxybenzenesulfonyl chloride by the method described for **11**. Free base: ¹H NMR (500 MHz, CDCl₃) δ 7.73 (m, 4 H, *o*-Ts, *o*-ArSO₂), 7.29 (d, 8 Hz, 2 H, *m*-Ts), 6.94 (d, 8 Hz, 2 H, *m*-ArSO₂), 4.01 (t, 6 Hz, 2 H, OCH₂), 2.96 (m, 4 H, CH₂NHSO₂), 2.42 (s, 3 H, TsCH₃), 2.34 (t, 6 Hz, 4 H, CH₂NCH₂Cy), 2.04 (d, 7 Hz, 2 H, CH₂Cy), 1.79 (quint, 8 Hz, 2 H, CH₂Et), 1.63 (m, 10 H, NCCCH₂CN, Cy), 1.49 (quint, 7 Hz, 2 H, CH₂Me), 1.34 (m, 1 H, CH), 1.15 (m, 2 H, Cy), 0.98 (t, 8 Hz, 3 H, CH₂CH₃), 0.78 (m, 2 H, Cy). ¹³C NMR (125 MHz, CDCl₃) δ 162.3, 143.1, 137.0, 131.2, 129.6, 129.1, 127.1, 114.6, 68.1, 62.3, 62.0, 53.0, 42.5, 35.6, 31.9, 31.0, 26.7, 26.6, 26.1, 26.0, 25.9, 21.5, 19.1, 13.8. **23**•HCl, mp 64–73 °C (dec). ¹H NMR (500 MHz, CD₃OD/TMS) δ 7.78 (d, 8 Hz, 2 H, *o*-Ts), 7.74 (d, 8 Hz, 2 H, *o*-ArSO₂), 7.40 (d, 8 Hz, 2 H, *m*-Ts), 7.07 (d, 8 Hz, 2 H, *m*-ArSO₂), 4.05 (m, 2 H, OCH₂), 3.31 (m, 2 H, CH₂NCH₂Cy), 3.22 (m, 2 H, CH₂NCH₂Cy), 2.94 (m, 2 H, CH₂Cy), 2.44 (m, 4 H, CH₂NHSO₂), 2.42 (s, 3 H, TsCH₃), 1.90 (quint, 8 Hz, 2 H, CH₂Et), 1.79 (m, 10 H, NCCCH₂CN, Cy), 1.70 (quint, 7 Hz, 2 H, CH₂Me), 1.51 (m, 1 H, CH), 1.38 (m, 2 H, Cy), 1.23 (t, 8 Hz, 3 H, CH₂CH₃), 1.00 (m, 2 H, Cy). ¹³C NMR (125 MHz, CD₃OD) δ 162.7, 143.6, 136.9, 131.1, 129.5, 128.8, 126.7, 114.5, 67.9, 59.6, 51.13, 51.07, 39.6, 33.2, 30.9, 30.3, 25.49, 25.45, 25.0, 23.43, 23.38, 20.1, 18.8, 12.7. IR (neat, cm⁻¹) 3285 (w), 3074 (w), 2930 (m), 2851 (m), 2594 (w), 1741 (w), 1598 (s), 1500 (m), 1320 (s), 1307 (s), 1256 (s), 1149 (s), 1100 (s), 968 (m), 827 (s), 705 (s), 660 (s). MS *m/z* 594 (MH⁺). Anal. Calcd. for C₃₀H₄₇N₃O₅S₂•HCl: C, 57.17; H, 7.68; N, 6.67. Found: C, 57.19; H, 8.00; N, 6.43.

***N'*-(*p*-(Dimethylamino)benzenesulfonyl)-*N'*-(*p*-toluenesulfonyl)-[*N,N*-bis(3-aminopropyl)cyclohexylmethamphetamine] (24):**

Prepared from **8**²³ and *p*-(dimethylamino)benzenesulfonyl chloride^{62,63} by the method described for **11**. Free base: ¹H NMR (500 MHz, CDCl₃) δ 7.74 (d, 8 Hz, 2 H, *o*-Ts), 7.68 (d, 8 Hz, 2 H, *o*-ArSO₂), 7.31 (d, 8 Hz, 2 H, *m*-Ts), 6.68 (d, 8 Hz, 2 H, *m*-ArSO₂), 3.05 (s, 6 H, (CH₃)₂N), 2.99 (t, 7 Hz, 2 H, CH₂NHSO₂), 2.95 (t, 7 Hz, 2 H, CH₂NHSO₂), 2.43 (s, 3 H, CH₃), 2.37 (t, 6 Hz, 4 H, CH₂NCH₂Cy), 2.05 (d, 7 Hz, 2 H, CH₂Cy), 1.63 (m, 10 H, CCH₂C, Cy), 1.34 (m, 1 H, CH), 1.15 (m, 2 H, Cy), 0.81 (m, 2 H, Cy). ¹³C NMR (125 MHz, CDCl₃) δ 152.8, 143.1, 137.1, 129.6, 128.9, 127.1, 110.9, 53.2, 52.9, 40.1, 31.9, 26.6, 26.0, 25.8, 21.5. **24**•2HCl, mp 73–93 °C (dec). ¹H NMR (500 MHz, CDCl₃) δ 8.08 (d, 8 Hz, 2 H, *o*-Ts), 7.95 (d, 8 Hz, 2 H, *o*-ArSO₂), 7.77 (d, 8 Hz, 2 H, *m*-Ts), 7.29 (d, 8 Hz, 2 H, *m*-ArSO₂), 3.28 (s, 3 H, (CH₃)₂N), 3.24 (s, 3 H, (CH₃)₂N), 3.18 (d, 7 Hz, 2 H, CH₂Cy), 2.99 (m, 4 H, CH₂NHCH₂Cy), 2.41 (m, 4 H, CH₂NHSO₂), 2.40 (s, 3 H, TsCH₃), 2.13 (m, 10 H, CCH₂C, Cy), 1.89 (m, 1 H, CH), 1.69 (m, 2 H, Cy), 1.13 (m, 2 H, Cy). ¹³C NMR (125 MHz, CDCl₃) δ 143.6, 137.0, 129.5, 128.5, 126.7, 112.3, 105.0, 59.7, 51.2, 51.0, 39.6, 33.2, 30.3, 25.5, 25.0, 23.4, 23.3, 20.1. IR (neat, cm⁻¹) 3270 (w), 3062 (w), 2924 (m), 2851 (m), 2576 (w), 1735 (m), 1595 (s), 1512 (m), 1436 (m), 1369 (s), 1304 (s), 1225 (m), 1145 (s),

1081 (s), 940 (m), 815 (s), 773 (m), 708 (m), 644 (s). MS m/z 565 (MH^+). Anal. Calcd. for $C_{28}H_{44}N_4O_4S_2 \cdot 2HCl$: C, 52.74; H, 7.27; N, 8.79. Found: C, 52.36; H, 6.89; N, 9.00.

Procedure for synthesis of 25–38 by macrocyclization. Following is a representative example: 1-(benzenesulfonyl)-9-benzyl-3-methylene-5-(*p*-toluenesulfonyl)-1,5,9-triazacyclododecane (25).

A mixture of 0.11 g (0.21 mmol) of **9**, 0.15 g (0.52 mmol) of 2-methylene-1,3-propanebis(*tert*-butylcarbonate), 5.4 mg (0.014 mmol) of 1,4-bis(diphenylphosphinobutane) (dppb), 60 mg (0.007 mmol) of tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$), 12.5 mg (0.120 mmol) of Na_2CO_3 and 11 mL of anhydrous AN was stirred and heated under reflux for 24 h then allowed to cool to room temperature. The reaction mixture was concentrated to dryness by rotary evaporation and the residue was dissolved in 15 mL of DCM. The solution was washed with sat. aq. $NaHCO_3$ (2×7 mL) and 7 mL of sat. aq. NaCl, dried, filtered and rotary evaporated to dryness. The crude product was purified by chromatotron chromatography (25:75 (v/v) ethyl acetate/hexane) giving 62 mg (52 %) of pure **25**. 1H NMR (400 MHz, $CDCl_3/TMS$) δ 7.78 (d, 8 Hz, 2 H, *o*- $PhSO_2$), 7.66 (d, 8 Hz, 2 H, *o*-Ts), 7.58 (m, 1 H, *p*- $PhSO_2$), 7.52 (m, 2 H, *m*- $PhSO_2$), 7.31 (d, 8 Hz, 2 H, *m*-Ts), 7.23 (m, 3 H, *m,p*-Bn), 7.14 (m, 2 H, *o*-Bn), 5.21 (s, 2 H, $C=CH_2$), 3.89 (s, 2 H, H2/4), 3.83 (s, 2 H, H4/2), 3.39 (s, 2 H, CH_2Ph), 3.16 (t, 7 Hz, 2 H, H6/12), 3.11 (t, 7 Hz, 2 H, H12/6), 2.43 (s, 3 H, CH_3), 2.37 (m, 4 H, H8,10), 1.65 (m, 4 H, H7,11). ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.5, 139.2, 138.8, 138.3, 135.4, 132.6, 129.8, 129.2, 128.8, 128.2, 127.3, 127.2, 127.0, 116.2, 59.0, 51.3, 50.7, 49.6, 49.5, 44.2, 43.9, 24.6, 24.4, 21.5. A mixture of 40 mg of **25** and 10 mL of 2 N HCl in MeOH/ H_2O was stirred for 5 h, concentrated by rotary evaporation and dried *in vacuo*. The residue was triturated with ether and dried *in vacuo*, giving 41.4 mg of pure **25**•HCl, mp 122 °C (dec). 1H NMR (400 MHz, $CDCl_3$) δ 12.37 (bs, 1 H, NH^+), 7.75 (d, 8 Hz, 4 H, *o*- $PhSO_2$, *o*-Ts), 7.60 (m, 5 H, *m,p*- $PhSO_2$, *o*-Bn), 7.45 (m, 3 H, *m,p*-Bn), 7.34 (d, 8 Hz, 2 H, *m*-Ts), 5.37 (s, 2 H, $C=CH_2$), 4.14 (s, 2 H, CH_2Ph), 3.73 (m, 4 H, H2,4), 3.39 (m, 2 H, H8/10), 3.17 (m, 6 H, H8/10,6,12), 2.45 (s, 3 H, CH_3), 2.29 (m, 2 H, H7/11), 2.00 (m, 2 H, H7/11). ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.4, 141.5, 136.5, 133.38, 133.35, 130.9, 130.1, 130.0, 129.43, 129.40, 128.8, 127.51, 127.5, 119.5, 58.6, 52.9, 52.7, 48.6, 48.5, 46.2, 21.5, 20.3, 20.2. IR (neat, cm^{-1}) 3059 (w), 2927 (w), 1598 (w), 1473 (m), 1333 (s), 1157 (s), 1088 (m), 950 (w), 915 (m), 733 (s), 690 (s), 674 (m), 653 (m), 580 (s), 545 (m), 534 (m). MS m/z 568 (MH^+). Anal. Calcd. for $C_{30}H_{37}N_3O_4S_2 \cdot HCl \cdot H_2O$: C, 57.91; H, 6.48; N, 6.75. Found: C, 57.73; H, 6.41; N, 6.59.

1-(*p*-Butoxybenzenesulfonyl)-9-cyclohexylmethyl-3-methylene-5-(*p*-toluenesulfonyl)-1,5,9-triazacyclododecane (39):

Prepared by the method described for **25**. Free base: 1H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, 8 Hz, 2 H, *o*-Ts), 7.67 (d, 8 Hz, 2 H, *o*- $ArSO_2$), 7.33 (d, 8 Hz, 2 H, *m*-Ts), 6.98 (d, 8 Hz, *m*- $ArSO_2$), 5.18 (s, 2 H, $C=CH_2$), 4.03 (t, 6 Hz, 2 H, OCH_2), 3.81 (s, 2 H, H2/4), 3.77 (s, 2 H, H4/2), 3.15 (m, 4 H, H6,12), 2.44 (s, 3 H, Ts CH_3), 2.26 (m, 4 H, H8,10), 1.96 (d, 7 Hz, 2 H, CH_2Cy), 1.80 (m, 2 H, CH_2Et), 1.62 (m, 10 H, H7,11, Cy), 1.52 (m, 2 H, CH_2Me), 1.25 (m, 1 H, CH), 1.15 (m, 2 H, Cy), 0.98 (t, 8 Hz, 3 H, CH_2CH_3), 0.70 (m, 2 H, Cy). ^{13}C (100 MHz, $CDCl_3$) 162.5, 143.4, 140.0, 135.7, 129.8, 129.7, 129.3, 127.2, 116.5, 114.7, 68.1, 62.1, 51.1, 50.6, 50.4, 50.3, 44.2, 44.0, 35.9, 31.9, 31.0, 26.8, 26.0, 24.5, 24.3, 21.5, 19.1,

13.8. **39**•HCl, mp 92–97 °C (dec). ¹H NMR (400 MHz, CD₃OD) δ 7.64 (d, 8 Hz, 2 H, *o*-ArSO₂), 7.60 (d, 8 Hz, 2 H, *o*-Ts), 7.34 (d, 8 Hz, 2 H, *m*-Ts), 7.02 (d, 8 Hz, *m*-ArSO₂), 5.30 (s, 1 H, C=CH₂), 5.29 (s, 1 H, C=CH₂), 3.99 (t, 6 Hz, 2 H, OCH₂), 3.64 (s, 4 H, H_{2,4}), 3.35 (m, 2 H, H_{8/10}), 3.23 (m, 2 H, H_{8/10}), 3.11 (m, 4 H, H_{6,12}), 2.99 (d, 7 Hz, 2 H, CH₂Cy), 2.36 (s, 3 H, TsCH₃), 1.92 (m, 2 H, CH₂Et), 1.70 (m, 10 H, H_{7,11}, Cy), 1.42 (m, 2 H, CH₂Me), 1.27 (m, 1 H, CH), 1.15 (m, 2 H, Cy), 0.97 (m, 2 H, Cy), 0.90 (t, 8 Hz, 3 H, CH₂CH₃). ¹³C (100 MHz, CD₃OD) 163.2, 144.4, 142.7, 133.4, 129.7, 129.6, 127.5, 127.4, 118.4, 114.7, 70.0, 60.5, 52.6, 48.5, 33.0, 30.8, 30.2, 25.5, 25.1, 25.0, 20.1, 20.0, 19.4, 18.8, 12.7. IR (neat, cm⁻¹) 3089 (w), 3086 (w), 2921 (m), 2851 (m), 1732 (m), 1595 (m), 1494 (m), 1338 (s), 1252 (s), 1188 (m), 1142 (m), 1109 (m), 1026 (m), 999 (m), 950 (m), 900 (m), 860 (m), 827 (m), 705 (m), 550 (m). MS *m/z* 646 (MH⁺). Anal. Calcd. for C₃₄H₅₁N₃O₅S₂•HCl•1/3CH₂Cl₂: C, 58.03; H, 7.47; N, 5.91. Found: C, 57.85; H, 7.35; N, 5.67.

9-Cyclohexylmethyl-1-(*p*-(dimethylamino)benzenesulfonyl)-3-methylene-5-(*p*-toluenesulfonyl)-1,5,9-triazacyclododecane (40).

Prepared by the method described for **25**. Free base: ¹H NMR (400 MHz, CDCl₃) δ 7.68 (d, 8 Hz, 2 H, *o*-Ts), 7.60 (d, 8 Hz, 2 H, *o*-ArSO₂), 7.31 (d, 8 Hz, 2 H, *m*-Ts), 6.68 (d, 8 Hz, 2 H, *m*-ArSO₂), 5.18 (m, 2 H, C=CH₂), 3.86 (s, 2 H, H₄), 3.70 (s, 4 H, H₂), 3.24 (t, 7 Hz, 2 H, H₆), 3.06 (s, 6 H, (CH₃)₂N), 3.04 (m, 2 H, H₁₂), 2.44 (s, 3 H, TsCH₃), 2.28 (t, 5 Hz, 2 H, H₈), 2.22 (t, 5 Hz, 2 H, H₁₀), 1.96 (d, 7 Hz, 2 H, CH₂Cy), 1.64 (m, 10 H, H_{7,11}, Cy), 1.54 (m, 2 H, Cy), 1.15 (m, 1 H, CH), 0.68 (m, 2 H, Cy). ¹³C (100 MHz, CDCl₃) δ 152.7, 143.2, 138.2, 136.1, 129.7, 129.1, 127.2, 123.2, 116.3, 110.9, 62.2, 52.0, 50.6, 50.1, 49.7, 44.5, 43.6, 40.0, 35.9, 31.8, 26.8, 26.0, 24.9, 23.9, 21.5. **40**•HCl, mp 111–113 °C (dec). ¹H NMR (400 MHz, CD₃OD) δ 7.69 (d, 8 Hz, 2 H, *o*-Ts), 7.64 (d, 8 Hz, 2 H, *o*-ArSO₂), 7.44 (d, 8 Hz, 2 H, *m*-Ts), 6.96 (d, 8 Hz, *m*-ArSO₂), 5.37 (m, 2 H, C=CH₂), 3.71 (m, 4 H, H_{2,4}), 3.43 (m, 2 H, H_{8/10}), 3.33 (m, 2 H, H_{8/10}), 3.29 (s, 6 H, (CH₃)₂N), 3.17 (m, 4 H, H_{6,12}), 2.97 (d, 7 Hz, 2 H, CH₂Cy), 2.44 (s, 3 H, TsCH₃), 2.00 (m, 4 H, H_{7,11}), 1.79 (m, 8 H, Cy), 1.15 (m, 1 H, CH), 0.68 (m, 2 H, Cy). ¹³C (100 MHz, CDCl₃) δ 144.4, 142.9, 133.4, 129.7, 129.2, 127.4, 118.2, 112.1, 70.0, 60.5, 52.8, 52.7, 48.6, 39.6, 33.0, 30.6, 30.2, 25.5, 25.1, 25.0, 20.1, 20.0, 19.9. IR (neat, cm⁻¹) 2927 (w), 2845 (w), 1735 (w), 1595 (s), 1515 (w), 1350 (s), 1142 (s), 1148 (m), 944 (w), 913 (w), 870 (w), 782 (s), 711 (w), 647 (s). MS *m/z* 617 (MH⁺). Anal. Calcd. for C₃₂H₄₈N₄O₄S₂•2HCl•2H₂O: C, 52.95; H, 7.50; N, 7.72. Found: C, 53.30; H, 7.33; N, 7.93.

9-Benzyl-3-methylene-1-(*p*-thiomethoxybenzenesulfonyl)-5-(*p*-toluenesulfonyl)-1,5,9-triazacyclododecane (41).

A solution of 0.50 g (0.85 mmol) of **37** and 0.30 g (1.1 mmol) of tetrabutylammonium chloride in 5 mL of anhydrous THF was added under nitrogen by cannula to a stirred suspension of 75 mg (1.1 mmol) of sodium thiomethoxide in 5 mL of anhydrous THF. The mixture was stirred overnight then 5 mL of MeOH was added. The solvents were removed by rotary evaporation. A solution of the residue in 50 mL of ethyl acetate was washed with 5% aq. NaOH, dried and rotary evaporated to afford 0.6 g of a dark brown oil. Alumina column chromatography (25:75 (v/v) ethyl acetate/hexane) gave the desired product, which was stirred with 50 mL of a solution of 2 N HCl in MeOH/H₂O for 4 h. Rotary evaporation

gave a residue that was triturated with ether and dried *in vacuo*, yielding 0.31 g (59%) of **41**•HCl. ¹H NMR (500 MHz, CD₃OD) δ 7.77 (d, 8 Hz, 2 H, *o*-Ts), 7.67 (d, 8 Hz, 2 H, *o*-ArSO₂), 7.56 (m, 5 H, Bn), 7.44 (d, 8 Hz, *m*-Ts), 7.13 (d, 8 Hz, 2 H, *m*-ArSO₂), 5.38 (s, 2 H, C=CH₂), 4.35 (s, 2 H, CH₂Ph), 3.88 (s, 3 H, SCH₃), 3.74 (s, 4 H, H_{2,4}), 3.42 (m, 2 H, H_{8/10}), 3.32 (m, 2 H, H_{8/10}), 3.16 (m, 4 H, H_{6/12}), 2.43 (s, 3 H, TsCH₃), 2.02 (m, 4 H, H_{7,11}). ¹³C NMR (125 MHz, CD₃OD) δ 163.7, 142.7, 133.4, 130.4, 129.9, 129.7, 129.6, 129.5, 129.2, 127.7, 127.4, 125.2, 114.3, 65.4, 58.1, 54.9, 52.3, 23.8, 20.14, 20.06, 14.0, 13.2, 7.8. IR (neat, cm⁻¹) 2924 (s), 2851 (m), 1735 (s), 1598 (s), 1670 (m), 1650 (m), 1347 (s), 1259 (s), 1220 (m), 1158 (s), 1103 (m), 1026 (m), 1002 (w), 980 (w), 941 (m), 901 (m), 837 (m), 770 (s), 702 (m), 647 (m). MS *m/z* 599 (100%, MH⁺-CH₃), 614 (30%, MH⁺). Anal. Calcd. for C₃₁H₃₉N₃O₄S₃•HCl: C, 57.26; H, 6.20; N, 6.46. Found: C, 57.08; H, 6.44; N, 6.84. Free base **41**: ¹H NMR (500 MHz, CDCl₃) δ 7.77 (d, 8 Hz, 2 H, *o*-Ts), 7.67 (d, 8 Hz, 2 H, *o*-ArSO₂), 7.32 (d, 8 Hz, *m*-Ts), 7.24 (m, 5 H, Bn), 7.05 (d, 8 Hz, 2 H, *m*-ArSO₂), 5.24 (s, 2 H, C=CH₂), 3.88 (s, 3 H, SCH₃), 3.82 (s, 4 H, H_{2,4}), 3.45 (s, 2 H, CH₂Ph), 3.13 (m, 4 H, H_{6/12}), 2.43 (s, 3 H, TsCH₃), 2.10 (m, 4 H, H_{8,10}), 1.67 (m, 4 H, H_{7,11}). ¹³C NMR (125 MHz, CDCl₃) δ 143.1, 137.0, 131.6, 129.6, 129.4, 129.2, 128.6, 128.2, 127.6, 127.4, 127.1, 125.4, 114.2, 58.5, 55.6, 51.5, 45.7, 41.8, 25.7, 21.5, 14.8, 8.6.

9-Benzyl-3-methylene-1-(*p*-toluenesulfonyl)-1,5,9-triazacyclododecane (**49**).

A mixture of 0.73 g (1.2 mmol) of **27**, 0.55 mL (0.56 g, 3.7 mmol) of DBU in 25 mL of AN was stirred at room temperature for 15 min then 0.10 mL (0.11 g, 1.4 mmol) of 2-mercaptoethanol was added. The mixture was stirred at room temperature for 24 h and passed through a short silica gel column, eluting with AN, then the eluent was concentrated by rotary evaporation. A mixture of the residue, 40 mL of 2-propanol, 40 mL of H₂O and 1.5 g (18 mmol) of NaHCO₃ was stirred for 24 h, diluted with 50 mL of sat. aq. NaCl and 2 N aq. NaOH was added dropwise until the pH reached 14. The mixture was extracted with DCM (3 × 30 mL) and the combined extracts were dried (Na₂SO₄) then concentrated by rotary evaporation. The residue was stirred with 2 N HCl in MeOH/H₂O for 30 min then the mixture was concentrated by rotary evaporation and the residue was dried *in vacuo* then triturated with ether. A solution of the residue in 50 mL of DCM was washed with 10 % aq. Na₂CO₃ (3 × 20 mL) then with 20 mL of sat. aq. NaCl then dried and concentrated by rotary evaporation. Silica column chromatography (1:1 (v/v) ethyl acetate/hexane) gave 0.37 g (73 %) of pure **49**. ¹H NMR (500 MHz, CDCl₃) δ 7.64 (d, 8 Hz, 2 H, *o*-Ts), 7.27–7.16 (m, 7 H, *m*-Ts, Bn), 5.10 (s, 1 H, C=CH₂), 5.08 (s, 1 H, C=CH₂), 3.95 (s, 2 H, H₂), 3.36 (s, 2 H, H₄), 3.20 (t, 6 Hz, 2 H, H₁₂), 3.18 (s, 2 H, CH₂Ph), 2.68 (t, 6 Hz, 2 H, H₁₀), 2.47 (t, 6 Hz, 2 H, H₈), 2.39 (s, 3 H, CH₃), 2.14 (t, 6 Hz, 2 H, H₆), 1.55 (m, 2 H, H₁₁), 1.44 (quint, 7 Hz, 2 H, H₇). ¹³C NMR (125 MHz, CDCl₃) δ 129.9, 129.8, 129.5, 128.8, 128.7, 128.2, 127.7, 127.2, 126.8, 59.0, 58.6, 58.4, 53.4, 49.2, 21.5. **49**•2HCl, mp 190 °C (dec). ¹H NMR (500 MHz, CDCl₃) δ 11.31 (bs, 1 H, NH⁺), 9.89 (bs, 2 H, NH₂⁺), 7.71 (m, 2 H, *o*-Ts), 7.56 (m, 2 H, *o*-Bn), 7.38 (m, 3 H, *m,p*-Bn), 7.32 (d, 8 Hz, 2 H, *m*-Ts), 5.76 (s, 1 H, C=CH₂), 5.46 (s, 1 H, C=CH₂), 4.46 (s, 2 H, H₄), 4.15–3.31 (m, 10 H, H_{2,8,10,12}, CH₂Ph), 2.41 (s, 3 H, CH₃), 2.30 (m, 2 H, H₇), 2.03 (m, 2 H, H₁₁). ¹³C NMR (125 MHz, CDCl₃) δ 144.6, 135.4, 132.6, 131.3, 130.1, 130.0, 129.3, 128.9, 127.6, 123.0, 58.9, 54.8, 48.1, 47.7, 45.6, 44.2, 42.6, 29.7, 21.7, 17.1. IR (neat, cm⁻¹) 3389 (m), 2955 (m), 2585 (m), 1705 (w), 1595 (m), 1454 (s), 1338 (s), 1158 (s), 1106 (m), 1087 (m), 1026 (w), 997 (m), 941 (m), 916 (m), 812 (m), 800

(m), 736 (m), 693 (s), 648 (m). MS m/z 428 (MH^+). Anal. Calcd. For $C_{24}H_{33}N_3O_2S \cdot 2HCl \cdot CH_2Cl_2 \cdot H_2O$: C, 49.76; H, 6.51; N, 6.96. Found: C, 49.68; H, 6.95; N, 7.26.

9-Cyclohexylmethyl-1-(*p*-hydroxybenzenesulfonyl)-3-methylene-5-(*p*-toluenesulfonyl)-1,5,9-triazacyclododecane (51).

A solution of 1.94 g (3.2 mmol) of **1** in 150 mL of anhydrous DCM was stirred at room temperature under nitrogen as 45 mL of 1.0 M BBr_3 in DCM was added dropwise. The mixture was stirred at room temperature for 21 h then cooled to 0 °C and 150 mL of NH_4OH (30 wt% in H_2O) was added slowly. The resulting mixture was stirred vigorously at room temperature for 48 h then the layers were separated. The organic layer was washed with H_2O (2×120 mL), dried, filtered, concentrated by rotary evaporation and dried *in vacuo*. Column chromatography on alumina (9:1 (v/v) EtOAc/MeOH) gave 1.72 g of partially protonated **51**. A solution of this crude product in 15 mL of DCM was stirred with 30 mL of sat. aq. NH_4OH for 0.5 h then the layers were separated. The organic layer was washed with water (2×20 mL), dried, filtered, concentrated by rotary evaporation and dried *in vacuo* to give 1.4 g (74%) of **51** as a yellow solid. 1H NMR (500 MHz, $CDCl_3$) δ 7.67 (d, 8 Hz, 2 H, *o*-Ts), 7.65 (d, 9 Hz, 2 H, *o*-ArSO₂), 7.33 (d, 8 Hz, 2 H, *m*-Ts), 6.93 (d, 9 Hz, 2 H, *m*-ArSO₂), 5.18 (s, 2 H, C=CH₂), 3.80 (s, 2 H, H₂/4), 3.78 (s, 2 H, H₄/H₂), 3.15 (m, 4 H, H₆,12), 2.44 (s, 3 H, CH₃), 2.29 (m, 4 H, H₈,10), 1.98 (d, 7 Hz, 2 H, CH₂Cy), 1.63 (m, 8 H, H₇,11,Cy), 1.26 (s, 1 H, Cy), 1.12 (m, 4 H, Cy), 0.70 (m, 2 H, Cy). ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.4, 143.6, 138.3, 135.4, 129.8, 129.6, 127.3, 116.7, 116.2, 62.1, 51.4, 50.7, 50.2, 50.0, 44.7, 44.5, 35.7, 31.9, 26.7, 26.0, 24.2, 24.0, 21.5. IR (neat, cm^{-1}) 3383 (w), 2927 (w), 2857 (w), 2802 (w), 1580 (w), 1453 (w), 1331 (w), 1155 (m), 1090 (w), 908 (s), 729 (s). MS m/z 590 (MH^+). Anal. Calcd for $C_{30}H_{43}N_3O_5S_2$: C, 61.09; H, 7.35; N, 7.12. Found: C, 59.62; H, 7.50; N, 7.14.

Synthesis of compounds 58–61.

Following is a representative example: **9-benzyl-3-methylene-5-(*p*-methoxybenzoyl)-1-(*p*-toluenesulfonyl)-1,5,9-triazacyclododecane (58)**. To a cold (0 °C) solution of 0.12 g (0.29 mmol) of **49** in 5 mL of DCM were added 54 mg (0.68 mmol) of pyridine and 0.14 g (0.82 mmol) of 4-methoxybenzoyl chloride. The mixture was stirred at room temperature for 48 h then concentrated by rotary evaporation. The resulting viscous yellow oil was shaken with 10 mL of water and 10 mL of $CHCl_3$ then the layers were separated. The aqueous layer was extracted with $CHCl_3$ (2×10 mL). The combined organic solutions were washed with 10 mL of 15 % aq. NaOH, dried, filtered and concentrated by rotary evaporation. The residue was dried *in vacuo* then chromatographed on silica gel (20:80 (v/v) ethyl acetate/hexane), giving 118 mg (73 %) of **58** as a colorless oil. 1H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, 8 Hz, 2 H, *o*-Ts), 7.36 (d, 8 Hz, 2 H, *o*-ArCO), 7.31 (d, 8 Hz, 2 H, *m*-Ts), 7.20 (m, 5 H, Bn), 6.85 (d, 8 Hz, 2 H, *m*-ArCO), 5.22 (s, 2 H, C=CH₂), 4.24 (s, 2 H, H₂/4), 3.81 (s, 3 H, OCH₃), 3.58 (s, 2 H, H₄/2), 3.45 (s, 2 H, CH₂Ph), 2.91 (m, 4 H, H₆,12), 2.61 (t, 6 Hz, 2 H, H₈/10), 2.43 (s, 3 H, TsCH₃), 2.36 (t, 6 Hz, 2 H, H₁₀/8), 1.99 (quint, 2 H, H₇/11), 1.78 (quint, 7 Hz, 2 H, H₁₁/7). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3, 160.7, 143.8, 139.3, 129.8, 128.8, 128.4, 128.1, 127.6, 126.9, 113.7, 58.7, 56.1, 55.3, 51.1, 48.6, 46.3, 27.0, 21.5. The free base was stirred with 30 mL of 2 N HCl in MeOH/ H_2O then solvent was removed by rotary

evaporation. The residue was dried *in vacuo*, triturated with ether then dried *in vacuo* to give 126 mg (73 % overall) of pure **58**•HCl, mp 149–151°C (dec). ¹H NMR (500 MHz, CD₃OD) δ 7.69 (d, 8 Hz, 2 H, *o*-Ts), 7.56 (m, 2 H, *o*-Bn), 7.48 (m, 3 H, *m,p*-Bn), 7.41 (m, 4 H, *m*-Ts, *o*-ArCO), 6.98 (d, 9 Hz, 2 H, *m*-ArCO), 5.42 (s, 1 H, C=CH₂), 5.18 (s, 1 H, C=CH₂), 4.36 (s, 2 H, CH₂Ph), 3.82 (s, 4 H, H₂/4), 3.42 (m, 4 H, H₈,10), 3.28 (s, 3 H, OCH₃), 3.22 (m, 4 H, H₆,12), 2.42 (s, 3 H, TsCH₃), 2.10 (m, 2 H, H₇/11), 1.96 (m, 2 H, H₁₁/7). ¹³C NMR (125 MHz, CD₃OD) δ 173.7, 161.4, 144.3, 134.6, 130.9, 129.9, 129.8, 129.2, 129.1, 128.2, 127.4, 127.1, 116.1, 113.7, 58.9, 55.9, 54.5, 20.2. IR (neat, cm⁻¹) 3343 (w), 3071 (w), 2964 (w), 2918 (w), 1836 (w), 1635 (s), 1610 (m), 1509 (w), 1448 (m), 1408 (m), 1375 (m), 1329 (m), 1292 (m), 1237 (s), 1182 (w), 1152 (m), 1026 (m), 996 (m), 974 (m), 886 (m), 835 (s), 806 (m), 778 (m), 739 (s), 696 (s), 675 (s), 586 (m), 571 (m). MS *m/z* 562 (MH⁺). Anal. Calcd. for C₃₂H₃₉N₃O₄S•HCl•3H₂O: C, 58.93; H, 7.11; N, 6.44. Found: C, 58.93; H, 6.71; N, 6.50.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

AIDS	acquired immunodeficiency syndrome
AN	acetonitrile
b	broad
CADA	cyclotriazadisulfonamide
CC₅₀	50% cytotoxic concentration
CD4	complex of differentiation 4
CHO	Chinese hamster ovary
d	doublet
DABCO	1,4-diazabicyclo[2.2.2]octane
dba	dibenzylideneacetone
DBU	8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DEAE-Dextran	diethylaminoethyl dextran

DMF	<i>N,N'</i> -dimethylformamide
dppb	1,4-bis(diphenylphosphino)butane
EDG	electron donating group
ER	endoplasmic reticulum
ESI	electrospray ionization
EWG	electron withdrawing group
FBS	fetal bovine serum
GUI	graphical user interface
h	hour(s)
HBA	hydrogen bond acceptor
HBD	hydrogen bond donor
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPLC	high pressure liquid chromatography
HIV	human immunodeficiency virus
IC₅₀	50% inhibitory concentration
IR	infrared spectroscopy
m	medium or multiplet
MFI	mean fluorescence intensity
min	minute(s)
Morph	morpholine
MS	mass spectrometry
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt
NMR	nuclear magnetic resonance
PES	phenazine ethosulfate
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
Phth	phthalimide
Pyrr	pyrrole

q	quartet
QSAR	quantitative structure-activity relationship
quint	quintet
RLU	relative luminescence unit
s	singlet or strong
SAR	structure-activity relationship
SIV	simian immunodeficiency virus
SOD	superoxide dismutase
SP	signal peptide
t	triplet
TCID₅₀	50% tissue culture infective dose
THF	tetrahydrofuran
tlc	thin-layer chromatography
TOF	time of flight
w	weak
YFP	yellow fluorescent protein

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Figure 1.

(a) Structure of lead compound CADA. (b) General structure of previously synthesized CADA compounds with various side arms (Ar¹ and Ar²) and tail groups (R). (c) Structure of potent analog VGD020. (d) Proposed unsymmetrical “two-site” binding model, with the Y-substituted benzenesulfonyl side arm, of interest in the current study, occupying site 1.

Figure 2.

(a) Correlation between anti-HIV-1 (NL4.3) activities in MT-4 cells and CD4 expression/down-modulation in CHO•CD4-YFP cells for 27 compounds (**1**, **26** – **33**, **35**, **37** – **41**, **43** – **45**, **47**, **48**, **50**, **52**, **55**, **56**, **58**, **59**, and **63**). Mean IC_{50} values from Table 1 and 2 were used, with $pIC_{50} = -\log_{10}(IC_{50})$. (b) Antiviral activities in TZM-bl cells infected with X4- (NL4.3) and R53tropic (BaL) HIV-1 strains correlate well for 14 compounds (CADA, **1**, **26** – **33**, **43**, **45**, **58**, and **59**). The pIC_{50} values are plotted as calculated from mean IC_{50} values of 3 independent experiments (see Table S1 in Supporting Information).

Figure 3.

Orientations and relative sizes of in-plane dipole moment components in arenesulfonamide side arms, represented by *N,N*-dimethylbenzenesulfonamides. (a) Dipole induced by sulfonyl group. (b) Enhanced dipole produced by *p*-EDG. (c) Dipole produced by *p*-EWG stronger than sulfonyl. (d) Dipole produced by *o*-EWG.

Figure 4.

(a) Alignment of model *N,N*-dimethylsulfonamides with the Cartesian coordinate system. The ring is in the *xy* plane, the S-C bond is along the *x* axis, and the S-N bond is in the *xz* plane. (b) Energy-minimized structure of side-arm model of **25** (*R* = H) and orientation of the calculated dipole (μ_{tot} : large red arrow pointing from the negative charge to the positive charge).

Figure 5. Plot of the x component of dipole moments calculated for 16 model arenesulfonamides vs. pIC₅₀ values for CD4 down-modulation by the corresponding CADA compounds (CADA, 25–34, 37, 38, 41, 50 and 56).

* Yield as HCl salt

Scheme 1.

Scheme 2.

Scheme 3.

Scheme 4.

Table 1.

CD4 Down-Modulating and anti-HIV Potencies and Cytotoxicities of Benzyl-Tailed CADA Compounds

Compd ^a	Struct. ^b	IC ₅₀ CD4 (μM) ^c	IC ₅₀ HIV (μM) ^e	CC ₅₀ (μM) ^f
		mean ± SD	mean ± SD	
25	Sch. 1	1.46 ± 0.05	> 50	> 150
26	Sch. 1	0.55 ± 0.17	1.35 ± 0.20	> 150
27	Sch. 1	5.25 ± 1.10	35.9 ± 16.7	> 150
28	Sch. 1	0.70 ± 0.09	27.7 ± 7.20	> 150
29	Sch. 1	1.20 ± 0.01	3.26 ± 1.06	> 150
30	Sch. 1	1.35 ± 0.30	7.00 ± 1.99	> 150
31	Sch. 1	0.31 ± 0.06	0.86 ± 0.24	> 150
32	Sch. 1	0.17 ± 0.08	0.62 ± 0.13	> 150
33	Sch. 1	1.06 ± 0.12	14.2 ± 1.70	> 150
34	Sch. 1	2.02 ± 0.29	> 50	> 150
35	Sch. 1	1.58 ± 0.20	12.77 ± 3.17	> 150
36	Sch. 1	3.17 ± 0.64	> 50	> 150
37	Sch. 1	0.92 ± 0.11	4.66 ± 0.06	> 150
38	Sch. 1	0.79 ± 0.10	> 50	> 150
41	Sch. 1	0.23 ± 0.05	1.44 ± 0.14	> 150
43	Sch. 2	2.12 ± 0.39	6.93 ± 1.41	70.3
44	Sch. 2	1.02 ± 0.09	6.22 ± 1.05	> 150
45	Sch. 2	2.42 ± 0.42	7.72 ± 2.00	63.4
49	Sch. 2	>50 (20%) ^d	n.d.	> 150
50	Sch. 3	0.29 ± 0.09	0.55 ± 0.09	> 150
55	Sch. 3	3.58 ± 0.54	2.84 ± 0.73	13.6
56	Sch. 3	0.46 ± 0.07	0.55 ± 0.07	> 150
57	Sch. 3	6.28 ± 2.04	> 50	106
58	Sch. 4	4.22 ± 0.84	3.86 ± 0.81	23.6
59	Sch. 4	3.78 ± 2.38	3.95 ± 0.68	19.9
60	Sch. 4	>50 (39%) ^d	> 50	> 150
61	Sch. 4	7.32 ± 1.70	> 10	15.8
62	Sch. 4	>50 (15%) ^d	> 10	13.7
63	Sch. 4	1.08 ± 0.10	8.99 ± 3.26	> 150

^aCompounds were used as HCl salts.^bLocation of structural diagram.^cIC₅₀: inhibitory concentration 50%; concentration at which 50% down-modulation of CD4 expression measured in CD4-YFP transfected CHO cells after 24 h of drug treatment. Values are the mean ± standard deviation with n = 3.^dActivity of compound is too weak to calculate IC₅₀ value. Activity is represented as percentage CD4 down-modulation measured at highest (50 μM) compound concentration used.

e IC₅₀: inhibitory concentration 50%; concentration at which 50% reduction of HIV-1 NL4.3 (X4) replication in MT-4 cells was measured. Values are mean $\pm$ standard deviation with n = 2 or 3, n.d.: not determined.

f CC₅₀: cytotoxic concentration 50%; concentration required to reduce viability of MT-4 cells by 50%.

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Table 2.

CD4 Down-Modulating and anti-HIV Potencies and Cytotoxicities of Cyclohexylmethyl-Tailed Compounds

Compd ^a	Struct. ^b	IC ₅₀ CD4 (μM) ^c	IC ₅₀ HIV (μM) ^d	CC ₅₀ (μM) ^e
		mean ± SD	mean ± SD	
1	Fig. 1	0.15 ± 0.01	0.49 ± 0.06	99
39	Sch. 1	1.10 ± 0.13	1.95 ± 0.24	13.2
40	Sch. 1	0.063 ± 0.005	0.18 ± 0.03	44
46	Sch. 2	1.00 ± 0.15	> 10	10.6
47	Sch. 2	0.18 ± 0.01	1.25 ± 0.40	48
48	Sch. 2	0.13 ± 0.01	0.36 ± 0.02	>150
51	Sch. 3	1.14 ± 0.02	n.d.	>150
52	Sch. 3	0.19 ± 0.01	0.62 ± 0.19	19.6
53	Sch. 3	0.75 ± 0.06	n.d.	32.2
54	Sch. 3	>50	n.d.	11.6

^aCompounds were used as HCl salts.^bLocation of structural diagram.^cIC₅₀: inhibitory concentration 50%, i.e., concentration at which 50% down-modulation of CD4 expression was measured in stably CD4-YFP transfected CHO cells after 24 h of drug treatment. Values are the mean + standard deviation with n = 3.^dIC₅₀: inhibitory concentration 50%; concentration at which 50% reduction of HIV-1 NL4.3 (X4) replication in MT-4 cells was measured. Values are mean ± standard deviation with n = 2 or 3, n.d.: not determined.^eCC₅₀: cytotoxic concentration 50%; concentration required to reduce viability of MT-4 cells by 50%.

Table 3.Dipole Moments of 23 *N,N*-Dimethylsulfonamides Modeling Side Arms of Benzyl-Tailed CADA Compounds

Compd ^a	Struct. ^b	pIC ₅₀ ^c	Side arm dipole moment (Debye)			
			μ_x	μ_y	μ_z	μ_{tot}
CADA*	Fig. 1	6.40	3.47	0.90	3.37	4.91
50*	Sch. 3	6.54	3.92	-0.29	3.30	5.13
25*	Sch. 1	5.84	2.81	-0.90	3.40	4.50
26*	Sch. 1	6.26	3.21	-0.60	3.35	4.68
27*	Sch. 1	5.28	-1.24	0.00	3.95	4.14
28*	Sch. 1	6.15	3.20	-2.86	3.64	5.63
29*	Sch. 1	5.92	0.23	-1.10	3.89	4.05
30*	Sch. 1	5.87	3.68	-3.80	2.08	5.69
31*	Sch. 1	6.51	3.56	0.55	3.25	4.78
32*	Sch. 1	6.77	6.09	0.85	3.19	6.93
33*	Sch. 1	5.97	3.52	-1.17	3.15	4.87
34*	Sch. 1	5.69	-0.16	2.54	3.47	4.30
36	Sch. 1	5.50	-5.20	-1.51	3.13	6.26
37*	Sch. 1	6.04	1.26	-0.91	3.42	3.76
38*	Sch. 1	6.10	4.19	1.01	3.39	5.48
41*	Sch. 1	6.64	3.35	0.39	3.31	4.73
43	Sch. 2	5.66	2.22	1.61	2.66	3.82
44	Sch. 2	5.99	3.67	0.17	4.20	5.58
45	Sch. 2	5.62	5.03	-0.79	4.14	6.56
55	Sch. 3	5.45	3.19	0.40	3.32	4.62
56*	Sch. 3	6.34	3.41	0.25	3.32	4.77
57	Sch. 3	5.20	5.02	-1.04	6.01	7.90
63	Sch. 4	5.97	-0.97	-0.35	3.19	3.36

^aCompounds were used as HCl salts; asterisks indicate compounds included in the final correlation (Fig. 4).^bLocation of structural diagram.^cIC₅₀: inhibitory concentration 50%, i.e., concentration (μ M) at which 50% down-modulation of CD4 expression was measured in stably CD4-YFP transfected CHO cells after 24 h of drug treatment; pIC₅₀ = $-\log(\text{IC}_{50})$ based on the IC₅₀ values for CD4 down-modulation as listed in Table 1.