ACS Medicinal Chemistry Letters

Letter

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Discovery of Tetrahydroisoquinoline-Based CXCR4 Antagonists

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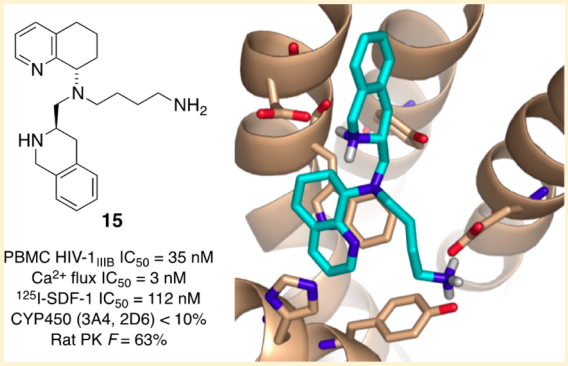
Supporting Information

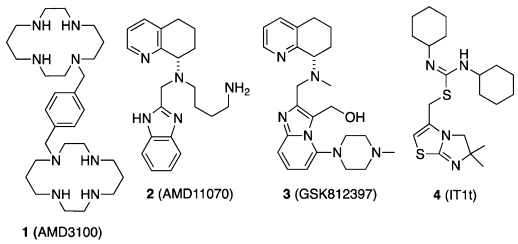
ABSTRACT: A de novo hit-to-lead effort involving the redesign of benzimidazole-containing antagonists of the CXCR4 receptor resulted in the discovery of a novel series of 1,2,3,4-tetrahydroisoquinoline (TIQ) analogues. In general, this series of compounds show good potencies (3−650 nM) in assays involving CXCR4 function, including both inhibition of attachment of X4 HIV-1_{IIIB} virus in MAGI-CCR5/CXCR4 cells and inhibition of calcium release in Chem-1 cells. Series profiling permitted the identification of TIQ-(*R*)-stereoisomer **15** as a potent and selective CXCR4 antagonist lead candidate with a promising in vitro profile. The drug-like properties of **15** were determined in ADME in vitro studies, revealing low metabolic liability potential. Further in vivo evaluations included pharmacokinetic experiments in rats and mice, where **15** was shown to have oral bioavailability (*F* = 63%) and resulted in the mobilization of white blood cells (WBCs) in a dose-dependent manner.



KEYWORDS: *Tetrahydroisoquinolines, CXCR4 receptor, CXCR4 antagonists, HIV, WBC mobilization*

The chemokine receptor CXCR4 belongs to class A of G-protein coupled receptors (GPCRs) and is broadly expressed on most hematopoietic cell types including neutrophils, monocytes and macrophages, immature and mature T and B-lymphocytes, hematopoietic and endothelial progenitor stem cells, vascular endothelial cells, neurons, neuronal stems cells, microglia, and astrocytes.[1] The natural ligand for CXCR4 is the chemokine CXCL12 (SDF-1, stromal cell-derived factor-1), a key regulator of hematopoietic stem cell (HSC) homing and retention in the bone marrow.[2,3] Importantly, the CXCR4/CXC12 axis synchronizes many essential physiological roles, such as homeostatic regulation of leukocyte trafficking, hematopoiesis, and embryonic development.[4−6] However, from a historical perspective, it was the discovery elucidating CXCR4 as a coreceptor used along with CD4 by T cell-tropic (X4) HIV-1 strains for cellular entry that catalyzed the observed cornucopia of research.[7,8] This seminal discovery was concurrent with elucidation of the mechanism of the anti-HIV-1 bicyclam AMD3100, which a priori became the first small molecule CXCR4 antagonist. Although AMD3100 (**1**, Chart 1, Plerixafor) entered the clinic as an antiviral treatment of X4 HIV-1 strains, during clinical trials it was shown to mobilize various hematopoietic cells, including CD34+ cells. Through further clinical investigations the compound was subsequently approved by the FDA for use in mobilizing hematopoietic stem cells (HSCs) for autologous transplants in patients with non-Hodgkin's lymphoma and multiple myeloma.[9] This unexpected finding resulted in unprecedented therapeutic use and prompted a paradigm shift in CXCR4 research, significantly expanding the breadth and scope from both biological and medicinal chemistry standpoints. More recent CXCR4 discoveries include the involvement of the CXCR4/CXCL12 axis in the progression of 23 different types of cancer, where the interaction between CXCR4 and CXCL12 promotes metastasis, angiogenesis, and tumor growth via direct and indirect mechanisms.[10−14]

Chart 1. Selected Small-Molecule CXCR4 Antagonists



The unique biological properties of the CXCR4/CXCL12 axis and further research with AMD3100 (**1**) prompted a surge in activity around small-molecule CXCR4 antagonists with

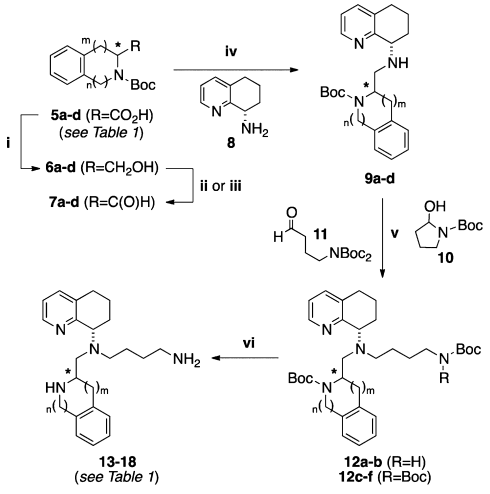
Received: May 14, 2013
Accepted: September 5, 2013

   dx.doi.org/10.1021/ml400183q | *ACS Med. Chem. Lett.* XXXX, XXX, XXX−XXX

several entering clinical trials (Chart 1).[1,14,15] Systematic efforts to replace the cyclam rings of AMD3100 with moieties that retain basic character, while also reducing the molecular weight and overall charge, resulted in the disclosure of a series of compounds exemplified by AMD11070 (**2**).[16] Noteworthy are three structural modifications shown to be effective as cyclam pharmacophore replacements: (i) a chiral tetrahydroquinoline (THQ); (ii) a basic heterocycle; and (iii) a butyl amine chain. As AMD11070 (**2**) was the first oral CXCR4 antagonist to enter the clinic and advance to phase II clinical trials, it paved the way for other efforts, which produced preclinical leads, such as the hybrid-piperazine (**3**) and isothiourea series (**4**).[14,17−19] Herein, we describe our efforts leading to discovery of a series of novel and potent CXCR4 antagonists that contain a 1,2,3,4-tetrahydroisoquinoline (TIQ) ring.

In light of the development of AMD11070 as an oral HIV treatment, our initial focus was on replacement of the benzimidazole, as we hypothesized this subunit to be the likely source of CYP450 isozyme (3A4, 2D6) inhibition activities observed with this compound.[20,21] We settled on using isomeric fused phenyl piperidines because they are substructures found in many drug-like compounds and GPCR ligands, as well as synthetically accessible starting from amino acids or suitable derivatives via the Pictet−Spengler reaction.[22,23] The (*S*)-tetrahydroquinoline headpiece previously disclosed for **2** was retained due to observed benefits for CXCR4 potency.[16] Our first goal was to synthesize a group of compounds in which the isomeric heterocycle and additional stereogenic center are variable (**13−18**, Scheme 1 and Table 1).

Scheme 1[a]



[a]Reagents: (i) BH_3−Me_2S, THF (2.0 M); (ii) Dess Martin periodinane, DCM; (iii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; (iv) **8**, $NaBH(OAc)_3$, 1,2-DCE; (v) **10** or **11**, $NaBH(OAc)_3$, 1,2-DCE; (vi) DCM, TFA.

The group of compounds **13−18** can be synthesized according to the sequence shown in Scheme 1 from readily available Boc-protected amino acid based tetrahydroquinoline (THQ, **5a**) and tetrahydroisoquinoline (TIQ, **5b−d**) building blocks. Borane dimethyl sulfide reduction of chiral or racemic Boc protected amino carboxylic acids **5a−d** provided the corresponding alcohols (**6a−d**). Oxidation to the aldehydes (**7a−d**), followed by reductive amination with **8** afforded the racemic or chiral enriched secondary amines **9a−d**. Reductive amination

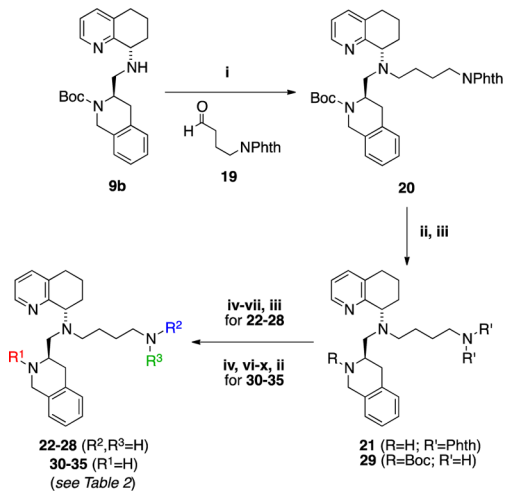
Table 1. MAGI Assay Results for Compounds 13−18

Starting Acid (**5a-d**)	Product Cmpd. No.-(*)	MAGI-HIV1$_{IIIB}$ IC_{50} (μM)[a]	MAGI-TC_{50} (μM)[a]
5a-*rac*	**13**-(*R*)	>30	>30
	14-(*S*)	>30	>30
5b-(*R*) / **5c**-(*S*)	**15**-(*R*)	0.005	>10
	16-(*S*)	1.36	>10
5d-*rac*	**17**-(*R*)	0.25	>30
	18-(*S*)	0.45	>30

[a]All assays were performed in duplicate.

with Boc protected amino butyraldehydes **10** or **11** furnished advanced intermediates **12a−f**. This was followed by Boc deprotection to furnish the final compounds **13−18**. The stereochemistry of these compounds was determined and confirmed by a combination of proof by synthesis, assignment of spectroscopic and chromatographic properties and an X-ray crystal structure of Boc derivative **29** (Scheme 2, Supporting Information).

Scheme 2[a]



[a]Reagents: (i) **19**, $NaBH(OAc)_3$, 1,2-DCE; (ii) TFA, DCM; (iii) H_2NNH_2, MeOH; (iv) R^1CHO, $NaBH(OAc)_3$, 1,2-DCE; (v) *N*-($ClCH_2CO$)−morpholine, K_2CO_3, DMF; (vi) $(CH_2O)_n$, $NaBH(OAc)_3$, DCM; (vii) acetone, $NaBH(OAc)_3$, DCM; (viii) AcCl, Et_3N, DCM; (ix) KNCO, 1 N HCl; (x) Me_2NCOCl, NEt_3, DCM.

Initial evaluation of these compounds against CXCR4 mediated effects was performed in the viral attachment assay with HIV-1_{IIIB} (Table1) in CCR5/CXCR4-expressing HeLa-CD4-LTR-β-gal (MAGI) cells measuring each compound's ability to block potential viral entry as well as cellular toxicological properties.[24] The stereochemistry, inclusion and location of fusion of the aromatic ring all affected potency. Unlike previous quinoline and isoquinoline heterocyclic

dx.doi.org/10.1021/ml400183q | ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

replacements, the action of the tetrahydroquinoline and tetrahydroisoquinoline moieties indicated that both the placement of the phenyl ring and the need for the nitrogen atom basicity are important.[25] Surprisingly, in the case of the tetrahydroquinolines **13** and **14**, the compounds showed no effect against viral entry in the MAGI assay up to 30 μM indicating the importance of the basicity of the nitrogen heterocycle. In comparison, tetrahydroisoquinolines **15**−**18** showed a range of antiviral potencies with therapeutic selectivity and two important features to note. First, the site of the phenyl ring fusion to the piperidine ring had dramatic effects on potency, with the distal phenyl ring (*R*)-isomer **15** being 50−100-fold more potent than the isomeric compounds **17** and **18** with the phenyl adjacent to the extending carbon. The second important activity relationship was evident in the dramatic effect on potency that the stereochemistry on the tetrahydroisoquinoline demonstrated. The respective isomers with the (*R*) configuration at the TIQ ring stereocenter (**15** and **17**) are the most potent analogues, and in the case of **15**, 100-fold or more potent than the compounds bearing the alternative stereochemistry (**16** and **18**). The stereochemical preference in ligand design also led to the development of the (*S*)-tetrahydroquinoline (**8**) ring in CXCR4 antagonists.[16] Given these results, compound **15** was chosen for further medicinal chemistry and biological studies.

We first focused on making conservative independent modifications to (*R*)-TIQ compound **15** (Scheme 2). Our efforts involved modifications on the TIQ ring and butyl amine side chain nitrogen atoms. Generally, these compounds (**22**−**28** and **30**−**35**, Table 2) can be synthesized as shown in Scheme 2 from previously generated intermediate **9b**. Reductive amination with **19** provided the orthogonally protected advanced intermediate **20**. To access compounds with modification at the TIQ nitrogen (**22**−**28**, R^1), Boc removal gave advanced intermediate **21** followed by either a reductive amination or N-alkylation. Hydrazine removal of the phthalamide protecting group provided the final compounds. Alternatively, to access compounds with modification at the butyl amine nitrogen (**30**−**35**, R^2 and R^3), phthalamide removal on **20** to give **29**, followed by reductive amination or N-acylation and subsequent Boc removal provided the final compounds.

Table 2. Assay Results for Compounds 15 and 22−35

Cmpd. No.	R^1	R^2	R^3	MAGI-HIV1$_{IIIB}$ IC_{50} (μM)[a,b]	Ca^{2+} Flux IC_{50} (μM)[b]
15	H	H	H	0.005	0.003
22		H	H	0.06	0.056
23		H	H	0.07	0.35
24		H	H	0.06	0.66
25		H	H	0.04	0.49
26		H	H	0.08	0.57
27	Me	H	H	0.02	0.039
28	*i*-Pr	H	H	0.02	0.14
30	H	Me	Me	0.03	0.008
31	H	H	*i*-Pr	0.04	0.007
32	H	H		0.12	0.001
33	H	H	Ac	0.29	0.004
34	H	H		0.21	0.003
35	H	H		0.42	0.18

[a]All compunds had TC_{50} values >10 μM. [b]All assays were run in duplicte.

The data used to elucidate the structure−activity relationships (SAR) of these series was generated by a combination of two assays, namely, blockade of HIV-1_{IIIB} attachment via the CXCR4 receptor in MAGI cells, as well as the inhibition of CXCL12 induced calcium (Ca^{2+}) flux/release in Chem-1 cells (Table 2). The compounds revealed a range of potencies and divergent SAR. All compounds were put in both agonist and antagonist modes in the calcium flux assay, and none showed any agonist activity, while showing complete blockade of SDF-1 induced calcium flux at various potencies. Substitution at the tetrahydroisoquinoline nitrogen with H-bond acceptors or aliphatic moieties all resulted in a slight loss of potency (**22**-**28**, 2−10-fold) in the MAGI attachment assay when compared to **15** (Table 2). As a trend, bulkier heterocycles (compounds **22**−**26**) decreased potency more than the smaller groups (**27**−**28**). A similar trend was exhibited for CXCL12 induced Ca^{2+} flux; however, the effect was much more dramatic. While there was generally a 2−10-fold decrease in anti-HIV potency compared to **15**, there was a 10−100-fold decrease in the compounds' abilities to block CXCL12 induced Ca^{2+} flux. For example, some agents with bulky heterocycles (**23**−**26**) were ∼100-fold less active than **15** in the calcium flux assay versus ∼10-fold less active in the MAGI, while with the 2-pyridyl group (**22**) and the methyl and isopropyl alkyl groups (**27**, **28**), less of a difference was observed. With regard to CXCR4 antagonist activity, this trend is supportive of the importance of an H bond donor at the TIQ nitrogen position and the need to retain basicity.

Regarding the SAR of the carbamide and *N*-alkyl butyl amine moieties (**30**−**35**), the simple N-alkylated compounds **30** and **31** retained both potent HIV activity and CXCL12 induced Ca^{2+} flux, although slightly less potent in the MAGI assay (2−5-fold). To our surprise, the 2-pyridyl and carbamide based derivatives (**32**−**34**) were 40−80-fold less potent than **15** in the MAGI assay but remained equipotent in blocking CXCL12 induced Ca^{2+} flux. These results potentially signify that basicity and steric environment on the nitrogen of the butyl amine side chain may be important for anti-HIV activity but not for normal CXCL12 mediated receptor signaling since nearly all substitutions (**30**−**34**) resulted in potent Ca^{2+} flux inhibition, with the only exception being the dimethyl urea (**35**). Although the complete nature of this difference is not well understood, taken together with the TIQ nitrogen substitution effects, it is both significant and novel from the perspective of antagonist design.

We next undertook a computational docking study in order to better understand this preliminary SAR and develop a working hypothesis on how our compounds interact with the CXCR4 receptor. Using the recently reported CXCR4 receptor crystal structure, we developed a computational model of **15** bound to CXCR4.[26] Because of the importance of the

dx.doi.org/10.1021/ml400183q | ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

interaction of IT1t with Asp97 and Glu288 in the crystal structure, we hypothesized that **15** would interact with the same residues. Thus, we flexibly docked compound **15** into the IT1t subsite of CXCR4 (PDB ID 3ODU).[27] In our best pose, the positively charged primary butyl amine of **15** interacts with the Glu288 carboxylate via a salt bridge and forms a hydrogen bond with the Tyr116 side chain phenol oxygen at the bottom of the pocket, while the protonated TIQ ring nitrogen forms a salt bridge with the Asp97 carboxylate (Figure 1). The THQ

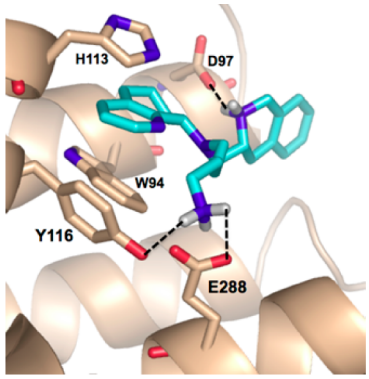


Figure 1. Docking pose of compound **15** in the IT1t/3ODU crystal structure showing hydrogen bonding and key amino acids.

moiety is situated in a hydrophobic pi-stacking pocket comprising mainly the indole of Trp94 and the imidazole of His113. The key role of the TIQ nitrogen in salt bridge formation is consistent with the SAR of the compounds in Table 1, where activity drops off significantly for the nonbasic tetrahydroquinolines **13** and **14**. Furthermore, the SAR from the alteration of substitution on both nitrogen atoms of **15** (Table 2) provides some support for this model. Since Glu288 is a key residue for HIV interaction while Asp97 is more important for SDF-1 function, the modifications of the TIQ nitrogen (**22**–**28**), which reduce calcium flux potency more than HIV activity, are consistent with altering the Asp97-TIQ interaction while maintaining the integrity of the Glu288-butyl amine interaction.[26,28] The modifications of the butyl amine side chain that alter the basicity (**32**–**34**), which reduce HIV blocking effects while maintaining calcium flux potency, are consistent with maintaining the TIQ nitrogen-Asp97 interaction and altering the butyl amine-Glu288 interaction.

As lead compound **15** was identified as the most potent in both assays, we decided to examine other in vitro CXCR4 based properties of the compound (Table 3). As a confirmation of antiviral properties, screening in peripheral blood mononuclear cells (PBMCs), resulted in IC_{50} and IC_{90} values of 35 and 240 nM, respectively, for blockage of infection of $HIV1_{IIIB}$. Similar to the TC_{50} result observed in the MAGI assay, at these concentrations cytotoxicity was not observed in PBMCs, resulting in a therapeutic index of >1000 (TC_{50} = 47 μM). Concerning anti-HIV activity, the potencies of **15**, AMD3100 (**1**), and 11070 (**2**) are comparable and equal to both the literature and in-house assays (30–40 nM for all three compounds). Further validation of CXCR4 antagonist behavior was provided by a competitive binding assay and other functional assays. Competitive binding studies with radiolabeled ^{125}I-SDF-1 showed displacement of the chemokine with an IC_{50} of 112 nM for **15**. In the forskolin/CXCL12 stimulated cAMP production assay, a measure of Gα_i signaling, **15** inhibited cAMP production, with an IC_{50} value of 19 nM. Compound **15** also inhibited β-arrestin recruitment with an IC_{50} value of 15 nM. Finally, to measure chemokine family and type selectivity, compound **15** was screened in a panel of chemokine receptor assays in both CXCR and CCR types with no observed cross-activity up to 10 μM indicating high selectivity toward CXCR4.

In an effort to assess the drug potential of compound **15**, several ADME based tests were performed (Table 3). In terms of physical parameters, the aqueous solubility of the free base was investigated by nephelometry and found to be >100 μg/mL at physiological pH (7.4). The metabolic stability of compound **15** was assessed in mouse, rat, and human liver microsomes, where it exhibited species-dependent stability behavior. In both rodents, lower half-lives (20–30 min) and higher clearances were observed. In human liver microsomes, the result was a longer half-life ($t_{1/2}$ > 60 min) and lower clearance rate. Also, the human CYP450 inhibition for isozymes 2C19, 2D6, and 3A4 by compound **15** was found to be minimal (<10%, Table 3). These results are significant, as AMD11070 (**2**) has been shown to inhibit both 3A4 and 2D6 isozymes at levels with low therapeutic difference and to alter drug levels in humans with the known CYP inhibitors midazolam (CYP3A4) and dextromethorphan (CYP2D6).[21] On the basis of the

Table 3. Data Summary of CXCR4 Biological and ADME Assays on Compound 15

CXCR4 biology or ADME assay	result(s)
inhibition of infection of $HIV\text{-}1_{IIIB}$ virus in PBMCs	IC_{50} = 0.035 μM/IC_{90} = 0.24 μM
cytotoxicity in PBMCs/therapeutic Index	TC_{50} = 47 μM/TI = 1334
[^{125}I]-SDF-1α displacement Chem-1 cells	IC_{50} = 0.12 μM
effect on SDF-1 blockade of cAMP	EC_{50} = 0.019 μM
blockade of SDF-1 induced β-arrestin-2	IC_{50} = 0.015 μM
chemokine family selectivity panel: CCR1,2b,3,4,5,6,7,8,9/CXCR1,2,3,5,6	<10% @ 10 μM
aqueous solubility (pH 7.4)	>274 μM/>100 μg/mL
liver microsomal digestion by species:	CL_{int} (mL/min/mg)/$t_{1/2}$
Mouse	0.064/22 min
Rat	0.035/39 min
Human	<0.02/>60 min
human CYP450 [3A4, 2D6, 2C19] % inhibition	6%, 8%, 0% @ 1 μM
rat PK (i.v.-2 mg/kg): $t_{1/2}$, AUC (0–∞)	1.2 h, 108 h·ng/mL
rat PK (p.o.-10 mg/kg): $t_{1/2}$, AUC (0–∞)	6 h, 328 h·ng/mL
F	63%

dx.doi.org/10.1021/ml400183q | *ACS Med. Chem. Lett.* XXXX, XXX, XXX–XXX

excellent potency and encouraging in vitro metabolism data, **15** was profiled in rat pharmacokinetic (PK) studies dosed both intravenously and by oral gavage. The area under the curve values (328 h·ng/mL for 10 mg/kg p.o. and 108 h·ng/mL for 2 mg/kg i.v.) were calculated for the experiments. Normalizing for dose levels, this translates into a good oral bioavailability value (F = 63%).

Clinical trial observations with AMD3100 (**1**) led to its development as a mobilization agent of hematopoietic progenitor (CD34+) cells. Therefore, we became interested in measuring the mobilization effects of compound **15**. We chose white blood cell (WBC) mobilization studies in mice as a proxy for CD34+ mobilization since both phase I human studies and preclinical mouse studies with AMD3100 showed that the WBCs mobilized into the peripheral blood exhibited almost identical kinetics and egress to CD34+ cells.[29] The experiments for **15** were performed by giving the compound to Harlan mice subcutaneously at each of three different dose levels (5, 10, and 20 mg/kg) and taking blood samples at multiple time points followed by measurement of both WBC and drug levels. The WBC response of **15** was found to be dose dependent over the dose range used (Figure 2, solid lines).

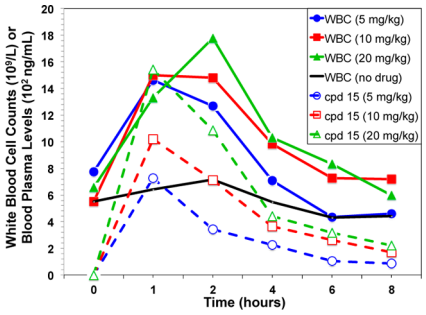


Figure 2. Plots of WBC counts (solid lines) and drug-plasma levels (dotted lines) versus time for three subcutaneous dose levels of compound **15** in mice.

Two hours after injection, total circulating WBC counts increased and reached a maximum for each dose. This corresponds to increases of 1.6-fold at 5 mg/kg, 2.3-fold at 10 mg/kg, and 2.7-fold at 20 mg/kg. Maximal WBC mobilization occurred at the one to two hour time range, similar to the slight delay observed in mobilization studies with AMD3100.[29] Furthermore, this data shows that the WBC response seems dose-related, with the 10 and 20 mg/kg doses giving similar responses that appear to be near maximal. When measuring drug levels (Figure 2, dotted lines), maximal amounts were seen at the one-hour time point for all three doses, while WBC mobilization occurred at the one to two-hour time range. The maximum plasma concentrations of **15** were as follows: 1542, 1021, and 725 ng/mL for the 20, 10, and 5 mg/kg doses, respectively (Supporting Information). The lowest concentrations measured were at the 6 to 8 h time points (87−318 ng/mL range), where little to no WBC mobilization was observed.

In summary, a novel series of highly potent and selective CXCR4 antagonists based on a chiral tetrahydroisoquinoline ((***R***)-TIQ) scaffold (**15**, **22**−**28**, and **30**−**35**) has been identified through a hit-to-lead effort focused on benzimidazole replacements. This novel series makes use of a GPCR chemotype with a chiral linkage that may exploit unique and efficient contacts with amino acid residues in the receptor. The motif may also provide compounds with unique biological selectivity and initial modifications to the TIQ nitrogen and butyl amine side chain provide tantalizing insights for the potential design of T-tropic HIV selective antagonists that do not interfere with SDF-1 based receptor signaling, as well as more potent antagonists of the CXCR4/CXCL12 axis. On the basis of the encouraging in vivo and in vitro properties of compound **15**, it has become the target for further lead optimization studies and future communications.[30]

■ ASSOCIATED CONTENT

Supporting Information

Experimental and characterization data for all new compounds and all biological data, computational docking studies, and the X-ray of compound **29** is provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors and all have given approval to the final version.

Funding

We acknowledge the use of shared instrumentation provided by grants from the NIH and the NSF.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Open Eye Scientific Software for generous provision of software enabling several molecular design tasks.

■ ABBREVIATIONS

GPCR, G-protein coupled receptor; CXCR4, CXC chemokine receptor 4; TIQ, tetrahydroisoquinoline; THQ, tetrahydroquinoline; Boc, *t*-butyloxycarbonyl; CXCL12, CXC chemokine ligand 12; SDF-1, stromal cell-derived factor 1; HIV_{IIIB}, Human Immunodeficiency Virus strain IIIB; PBMC, peripheral blood mononuclear cells; WBC, white blood cells; HSC, hematopoietic stem cells; Phth, phthalimide; MAGI, multinuclear activation of a galactosidase indicator; ADME, absorption−distribution−metabolism−excretion; CYP450, cyto-chrome P450; cAMP, cyclic adenosine monophosphate; AUC, area under the curve; PK, pharmacokinetic; i.v., intravenous; p.o., oral gavage; DCE, 1,2-dichloroethane; DCM, dichloromethane; TFA, trifluoroacetic acid

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