Letter

# Discovery of N-Alkyl Piperazine Side Chain Based CXCR4 Antagonists with Improved Drug-like Properties

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**S** Supporting Information
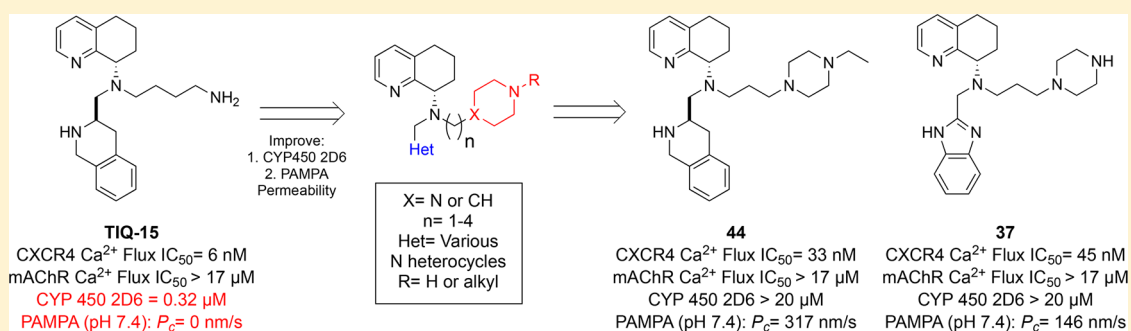
**ABSTRACT:** A novel series of CXCR4 antagonists with piperidinyl and piperazinyl alkylamine side chains designed as butyl amine replacements are described. Several of these compounds showed similar activity to the parent compound TIQ-15 (**5**) in a SDF-1 induced calcium flux assay. Preliminary structure−activity relationship investigations led us to identify a series containing N-propyl piperazine side chain analogs exemplified by **16** with improved off-target effects as measured in a muscarinic acetylcholine receptor (mAChR) calcium flux assay and in a limited drug safety panel screen. Further efforts to explore SAR and optimize drug properties led to the identification of the N'-ethyl-N-propyl-piperazine tetrahydroisoquinoline derivative **44** and the N-propyl-piperazine benzimidazole compound **37**, which gave the best overall profiles with no mAChR or CYP450 inhibition, good permeability in PAMPA assays, and metabolic stability in human liver microsomes.

**KEYWORDS:** CXCR4 receptor, CXCR4 antagonists, piperazine, tetrahydroisoquinoline, muscarinic receptor, calcium flux, liver microsomes, PAMPA, CYP450

The C−X−C chemokine receptor type 4 (CXCR4) is a seven transmembrane G-protein coupled receptor (GPCR) and is classified as a family I or rhodopsin-like GPCR family.[1,2] The endogenous ligand CXCL12, or stromal cell-derived factor-1 (SDF-1), plays a vital role in the homing of hematopoietic stem cells (HSCs) to the bone marrow and HSC quiescence.[3−5] When SDF-1 binds to CXCR4, it activates different signaling pathways, which lead to biological responses such as chemotaxis, cell survival and proliferation, intracellular calcium flux, and gene transcription.[6] Levels of CXCR4 expression are low in healthy tissues, but this receptor is upregulated in ≥48 cancer types and has further been demonstrated as a prognostic marker in breast, lung, ovarian, prostate, and colorectal cancers, as well as in hematological cancers like leukemia.[7] Organs and tissues that express high levels of SDF-1, such as the liver, lung, bone marrow, and lymph nodes, attract the migration of CXCR4-expressing cancer cells in cancer metastasis.[7]

Previous research of CXCR4 antagonists has resulted in several types of small molecule inhibitors such as AMD3100 (**1**,

Plerixafor), AMD11070 (**2**), GSK812397 (**3**), and IT1t (**4**) (Figure 1). Of these the first FDA approved CXCR4 antagonist drug was Plerixafor (**1**, AMD3100), which was first developed as an anti-HIV agent functioning as a potent antagonist, but drug toxicity prevented it from being administered daily.[8,9] Aside from anti-HIV activity, **1** also showed the mobilization of hematopoietic stem cells (HSCs). Clinical investigations of Plerixafor led to its FDA-approval for hematopoietic stem cell mobilization in non-Hodgkin's lymphoma and multiple myeloma patients with autologous transplants.[10] The poor oral bioavailability displayed by **1** prompted the discovery of **2** as the first orally active CXCR4 antagonist, which is currently in clinical trials.[6,11−13] Efforts to increase the potency by modifying structural features of **2** resulted in the novel piperazine-containing scaffold exemplified by **3** (GSK812397).
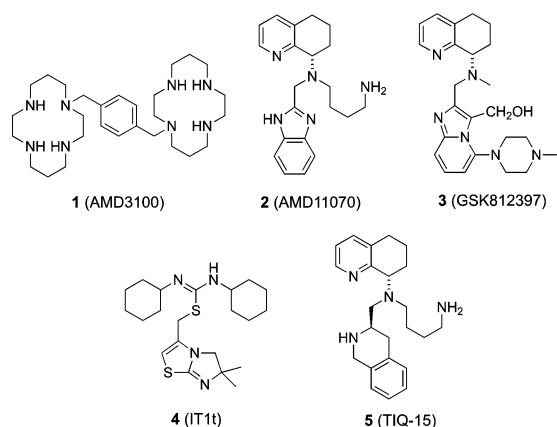
**Figure 1.** Selected small molecule CXCR4 antagonists.

Alternatively, the isothiourea series typified by **4** (IT1t) was discovered through high throughput screening efforts.[12−15] As the benzimidazole ring of **2** was considered to be the most likely source of reported off-target activity, much effort was exerted to replace this motif. Our lab discovered that a tetrahydroisoquinoline ring, exemplified by our parent compound TIQ-15 (**5**), was a suitable replacement of the benzimidazole core of **2**. As previously reported, **5** is a potent and selective CXCR4 antagonist with good drug-like properties.[16] Our efforts to improve the ADMET properties of compound **5**, mainly the CYP450 2D6 inhibition and poor cell permeability, by structural changes to the *n*-butyl amine side chain have recently been reported.[17,18] In this Letter, we describe synthetic efforts focused on replacement of the *n*-butylamine side chain with various alkyl-substituted heterocyclic side chains, such as alkyl piperidines and piperazines, and replacement of the tetrahydroisoquinoline ring with different heterocycles with the same goals of improving CYP 2D6 inhibition and low permeability.

Synthetic modifications to the lead tetrahydroisoquinoline scaffold exemplified by **5** began with key intermediate **6**.[16] We first synthesized several piperidine and piperazine side chain analogs with varying carbon chain lengths, similar to the optimization efforts of the butylamine side chain of **2**.[11] Reductive amination reaction of **6** with Boc protected piperidinyl aldehydes (**7**, **8**) and Boc protected piperazinyl aldehydes (**9**, **10**) gave compounds **12a−c** and **12e**, respectively (Scheme 1). Subsequent removal of the Boc group with trifluoroacetic acid gave desired products **13−15** and **17**. Alternatively, an alkylation reaction of **6** was necessary for the synthesis of propyl piperazine intermediate **12d**. The secondary amine **6** was treated with alkyl bromide **11** to give **12d**, followed by deprotection of the Boc groups to yield **16**. A series of enantiomerically pure piperidines containing an ethyl linker (**20** and **21**) was also explored. These side chains closely resembled the parent butylamine side chain, but with limited free rotation. The protected ethylpiperidine intermediates **19a** and **19b** were prepared via a reductive amination of **6** with (*S*)-**18a** and (*R*)-**18b**, respectively. Protecting group removal from **19a** and **19b** yielded the ethylpiperidine analogs **20** and **21**.

Several *in vitro* pharmacological assays were used to screen these derivatives of **5**, and the results revealed a range of potencies and divergent structure−activity relationships (SAR). All compounds were tested as both agonists and antagonists in CXCR4 calcium flux assays, and none showed any agonistic activity (Table 1). In addition, compounds were screened

**Scheme 1**[a]
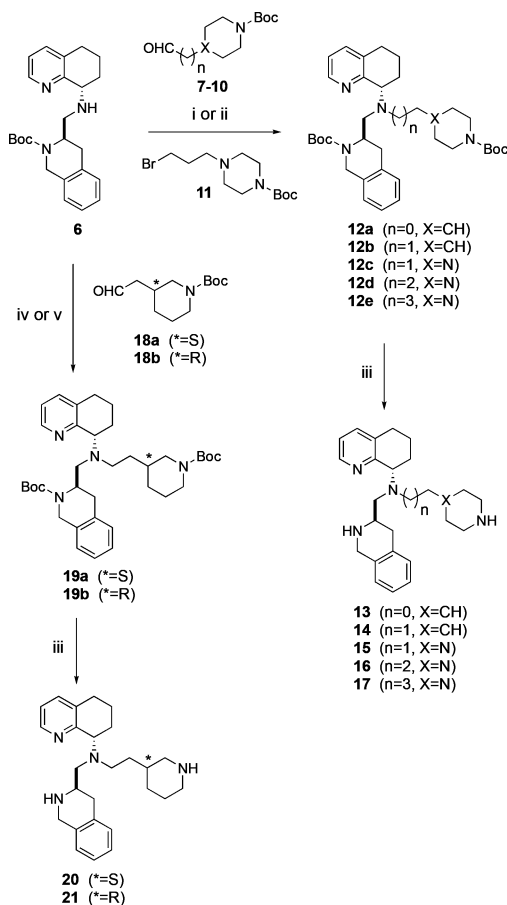


[a]Reagents: (i) **7** (*n* = 0, X = CH), **8** (*n* = 1, X = CH), **9** (*n* = 1, X = N), **10** (*n* = 3, X = N), NaBH(OAc)₃, DCE, 75−95% yield; (ii) *tert*-butyl-4-(3-bromopropyl)piperazine-1-carboxylate (**11**), DIPEA, MeCN, 65 °C, 73% yield; (iii) TFA, DCM, r.t. 35−48% yield; (iv) *tert*-butyl (*S*)-3-(2-oxoethyl)piperidine-1-carboxylate (**18a**), NaBH-(OAc)₃, DCE, 82% yield; (v) *tert*-butyl (*R*)-3-(2-oxoethyl)-piperidine-1-carboxylate (**18b**), NaBH(OAc)₃, DCE, r.t.

**Table 1. Biological Data of Compounds from Scheme 1**

| compd. No. | CXCR4 Ca²⁺ flux IC₅₀ (nM) | microsomal stability (H/R/M)[a] | CYP450 2D6 IC₅₀ (μM)[b] | PAMPA pH 7.4 $P_c$ (nm/s) |
|---|---|---|---|---|
| **5** | 6 | 77/37/17 | 0.32 | 0 |
| **13** | 65 | 86/65/13 | 1.87 | 0 |
| **14** | 150 | 66/17/32 | 1.04 | 19 |
| **15** | 553 | 96/62/79 | 4.21 | 10 |
| **16** | 17 | 100/100/92 | 2.14 | 0 |
| **17** | 66 | 82/15/96 | 1.43 | 14 |
| **20** | 161 | 75/28/23 | 0.03 | 0 |
| **21** | 16 | 93/55/56 | 0.11 | 55 |

[a]Liver microsomal stability measured as percent remaining by LCMS after 10 min in human (H), rat (R), and mouse (M) microsomes. [b]All compounds have mAChR Ca²⁺ flux IC₅₀ > 14 μM and CYP450 3A4 IC₅₀ > 12 μM.

against the muscarinic receptor to measure potential off-target GPCR activity, and all compounds were found to be inactive in this assay. Replacing the butylamine side chain with 4-methylpiperidine (**13**), 4-ethylpiperidine (**14**), (*S*)-3-ethyl-piperidine (**20**), or (*R*)-3-ethylpiperidine (**21**) resulted in robust CXCR4 inhibition, albeit with a slight loss in potency

(2–3-fold) versus **5**. We were encouraged to observe a decrease in CYP450 2D6 IC$_{50}$ values for **13** and **14** relative to **5**; however, **20** and **21** had slightly increased 2D6 activity. Also screened were the 2 through 4 carbon linked *N*-piperazines (**15**–**17**). Although all three had reduced CYP450 2D6 activity with IC$_{50}$ values up to 10-fold higher than **5**, propyl piperazine analog **16** proved to be the best in terms of CXCR4 potency. Overall, these efforts led to the discovery of compounds **21** and **16**, which exhibited the most potent CXCR4 antagonist activity of all compounds screened in this initial series. In comparing piperidines to piperazines, an increase in 2D6 for piperidine **21** and the lower calcium flux value of piperidine **13** showed the limitations in trying to optimize the piperidine series further. In contrast, we found that the piperazine side chain analogs (**15**–**17**) had better liver microsomal stability than the parent compound **5**, as well as piperidine analogs **13**, **14**, **20**, and **21**. In general, the piperidines were inferior compounds, having more problematic 2D6 potencies and liver microsomal stability across species. For example, ethyl piperazine **15** was less potent than the ethylpiperidine **14**, but a slight improvement in metabolic stability and CYP450 2D6 inhibition was observed. The propyl and butyl piperazines (**16**, **17**) had better on-target potency than ethyl piperazine **15** and also maintained good microsomal stability. Overall, propyl piperazine **16** was the best compound from these first generation results.

In order to further distinguish between the piperazine and piperidine side-chain series, we chose two compounds with the best profiles for each heterocycle type (**16**, **21**) as well as TIQ-15 (**5**) for comparison to test for additional non-CXCR4 pharmacology beyond the CYP450 2D6 and Muscarinic receptor (mAChR) assays. A limited panel of four assays (Table 2) were selected based on proteins that are known to

**Table 2. Off-Target Panel of Compounds 5, 16, and 21**

| off-target assay | IC$_{50}$ (µM) | | |
|---|---|---|---|
| | **5** | **16** | **21** |
| α-adrenergic 2A | 3.7 | >30 | 5.9 |
| μ-opioid | 14.9 | >30 | 11.3 |
| nAChR α1 | >30 | >30 | 11.3 |
| acetylcholine esterase | >30 | >30 | 10.7 |

interact with many nitrogen-based chemotypes (such as phenethyl amines, pyridines, piperazines, and piperidines) similar to those found in our compounds. As there are a vast number of possibilities for cross-pharmacology and our goal was not New Chemical Entity (NCE) candidate selection at this stage, this panel was very limited to convey potential liabilities. First, no responses were observed lower than 3 µM for all compounds indicating a therapeutic index of at least 100-fold compared to the CXCR4 Ca$^{2+}$ responses. In the two class A GPCRs (adrenergic, opioid) both **5** and **21** had similar responses in the single digit micromolar ranges but still with therapeutic indices at 100- to 1000-fold. Furthermore, compound **21** had similar responses in the nicotinic receptor and acetyl choline esterase with IC$_{50}$ values in the 10 µM range, while both **5** and **16** were devoid of this activity up to 30 µM. In comparison, the propyl piperazine **16** had the least off-target effects with all four assays showing no responses up to 30 µM. Although piperidine **21** had slightly improved PAMPA permeability, all four off-target effects in the 6–11 µM range indicating a less favorable profile even compared to TIQ-15. Since the piperazine based side chain had the best profile, we

selected compound **16** as a more selective agent for continued SAR exploration.

After finding that the propyl piperazine side chain delivered good CXCR4 activity, improved metabolic stability, and reduced off target activity, we focused our SAR on substituting different heterocycles for the bottom THIQ ring system. This effort was also designed to compare the different heterocycles used in CXCR4 medicinal chemistry with the novel propyl piperazine substitution versus the butyl amine. These included pyridine for phenyl swaps and various aromatic heterocyclic substitutions and the previously reported benzimidazole (as that found in **2**) and isoquinoline rings.[11,19] The synthesis of the THIQ and alternate heterocyclic scaffolds began with the alkylation of compound **22** with the side chain alkyl bromide **11** to yield the intermediate **23** (Scheme 2). Compound **23** was

**Scheme 2**[a]
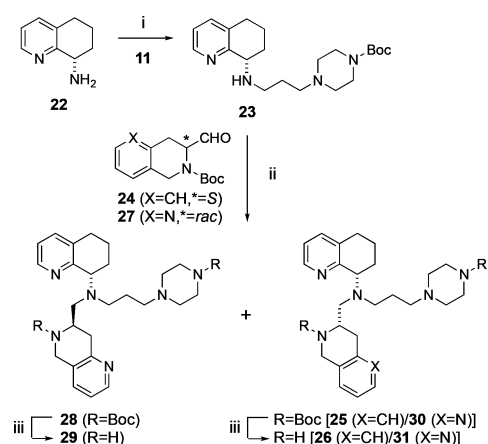


[a]Reagents: (i) **11**, DIPEA, MeCN, r.t., 75% yield; (ii) **24** or **27**, NaBH(OAc)$_3$, DCE, r.t., 16–26% yield; (iii) TFA, DCM, r.t., 85–98% yield.

then either used in a reductive amination reaction with its corresponding aldehyde to give compounds **25**, **28**, **30**, and **34** or alkylated with the corresponding chloride to give compounds **32**, **36**, or **38** (Schemes 2 and 3). Diastereomers **28** and **30** were readily separable by column chromatography. A final deprotection of the Boc group furnished the analogs **26**, **29**, **31**, **33**, **35**, **37**, and **39**.

First, the TIQ substitutions yielded further SAR. Pharmacological results of **26**, the epimer of **16**, demonstrated a nearly 100-fold reduction in CXCR4 activity, illustrating a preference for the *R*-isomer (Table 3), which was similarly observed during the discovery of TIQ-15.[16] The pyridine for phenyl swap (**29**, **31**) resulted in a compound (**29**) with 10-fold lower CXCR4 activity but a marked improvement in CYP 2D6. For the heteroaromatic substitution SAR, the pyrimidine (**33**), isoquinoline (**35**), benzimidazole (**37**), and benzothiazole (**39**) analogs were tested. The isoquinoline **35**, synthesized as a replacement for the TIQ ring on **16**, had similar CXCR4 and CYP450 2D6 potencies but with more potent muscarinic activity (3 µM versus >17 µM). The muscarinic activity, although not reported for the analogous *n*-butyl amine compound, are likely attributed solely to the isoquinoline ring.[19] Benzimidazole (**37**) had 3-fold lower CXCR4 activity compared to **35**, but showed no CYP450 2D6 or muscarinic activities. Benzothiazole (**39**) and pyrimidine (**33**) were much less active against CXCR4 with both compounds having single

**Scheme 3**[a]
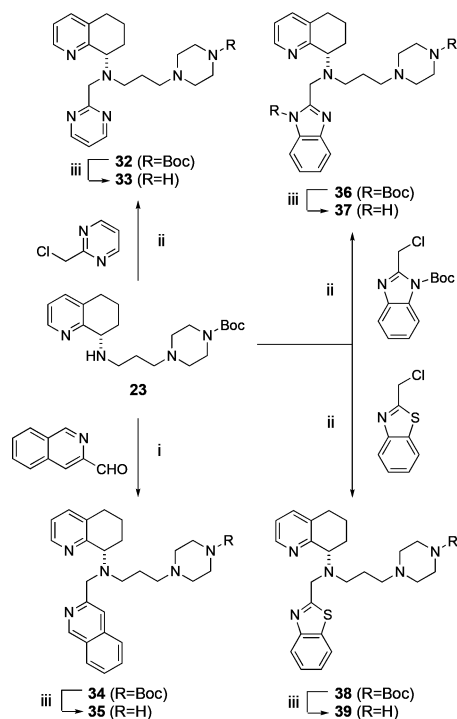


[a]Reagents: (i) isoquinoline-3-carbaldehyde, NaBH(OAc)$_3$, DCE, r.t.; (ii) N-Boc-2-chloromethylbenzimidazole,[11] 2-(chloromethyl)benzo-[d]thiazole, or 2-(chloromethyl)pyrimidine hydrochloride, DIPEA, MeCN, 65 °C; (iii) TFA, DCM, r.t., 22–52% yield over two steps.

**Table 3. Biological Data of Compounds from Schemes 2 and 3**

| compd. No. | CXCR4 Ca$^{2+}$ flux IC$_{50}$ (nM) | microsomal stability (H/R/M)[a] | CYP450 2D6 IC$_{50}$ ($\mu$M)[b,c] | PAMPA pH 7.4 $P_c$ (nm/s) |
|---|---|---|---|---|
| 2 | 5 | 63/<1/<1 | 1.64 | 86 |
| 26 | 1154 | 99/88/88 | 1.40 | 16 |
| 29 | 171 | 79/79/78 | >20 | 9 |
| 31 | 2639 | 100/87/63 | >20 | 0 |
| 33 | 4316 | 100/68/78 | >20 | 31 |
| 35 | 17 | 66/59/74 | 2.96 | 243 |
| 37 | 45 | 63/29/68 | >20 | 146 |
| 39 | 1199 | 50/21/45 | >20 | nd[d] |

[a]Liver microsomal stability measured as percent remaining by LCMS after 10 min in human (H), Rat (R), and mouse (M). [b]All compounds have mAChR Ca$^{2+}$ flux IC$_{50}$ > 17 $\mu$M except **35** (mAChR IC$_{50}$ = 3.2 $\mu$M). [c]All CYP450 3A4 values IC$_{50}$ > 20 $\mu$M, except **2** (>6.67 $\mu$M), **37** (12.7 $\mu$M), and **39** (9.4 $\mu$M). [d]nd = not determined.

digit micromolar activities in the calcium flux assay. One advantage to the aromatic heterocycle compounds was an improvement in PAMPA permeability, especially **35** and **37** showing >100 nm/s $P_c$ values. In this effort, the benzimidazole compound (**37**) had the best overall properties with slightly higher CXCR4 activity than **16** (2- to 3-fold) but no CYP450 2D6 inhibition and acceptable PAMPA permeability. Also, when comparing this n-propyl piperazine side chain compound (**37**) to AMD11070 (**2**), improvements in CYP450 2D6, liver microsomal stabilities, and PAMPA are observed with a 10-fold drop in CXCR4 activity. The majority of these trends were now consistent for both THIQ and benzimidazole substitutions (**16** and **37**).

Since compound **16** had low permeability observed in the PAMPA assay, we decided to continue to alter the structure of the propyl piperazine side chain of this compound by focusing on the terminal piperazine nitrogen. Altering the basicity of the piperazine nitrogen and reducing H-bond donor count have been shown to improve permeability in other efforts.[20] A series of N-substituted propyl piperazines **43–48** were synthesized to probe how substitution at the terminal piperazine might alter CXCR4 activity, metabolic stability, and permeability. The side chain benzyl 4-(3-bromopropyl)piperazine-1-carboxylate **40** was synthesized using Cbz protected piperazine and 1,3-dibromopiperazine (see Supporting Information). Alkylation of **6** with **40** gave intermediate **41** (Scheme 4), and deprotection

**Scheme 4**[a]
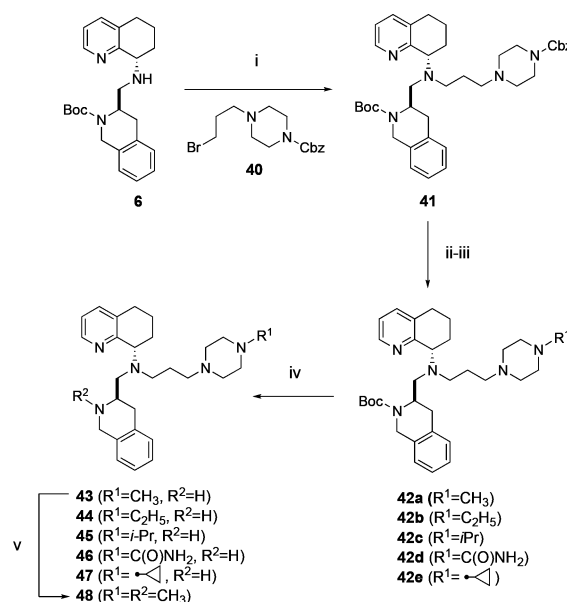


[a]Reagents (i) benzyl-4-(3-bromopropyl)piperazine-1-carboxylate) (**40**), DIPEA, MeCN, 65 °C, 78% yield; (ii) Pd(OH)$_2$, HCOONH$_4$, EtOH, reflux; (iii) HCHO (for **42a**), CH$_3$–CHO (for **42b**), acetone (for **42c**), NaBH(OAc)$_3$, DCE, r.t. or TMSNCO, DIPEA, THF (for **42d**) or (1-ethoxycyclopropoxy)trimethylsilane, AcOH, NaBH$_3$CN, MeOH (for **42e**), r.t.; (iv) TFA, DCM, 40–61% yield over three steps (v) H–CHO, NaBH(OAc)$_3$, DCE, r.t.

of the Cbz group with Pd/C followed by reductive amination with either formaldehyde, acetaldehyde, acetone, or (1-ethoxycyclopropoxy)trimethylsilane furnished compounds **42a–c** and **42e**, respectively, whereas reaction with (trimethylsilyl)isocyanate resulted in compound **42d**. Boc deprotection of compounds **42a–e** provided compounds **43–47**. Reductive methylation of **43** gave compound **48**.

Compounds (**43–48**) were subsequently assessed for CXCR4 and mAChR inhibition, microsomal stability, CYP450 2D6 inhibition, and PAMPA permeability. A methyl substitution on piperazine **43** resulted in an almost 10-fold loss in CXCR4 potency, increased muscarinic activity, and a reduction in microsomal stability. In contrast, the CYP450 2D6 liability saw a slight improvement. The biggest change was the large enhancement in PAMPA permeability, which increased 100-fold versus **16**. The compound with both the piperazine nitrogen and THIQ nitrogen methylated (**48**) was less active in the CXCR4 calcium flux assay by 20-fold but again saw an improvement in PAMPA permeability as compared to

**16**. The effect on PAMPA permeability in reduction of H-bond donor count, from two on **16** to one on **43** and then none on **48**, showed the biggest improvement came from capping the piperazine N−H. The $P_c$ values for **43** and **48** were not substantially different (Table 4, 360 versus 513 nm/s) but over

**Table 4. Biological Data of Compounds from Scheme 4**

| compd No. | CXCR4 Ca²⁺ flux IC$_{50}$ (nM) | microsomal stability (H/R/M)[a] | CYP450 2D6 IC$_{50}$ ($\mu$M)[b] | PAMPA pH 7.4 $P_c$ (nm/s) |
|---|---|---|---|---|
| 43 | 125 | 72/10/53 | 5.60 | 360 |
| 44 | 33 | 59/1/19 | >20 | 317 |
| 45 | 62 | 46/4/58 | 6.65 | 344 |
| 46 | 959 | 79/4/42 | 4.14 | 67 |
| 47 | 50 | 21/2/3 | 6.04 | 505 |
| 48 | 536 | 60/2/43 | 5.14 | 513 |

[a]Liver microsomal stability measured as percent remaining by LCMS after 10 min in human (H), rat (R), and mouse (M). [b]All compounds have mAChR Ca²⁺ flux IC$_{50}$ > 17 $\mu$M, except **43** (6.56 $\mu$M) and **46** (4.98 $\mu$M). All compounds have CYP450 3A4 IC$_{50}$ > 20 $\mu$M.

100-fold better than **16**. Showing great improvement in the PAMPA permeability, we continued our focus on the terminal piperazine nitrogen. The ethyl-substituted piperazine **44** had better CXCR4 potency than **43**, but a decrease in microsomal stability across all three species was observed. However, this compound was devoid of CYP450 2D6 inhibition and additionally retained the improvement in PAMPA permeability (Table 4). Further alkyl group substitutions, including isopropyl (**45**) and cyclopropyl (**47**) motifs, had similar results to **43**, exhibiting less potent CXCR4 inhibition and no improvement in CYP450 2D6 or liver microsomal stability. Urea substituted piperazine **46** demonstrated a 50-fold potency loss and also introduced moderate muscarinic activity. Surprisingly, this compound (**46**) also showed lower permeability by 10-fold versus the alkylated analogs (**43−45**, **47**, **48**) despite the nonbasic terminal piperazine nitrogen. Overall, the ethyl analog **44** was the best compound in this series, having similar potency to **16** and greatly improved CYP450 2D6 and PAMPA properties.

In conclusion, a novel series of highly potent and selective CXCR4 antagonists featuring N-alkyl-substituted heterocycles as replacements of the butylamine side chain of lead compound TIQ-15 (**5**) was presented. The SAR around these side chain replacements led to the N-propyl piperazine compound **16**, which not only retained CXCR4 activity and improved metabolic stability in human, rat, and mouse liver microsomes, but also reduced CYP450 2D6 activity, as compared to **5**. In addition, compound **16** had fewer off-target liabilities than the best piperidine compound **21** or **5** (TIQ-15; Table 2). We were also able to replace the THIQ bottom ring with various heterocycles using the newly discovered n-propyl piperazine side chain with mixed results showing both the THIQ and benzimidazole rings having the best overall properties. In fact, the benzimidazole compound **37** had eliminated CYP450 2D6 activity with only a 3-fold drop in CXCR4 potency but did increase CYP450 23A4 activity. Also, compound **37** had slightly improved PAMPA permeability compared to **16**, likely due to the lower basicity of the benzimidazole versus THIQ rings. The N-alkylations of the piperazine nitrogen on **37** were not carried out as it already had optimal properties and the CXCR4 activity was already significantly higher than TIQ-15 and **2** (~10-fold). In contrast, substituting the distal piperazine nitrogen of **16** in

order to improve permeability was merited, providing a greater than 100-fold increase in $P_c$ values in the PAMPA assay. This led to identification of the N′-ethyl-substituted N-propyl piperazine compound **44**, which provided the best overall combination of properties. In comparison to the starting butyl amine compound **5** (TIQ-15), compound **44** was equivalent or superior in all respects with the exception of CXCR4 activity being 5-fold less potent. However, with good CXCR4 potency, reduced off-target effects, good human liver microsomal stability, PAMPA permeability, and greatly reduced CYP450 2D6 activity, compounds **37** and **44** offer improved selections to progress into lead optimization.

When comparing the newly discovered n-propyl-piperazine side chain to our other n-butyl amine structural changes, we can directly determine that this current effort provides the best overall compound identified in **44**. Previously, one effort found a cyclo-hexyl amine compound with similar CXCR4 potency but lower 2D6 activity and PAMPA permeability than **44**.[17] Our other work found that substituting the butyl chain with methyl groups and unsaturation coupled with nitrogen substitutions provided compounds similar in CXCR4 activity and PAMPA permeability to **44** but lower 2D6 activity.[18] In the latter case, it was the nitrogen substitutions not structural changes on the butyl amine chain that led to the improvements over TIQ-15. Many of these previously described structural variations (gem-dimethyls, unsaturation, nitrogen pyranyl groups) were not attempted in the current series due to the quick identification of compounds **37** and **44**; however, our interest in these modifications may lead to further improvements. Future efforts, including pharmacokinetic studies and evaluation in models of immuno-oncology, will be reported in due course.

## ■ ASSOCIATED CONTENT

**Ⓢ Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.8b00030.

> Experimental and characterization data for all new compounds and all biological data (PDF)

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**Author Contributions**

The manuscript was written by Y.A.T. and through contributions of all authors. All authors have given approval to the final version of the manuscript.

**Notes**

The authors declare the following competing financial interest(s): D.C.L. is the principle investigator on a research grant from Bristol-Myers Squibb Research and Development to Emory University. D.C.L., L.J.W., Y.A.T., E.J.M., E.J., H.H.N.,

## ABBREVIATIONS

CXCR4, CXC chemokine receptor type 4; GPCR, G protein-coupled receptor; CXCL12, CXC chemokine ligand type 12; THQ, 5,6,7,8-tetrahydroquinoline; THIQ, 1,2,3,4-tetrahydroisoquinoline; HLM, human liver microsomes; RLM, rat liver microsomes; MLM, mouse liver microsomes; TFA, trifluoroacetic acid; DCE, 1,2-dichloroethane; mAChR, muscarinic acetylcholine receptor; STAB-H, sodium triacetoxyborohydride; PAMPA, parallel artificial membrane permeability assay

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