CHEMMEDCHEM

CHEMISTRY ENABLING DRUG DISCOVERY

Accepted Article

Title: Synthesis, Structure-activity Relationship Studies and ADMET Properties of 3-aminocyclohex-2-en-1-ones as Chemokine Receptor 2 (CXCR2) Antagonists

Authors: Weiyang Dai, Wenmin Chen, Bikash Debnath, Wu Yong, and Nouri Neamati

To be cited as: *ChemMedChem* 10.1002/cmdc.201800027

Link to VoR: http://dx.doi.org/10.1002/cmdc.201800027

A Journal of

ChemPubSoc Europe

WILEY-VCH

www.chemmedchem.org

Synthesis, Structure-activity Relationship Studies and ADMET Properties of 3-aminocyclohex-2-en-1-ones as Chemokine Receptor 2 (CXCR2) Antagonists

Weiyang Dai, [a,b] Wenmin Chen, [a] Bikash Debnath, [a] Yong Wu, *[b] and Nouri Neamati*[a]

[a] W. Dai, Dr. W. Chen, Dr. B. Debnath and Prof. N. Neamati*
Department of Medicinal Chemistry, College of Pharmacy
University of Michigan
1600 Huron Parkway, Ann Arbor, MI, USA
E-mail: neamati@med.umich.edu

[b] W. Dai and Prof. Y. Wu*
Key Laboratory of Drug Targeting and Drug Delivery System of Ministry of Education, West China School of Pharmacy
Sichuan University
No.17 People's South Road, Chengdu, 610041, P. R. of China

Supporting information for this article is given via a link at the end of the document.

Abstract: Herein, we describe the synthesis and structure-activity relationship of 3-aminocyclohex-2-en-1-one derivatives as novel CXCR2 antagonists. Thirteen out of 44 derivatives inhibit CXCR2 down-stream signaling in a Tango assay specific for CXCR2 with IC_{50} values less than 10 μM. *In silico* ADMET prediction suggests that all active compounds possess drug-like properties. None of these compounds show cytotoxicity, suggesting their potential application in inflammatory mediated diseases. A structure-activity relationship (SAR) map has been generated to gain better understanding of their binding mechanism to guide further optimization of these new CXCR2 antagonists.

Introduction

The CXC chemokine receptor CXCR1 and CXCR2 are members of class A (rhodopsin-like) family of seven transmembrane G-protein-coupled receptors[1] expressed on inflammatory cells such as neutrophils, monocytes, T-lymphocytes, and basophils as well as on keratinocytes, endothelial, fibroblasts, central nervous system (CNS) neurons and melanoma cells[2]. These receptors are activated by chemokines CXCL8 (Interleukin-8, IL-8), NAP2, CXCL1 (GRO-α), GRO-β, GRO-γ, ENA-78, γIP-10, I-TAC[3]. Chemokine CXCL1 is selective for CXCR2, whereas, CXCL8 can activate both CXCR1 and CXCR2[4]. Upon chemokine binding, CXCR1/2 couples to pertussis toxin-sensitive G-protein via Gαi subunit to regulate signaling cascades that mediate neutrophil activation and chemotaxis. The G-protein signaling is tightly regulated by rapid desensitization of the receptor. Receptor internalization is one of the desensitization processes. CXCR2 is more rapidly internalized and at low ligand concentrations than the CXCR1[5]. CXCR2 plays a significant role in the activation and recruitment of neutrophils at the sites of inflammation in several inflammatory diseases including chronic obstructive pulmonary disease (COPD), arthritis, asthma, psoriasis as well as CNS demylinating disorders[6]. CXCR2 signaling is also implicated in tumorigenesis and metastasis[7]. Therefore, discovery of novel peptides and small molecule inhibitors of CXCR2 antagonists emerges as a promising therapy for the treatment of various inflammatory disorders[8]. Serveral small molecule CXCR2 inhibitors with different scaffolds have been reported, including diaryl ureas, boronic acids, squaramides, pyrimidines and ketoprofen derivatives (Figure 1). Some CXCR2 inhibitors have advanced to clinical trials. Reparixin is a ketoprofen derivative being investigated in prevention and treatment of delayed graft function and pancreatic islet transplantation[9]. Additionally, a combination of ketoprofen and paclitaxel inhibited brain tumor metastasis[10]. Reparixin is now in Phase II for both breast cancer and ischemia-reperfusion injury. A potent and selective CXCR2 diarylurea antagonist SB225002 inhibits CXCL8-induced neutrophil migration[11], and its analog SB656933, has advanced into clinical trials for COPD and cystic fibrosis[12]. Results from these trials demonstrated that CXCR2 inhibitors can effectively suppress ozone and LPS- mediated lung inflammation in healthy subjects by reducing sputum neutrophils. Another CXCR2 inhibitor, SCH527123[13], was tested in a phase II clinical trial for the oral treatment of moderate to severe COPD and asthma[14]. Bicyclic thiazolopyrimidine compounds exemplified by AZD-8309 effectively inhibited the increase of LPS-induced neutrophil recruitment in the nasal lavage of healthy patients[15]. Its analog AZ13381758 decreased metastases and augmented immunotherapy response in a mouse model of pancreatic cancer[16]. Another bicyclic thiazolopyrimidine small molecule AZD5069 is also in clinical trials for COPD, asthma and advanced solid tumors. SX-517 is another small molecule CXCR2 antagonist with unique boronic acid moiety that effectively inhibited CXCL1-induced Ca^{2+} flux in human PMNs[17]. Its analog SX-682 decreased metastases and augmented immunotherapy in a mouse model of prostate cancer and has advanced to Phase I clinical trial for melanoma [18]. However, there is not yet an approved CXCR2 antagonist on the market. We previously reported the discovery of a novel phenylcyclohex-1-enecarbothioamide derivative CX4338 that inhibits CXCL8-mediated chemotaxis through selective regulation of CXCR2-mediated signaling[19]. It selectively blocks CXCR2/β-arrestin-2 signaling and receptor internalization. CX4338 also showed inhibition of CXCL8-induced chemotaxis in CXCR2-overexpressing cells and human neutrophils and significantly reduced neutrophils in bronchoalveolar lavage induced by LPS in murine studies. Therefore, CX4338 is a good hit compound for further investigation and optimization. Herein, we describe our effort

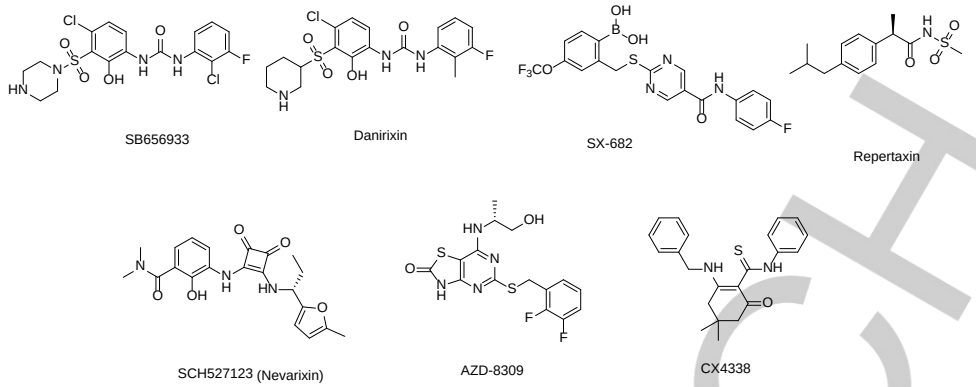


Figure 1. CXCR2 receptor antagonists

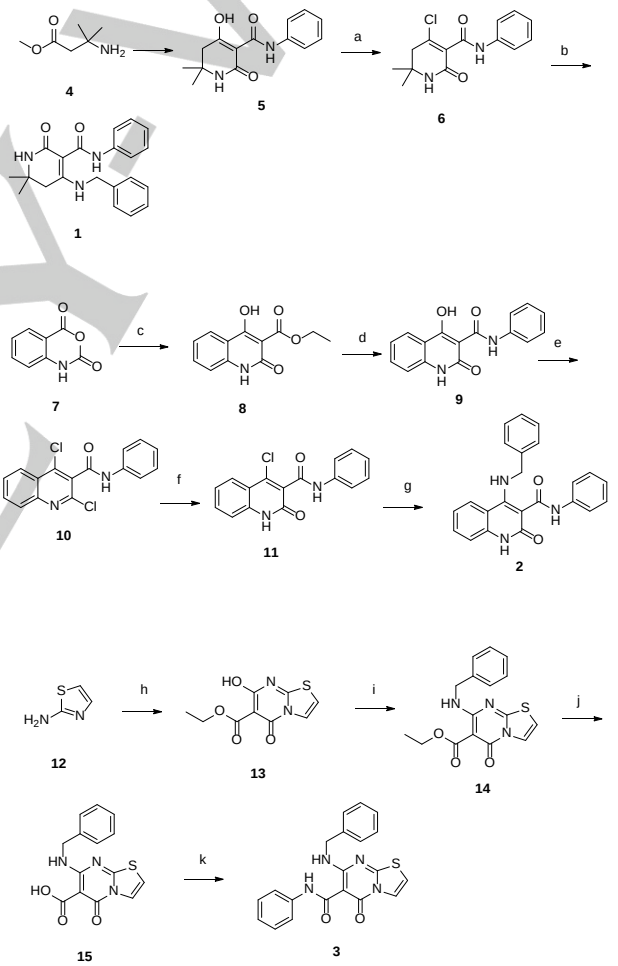
towards modifications of CX4338 to establish a SAR for CXCR2 inhibition.

Results and Discussion

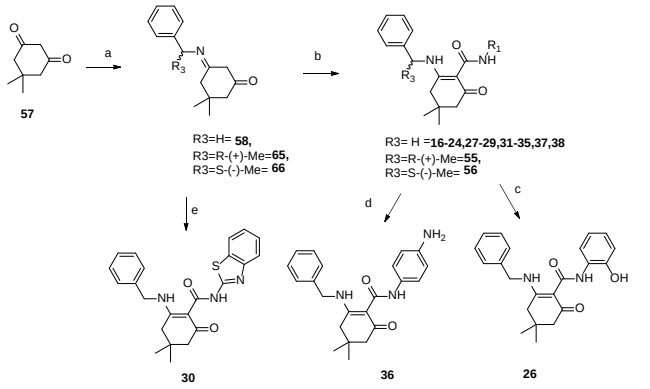
Chemistry

Different synthetic methods were applied to prepare compounds **1-3** with various core structures (Scheme 1). The synthesis of **1** started from methyl 3-amino-3-methylbutanoate **4** in three steps to generate **5** using an established method [20]. Chlorination of **5** occurred by exposure to $POCl_3$ at room temperature to yield mono chloro product **6.** Compound **1** was obtained by coupling the chlorinated product **6** with benzyl amine in DCM. Quinolinone **2** was prepared from isatoic anhydride **7** treating with NaH and diethyl malonate in DMF to provide the intermediate **8,** which was heated with aniline to yield **9.** The chlorination of **9** was non-selective yielding the bichloro intermediate **10** by refluxing of **9** with $POCl_3$. Then chlorine at the 2-position of compound **10** was subsequently converted to keto in AcOH with AcONa to give **11**. 4-Chloroquinolinone intermediate **11** was then heated with benzyl amine and triethylamine in acetonitrile to provide **2** in a 66% yield. Synthesis of **3** started from the cyclization of 2-animothiozole with excess triethyl methanetricarboxylate to afford thiazolopyrimidine intermediate **13.** Then **13** was heated under reflux with TsCl and followed by coupling with benzyl amine to provide **14**. Compound **14** was hydrolyzed with aqueous LiOH for 20 h. Amidation with aniline by EDCI and HOBt in DCM under reflux condition furnished **3** in a 51% yield.

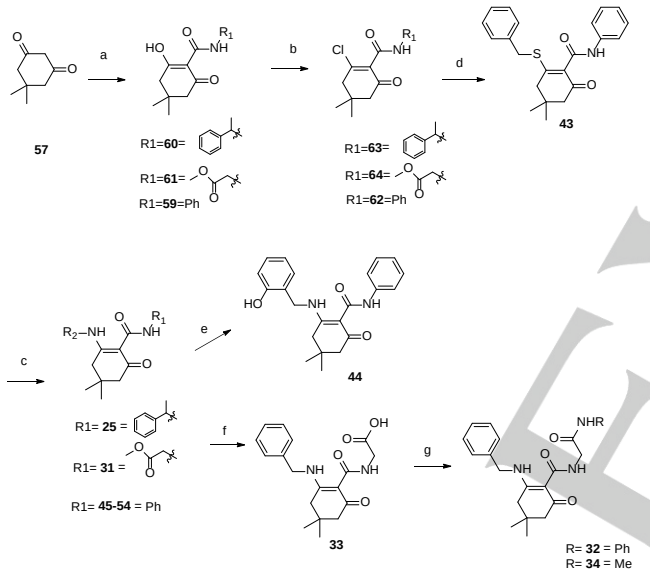
Scheme 2 describes the synthesis of **16-24, 26-38, 55-56** (structures in Table 2-4 and Figure 3). Starting with commercially available 5,5-dimethylcyclohexane-1,3-dione **57**, which was stirred with benzylamine in toluene at 80 °C to afford 5-(benzylimino)-3,3-dimethylcyclohexanone **58.** To facilitate the reaction progress, anhydrous sodium sulfate was added to abosorb generated water and removed by filtration upon completion of the reaction. The crude product of **58** was further purified by recrystallization with hexane/toluene 1:1. The resultant pure product **58** was exposed to various commercially available isocyanates to generate diverse *β*-enamioketone(**16-25, 27, 28, 35, 40-42**). Corresponding isocyanates **29, 37, 38,**



Scheme 1. *Reagents and conditions*: (a) Phosphorus oxychloride, DIEA, rt, 18h, 56%; (b) benzylamine, TEA, DCM, rt, 3h, 52%. (c) diethyl malonate, NaH, DMF, reflux, 5 h,70%; (d) 160°C,10 min; (e) phosphorus oxychloride, 110 °C, 2 h, 89%; (f) AcONa, AcOH, 120°C, 20 h, 57%; (g) benzylamine, TEA, DCM, rt, 4 h, 66%. (h) triethyl methanetricarboxylate, xylene, reflux, 5 h, 60%; (i) *p*-toluenesulfonyl chloride, TEA, acetonitrile, reflux, 3 h, benzyl amine, reflux, 2 h, 69%. (j) LiOH, MeOH, H_2O, 40°C, 20 h, 70%. (k) aniline, EDCI, HOBt, TEA, DCM, 40°C, 24 h, 51%.



Scheme 2. *Reagents and conditions*: (a) Sodium sulfate anhydrous, toluene, 80°C, 5 h, 86~97%; (b) R1NCO, 125°C, 1.5 h, 44%~81%. (c) BBr3, DCM, -78°C, 30 min, rt, 2h, 52%; (d) Tin(II) dichloride, EtOH, HCl,78°C,3 h, 43%; (e) 2-Isocyanatobenzothiazole, EtOH, microwave, 120°C, 30 min, 12%;



Scheme 3. *Reagents and conditions*: (a) Acetone/acetonitrile, TEA, rt or reflux, overnight, 31~85%; (b) oxalyl chloride, DMF, rt, overnight. 4 h,76%; (c) R2NH2, rt, TEA, DCM, 4 h, 29~78%; (d) benzyl mercaptan, 40°C, TEA, acetonitrile, 31%; (e) BBr3, DCM, -78°C, 30 min, rt, 2h, 30%; (f) LiOH, MeOH, H2O, rt, 12 h, 89%; (g) aniline/methylamine hydrochloride, EDCI, HOBt, TEA, DCM, rt, 12 h, 43~57%

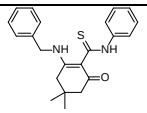
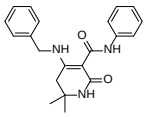
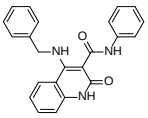
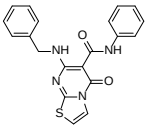
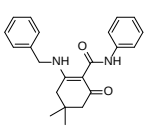
39 were prepared with substituted anilines and triphosgene in refluxing toluene. Heating the mixture of 2 equiv of isocyanate and 1 equiv of **58** under neat condition gave the desired products. The yields are dependent on the reactivity of the isocyanates (44%~81%) and were decreased when longer reaction time was applied. Substrates containing hydroxyl or carboxylic ester cannot react under this condition. For most compounds, ethanol was added after the reaction was completed to precipitate the crude products as white solids in moderate to excellent purity by recrystallization. Microwave irradiation of 2-isocyanatobenzothiazole with **58** at 130 °C in ethanol was conducted to give **30** (12%). Compound **27** was treated with boron tribromide to give corresponding hydroxyl products **26**. Optical isomers **55** and **56** were prepared by the same scheme using commercialy available *R*(+)- and *S*-(-)1-phenylethylamine as starting material.

Another efficient parallel synthetic approach was used for the installation of R_2 groups to generate compounds **25, 31-34, 43-54** (Scheme 3). Starting material 5,5-dimethyl- cyclohexane-1,3-dione **58** and corresponding isocyanates were stirred with 3 equiv of TEA in acetone overnight to give intermediate **59, 60** and **61** which were subsequently chlorinated with oxalyl chloride and catalytic amounts of DMF**.** The resulting intermediates (**62, 63,** and **64**) were coupled with the corresponding substituted amines or thioalcohol to obtain final products **43-54.** Compounds **47** were treated with boron tribromide to give products **44**. Compound **31** was obtained from **33** using LiOH and then conjugated with aniline or methylamine by EDCI/HOBt to provide **32** and **34.**

In vitro biological evaluation and SAR studies

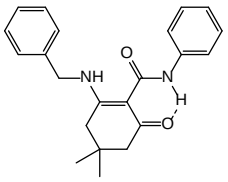
To establish a robust SAR, CX4338 was conceptualized as of having three structural units: (1) cyclohexanone core; (2) phenylthioamide moiety; and (3) benzyl amino functionality. All compounds were initially tested at 10 μM in a CXCR2 specific Tango assay as previously described[19]. Initial SAR study was based on a scaffold hopping strategy by keeping two side chains as well as the carbonyl group on the ring (Table 1). Bicyclic core including thiazolopyrimidinone and quinolinone **(2, 3**) resulted in a loss of activity. Similarly, simple replacement of one carbon of cyclohexanone with a heteroatom (e.g.

Table 1. IC_{50} values of CXCR2 of compounds **1, 2, 3** and **16** in CXCR2 Tango assay

Cpd	Structure	[a]IC_{50} (μM)[a]
CX4338		4.4±1.1
1		>10
2		>10
3		>10
16		4.2±1.1

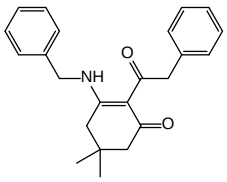
[a]CXCR2 inhibition was determined using the CXCR2 Tango assay. Values are the mean ± SD of at least three independent determinations.

compound **1)**, caused a significant decrease in activity. Based on these results, we postulate that the cyclohexanone moiety possibly occupies a hydrophobic pocket with a moderate size, which couldn't accommodate groups with polar properties or bulky substituent. The thiocarboxamide of CX4338 was replaced with bioisosteres because of its high chemical reactivity and potential *in vivo* toxicity[21]. Replacement of the thiocarboxamide moiety with carboxamide resulted in **16**, that maintained CXCR2-inhibiory potency. Because of its improved chemical stability and synthetic accessibility, the cyclohexene-1-carboxamide can serve as a new CXCR2 antagonist template. Compound **16** and CX4338 are not very soluble in protic solvent during synthesis. It is likely that an intramolecular H-bond is formed, as observed in many β-ketoamides[22] (Figure 2). Compared with **16**, an analog CX1142 with a methylene connection lacking intramolecular H-bond, showed 8 fold decreased potency (IC_{50} = 32.0 ± 5.5 μM). This suggested that the intramolecular H-bond may restrict the compound in a favorable conformation and it is important for binding. Considering intramolecular H-bond may improve membrane and blood-brain barrier (BBB) permeability[23], such inhibitors can be optimized to treat CNS tumors and inflammatory diseases.



16

CXCR2 IC_{50}=4.2 ±1.1μM



CX1142

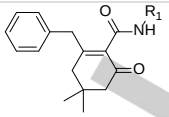
CXCR2 IC_{50}=32.0 ±5.5 μM

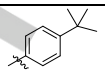
Figure 2. Intramolecular H-bond is important for CXCR2 inhibition

Our initial SAR efforts focused on phenylamide moiety (Table 2). Elaboration of benzene ring with chlorine atoms showed preference for the substituents at 4-position (**18**, IC_{50} = 6.7 μM) over the 3-position (**17**, IC_{50} > 50 μM) and bi-substitution (**19**, IC_{50} = 7.0 μM; **20**, IC_{50} = 3.3 μM) favored over mono-substitution. Thus, we synthesized additional analogs with 4-position substitutions. Interestingly, The classic isosteric replacement of 4-choloro with 4-methyl resulted in a totally inactive analog **21** but 4-methoxyl analog retained potency (**22**, IC_{50} = 5.0 μM). The extended benzyl analogues represented by **24** also retained potency (IC_{50} = 5.7 μM). This suggests that there is some space to accommodate large groups in the binding pocket. For example, the 4-tert-butyl analog **23 (**IC_{50} = 5.0 μM) may bind to this pocket. Adding an extra methyl group on **24** to give *α*-benzylmethyl analog **25** resulted in loss of activity. It is possible that the decrease in potency is due to *α*-benzylmethyl group forming a non-coplanar conformation whereas the binding pocket can only accommodate flat groups. Further decorations at 2-position (**21**, **27**, **28**) to increase out-of-plane steric hindrance resulted in loss of activity.

In order to extend our SAR efforts, we examined heterocyclic derivatives **29**, **30** as well as glycine derivatives **31**, **32**, **33**, **34**

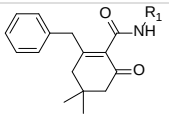
Table 2. IC_{50} values of CXCR2 of compounds **16-28** in CXCR2 Tango assay.

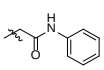
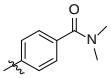
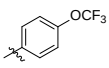
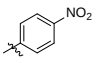


Cpd	R_1	[a]IC_{50} (μM)	Cpd	R_1	[a]IC_{50} (μM)
16		4.2±1.1	**23**		5.0±2.1
17		>50	**24**		5.7±0.8
18		6.7±2.3	**25**		>10
19		7.0±2.7	**26**		>10
20		3.3±0.7	**27**		>50
21		>50	**28**		>30
22		5.0±0.1			

[a] CXCR2 inhibition was determined using the CXCR2 Tango assay. Values are the mean ± SD of at least three independent determinations

Table 3. IC_{50} values of CXCR2 of compounds **29-42** in CXCR2 Tango assay



Cpd	R_1	[a]IC_{50} (μM)	Cpd	R_1	[a]IC_{50}(μM)
29		>10	**36**		>10
30		>10	**37**		27.7±9.0
31		>10	**38**		>10
32		>10	**39**		>10
33		>10	**40**		5.2±0.2
34		>10	**41**		2.9±0.4
35		>10	**42**		2.5±0.9

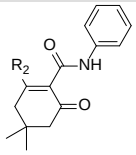
[a] CXCR2 inhibition was determined using the CXCR2 Tango assay. Values are the mean ± SD of at least three independent determinations

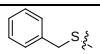
to explore potential H-bonding. However, these modifications led to inactive compounds (Table 3), suggesting that thebenzene ring contributes to potency. Therefore, changing

electron density of the ring may improve potency. Thus, a series of derivatives with lipophilic/hydrophilic, electron-withdrawing/electron-donating groups were synthesized (**35**-**42**). The results showed that lipophilic substitutions produced active inhibitions (**40**, **41**, **42**). Improved potency was shown in compounds with trifluoromethyl group on either 3-position (**41**, IC_{50} = 2.9 μM) or 4-position (**42**, IC_{50} = 2.5 μM). We postulate that a electron-deficient group positioned in a hydrophobic pocket may contribute to improved potency.

Next, our SAR efforts involved the modification of the benzyl amino moiety. The sulfur ether replacement of NH moiety was inactive, indicating that the NH is also important for retaining potency (Table 4). Considering that the phenylamide moiety is well tolerated with lipophilic groups and possibly sit in a hydrophobic pocket, we hypothesized that benzyl amine moiety is more likely to be solvent exposed. Many CXCR2 clinical candidates possess hydroxyl near free NHs (SB656933, SCH527123, AZD-8309, Figure 1). Therefore, we synthesized a series of derivatives with hydroxyl groups on different positions (**44-46**). Unfortunately, none of these derivatives were active. Interestingly, the 2-methoxyl derivative **47** retained potency. Since 2-methoxyl could cause the restricted rotation of the benzene ring, we designed and examined analogs with other bulky groups. Carboxylate derivative **48** lost activity while *α*-benzylmethyl was active (**49**, IC_{50} = 4.2 μM). Installing a hydroxyl on the methyl of **49** reduced potency (**50**, IC_{50} = 15.2 μM). That means moderate size groups can be well accommodated and bulky groups (e.g. **48)** were not tolerated. All efforts to improve aqueous solubility by replacing phenyl moiety with pyridine **51**-**52,** 3-benzamide **53** and isoxozole **54** led to reduced potency, reinforcing the narrow requirement of allowable lipophilicity.

Table 3. IC_{50} values of CXCR2 of compounds **43-54** in CXCR2 Tango assay



Cpd	R_2	[a]IC_{50} (μM)	Cpd	R_2	[a]IC_{50} (μM)
43		>10	**49**		4.2±1.9
44		11.1 ±6.4	**50**		15.2 ±4.8
45		>10	**51**		>10
46		>10	**52**		>10
47		6.1±1.6	**53**		>10
48		>10	**54**		>10

[a] CXCR2 inhibition was determined using the CXCR2 Tango assay. Values are the mean ± SD of at least three independent determinations

On the basis of above preliminary SAR results, we examined whether the potency gains from modifications of phenylamide and benzyl amine moieties would be additive. We combined the most favorable 3-trifluoromethyl-4-chloro phenylamide moiety and the privileged *α*-benzylmethyl amine moiety to obtain isomers **55** and **56**. The resulting hybrid compounds did not show improved potency over **42**. Although the cLogP of **55** is slightly higher than **42**, its out-of-plane methyl substitution reduces crystal packing, and to some extend can increase solubility.

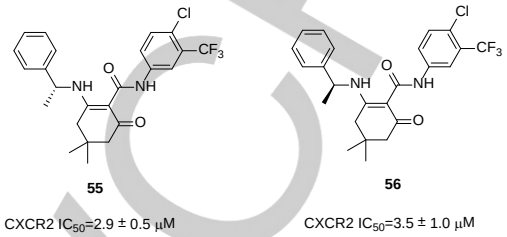


Figure 3. IC_{50} values of CXCR2 of compounds **55-56** in CXCR2 Tango assay

Since there is no crystal structure for CXCR2 and establishing a robust docking model for the ligands bound with GPCR receptor is challenging, ligand-based drug design guided by SAR is a potential strategy for designing new CXCR2 inhibitors. In this study, we made extensive modification of the structure of 3-aminocyclohex-2-en-1-ones to develop a SAR model. As shown in Figure 4, three hydrophobic sites are identified that makes the molecule rather lipophilic, indicating its binding site is dominantly hydrophobic. Incorporating nitrogen atom into the hexane ring or replacing it with fused aromatic rings is detrimental to potency and may not be well tolerated. EWG on ring A enhances activity and a lipophilic benzene ring is preferred on ring B. The NH group on the right side is probably involving in the formation of intramolecular H-bond to minimize the energy of active conformation, and consequently serves as an essential moiety for activity. The NH group on the left side may form H-bond with the receptor and is also important for activity.

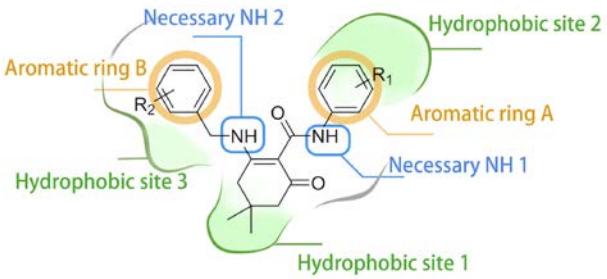


Figure 4. The SAR map of 3-aminocyclohex-2-en-1-ones.

To assess selectivity, all compounds were evaluated in the CXCR4 Tango assay. All compounds were inactive at 10 μM in a Tango assay specific for CXCR4 (Supplementary Table 1),

indicating good selectivity for CXCR2 over CXCR4. Additionally, these compounds are tested in a MTT assay and a colony formation assay to evaluate their cytotoxicity (Supplementary Table 2, Supplementary Table 3, Supplementary Figure 1). These compounds show low toxicity on OVCAR8 cells (MTT IC_{50} > 50 µM) and CXCR2-U2OS cells (MTT IC_{50} > 30 µM) suggesting that cytotoxicty did not contribute to CXCR2 inhibition.

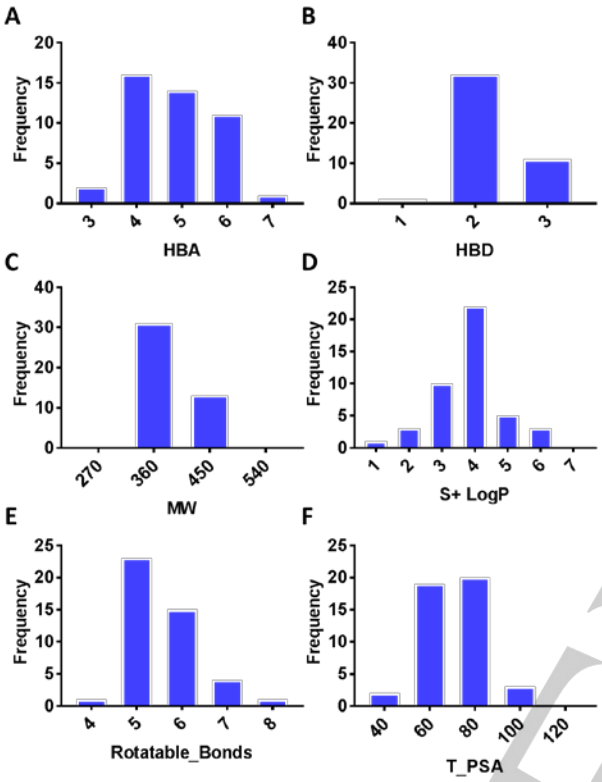


Figure 5. ADMET properties of CX4338 analogs. Frequency distribution plots for HBA (A), HBD (B), molecular weight (C), LogP (D), number of rotatable bonds (E) and topological polar surface area (F).

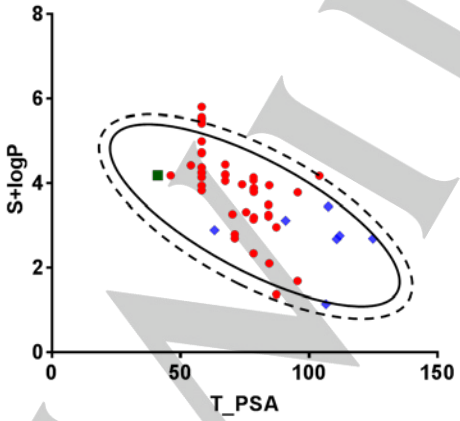


Figure 6. Topological polar surface area in Å^2 for each of CX4338 (green), its analogs (red) and CXCR2 antagonists in clinical trials (blue) is plotted against their corresponding calculated LogP.

ADMET properties

ADMET Predictor 8.0[24] was used to caculat select ADMET properties. We calculated various PK properties like topological polar surface area (T_PSA), LogP (S+logP)，molecular weight

Table 5. ADMET risk descriptors of CX4338 analogs and clinical trial compound

Identifier	Absn_Risk[a]	ADMET_Risk[b]	CYP_Risk[c]	TOX_Risk[d]	Rule Of 5[e]
Desired values[f]	<3.5	<7.5	<2.5	<3.3	≤1
CX4338	1.59	5.14	3.55	0	0
16	0.45	2.13	1.67	0	0
17	1.82	3.77	1.95	0	0
18	1.94	3.62	1.68	0	0
18	2.00	3.62	1.62	0	1
20	2.00	3.63	1.63	0	1
22	0.55	1.78	1.22	0	0
23	1.91	3.91	2.00	0	1
24	0.33	2.44	2.11	0	0
28	1.27	3.02	1.75	0	0
40	1.94	4.00	1.48	0.17	0
41	2.00	5.22	2.00	0	0
42	2.01	5.77	2.00	0	1
44	0.57	0.74	0.17	0	0
47	0.69	2.25	1.56	0	0
49	1.03	2.90	1.87	0	0
55	2.15	6.14	2.00	0.09	1
56	2.15	6.14	2.00	0.09	1
SB656933	0.71	1.23	0.52	0	0
Repertaxin	0.00	0.68	0.68	0	0
SCH527123	0.00	1.21	0.21	1	0
AZD-8309	0.00	6.00	3.00	3	0
DF2156A	0.00	2.00	0.00	2	0
Danirixin	1.59	1.81	0.22	0	0
AZD5069	2.79	6.75	1.96	2	0

[a] Absorption risk model: eight rules based on descriptors and predicted properties that contribute directly to the absorption of drugs. [b] ADMET risk model: all of the risk models combined and two others such as low unbound fraction and high steady-state volume of distribution. [c] CYP risk model: eight rules based on predicted enzymatic clearances and CYP inhibition. [d] Toxicity risk model: seven rules based on calculated toxicities including mutational risk model. [e] Lipinski's Rule of 5 violation count [i] Desired values are reported in the tutorial of the ADMET Predictor.

polar surface area (T_PSA), LogP (S+logP)，molecular weight (MW), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), number of rotatable bonds (N_FrRotB), Lipinski's rule of 5 violation (Ruleof5), risks descriptors including abosoption risk (Absn_Risk), AMET_risk, cytochrome P450 metabolism risk (CYP_Risk) and toxicity risk (Tox_risk). Figure 5 presents frequency distribution plots MW, LogP (S+ LogP), HBA, HBD, T_PSA in $A^{\circ 2}$, Rota_bonds of CX4338 analogs. Number of rotatable bonds represents conformational space of a molecule. The conformational behaviors directly or indirectly affect pharmacodynamics as well as pharmacokinetic properties. Too many rotatable bonds (optimal value ≤ 7) are not desirable for molecule to be drug-like[25]. Similar frequency plots of CXCR2 antagonists in clinical trials are presented in Supplementary Figure 2. CX4338 analogs showed similar ADMET properties to CXCR2 antagonists in clinical trials and obey Lipinski's rule. Figure 6 shows a T_PSA vs LogP plot (Egan plot) suggesting over 90% of the compounds are within desirable range of T_PSA (<140) as well as S+ logP (< 5) values. Egan plot represents prediction of drug absorption and considers correlations between these two parameters to oral absorption. On the basis of Egan et al absorption model[26], the outer ellipse represents a 99% confidence, whereas the inner ellipse a 95% confidence. T_PSA vs S+logP plots for optimized analogs (red points) are shifted from CXC4338 (green point) towards clinical trial antagonists (blue points) indicating that our lead optimization strategy is reasonable. Table 5 summarizes risk descriptors for absorption, CYP inhibition, toxicity and overall ADMET properties of the active compounds. Most of the compounds are predicted to be well tolerated as values are well below the threshold level of the risk descriptors.、

Conclusions

In the present study, we describe our initial SAR studies of a novel scaffold of CXCR2 inhibitor CX4338. Scaffold hopping was conducted to optimize the core structure. Compound **16** was synthesized by replacing sulfur with oxygen. Then, a series of 3-aminocyclohex-2-en-one derivatives were synthesized to select optimized ring substituents. Thirteen compounds showed IC_{50} values < 10 μM. We observed that both of the benzene rings as well as two NH groups are critical moieties for potency. Trifluromethylphenyl substituent on carboxamide moiety and *α*-benzylmethyl amino moiety improve the activity. All active compounds show selectivity against CXCR2 over CXCR4 and do not exhibit cytotoxicity. Compound **55** (IC_{50} = 2.9 ± 0.4 μM) and **42** (IC_{50} = 2.5 ± 0.9 μM) are new antagonists with desirable properties. *In silico* ADMET predicted properties suggest that lead optimization is reasonable according to Egan plot, where properties of new analogs are improved towards the CXCR2 antagonists in clinical trials. Since this scaffold is new, our SAR studies provided valuable data for further optimization and of new CXCR2 antagonist for treating inflammatory diseases.

Experimental Section

Chemistry

All commercial reagents and anhydrous solvents were purchased and used without purification unless specified. Column chromatography was performed using a Biotage Isolera chromatography system by normal phase silica gel columns. NMR spectra were recorded on a Bruker Ultrashield 300 MHz or a Bruker Ascend 400 MHz NMR spectrometer. Chemical shifts (*δ*) are reported in parts per million (ppm) units relative to residual undeuterated solvent. Mass spectra were obtained on a Shimadzu LCMS-2020 liquid chromatography mass spectrometer using the electron spray ionization (ESI) method. HPLC was used to determine purity of biologically tested compounds by Shimadzu HPLC Test Kit C18 column (3 μm, 4.6 × 50 mm) under the following gradient elution condition: acetonitrile/water (10-95%), gradient time: 15 min. The purity was established by integration of the areas of major peaks detected at 254 nm and all final products are >95% pure as determined by the Shimadzu LCMS-2020. All spectral information is listed in the Supporting Information.

4-Chloro-6,6-dimethyl-2-oxo-N-phenyl-1,2,5,6-tetrahydropyridine-3-carboxamide 6

To 10 mL phosphorus oxychloride was added 4-hydroxy-6,6-dimethyl-2-oxo-*N*-phenyl-1,2,5,6-tetrahydropyridine-3-carboxamide **5** (200 mg, 0.769 mmol) and *N*,*N*-diisopropylethylamine (2 mL). The solution immediately turned red. The reaction mixture was stirred at room temperature for 18 h and solvent was removed under reduced pressure. Then, the resulting crude was extracted with EtOAc and 1N aqueous sodium bicarbonate for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, concentrated in vacuo to afford the crude compound. The crude was purified *via* silica gel chromatography to afford **6** (120 mg, 56% yield) as colorless oil.

4-(Benzylamino)-6,6-dimethyl-2-oxo-N-phenyl-1,2,5,6-tetrahydropyridine-3-carboxamide 1

To a solution of **6** (50 mg, 0.179 mmol) in DCM (10 mL) was added triethylamine (0.074 mL, 0.538 mmol) and benzyl amine (23 μL 0.215 mmol). The reaction mixture was stirred at room temperature for 3 h. Then, 10 mL 1N aqueous HCl was added and the solution was extracted with EtOAc for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo and the crude was purified via silica gel chromatography to afford **1** (33 mg, 52% yield).

Ethyl-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylate 8

To a solution of isatoic anhydride **7 (**1.0 g, 6.13 mmol**)** in anhydrous DMF (20 mL) under argon was added sodium hydride (0.49 g, 60% in mineral oil, 12.3 mmol) at -5 °C**.** The suspension was stirred for 20 min and then diethyl malonate (1.12 mL, 7.36 mmol) was added. The mixture was heated to reflux for 5 h. Then the mixture was cooled to 0 °C and was carefully added 1N aqueous hydrochloric (50 mL). The resulting solid was collected by filtration, washed with water and diethylether and then dried in vacuo to give the desired compound **8** (1.0 g, 70% yield) .

4-Hydroxy-2-oxo-N-phenyl-1,2-dihydroquinoline-3-carboxamide 9

Compound **8** (500 mg, 2.13 mmol) and aniline (191 μL, 2.13 mmol) were mixed in a round-bottom flask under argon. The

reaction mixture was stirred at 160 °C for 10 min. The mixture was cooled to room temperature. 10 mL ethanol was added and stirred for 30 min and filtered to give white solid **9** (480 mg,78% yield).

2,4-Dichloro-N-phenylquinoline-3-carboxamide 10
To 10 mL phosphorus oxychloride was added **9** (200 mg, 0.769 mmol) and the reaction mixture was stirred at 110 °C for 2 h and solvent was removed under reduced pressure. The resulting crude was directly used for next step without further purification (200 mg, 89% yield)

4-Chloro-2-oxo-N-phenyl-1,2-dihydroquinoline-3-carboxamide 11
To a solution of **10** (50 mg, 0.158 mmol) in acetic acid (5 mL) was added sodium acetate anhydrous (16 mg, 0.174 mmol). The reaction mixture was stirred at 120 °C for 20 h. The mixture was cooled to room temperature and poured into 20 ml water. The resulting solid was filtered and washed with water and recrystallized with hexane and ethyl acetate to provide white solid **11** (27mg, 57% yield).

4-(Benzylamino)-2-oxo-N-phenyl-1,2-dihydroquinoline-3-carboxamide 2
To a solution of **11** (20 mg, 0.0692mmol) in acetonitrile (10 mL) was added triethylamine (19 µL, 0.138 mmol) and benzyl amine (8 µL, 0.0761 mmol). The reaction mixture was heated to reflux for 4h. The reaction was quenched by 10 ml 1N HCl solution and extracted with EA for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo to afford the crude compound. The crude was purified *via* silica gel chromatography to afford **2** (17 mg, 66% yield) as a white solid.

Ethyl-7-hydroxy-5-oxo-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate 13
To a solution of 2-animothiozole **12** (1.0 g, 10.0 mmol) in xylene (20 ml) was added triethyl methanetricarboxylate (2.32 g, 30.0 mmol). The mixture was heated to reflux for 5h. Then solvent was removed under vacuum and the resulting brown solid was recrystallized with isopropanol to afford yellow solid **13** (1.44g, 60% yield).

Ethyl-7-(benzylamino)-5-oxo-5H-thiazolo[3,2-a]pyrimidine-6-carboxylate 14
To a solution of **13** (200 mg, 0.833 mmol) in acetonitrile (20 ml) was added triethylamine (460 µL, 3.34 mmol) and *p*-toluenesulfonyl chloride (158 mg, 0.833 mmol). The mixture was heated to reflux for 5 h. The mixture was cooled to room temperature and benzylamine was added (89 µL, 0.833 mmol). The solution was refluxed for another 2h. The reaction was quenched by 10 ml 1N HCl solution and extracted with EA for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo to afford the crude compound. The crude was purified via silica gel chromatography to afford **14** (189 mg, 69% yield) as a white solid.

7-(Benzylamino)-5-oxo-5H-thiazolo[3,2-a]pyrimidine-6-carboxylic acid 15
To a solution of **14** (50 mg, 0.152 mmol) in methanol (5 ml) was added lithium hydroxide (19 mg, 0.456 mmol) monohydrate and H_2O (1 ml). The reaction mixture was stirred at 40°C for 20 h. Then the mixture was cooled to room temperature, 20 ml 1N aqueous HCl was added and extracted with EA for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo to afford **15** (32 mg, 70% yield).

7-(Benzylamino)-5-oxo-N-phenyl-5H-thiazolo[3,2-a]pyrimidine-6-carboxamide 3
To a solution of **15** (20 mg, 0.0664 mmol) in DCM (10 mL) was added EDCI(19 mg, 0.0997 mmol), HOBt (13 mg, 0.0997 mmol), triethylamine (18 µL, 0.199 mmol) and aniline(9.4 µL ,0.0997 mmol). The reaction mixture was stirred at 40 °C for 24 h. Then 10 ml 1N aqueous HCl was added and the solution was extracted with DCM for 3 times. The combined organic layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo and the crude was purified via silica gel chromatography to afford **3** (13 mg, 51% yield) as a white solid.

5-(Benzylimino)-3,3-dimethylcyclohexanone 58
To a solution of 5,5-dimethylcyclohexane-1,3-dione **57** (2.0 g, 14.2 mmol) in toluene (20 mL) was added 2.00 g sodium sulfate anhydrous and benzylamine (1.86 mL, 16.9 mmol). The reaction mixture was stirred at 80 °C for 5 h. The mixture was filtered immediately at high temperature and washed with hot toluene 2 times. The filtrate was concentrated in vacuo and recrystallized with hexane/toluene 1:1 to afford **58** (3.16 g, 97% yield) as a light-yellow needle crystal.

(R)-5,5-Dimethyl-3-((1-phenylethyl)amino)cyclohex-2-en-1-one 65
To a solution of 5,5-dimethylcyclohexane-1,3-dione **57** (1.0 g, 7.10 mmol) in toluene (20 mL) was added 1.00g sodium sulfate anhydrous and (*R*)-(+)-1-phenylethylamine (1.03 mL， 8.52 mmol). The reaction mixture was stirred at 80 °C for 5 h. The mixture was filtered immediately at high temperature and washed with hot toluene 2 times. The filtrate was concentrated in vacuo and recrystallized with hexane and toluene to afford **65** (1.52 g, 88% yield) as a light-yellow needle crystal.

(S)-5,5-Dimethyl-3-((1-phenylethyl)amino)cyclohex-2-en-1-one 66
Compound **66** was prepared from 5,5-dimethylcyclohexane-1,3-dione **57** and (*S*)-(-)-1-phenylethylamine according to the same procedure as **65** to give light-yellow needle crystal, 1.49 g, in 86% yield.

General procedure I of the preparation of compounds 17-24,27-29,31-35,37,38, 55,56,
5-imino-3,3-dimethylcyclohexanone (1 eq.) and 2 eq. isocyanates were mixed in a round-bottom flask under argon. The reaction mixture was stirred at 125 °C for 2 h. The mixture was cooled to room temperature. Ethanol (5mL) was added and stirred for 30 min and filtered to give pure compounds.

2-(Benzylamino)-N-(3-chlorophenyl)-4,4-dimethyl-6-oxocyclohex-1-enecarboxamide 17

Compound **17** was prepared from **58** and 4-chlorophenyl isocyanate according to the general procedure I described above as a white solid, in 70% yield.

2-(Benzylamino)-4,4-dimethyl-6-oxo-N-phenylcyclohex-1-enecarboxamide 16
Compound **16** was prepared from **58** and phenyl isocyanate according to the general procedure I described above as a white solid, in 61% yield.

2-(Benzylamino)-N-(4-chlorophenyl)-4,4-dimethyl-6-oxocyclohex-1-enecarboxamide 18
Compound **18** was prepared from **58** and 4-chlorophenyl isocyanate according to the general procedure I described above as a white solid, in 66% yield.

2-(Benzylamino)-N-(3,5-dichlorophenyl)-4,4-dimethyl-6-oxocyclohex-1-enecarboxamide 19
Compound **19** was prepared from **58** and 3,5-dichlorophenyl isocyanate according to the general procedure I described above as a white solid, in 45% yield.

2-(Benzylamino)-4,4-dimethyl-6-oxo-N-(4-(trifluoromethyl)phenyl)cyclohex-1-enecarboxamide 40
Compound **40** was prepared from **58** and 4-(trifluoromethyl)phenyl isocyanate according to the general procedure I described above as a white solid, in 76% yield.

2-(Benzylamino)-N-(4-chloro-3-(trifluoromethyl)phenyl)-4,4-dimethyl-6-oxocyclohex-1-enecarboxamide 42
Compound **42** was prepared from **58** and 4-chloro-3-(trifluoromethyl)phenyl isocyanate according to the general procedure I described above as a white solid (yield 80%).

2-(Benzylamino)-N-(3,4-dichlorophenyl)-4,4-dimethyl-6-oxocyclohex-1-enecarboxamide 20
Compound **20** was prepared from **58** and 3,4-dichlorophenyl isocyanate according to the general procedure I described above as a white solid (yield 65%).

2-(Benzylamino)-N-(2-fluorophenyl)-4,4-dimethyl-6-oxocyclohex-1-enecarboxamide 28
Compound **28** was prepared from **58** and 2-fluorophenyl isocyanate according to the general procedure I described above as a white solid, in 72% yield.

2-(Benzylamino)-4,4-dimethyl-N-(4-nitrophenyl)-6-oxocyclohex-1-enecarboxamide 35
Compound **35** was prepared from **58** and (4-nitrophenyl isocyanate according to the general procedure I described above as a yellow solid. in 81% yield.

N-(4-Aminophenyl)-2-(benzylamino)-4,4-dimethyl-6-oxocyclohex-1-enecarboxamide 36
To a solution of **35** (40 mg, 0.102 mmol) in EtOH (10 mL) was added Tin (II) dichloride (114 mg, 0.508mmol) and 1 drop hydrochloric acid (36%). The reaction mixture was stirred at 78 °C for 3 h. The mixture was cooled to room temperature and pH was adjusted to 8 with sodium carbonate solution (1 N). The water solution was extracted with EtOAc. The combined organic layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo to afford the crude compound. This crude was purified via silica gel chromatography to afford **36** (16 mg, 43% yield) as an off white solid.

2-(Benzylamino)-4,4-dimethyl-6-oxo-N-(p-tolyl)cyclohex-1-enecarboxamide 21
Compound **21** was prepared from **58** and p-tolyl isocyanate according to the general procedure I described above as a white solid, in 43% yield.

2-(Benzylamino)-N-(4-methoxyphenyl)-4,4-dimethyl-6-oxocyclohex-1-enecarboxamide 22
Compound **22** was prepared from **58** and 4-methoxyphenyl isocyanate according to the general procedure I described above, in 47% yield.

2-(Benzylamino)-N-(4-(tert-butyl)phenyl)-4,4-dimethyl-6-oxocyclohex-1-enecarboxamide 23
Compound **23** was prepared from **58** and 4-(tert-butyl)phenyl isocyanate according to the general procedure I described above as a white solid, in 30% yield.

N-benzyl-2-(Benzylamino)-4,4-dimethyl-6-oxocyclohex-1-enecarboxamide 24
Mixed 5-(benzylimino)-3,3-dimethylcyclohexanone **58** (50 mg, 0.218 mmol) was mixed with benzyl isocyanate (58 µL, 0.436 mmol) in a round-bottom flask under argon. The reaction mixture was stirred at 125 °C for 3 h. The mixture was cooled to room temperature and acidified to pH 3 with hydrochloric acid (1 N). The acidic solution was extracted with EtOAc. The combined organic layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo to afford the crude compound. This crude was purified via silica gel chromatography to afford **24** (25 mg, 31% yield) as a white solid.

2-(Benzylamino)-N-(2-methoxyphenyl)-4,4-dimethyl-6-oxocyclohex-1-ene-1-carboxamide 27
Compound **27** was prepared from **58** and 4-methoxyphenyl isocyanate according to the general procedure I described above as a white solid (yield 51%).

2-(Benzylamino)-N-(2-hydroxyphenyl)-4,4-dimethyl-6-oxocyclohex-1-ene-1-carboxamide 26
A solution of **26 (**100 mg, 0.264 mmol) in anhydrous DCM (20 mL) was stirred at -78°C for 30 min, 1M BBr_3 solution in THF (1.32 ml, 1.32 mmol) was added dropwise. The reaction mixture was slowly warmed to room temperature and stirred for 2 h. Water(20 ml) was slowly added to quench the reaction. The mixture was extracted with DCM for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo to afford a clear oil. The crude was refluxed in 1M HCl ethanol solution for 2h to dissociate the boron complex. Solvent was then removed and the crude was purified via silica gel chromatography to afford **30** (50 mg, 52% yield) as a white solid.

General procedure II of the preparation of isocyanates 67-70

A solution of triphosgene (0.35 eq.) in anhydrous toluene (10 mL) was added anilines (1eq.) in anhydrous DCM (10 mL) dropwise at 0°C within 15 min. After addition, the reaction was refluxed for 8 h. The solvent was removed in vacuo and the resulting isocyanate was used directly without further purification.

3-Isocyanato-N,N-dimethylbenzenesulfonamide 67
Compound **67** was prepared from 3-amino-***N***,***N***-dimethylbenzenesulfonamide according to the general procedure II described above as a colorless oil.

3-Isocyanato-N,N-dimethylbenzamide 68
Compound **68** was prepared from 3-amino-N,N-dimethylbenzamide according to the general procedure II described above as a colorless oil.

4-Isocyanato-N,N-dimethylbenzamide 69
Compound **69** was prepared from 4-amino-N,N-dimethylbenzamide according to the general procedure II described above as a colorless oil.

3-Isocyanato-5-methylisoxazole 70
Compound **70** was prepared from 3-amino-5-methylisoxazole according to the general procedure II described above as a colorless oil.

2-Isocyanatobenzothiazole 71
Compound **71** was prepared from 2-aminobenzothiazole according to the general procedure II described above as a yellow solid.

2-(Benzylamino)-N-(3-(N,N-dimethylsulfamoyl)phenyl)-4,4-dimethyl-6-oxocyclohex-1-ene-1-carboxamide 37
Compound **37** was prepared from **58** and **67** according to the general procedure I described above as a white solid, in 60% yield.

3-(2-(Benzylamino)-4,4-dimethyl-6-oxocyclohex-1-ene-1-carboxamido)-N,N-dimethylbenzamide 38
Compound **38** was prepared from **58** and **68** according to the general procedure I described above as a white solid, in 43% yield.

4-(2-(Benzylamino)-4,4-dimethyl-6-oxocyclohex-1-ene-1-carboxamido)-N,N-dimethylbenzamide 39
Compound **39** was prepared from **58** and **69** according to the general procedure I described above as a white solid, in 51% yield.

2-(Benzylamino)-4,4-dimethyl-N-(5-methylisoxazol-3-yl)-6-oxocyclohex-1-ene-1-carboxamide 29
Compound **29** was prepared from **58** and **70** according to the general procedure I described above as a white solid, in 44% yield.

N-(Benzo[d]thiazol-2-yl)-2-(benzylamino)-4,4-dimethyl-6-oxocyclohex-1-ene-1-carbothioamide 30
To a solution of **58** (76 mg, 0.436 mmol) in EtOH (5 mL) added **71** (50 mg, 0.218 mmol) in a microwave tube. The mixture was reacted in microwave reactor at 120°C for 30 min, then solvent was removed and the crude was purified via silica gel chromatography to afford **36** as a white solid (11mg, 12% yield).

2-(Benzylamino)-4,4-dimethyl-6-oxo-N-(4-(trifluoromethoxy)phenyl)cyclohex-1-ene-1-carboxamide 40
Compound **40** was prepared from **58** and 4-(trifluoromethoxyl)phenyl isocyanate according to the general procedure I described above as a white solid. yield 44%.

(R)-N-(4-Chloro-3-(trifluoromethyl)phenyl)-4,4-dimethyl-6-oxo-2-((1-phenylethyl)amino)cyclohex-1-ene-1-carboxamide 55
Compound **55** was prepared from **65** and 4-chloro-3-(trifluoromethyl)phenyl isocyanate according to the general procedure I described above as a white solid, in 78% yield.

(S)-N-(4-Chloro-3-(trifluoromethyl)phenyl)-4,4-dimethyl-6-oxo-2-((1-phenylethyl)amino)cyclohex-1-ene-1-carboxamide 56
Compound **56** was prepared from **66** and 4-chloro-3-(trifluoromethyl)phenyl isocyanate according to the general procedure I described above as a white solid in 81%yield.

2-Hydroxy-4,4-dimethyl-6-oxo-N-(1-phenylethyl)cyclohex-1-ene-1-carboxamide 59
To a solution of 5,5-dimethylcyclohexane-1,3-dione (1.5 g, 10.7 mmol) in DCM (30 mL) was added triethylamine (5.9 ml, 32.1 mmol) and stirred for 10 min at 0 °C . Then a solution of phenyl isocyanate (1.91g, 16.50 mmol) in DCM (10 ml) was added dropwise. The reaction mixture was stirred at room temperature for 12 h. The reaction was quenched by 20 ml 1N HCl solution and extracted with DCM for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo to afford the crude compound. The crude was purified via silica gel chromatography to afford **59** (2.33 g, 85% yield) as a white solid..

2-Chloro-4,4-dimethyl-6-oxo-N-phenylcyclohex-1-ene-1-carboxamide 62
To a solution of 4,4-dimethyl-2,6-dioxo-N-phenylcyclohexane-1-carboxamide **59** (1.0 g, 3.86 mmol) in anhydrous DCM (20 mL) was added oxalyl chloride (3.30ml, 38.6mmol) and 0.05 ml DMF. The solution immediately became red. The reaction mixture was stirred at room temperature for 18 h and was quenched by carefully adding 2 ml water in an ice bath. Then the mixture was extracted with EtOAc and 1N aqueous sodium bicarbonate for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, concentrated in vacuo to afford the crude compound. This crude was purified via silica gel chromatography to afford **62** (0.82 g, 76% yield) as colorless oil.

2-Hydroxy-4,4-dimethyl-6-oxo-N-(1-phenylethyl)cyclohex-1-ene-1-carboxamide 60
To a solution of 5,5-dimethylcyclohexane-1,3-dione **57** (500 mg, 3.57 mmol) in acetonitrile (20 mL) was added triethylamine (1.97 ml,10.7 mmol) and stirred for 10 min at 0 °C . Then a solution of (1-isocyanatoethyl)benzene (786 g, 5.35 mmol) in DCM (10 ml) was added dropwise. The reaction mixture was heated to reflux for 12 h. The reaction was quenched by 20 ml

1N HCl solution and extracted with DCM for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo to afford the crude compound. The crude was purified via silica gel chromatography to afford **60** (317 mg, 31% yield) as a white solid.

2-Chloro -4,4-dimethyl-6-oxo-N-(1-phenylethyl)cyclohex-1-ene-1-carboxamide 63

To a solution of **60** (100 mg, 0.348 mmol) in anhydrous DCM (10 mL) was added oxalyl chloride (0.297 ml, 3.48 mmol) and 0.01 ml DMF. The solution immediately became red. The reaction mixture was stirred at room temperature for 18 h and was quenched by carefully adding 2 ml water in an ice bath. Then the mixture was extracted with EtOAc and 1N aqueous sodium bicarbonate for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, concentrated in vacuo to afford 105mg red oil. the resulting crude was used directly without further purification.

2-(Benzylamino)-4,4-dimethyl-6-oxo-N-(1-phenylethyl)cyclohex-1-ene-1-carboxamide 25

To a solution of **63** (50 mg, 0.163 mmol) in DCM (10 mL) was added triethylamine (0.068 ml, 0.491 mmol) and benzyl amine (21 µL, 0196 mmol). The reaction mixture was stirred at room temperature for 3 h. Then 10ml 1N aqueous HCl was added and the solution was extracted with EtOAc for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo and the crude was purified via silica gel chromatography to afford **25** (21 mg, 34% yield) as a white solid.

Methyl-(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-ene-1-carbonyl)glycinate 61

To a solution of 5,5-dimethylcyclohexane-1,3-dione **57**(500 mg, 3.57 mmol) in acetonitrile (20 mL) was added triethylamine (1.97 ml, 10.7 mmol) and stirred for 10 min at 0 °C. Then a solution of methyl 2-isocyanatoacetate (615 mg, 5.35 mmol) in DCM (10ml) was added dropwise. The reaction mixture was heated to reflux for 12 h. The reaction was quenched by 20 ml 1N HCl solution and extracted with DCM for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo to afford the crude compound. The crude was purified via silica gel chromatography to afford **61** (295 mg, 32% yield) as a white solid.

Methyl-(2-chloro-4,4-dimethyl-6-oxocyclohex-1-ene-1-carbonyl)glycinate 64

To a solution of **61** (100 mg, 0.392 mmol) in anhydrous DCM (10 mL) was added oxalyl chloride (0.335 ml, 3.92 mmol) and 0.01ml DMF. The solution immediately became red. The reaction mixture was stirred at room temperature for 18 h and was quenched by carefully adding 2 ml water in an ice bath. Then the mixture was extracted with EtOAc and 1N aqueous sodium bicarbonate for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, concentrated in vacuo to afford 120 mg red oil. the resulting crude was used directly without further purification.

Methyl-(2-(benzylamino)-4,4-dimethyl-6-oxocyclohex-1-ene-1-carbonyl)glycinate 31

To a solution of **64** (50 mg, 0.183 mmol) in DCM (10 mL) was added triethylamine (0.076 ml, 0.549 mmol) and benzyl amine(24 µL, 0.219 mmol). The reaction mixture was stirred at room temperature for 3 h. Then 10ml 1N aqueous HCl was added and the solution was extracted with EtOAc for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo and the crude was purified via silica gel chromatography to afford **31** (34 mg, 54% yield)as a white solid.

(2-(Benzylamino)-4,4-dimethyl-6-oxocyclohex-1-ene-1-carbonyl)glycine 33

To a solution of **31** (50 mg, 0.145 mmol)in methanol (5 ml) added lithium hydroxide (18 mg, 0.436 mmol)monohydrate and H_2O (1 ml). The reaction mixture was stirred at room temperature for 12 h. Then the mixture was cooled to room temperature and 20 ml 1N aqueous HCl was added and the suspension was stirred for 15 min. The precipitated white solid was collected by filtration and dried in vacuo to afford **33** (43 mg, 89% yield).

2-(Benzylamino)-4,4-dimethyl-6-oxo-N-(2-oxo-2-(phenylamino)ethyl)cyclohex-1-ene-1-carboxamide 32

To a solution of **33** (30 mg, 0.091 mmol) in DCM(10 ml) was added EDCI (27 mg, 0.136 mmol), HOBt (18 mg, 0.136 mmol), triethylamine (30 µL, 0.272 mmol) and aniline (10.5 µL ,0.109 mmol). The reaction mixture was stirred at room temperature for 12 h. Then 10ml 1N aqueous HCl was added and the solution was extracted with DCM for 3 times. The combined organic layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo and the crude was purified via silica gel chromatography to afford **32** (21 mg, 57% yield) as a white solid.

2-(Benzylamino)-4,4-dimethyl-N-(2-(methylamino)-2-oxoethyl)-6-oxocyclohex-1-ene-1-carboxamide 34

To a solution of **33** (30 mg, 0.091 mmol) in DCM(10ml) added EDCI (27 mg, 0.136 mmol), HOBt (18 mg, 0.136 mmol), triethylamine (30 µL, 0.272 mmol) and methylamine hydrochloride (7.4 mg ,0.109 mmol). The reaction mixture was stirred at room temperature for 12 h. Then 10 ml 1N aqueous HCl was added and the solution was extracted with DCM for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo and the crude was purified via silica gel chromatography to afford **34** (16 mg, 43% yield) as a white solid.

2-(Benzylthio)-4,4-dimethyl-6-oxo-N-phenylcyclohex-1-ene-1-carboxamide 43

To a solution of **62** (50 mg, 0.180 mmol) in acetonitrile (10 mL) was added triethylamine (0.075 ml, 0.540 mmol) and benzyl mercaptan (26 µL, 0.216 mmol). The reaction mixture was stirred at 40°C temperature for 4 h. Then the mixture was cooled to room temperature and 10 ml 1N aqueous HCl was added. The solution was extracted with EtOAc for 3 times. The combined organic layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo.

The crude was purified via silica gel chromatography to afford **43** as a yellow solid (20 mg, 31% yield).

General procedure III of the preparation of 45-54

To a solution of **62** (50 mg, 0.180 mmol) in DCM (10 mL) was added triethylamine (0.075 ml, 0.540 mmol) and amines (0.216 mmol). The reaction mixture was stirred at room temperature for 3 h. Then 10 ml 1N aqueous HCl was added and the solution was extracted with EtOAc for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in *vacuo* and the crude was purified via silica gel chromatography.

2-((2-Hydroxyphenyl)amino)-4,4-dimethyl-6-oxo-N-phenylcyclohex-1-ene-1-carboxamide 45

Compound **45** was prepared from **62** and 2-hydroxyaniline according to the general procedure III described above as a white solid. yield 44%.

2-((1-Hydroxypropan-2-yl)amino)-4,4-dimethyl-6-oxo-N-phenylcyclohex-1-ene-1-carboxamide 46

Compound **46** was prepared from **62** and 2-amino-1-propanol according to the general procedure III described above as a white solid in 44% yield.

2-(((5,5-Dimethyl-3-oxo-2-(phenylcarbamoyl)cyclohex-1-en-1-yl)amino)methyl)benzoate 48

Compound **48** was prepared from **62** and methyl 2-(aminomethyl)benzoate according to the general procedure III described above as a white solid in 56% yield.

2-((2-Hydroxy-1-phenylethyl)amino)-4,4-dimethyl-6-oxo-N-phenylcyclohex-1-ene-1-carboxamide 50

Compound **50** was prepared from **62** and *DL*-2-Phenylglycinol according to the general procedure III described above as a white solid in 33% yield.

2-((2-Methoxybenzyl)amino)-4,4-dimethyl-6-oxo-N-phenylcyclohex-1-ene-1-carboxamide 47

Compound **47** was prepared from **62** and methyl 2-methoxybenzylamine according to the general procedure III described above as a white solid in 70% yield.

2-((2-Hydroxybenzyl)amino)-4,4-dimethyl-6-oxo-N-phenylcyclohex-1-ene-1-carboxamide 44

A solution of **44 (**50 mg, 0.132 mmol) in anhydrous DCM (10 mL) was stirred at -78 °C for 30 min, 1M BBr_3 solution in THF (0.67 ml, 0.66 mmol) was added dropwise. The reaction mixture was slowly warmed to room temperature and stirred for 2 h. 20ml water was slowly added to quench the reaction. The mixture was extracted with DCM for 3 times. The combined organics layer was washed with brine, dried over sodium sulfate anhydrous, filtered, and concentrated in vacuo to afford a clear oil. The crude was refluxed in 1M HCl ethanol solution for 2h to dissociate the boron complex. Solvent was then removed and the crude was purified via silica gel chromatography to afford **19** (14 mg, 30% yield) as a white solid.

4,4-Dimethyl-6-oxo-N-phenyl-2-((pyridin-2-ylmethyl)amino)cyclohex-1-ene-1-carboxamide 51

Compound **51** was prepared from **62** and methyl pyridin-2-ylmethanamine according to the general procedure III described above as a white solid in 60% yield..

4,4-Dimethyl-6-oxo-N-phenyl-2-((pyridin-3-ylmethyl)amino)cyclohex-1-ene-1-carboxamide 52

Compound **52** was prepared from **62** and methyl pyridin-3-ylmethanamine according to the general procedure III described above as a white solid in 29% yield.

3-(((5,5-Dimethyl-3-oxo-2-(phenylcarbamoyl)cyclohex-1-en-1-yl)amino)methyl)-N,N-dimethylbenzamide 53

Compound **53** was prepared from **62** and methyl 2-(aminomethyl)-N,N-dimethylbenzamide according to the general procedure III described above as a white solid in 42% yield.

4,4-Dimethyl-6-oxo-N-phenyl-2-((1-phenylethyl)amino)cyclohex-1-ene-1-carboxamide 49

Compound **49** was prepared from **62** and methyl 1-phenylethan-1-amine according to the general procedure III described above as a white solid in 78% yield.

4,4-Dimethyl-2-(((3-methylisoxazol-5-yl)methyl)amino)-6-oxo-N-phenylcyclohex-1-ene-1-carboxamide 54

Compound **54** was prepared from **62** and methyl (5-methylisoxazol-3-yl)methanamine according to the general procedure III described above as a white solid in 67% yield.

ADMET properties calculation

ADMET properties and associated risk values were calculated using ADMET Predictor 8.0 (Simulation Plus, Inc.)[28]. The absorption risk model includes eight rules, each contributes a value of 1, based on descriptors and predicted properties that directly contributes to the absorption of drugs, such as molecular weight, H-bond donor, H-bond acceptor, polar surface area, number of rotatable bonds, etc. The CYP risk model is composed of eight rules, each contributes a value of 1, based on predicted enzymatic clearances and CYP inhibitions. The toxicity risk model comprises seven rules, each contributes a value of 1, based on calculated toxicities, such as hERG toxicity, acute toxicity in rats including the mutational risk model. The ADMET risk model comprises a total of 23 rules, each contributes a value of 1, which include all of these risk models and two others, such as low unbound fraction and high steady state volume of distribution. Descriptors BBB Filter and LogBB were presented by two blood-brain barrier models (a qualitative permeability model and a blood-brain partition coefficient model). Log*P* and topological polar surface area were also calculated.

Biology

Cell culture: Tango ™ CXCR2-bla and CXCR4-bla U2OS cells were purchased from Invitrogen (Carlsbad, CA, USA) and grown in McCoy5A supplemented with 10% dialized FBS, zeocin (200 μg·mL−1), hygromycin (50 μg·mL−1), geneticin (100 $\mu g \cdot mL^{-1}$), 1 mM sodium pyruvate, 0.1 mM non-essential amino acids and 25 mM HEPES. OVCAR8 cells were cultured in RPMI 1640 medium (Gibco) supplemented with 10% FBS

(Gibco). All of the cells were grown at 37 °C in a humidified atmosphere of 5% CO_2. All cell lines used were maintained in culture under 35 passages and tested regularly for Mycoplasma contamination using Plasmo Test (InvivoGen, San Diego, CA).

CXCR2/4 Tango assay: The compounds inhibition potency for stimulus mediated CXCR2/4 *β*-arrestin-2 recruitment was assayed by Tango™ assay (Thermo Fisher) as described previously[17]. CXCR2/4-bla (beta-lactamase) U2OS cells were genetically modified to stably overexpress CXCR2 or CXCR4 linked to a TEV protease site and a GAL4-VP16 transcription factor, *via* a reporter-gene system. These cells also stably express a β-arrestin-2/TEV protease fusion protein and a β-lactamase reporter gene. Upon corresponding stimulus (IL8 or SDF1-*α*) binding and resulting in CXCR2 or CXCR4 activation, the *β*-arrestin-2/TEV fusion protein is recruited to the receptor and cleaves the peptide linker that links CXCR2/4 to the GAL4-VP16 transcription factor. GAL4-VP16 now can enter the nucleus and promote the transcription of the *β*-lactamase gene. *β*-Lactamase activity is detected using a FRET-based fluorescence assay with CCF4-AM, a *β*-lactamase FRET substrate. CCF4-AM is cleaved in the presence of *β*-lactamase. The cleaved substrate excites at 409 nm and emits at 460 nm. In the absence of *β*-lactamase, CCF4-AM will not be cleaved and excites at 409 nm and emits at 540 nm. Thus, the activation of CXCR2/4 is directly correlated with the amount of cleaved *β*-lactamase substrate.
In each assay, CXCR2 or CXCR4-bla U2OS cells were seeded (11000/well) in 384-well tissue culture plates for 24 h in DMEM supplemented with 1% dialysis FBS. Cells were pretreated with various concentrations of inhibitors for 30 min prior to the addition of 12 nM of IL8 or 60 nM of SDF1-*α* and incubated for 5 h at 37 °C. Then *β*-Lactamase substrate (CCF4-AM dye) was loaded for 2 h, and plates were read on Clario Star microplate reader at 409 nm excitation and 464/530 nm emissions. Percent inhibition was calculated using the following formulas:
Ratio = cleaved (405/464)/uncleaved (409/530)
%inhibition = [1–((compound treated-unstimulated control)/(IL8/SDF stimulated-unstimulated control))] ×100%

MTT Assay: Cell proliferation was assessed by standard MTT assay. Briefly, cells were seeded in 96-well plates (3000 cells/well) and allowed to attach overnight. Cells were then continuously treated with compounds for 72 h. At the end of treatment, cells were incubated with MTT solution (at a final concentration of 0.5 mg/mL) for 4 h at 37°C. Cell supernatant was removed, and 100 µL of DMSO was added. Absorbance was read at 570 nm on a microplate reader (Molecular Devices, Sunnyvale, CA). The percentage of cell viability was calculated by the following formula:
(viable cells) % = (OD of drug-treated sample/OD of untreated sample)×100.
Concentration of the tested agent that is required for 50% inhibition of the cell viability is presented as IC_{50}.

Clonogenic assay. CXCR2-U2OS cells were seeded 300 cells per well into 96-well plates, and allowed to attach overnight before the addition of compounds. After 7-day contentious treatment, the medium was removed and crystal violet solution was added to fix and stain the colonies for 20 min. Crystal violet was removed and the colonies were washed with ddH_2O for three times. The colonies were imaged by iBRIGHT imaging system.

Acknowledgements

This study was funded by the Department of Defense Lung Cancer Research Program, Grant No. LC090363 (N. N.). W. Dai was supported by China Scholarship Council 201606240054

Keywords: chemokine receptors • CXCR2 antagonist • SAR • ADMET

References:

[1] P. M. Murphy, M. Baggiolini, I. F. Charo, C. A. Hébert, R. Horuk, K. Matsushima, L. H. Miller, J. J. Oppenheim, C. A. Power, *Pharmacol. Rev.* **2000**, *52*(1), 145-176.

[2] a)M. Baggiolini, B. Dewald, B. Moser, *Adv. Immunol.* **1993**, *55*, 97-179; b)L. Xu, D. Kelvin, G. Ye, D. Taub, A. Ben-Baruch, J. Oppenheim, J. Wang, *J. Leukocyte Biol.* **1995**, *57*(2), 335-342; c)C. Murdoch, P. N. Monk, A. Finn, *Cytokine* **1999**, *11*(9), 704-712; d)R. Horuk, A. W. Martin, Z.-x. Wang, L. Schweitzer, A. Gerassimides, H. Guo, Z.-h. Lu, J. Hesselgesser, H. D. Perez, J. Kim, *J. Immunol.* **1997**, *158*(6), 2882-2890.

[3] C. Bizzarri, A. R. Beccari, R. Bertini, M. R. Cavicchia, S. Giorgini, M. Allegretti, *Pharmacol. Ther.* **2006**, *112*(1), 139-149.

[4] F. Petersen, H.-D. Flad, E. Brandt, *J. Immunol.* **1994**, *152*(5), 2467-2478.

[5] H. Ha, B. Debnath, N. Neamati, *Theranostics* **2017**, *7*(6), 1543-1588.

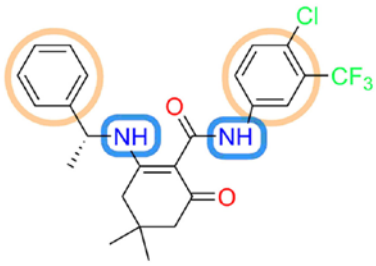
[6] a)V. M. Keatings, P. D. Collins, D. M. Scott, P. J. Barnes, *Am. J. Respir. Crit. Care Med.* **1996**, *153*(2), 530-534; b)K. Reich, V. Blaschke, C. Maurer, U. Lippert, C. Neumann, C. Garbe, P. Middel, G. Westphal, *J. Invest. Dermatol.* **2001**, *116*(2), 319-329; c)A. Kurdowska, J. M. Noble, I. S. Grant, C. R. Robertson, C. Haslett, S. C. Donnelly, *Crit. Care Med.* **2002**, *30*(10), 2335-2337; d)M. K. Schwarz, T. N. Wells, *Nat.Rev.Drug Disco.* **2002**, *1*(5), 347-358; e)C. Banks, A. Bateman, R. Payne, P. Johnson, N. Sheron, *J. Pathol* **2003**, *199*(1), 28-35; f)K. M. Beeh, O. Kornmann, R. Buhl, S. V. Culpitt, M. A. Giembycz, P. J. Barnes, *CHEST J.* **2003**, *123*(4), 1240-1247; g)H. Erdem, S. Pay, M. Serdar, İ. Şimşek, A. Dinç, U. Muşabak, A. Pekel, M. Turan, *Rheumatol. Int.* **2005**, *26*(2), 162-167; h)F. Lally, E. Smith, A. Filer, M. A. Stone, J. S. Shaw, G. B. Nash, C. D. Buckley, G. Ed Rainger, *Arthritis & Rheumatology* **2005**, *52*(11), 3460-3469; i)R. Chapman, J. Phillips, R. Hipkin, A. Curran, D. Lundell, J. Fine, *Pharmacol. Ther.* **2009**, *121*(1), 55-68; j)L. Donnelly, P. Barnes, *Drug Future* **2011**, *36*(6), 465; k)H. Xu, H. Lu, Z. Xu, L. Luan, C. Li, Y. Xu, K. Dong, J. Zhang, X. Li, Y. Li, G. Liu, S. Gong, Y. G. Zhao, A. Liu, Y. Zhang, W. Zhang, X. Cai, J. N. Xiang, J. D. Elliott, X. Lin, *ACS Med. Chem. Lett.* **2016**, *7*(4), 397-402.

[7] a)M. Clarke, T. Jamieson, C. Steele, M. Olson, M. Samuel, S. Das, O. Sansom, R. Nibbs, *Immunology* **2012**, *137*, 190; b)L. Han, B. Jiang, H. Wu, X. Wang, X. Tang, J. Huang, J. Zhu, *Med. Oncol.* **2012**, *29*(4), 2466-2472; c)T. Jamieson, M. Clarke, C. W. Steele, M. S. Samuel, J. Neumann, A. Jung, D. Huels, M. F. Olson, S. Das, R. J. Nibbs, *J.Clin.Invest.* **2012**, *122*(9), 3127; d)L. Gong, A. M. Cumpian, M. S. Caetano, C. E. Ochoa, M. M. De la Garza, D. J. Lapid, S. G. Mirabolfathinejad, B. F. Dickey, Q. Zhou, S. J. Moghaddam, *Mol. Cancer* **2013**, *12*(1), 154; e)H. Katoh, D. Wang, T. Daikoku, H. Sun, S. K. Dey, R. N. DuBois, *Cancer Cell* **2013**, *24*(5), 631-644.

[8] J. Busch-Petersen, *Curr. Top. Med. Chem.* **2006**, *6*(13), 1345-1352.

[9] M. Allegretti, R. Bertini, M. C. Cesta, C. Bizzarri, R. Di Bitondo, V. Di Cioccio, E. Galliera, V. Berdini, A. Topai, G. Zampella, *J. Med. Chem.* **2005**, *48*(13), 4312-4331.
[10] L. Brandolini, L. Cristiano, A. Fidoamore, M. De Pizzol, E. Di Giacomo, T. M. Florio, G. Confalone, A. Galante, B. Cinque, E. Benedetti, P. A. Ruffini, M. G. Cifone, A. Giordano, M. Alecci, M. Allegretti, A. Cimini, *Oncotarget* **2015**, *6*(41), 43375-43394.
[11] J. R. White, J. M. Lee, P. R. Young, R. P. Hertzberg, A. J. Jurewicz, M. A. Chaikin, K. Widdowson, J. J. Foley, L. D. Martin, D. E. Griswold, *J. Biol. Chem.* **1998**, *273*(17), 10095-10098.
[12] R. B. Moss, S. J. Mistry, M. W. Konstan, J. M. Pilewski, E. Kerem, R. Tal-Singer, A. L. Lazaar, C. Investigators, *J,Cyst.Fibros.* **2013**, *12*(3), 241-248.
[13] M. P. Dwyer, Y. Yu, J. Chao, C. Aki, J. Chao, P. Biju, V. Girijavallabhan, D. Rindgen, R. Bond, R. Mayer-Ezel, *J. Med. Chem.* **2006**, *49*(26), 7603-7606.
[14] P. Nair, M. Gaga, E. Zervas, K. Alagha, F. Hargreave, P. O'byrne, P. Stryszak, L. Gann, J. Sadeh, P. Chanez, *Clin. Exp. Allergy* **2012**, *42*(7), 1097-1103.
[15] R. Virtala, A. K. Ekman, L. Jansson, U. Westin, L.-O. Cardell, *Clin. Exp. Allergy* **2012**, *42*(4), 590-596.
[16] C. W. Steele, S. A. Karim, J. D. Leach, P. Bailey, R. Upstill-Goddard, L. Rishi, M. Foth, S. Bryson, K. McDaid, Z. Wilson, *Cancer Cell* **2016**, *29*(6), 832-845.
[17] D. Y. Maeda, A. M. Peck, A. D. Schuler, M. T. Quinn, L. N. Kirpotina, W. N. Wicomb, G. H. Fan, J. A. Zebala, *J. Med. Chem.* **2014**, *57*(20), 8378-8397.
[18] X. Lu, J. W. Horner, E. Paul, X. Shang, P. Troncoso, P. Deng, S. Jiang, Q. Chang, D. J. Spring, P. Sharma, *Nature* **2017**, 543(7647), 728.
[19] H. Ha, T. Bensman, H. Ho, P. M. Beringer, N. Neamati, *Br. J. Pharmacol.* **2014**, *171*(6), 1551-1565.
[20] S. Peukert, Y. Sun, R. Zhang, B. Hurley, M. Sabio, X. Shen, C. Gray, J. Dzink-Fox, J. Tao, R. Cebula, S. Wattanasin, *Bioorg. Med. Chem. Lett.* **2008**, *18*(6), 1840-1844.
[21] a)H. Hajovsky, G. Hu, Y. Koen, D. Sarma, W. Cui, D. S. Moore, J. L. Staudinger, R. P. Hanzlik, *Chem. Res. Toxicol.* **2012**, *25*(9), 1955-1963; b)M. Boyd, R. A. Neal, *Drug Metab. Disposition* **1976**, *4*(4), 314-322.
[22] P. E. Hansen, F. Duus, S. Bolvig, T. S. Jagodzinski, *J. Mol. Struct.* **1996**, *378*(1), 45-59.
[23] a)B. Kuhn, P. Mohr, M. Stahl, *J. Med. Chem.* **2010**, *53*(6), 2601-2611; b)A. Alex, D. S. Millan, M. Perez, F. Wakenhut, G. A. Whitlock, *MedChemComm* **2011**, *2*(7), 669-674.
[24] *ADMET Predictor 8.0, http://www.simulations-plus.com*
[25] G. Vistoli, A. Pedretti, B. Testa, *Drug Discovery Today* **2008**, *13*(7), 285-294.
[26] W. J. Egan, K. M. Merz, J. J. Baldwin, *J. Med. Chem.* **2000**, *43*(21), 3867-3877.

Entry for the Table of Contents



55

CXCR2 IC_{50}=2.9 ± 0.5 μM

CXCR4 IC_{50} > 10 μM

OVCAR8 MTT IC_{50} > 50 μM

New derivatives of 3-aminocyclohex-2-en-1-ones were synthesized and evaluated for their CXCR2 inhibition. Structure-activity relationship of these compounds was discussed. Several compounds display CXCR2 IC50 values less than 10 μM. they also show selectivity against CXCR2 and low cytotoxicty. *In silico* ADMET prediction suggests most active compounds possess good drug-like properties.