Synthesis of Novel Tetrahydroisoquinoline CXCR4 Antagonists with Rigidified Side-Chains

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Supporting Information

ABSTRACT: A structure–activity relationship study of potent TIQ15-derived CXCR4 antagonists is reported. In this investigation, the TIQ15 side-chain was constrained to improve its drug properties. The cyclohexylamino congener **15a** was found to be a potent CXCR4 inhibitor (IC_{50} = 33 nM in CXCL12-mediated Ca^{2+} flux) with enhanced stability in liver microsomes and reduced inhibition of CYP450 (2D6). The improved CXCR4 antagonist **15a** has potential therapeutic application as a single agent or combinatory anticancer therapy.

KEYWORDS: CXCR4, TIQ15, anticancer drugs, antagonist

One of the most commonly investigated chemokine receptors is CXCR4 (CXC chemokine receptor 4), a seven transmembrane G protein-coupled receptor whose only endogenous ligand is CXCL12 (CXC chemokine ligand 12), also known as stromal cell-derived factor 1 (SDF-1).¹ CXCL12 regulates the trafficking of CXCR4⁺ leukocytes and hematopoietic stem cells under normal physiological conditions (homeostasis). The binding of CXCL12 induces CXCR4⁺ leukocytes to migrate along chemokine gradients toward sites with high concentrations of CXCL12 (liver, lungs, kidneys, heart, skin, and bone marrow).² CXCR4 also plays a crucial role in pathological conditions, such as cancer, HIV/AIDS, and autoimmune diseases,³ as well as in important normal and aberrant physiological processes, such as chemotaxis, metastasis, angiogenesis, and tumor growth. In addition, CXCR4 is highly overexpressed in more than 23 hematopoietic and solid tumor types.⁴ Overexpression of CXCR4 in pancreatic adenocarcinoma,⁵ soft-tissue sarcoma,⁶ and ovarian cancers⁷ serves as biomarkers that indicate a poor prognosis for clinical outcome. The effects of CXCL12 binding to CXCR4 can be divided in two groups: (a) direct autocrine and paracrine effects, which activate specific pro-survival signaling pathways in the cancer cells, and (b) indirect effects, which include CXCR4⁺ cell migration to CXCL12 expressing sites, e.g., cancer metastasis.^{2,8} The CXCR4/CXCL12 axis accounts for approximately 75% of the metastatic phenotype of all cancers.⁹ Inhibition of the CXCL12 and CXCR4 interactions could decrease organ-targeted metastasis. For example, the small molecule CXCR4 inhibitor, AMD3100 (Figure 1), has been used in animal models to suppress metastasis.¹⁰ AMD3100 is also used for hematopoietic stem cell mobilization for

Figure 1. Small molecule CXCR4 antagonists.

transplantations in the case of non-Hodgkin lymphoma and multiple myeloma (FDA approved) and for chemotherapeutic sensitization in the treatment of hematologic cancers.^{11–13}

Cancer growth is promoted by several CXCR4 downstream signaling pathways, e.g., activation of MAPK (mitogen activated protein kinase) and PI3K (phosphoinositide 3 kinase)/AKT (protein kinase B) pathways that induce cell proliferation. Activation of MAPK also upregulates CXCR4 expression, creating a positive feedback loop for cancer progression.² Deactivation of canonical Wnt signaling pathway and activation of NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) also result in cell proliferation, but the mechanism of action is through suppression of cell apoptosis. In addition to stimulating cancer growth, CXCL12 binding induces intracellular calcium flux, and inhibition of this activity is a common measurement for CXCR4 antagonist potency. The small molecule CXCR4 antagonist, AMD3100, interrupts CXCR4 signaling, inhibiting the progression of a variety of

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cancers, including breast cancer,¹⁴ UV-induced skin cancer,¹⁵ prostate cancer,¹⁶ and ovarian cancer.¹⁷ In addition, promising results have been achieved using AMD3100 in combination with 1,3-bis(2-chloroethyl)-1-nitrosourea chemotherapy for glioblastomas.¹⁸ AMD3100 exhibited an anti-immunosuppression effect as a single agent in the case of CXCR4⁺/CXCL12⁺ ovarian cancer,¹⁹ as well as synergy with immunotherapeutics for CXCR4^{low} pancreatic ductal adenocarcinoma²⁰ and CXCR4⁺/CXCL12⁺ hepatocellular carcinoma.²¹ Taken together, these preclinical studies represent a proof of concept that CXCR4 antagonists can suppress CXCR4⁺ tumors, either as a single agent or in combination with immunotherapeutics.

Several potent small molecule CXCR4 antagonists (e.g., AMD3100, AMD11070, GSK812397, and IT1t; Figure 1) are known.²² Recently, TIQ15, a potent CXCR4 antagonist (IC₅₀ = 3 nM for CXCL12-induced Ca²⁺ flux and IC₅₀ = 5 nM for MAGI HIV-1_{IIIB}), was reported by our group. Similar activities were observed for inhibition of cAMP production (IC₅₀ = 19 nM) and β -arrestin recruitment (IC₅₀ = 15 nM), substantiating the applicability of the Ca²⁺ flux assay as an indicator of CXCR4 downstream signaling. TIQ15 showed a good safety profile with low cytotoxicity (TC₅₀ = 47 μ M; TC₅₀ = 50% cytotoxicity). In addition, this compound did not potently target other chemokine receptors, nor the muscarinic acetylcholine receptor (mAChR), which we used as a preliminary indication of off-target activity.

Although TIQ15 exhibited good metabolic stability in human liver microsomes, stability in rodent liver microsomes was unsatisfactory, effectively precluding the use of preclinical rodent models for studying *in vivo* efficacy. In addition, reinvestigation of CYP450 inhibitory activity revealed a significant inhibition of CYP450 (2D6) (IC₅₀ = 320 nM), which would severely limit its use in combination therapies.

Since most oral drugs are absorbed by passive transcellular absorption, the use of a parallel artificial membrane permeability assay (PAMPA) seemed well-suited for assessing the permeability of our compounds.²³ While the studies by Truax et al. reported good water solubility for TIQ15 (>100 μ g/mL in pH 7.4 buffer) and good oral availability in rats (*F* = 63%),²² TIQ15 exhibited low intestinal permeability (PAMPA values close to zero), probably as a consequence of the primary and secondary amino groups, which are protonated at physiological pH. Thus, we sought to reduce the pK_a of the nitrogens to increase compound lipophilicity and permeability. Simultaneously, the use of bulky groups to hinder solvation of the protonated amine might improve intestinal permeability.

Since prior studies suggested that rigidification of the butylamine side-chain of the structurally related CXCR4 antagonist, AMD11070, enhanced its activity, we decided to assess the effects of rigidifying it in our series.²⁴ Compounds in which an olefin was introduced to increase rigidity were synthesized from the common intermediate, secondary amine **1**, and the respective side-chains **2a–i**, **8a–d**, and **12**. The diastereomerically pure amine **1** was synthesized following the procedure developed in our group,²² and the side-chain precursors were either purchased or synthesized as described in the Supporting Information (Scheme 1). The alkenyl side-chains were attached by alkylation of **1** with bromides **2a–g**, followed by removal of the phthalimide and Boc protecting groups to afford the final compounds **4a–g** in good yields. The compounds **4h–i** were synthesized by alkylation of **1** with either (*E*)- or (*Z*)-((4-bromo-3-fluoro-2-methylbut-2-en-1-yl)-oxy)(*tert*-butyl)dimethylsilane (**2h–i**), followed sequentially by

Scheme 1. Synthesis of Compounds 4a–i, 6, and 7^a

^aReagents and conditions: (i) 0.1 equiv of KI, 1.5 equiv of Et(*i*Pr)₂N, acetonitrile, 60–78%; (ii) 1.5 equiv of 1 M *n*-Bu₄N⁺F[−] (THF), 53%; (iii) 1.2 equiv of phthalimide, 1.15 equiv of PPh₃, 1.2 equiv of DIAD, THF, 78–100%; (iv) 8 equiv of 24 wt % N₂H₄ in water, MeOH, quant.; (v) 30 equiv of CF₃COOH, CH₂Cl₂, 43–100%; (vi) 1.3 equiv of 4,4-difluorocyclohexanone, 1.2 equiv of CH₃COOH, 1.5 equiv of NaBH(OAc)₃, DCE, 81%; (vii) 1.3 equiv of 85 wt % (CH₃)₃SiNCO, 2 equiv of Et(*i*Pr)₂N, THF, 86%.

TBS deprotection, Mitsunobu reaction with phthalimide, and global deprotection. Compounds **6** and **7** were prepared from amine **5** by reductive amination with 4,4-difluorocyclohexanone or by a reaction with (CH₃)₃SiNCO, respectively, followed by CF₃COOH-induced Boc deprotection.

Next, saturated carbocyclic side-chains with increased rigidity were installed. Alkyl halides were found to react significantly slower in S_N2 reactions as compared to allyl halides; thus, we employed reductive amination reactions for the synthesis of these analogs. To that end, compounds **10a–d** were prepared by reductive amination of amine **1** with aldehydes **8a–d** as shown in Scheme 2.

Scheme 2. Synthesis of Compounds 10a–d and 15a–b^a

^aReagents and conditions: (i) 1.3 equiv of CH₃COOH, 1.25 equiv of STAB, DCE, 76–94%; (ii) 8 equiv of 24 wt % N₂H₄ in water, MeOH, quant.; (iii) 30 equiv of CF₃COOH, CH₂Cl₂, 49–100%; (iv) 1.5 equiv of CH₃COOH, 1.5 equiv of NaBH(OAc)₃, DCE, 72%; (v) 1.5 equiv of Ti(O*i*Pr)₄, 2 equiv of NaBH(OAc)₃, CH₂Cl₂, 92–94%.

Table 1. Strained TIQ-15 analogs

entry	compd	CXCR4 Ca ²⁺ flux IC ₅₀ , nM ^a	mAChR M3 Ca ²⁺ flux IC ₅₀ , μM	PAMPA, nm/s		CYP450 (2D6) IC ₅₀ , μM	CYP 450 (3A4 BFC) IC ₅₀ , μM	metabolic stability, % remaining		
				pH = 7.4	pH = 5.5			mouse	rat	human
1	TIQ15	6.3	>30	0	6	0.32	>20	17	37	77
2	4a	10	0.61	570	39	0.33	13	4	4	100
3	4b	130	>16	830	260	0.51	9.3	11	34	100
4	4c	230	23	^e	170	0.18	11	2	1	65
5	4d	8.6	0.032	11	4	<0.027	>20	2	1	100
6	4e	28	1.1	250	83	0.088	>20	2	2	96
7	4f	80	9.6	550	280	0.19	13	1	6	51
8 ^b	4g	1,800	>17	700	0	0.11	1.8	15	61	35
9	4h	28	0.77	490	50	0.059	15	2	2	100
10 ^c	4i	44	8.8	220	0	0.14	2.4	61	72	97
11 ^d	6	98	11	13	0	0.54	12	3	1	44
12	7	210	>16	110	13	0.47	19	2	2	73

^aSee Supporting Information for standard deviations. ^bIC₅₀ = 18 μM CYP450 (2C8). ^cIC₅₀ = 19 μM CYP450 (2C19). ^dSome agonist activity. ^eValue was not obtained.

Applying the same synthetic approach, no desired product was detected in the synthesis of **15a** and **15b** as a result of a bulky cyclohexyl side chain. The order of reductive aminations was therefore changed. Instead of reductive amination between secondary amine **1** and cyclohexanone **12**, the cyclohexanone was allowed to react with amine **11** to afford *cis*- and *trans*-amines **13a–b**. The *trans*-geometry was assigned from X-ray crystal structure of **13a** (see Supporting Information). Then, amine **13a–b** was employed in a reductive amination with the more reactive and less bulky aldehyde **14**. This reductive amination required the use of Ti(OiPr)₄ to facilitate formation of the imine intermediate, which was reduced *in situ* by NaBH(OAc)₃. The desired compounds **15a–b** were obtained after Boc deprotection.

Table 1 shows the results of our investigation into the effects of various alkenyl side-chains on TIQ15. The degree of CXCR4 antagonism was measured by inhibition of CXCL12-mediated intracellular calcium flux. Muscarinic receptor (mAChR) off-target activity was similarly measured using the same cell line (CCRF-CEM). The introduction of *Z*- or *E*-alkene isomers rigidified the butyl amine side-chain of TIQ15. The decrease of CXCR4 Ca²⁺ flux activity for the *E*-isomer **4b** suggested that the active binding conformation of TIQ15 is closer to the *cis*- and *gauche*-conformations. Further study of the drug properties for both isomers revealed negligible alteration of the metabolic stability in liver microsomes. Significant improvement of the PAMPA values, however, was observed due to lower basicity of allyl amines. Unfortunately, the introduction of the alkene motif slightly increased inhibition of the CYP450 (3A4 BFC) isoform as well as mAChR inhibition. In contrast, other CYP450 isoforms (1A2, 2C19, 2C9, 3A4 BZR, 2C8, 2B6, 2A6) were not inhibited. As a result of its potency, the *Z*-isomer of **4a** was chosen for further investigation. Gratifyingly, cyclohexenyl analog (**4c**) showed dramatic improvement in off-target mAChR activity but, unfortunately, at the expense of CXCR4

inhibition. The loss of CXCR4 activity suggested either a conformational change to a less active binding pose or increased steric interaction with the receptor in the binding pocket due to the cyclohexenyl ring.

To avoid steric congestion, the *Z*-alkene was substituted with a smaller methyl group (**4d** and **4e**). A slight improvement in CXCR4 activity was achieved in the case of 3-methyl substituted analog **4d**. Unfortunately, the methyl group increased the inhibitory activity of CYP450 (2D6) and the binding affinity toward mAChR to 30 nM range, while no other CYP450 isoforms were inhibited. To avoid CYP450 (2D6) inhibition, it has been suggested to lower aliphatic amine p*K*_A, overall lipophilicity, and topographical polar surface area.²⁵ To achieve these properties, fluorine substitution was proposed (entries 7–10). The substitution would lower the p*K*_A of the terminal amine, which would also likely improve the PAMPA values, which suffered after introducing the methyl substitution. Despite improvements in mAChR activity and permeability values (Table 1, **4f–i**), the CYP450 (2D6) values stayed in nanomolar range and CYP450 (3A4) values went to single digit micromolar range. In addition, a 3-fluoro substitution decreased the CXCR4 Ca²⁺ flux activity. Interestingly, the fluoro substitution on **4i** increased the metabolic stability in liver microsomes in mice and rats. Alternatively, a general trend of multiple CYP inhibition was recognized for the *trans*-fluoro alkenes **4g** and **4i**.

Our next approach involved modifying the terminal amino group by introducing heteroatom-containing motifs, which usually reduce the activity against CYP450 enzymes.²⁶ 4,4-Difluorocyclohexyl and urea groups were installed, moving mAChR activity into the micromolar range. Unfortunately, these modifications decreased CXCR4 activity. Although the PAMPA values were better for alkene side-chain analogs, the continued off-target activity required further investigation into other constrained side chains. Replacing unsaturation with

Table 2. Drug Properties of Cyclic Side-Chain Congeners of TIQ15

entry	compd	CXCR4 Ca ²⁺ flux	mAChR M3 Ca ²⁺ flux	PAMPA, nm/s		CYP450 (2D6)	CYP 450 (3A4 BFC)	metabolic stability, % remaining		
		IC ₅₀ , nM ^a	IC ₅₀ , μM	pH = 7.4	pH = 5.5	IC ₅₀ , μM	IC ₅₀ , μM	mouse	rat	human
1 ^b	10a	6.4	>33	130	8	0.54	>20	1	1	100
2	10b	13	13	<i>d</i>	88	0.27	>20	4	1	100
3	10c	280	>33	12	0	0.76	10	44	51	39
4 ^c	10d	340	10	<i>d</i>	0	12	12	92	86	63
5	15a	34	>33	37	6	2.5	>20	77	71	95
6	15b	1,800	>33	0	10	0.081	>20	56	24	86

^aSee Supporting Information for standard deviations. ^b $Y_{\max}$ for mAChR is 51%. ^cIC₅₀ = 9.1 μM CYP450 (2C19), 4.1 μM CYP450 (2C8). ^dValue was not obtained.

saturation and decreasing lipophilicity usually improves the metabolic stability of compounds metabolized by CYP450 (2D6) and CYP450 (3A4), the enzymes responsible for most of drug metabolism.²⁶

Next, CXCR4 inhibitory activity of carbocyclic side-chain analogs were investigated (Table 2). In order to maintain the favored *cis*-configuration, a cyclopropane ring was introduced (compounds 10a and 10b). Both of the *cis*-isomers were synthesized and resolved, and both were found to be potent CXCR4 antagonists. The absolute stereochemistry of the cyclopropane rings was not assigned due to almost identical activity profiles for both diastereomers. mAChR activity was greatly improved, although the PAMPA values slightly suffered. The absence of a double bond avoided the inhibition of CYP450 with the exception of CYP450 (2D6). Next, the cyclopropanes were replaced by cyclohexanes in hopes of improving the permeability and to decrease the affinity toward CYP450 (2D6). To avoid introducing new stereocenters, 1,4-cyclohexanes were selected. The *trans*-cyclohexane 15a was more potent than the *cis*-analog 15b in CXCR4 Ca²⁺ flux assay, although 15a was 4-fold less active compared to cyclopropyl congeners. No mAChR activity was observed for 15a, and the CYP450 (2D6) inhibitory activity was greatly improved. Interestingly, the inhibitory activity of 15b at CYP450 (2D6) was much higher, most likely due to the relative positions of the axial amino group and the lipophilic cyclohexyl ring. Compound 15a stands out due to its metabolic stability in rat and mouse liver microsomes, allowing evaluation of preclinical antitumor efficacy of this compound in animal models. The introduction of one or two methylene spacers in the side-chain (compounds 10c and 10d) did not improve the overall drug profile.

Extensive structure–activity relationship studies of TIQ15 side chains were performed in the course of this study. Rigidification of the side-chain by an alkene moiety and the measurements of the CXCR4 activity suggest that the *cis* geometry is closer to the bioactive binding pose. Although the alkene moiety improved cell permeability, persistent off-target activity countered the positive effects. Allylic amine analogs also exhibited poor liver microsomal activity in all three animal models, which was resolved when alkenes were replaced with cyclohexanes, leading to potent compound 15a. The TIQ15

congener 15a has high liver microsome stability and improved off-target profile such as inhibition of CYP450 (2D6). The low intestinal permeability of 15a could potentially be improved by applying a prodrug strategy on the terminal amino group.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchemlett.7b00406.

Experimental details for all compound synthesis, bioassays, and compound characterization (PDF)

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E.J., E.J.M., R.J.W., H.H.N., Y.A.T., B.M.K., V.M.T., M.B.K., K.M.K., L.J.W., and D.C.L. synthesized and characterized the compounds discussed in this manuscript. T.W., C.S.S., M.E.C., and G.M.S. designed and performed all biological assays. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

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■ ABBREVIATIONS

MAGI, multinuclear activation of galactosidase inhibitor assay; HIV1_{IIIIB}, CXCR4 tropic HIV isolate; cAMP, cyclic adenosine monophosphate; DIAD, diisopropyl azodicarboxylate; Boc, *tert*-butoxycarbonyl; Phth, phthalimide; TBS, *tert*-butyldimethylsilyl; DCE, 1,2-dichloroethane

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