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

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Herein we describe the synthesis and structure–activity relationships of 3-aminocyclohex-2-en-1-one derivatives as novel chemokine receptor 2 (CXCR2) antagonists. Thirteen out of 44 derivatives were found to inhibit CXCR2 downstream 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 significant 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 receptors CXCR1 and CXCR2 are members of the class A (rhodopsin-like) family of seven-transmembrane G-protein-coupled receptors (GPCRs)[1] expressed on inflammatory cells such as neutrophils, monocytes, T-lymphocytes, and basophils, as well as keratinocytes, endothelial cells, 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, and I-TAC.[3] The 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 the $G_{\alpha i}$ 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 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 demyelinating disorders.[6] CXCR2 signaling is also implicated in tumorigenesis and metastasis.[7] Therefore, the discovery of novel peptides and small-molecule inhibitors of CXCR2 antagonists has emerged as a promising approach for the treatment of various inflammatory disorders.[8] Several small-molecule CXCR2 inhibitors with various 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 under investigation for the prevention and treatment of delayed graft function and pancreatic islet transplantation.[9] Additionally, a combination of ketoprofen and paclitaxel was shown to inhibit brain tumor metastasis.[10] Reparixin is now in phase II trials for both breast cancer and ischemia-reperfusion injury. A potent and selective CXCR2 diarylurea antagonist, SB225002, inhibits CXCL8-induced neutrophil migration,[11] and its analogue, 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 decreasing 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 lipopolysaccharide (LPS)-induced neutrophil recruitment in the nasal lavage of healthy patients.[15] Its analogue, AZ13381758, decreased metastases and augmented immunotherapy response in a mouse model of pancreatic cancer.[16] Another bicyclic thiazolopyrimidine small molecule, AZD-5069, is also in clinical trials for COPD, asthma, and advanced solid tumors. SX-517 is another small-molecule CXCR2 antagonist with a unique boronic acid moiety that effectively inhibited CXCL1-induced Ca^{2+} flux in human polymorphonuclear neutrophils (PMNs).[17] Its analogue, SX-682, decreased metastases and augmented immunotherapy in a mouse model of prostate cancer and has advanced to phase I clinical trials 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, which inhibits CXCL8-mediated chemotaxis through selective regulation of

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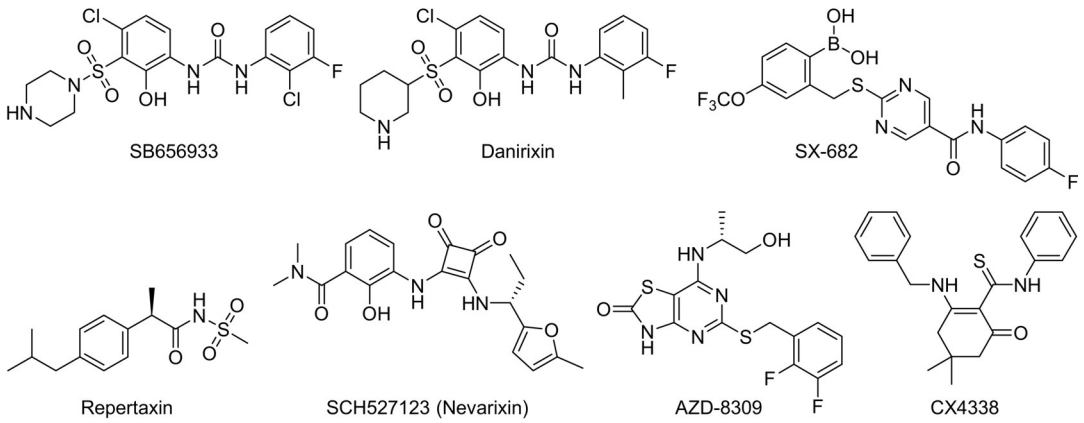


Figure 1. CXCR2 receptor antagonists.

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 decreased 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 toward modifications of CX4338 to establish a SAR for CXCR2 inhibition.

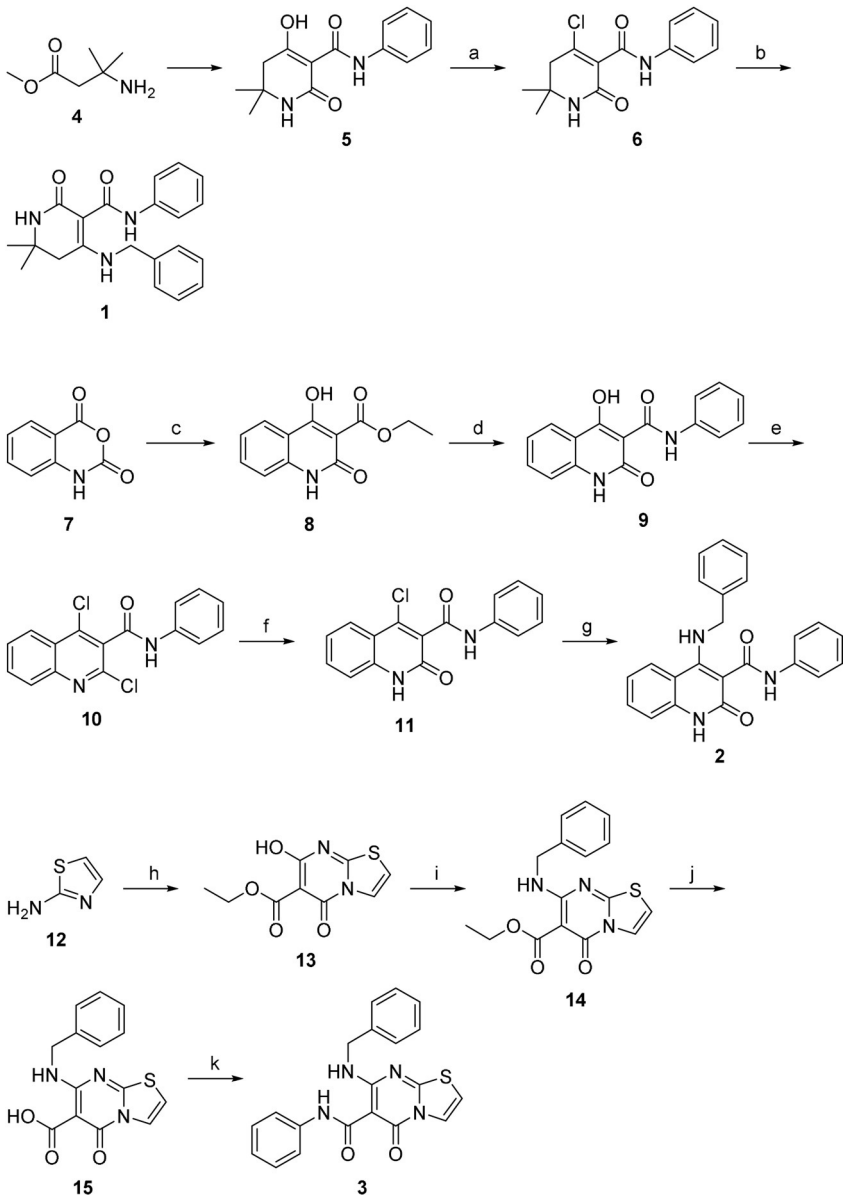
Results and Discussion

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 phosphoryl chloride at room temperature to yield monochloro product **6**. Compound **1** was obtained by coupling the chlorinated product **6** with benzylamine in dichloromethane. Quinolinone **2** was prepared from isatoic anhydride **7** by treatment with sodium hydride and diethyl malonate in DMF to provide intermediate **8**, which was heated with aniline to yield **9**. The chlorination of **9** was nonselective, yielding the dichloro intermediate **10** by refluxing **9** with phosphoryl chloride. The chlorine atom at the 2-position of compound **10** was subsequently converted into keto in acetic acid with sodium acetate to give **11**. The 4-chloroquinolinone intermediate **11** was then heated with benzylamine and triethylamine in acetonitrile to provide **2** in a 66% yield. Synthesis of **3** started from the cyclization of 2-aminothiozole with excess triethyl methanetricarboxylate to afford thiazolopyrimidine intermediate **13**. Compound **13** was then heated under reflux with *p*-toluenesulfonyl chloride followed by coupling with benzylamine to provide **14**. Compound **14** was hydrolyzed with aqueous lithium hydroxide for 20 h. Amidation with aniline by EDCI and HOBt in dichloromethane under reflux furnished compound **3** in 51% yield.

Scheme 2 illustrates the synthesis of compounds **16**–**24**, **26**–**38**, and **55**–**56** (structures in Tables 2–4 and Figure 3 below). Commercially available 5,5-dimethylcyclohexane-1,3-dione **57** was stirred with benzylamine in toluene at 80 °C to afford 5-(benzylimino)-3,3-dimethylcyclohexanone **58**. To facilitate reaction progress, anhydrous sodium sulfate was added to absorb generated water and was removed by filtration upon completion of the reaction. The crude product **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-enamino ketones (**16**–**24**, **27**, **28**, **35**, and **40**–**42**). The corresponding isocyanates **67**–**71** were prepared with substituted anilines and triphosgene in toluene under reflux. Heating the mixture of 2 equiv of isocyanate and 1 equiv of **58** under neat conditions gave the desired products. The yields depend on the reactivity of the isocyanates (44–81%) and were found to decrease with longer reaction times. Substrates containing hydroxy or carboxylic esters cannot react under these conditions. 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 the corresponding hydroxy products **26**. Optical isomers **55** and **56** were prepared by the same scheme using commercially 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**, and **43**–**54** (Scheme 3). The starting material 5,5-dimethylcyclohexane-1,3-dione **57** 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 **33** was obtained from **31** using lithium hydroxide and then conjugated with aniline or methylamine by EDCI/HOBt to provide compounds **32** and **34**.

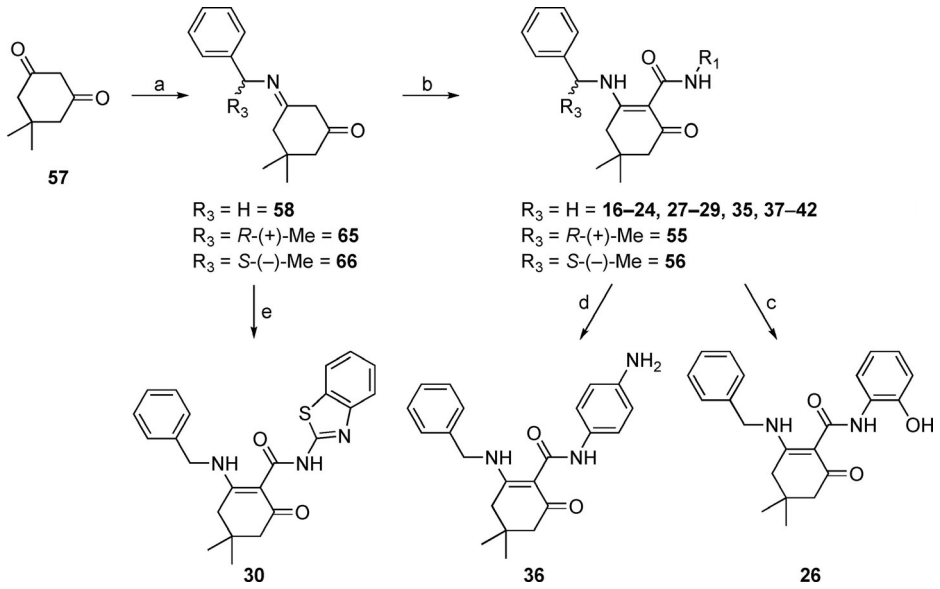


Scheme 1. Reagents and conditions: a) aniline, $POCl_3$, DIEA, RT, 18 h, 56%; b) benzylamine, TEA, CH_2Cl_2, RT, 3 h, 52%; c) diethyl malonate, NaH, DMF, reflux, 5 h, 70%; d) aniline, 160 °C, 10 min; e) $POCl_3$, 110 °C, 2 h, 89%; f) AcONa, AcOH, 120 °C, 20 h, 57%; g) benzylamine, TEA, CH_3CN, RT, 4 h, 66%; h) triethyl methanetricarboxylate, xylene, reflux, 5 h, 60%; i) *p*-TsCl, TEA, acetonitrile, reflux, 3 h, benzylamine, reflux, 2 h, 69%; j) LiOH, MeOH, H_2O, 40 °C, 20 h, 70%; k) aniline, EDCI, HOBt, TEA, CH_2Cl_2, 40 °C, 24 h, 51%.

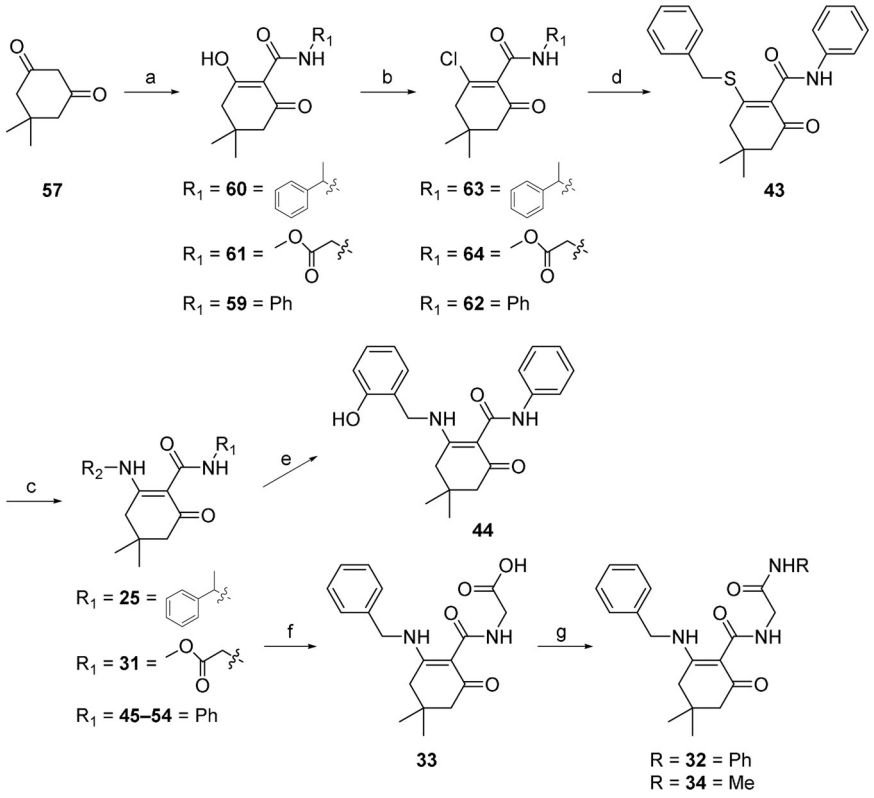
In vitro biological evaluation and SAR studies

To establish a robust SAR, CX4338 was conceptualized as having three structural units: 1) a cyclohexanone core, 2) a phenylthioamide moiety, and 3) a benzylamino functionality. All compounds were initially tested at 10 μM in a CXCR2-specific Tango assay as previously described.[19] The 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 cores, including thiazolopyrimidinone and quinolinone (compounds **2** and **3**), resulted in a loss of activity. Similarly, simple replacement of one carbon atom in the cyclohexanone ring with a heteroatom (e.g. compound **1**), caused a significant decrease in activity. Based on these results, we postulate that the cyclohexanone moiety occupies a hydrophobic pocket with a moderate size, which cannot accommodate groups with polar properties or bulky substituents. The thiocarboxamide group 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 compound **16**, which 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 hydrogen bond is formed, as observed in many β-ketoamides[22] (Figure 2). Compared with **16**, an analogue CX1142 with a



Scheme 2. Reagents and conditions: a) benzylamine, anhydrous sodium sulfate, toluene, 80 °C, 5 h, 86–97 %; b) R_1NCO, 125 °C, 1.5 h, 44–81 %; c) BBr_3, CH_2Cl_2, −78 °C, 30 min, RT, 2 h, 52 %; d) tin(II) dichloride, EtOH, HCl, 78 °C, 3 h, 43 %; e) 2-isocyanatobenzothiazole, EtOH, microwave, 120 °C, 30 min, 12 %.

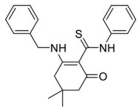
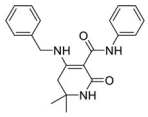
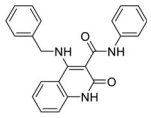
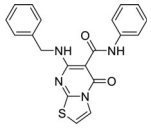
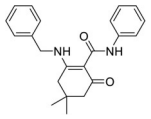


Scheme 3. Reagents and conditions: a) isocyanates, acetone/acetonitrile, TEA, RT or reflux, overnight, 31–85 %; b) oxalyl chloride, DMF, RT, overnight, 76 %; c) R_2NH_2, RT, TEA, CH_2Cl_2, 4 h, 29–78 %; d) benzyl mercaptan, 40 °C, TEA, acetonitrile, 31 %; e) BBr_3, CH_2Cl_2, −78 °C, 30 min, RT, 2 h, 30 %; f) LiOH, MeOH, H_2O, RT, 12 h, 89 %; g) aniline/methylamine hydrochloride, EDCI, HOBt, TEA, CH_2Cl_2, RT, 12 h, 43–57 %.

methylene connection lacking an intramolecular hydrogen bond, showed eightfold decreased potency (IC_{50} = 32.0 ± 5.5 μM).[19] This suggested that the intramolecular hydrogen bond may restrict the compound in a favorable conformation important for binding. Considering that an intramolecular hydrogen bond may improve membrane and blood–brain barrier (BBB) permeability,[23] such inhibitors can be optimized to treat CNS tumors and inflammatory diseases.

Our initial SAR efforts focused on the phenylamide moiety (Table 2). Elaboration of the benzene ring with chlorine atoms

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

Compd	Structure	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 by CXCR2 Tango assay; values are the mean ± SD of at least three independent determinations.

showed preference for the substituents at the 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) was favored over

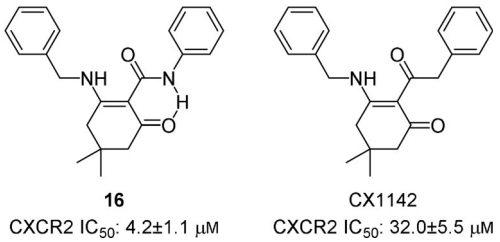
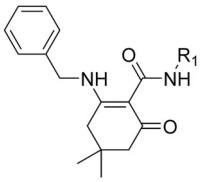


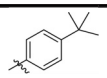
Figure 2. Intramolecular hydrogen bonding is important for CXCR2 inhibition.

mono-substitution. Thus, we synthesized additional analogues with 4-position substitutions. Interestingly, the classic isosteric replacement of 4-choloro with 4-methyl resulted in the totally inactive analogue **21**, whereas the 4-methoxy analogue 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 analogue **23** (IC_{50} = 5.0 μM) may bind to this pocket. Adding an extra methyl group on **24** to give α-benzylmethyl analogue **25** resulted in loss of activity. It is possible that the decrease in potency is due to an 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.

To extend our SAR efforts, we examined heterocyclic derivatives **29** and **30** as well as glycine derivatives **31**, **32**, **33**, and

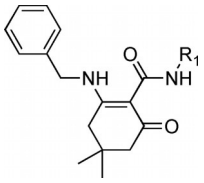
Table 2. IC_{50} values for CXCR2 of compounds **16**–**28**.

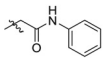
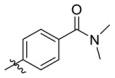
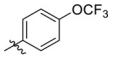
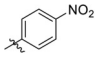


Compd	R_1	IC_{50} [μM][a]	Compd	R_1	IC_{50} [μM][a]
16		4.2 ± 1.1	**23**		5.0 ± 2.1
17		> 50	**24**		5.7 ± 0.8
18		6.7 ± 2.3	**25**		> 10
19		7.0 ± 2.7	**26**		> 10
20		3.3 ± 0.7	**27**		> 50
21		> 50	**28**		> 30
22		5.0 ± 0.1			

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

Table 3. IC_{50} values for CXCR2 of compounds **29**–**42**.



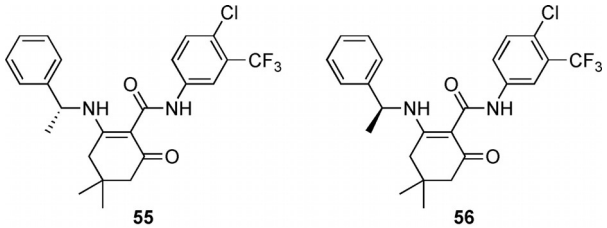
Compd	R_1	IC_{50} [μM][a]	Compd	R_1	IC_{50} [μM][a]
29		>10	**36**		>10
30		>10	**37**		27.7±9.0
31		>10	**38**		>10
32		>10	**39**		>10
33		>10	**40**		5.2±0.2
34		>10	**41**		2.9±0.4
35		>10	**42**		2.5±0.9

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

34 to explore potential hydrogen bonding. However, these modifications led to inactive compounds (Table 3), suggesting that the benzene 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 inhibitors (**40**, **41**, and **42**). Improved potency was shown in compounds with a trifluoromethyl group at either the 3-position (**41**, IC_{50} = 2.9 μM) or 4-position (**42**, IC_{50} = 2.5 μM). We postulate that an electron-deficient group positioned in a hydrophobic pocket may contribute to improved potency.

Next, our SAR efforts involved modification of the benzylamino moiety. The sulfur ether replacement of the NH moiety was inactive, indicating that NH is also important for retaining potency (Table 4). Considering that the phenylamide moiety is well tolerated with lipophilic groups and possibly sits within a hydrophobic pocket, we hypothesized that the benzylamine moiety is more likely to be solvent exposed. Many CXCR2 clinical candidates possess hydroxy groups near free NHs (SB656933, SCH527123, AZD-8309, Figure 1). Therefore, we synthesized a series of derivatives with hydroxy groups at different positions (**44**–**46**). Unfortunately, none of these derivatives were active. Interestingly, the 2-methoxy derivative **47** retained potency. Because 2-methoxy could restrict the rotation of the benzene ring, we designed and examined analogues with other bulky groups. Carboxylate derivative **48** lost activity, whereas α-benzylmethyl was active (**49**, IC_{50} = 4.2 μM). Installing a hydroxy group on the methyl of **49** decreased potency (**50**, IC_{50} = 15.2 μM). This means that moderately sized groups can be well accommodated, and bulky groups (e.g., **48**) are not tolerated. All efforts to improve aqueous solubility by replacing the phenyl moiety with pyridine **51**–**52**, 3-benzamide **53,** and isoxazole **54** led to decreased potency, reinforcing the narrow requirement of allowable lipophilicity.

On the basis of the above preliminary SAR results, we examined whether the potency gains from modifications of phenylamide and benzylamine moieties would be additive. We combined the most favorable 3-trifluoromethyl-4-chlorophenylamide moiety and the privileged α-benzylmethylamine moiety to obtain isomers **55** and **56** (Figure 3). The resulting hybrid compounds did not show improved potency over **42**. Although the cLog*P* value of **55** is slightly higher than that of **42**, its out-of-plane methyl substitution decreases crystal packing, and to some extent can increase solubility.

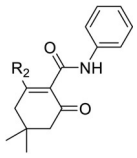


55 CXCR2 IC_{50}: 2.5±0.6 μM

56 CXCR2 IC_{50}: 3.1±0.8 μM

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

Table 4. IC_{50} values for CXCR2 of compounds **43–54**.



Compd	R_2	IC_{50} [μM][a]	Compd	R_2	IC_{50} [μM][a]
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 by CXCR2 Tango assay; values are the mean±SD of at least three independent determinations.

Because 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 modifications 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 a nitrogen atom into the hexane ring or replacing it with fused aromatic rings is detrimental to potency and may not be well tolerated. An electron-withdrawing group on ring A enhances activity, and a lipophilic benzene ring is preferred on ring B. The NH group on the right side is probably involved in the formation of an intramolecular hydrogen bond to minimize the energy of the active conformation, and consequently serves as an essential moiety for activity. The NH group on the left side may form a hydrogen bond with the receptor and is also important for activity.

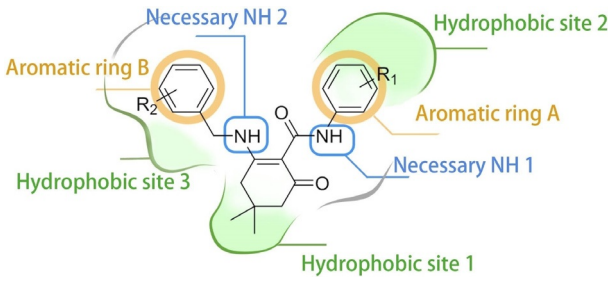


Figure 4. The SAR map of 3-aminocyclohex-2-en-1-ones.

To assess selectivity, all compounds were evaluated in a CXCR4 Tango assay. All compounds were found to be inactive at 10 μM in a Tango assay specific for CXCR4 (Supporting Information (SI) Table 1), indicating good selectivity for CXCR2 over CXCR4. Additionally, these compounds were tested in an MTT assay and a colony-formation assay to evaluate their cytotoxicity (SI Tables 2 and 3 and SI 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 cytotoxicity does not contribute to CXCR2 inhibition.

ADMET properties

ADMET Predictor 8.0[24] was used to calculate select ADMET properties. We calculated various pharmacokinetics (PK) properties such as topological polar surface area (T_PSA), Log*P* (S+LogP), molecular weight (MW), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), number of rotatable bonds (Rotatable_Bonds), Lipinski's rule of five violations (RuleOf5), and risk descriptors including absorption risk (Absn_Risk), ADMET_Risk, cytochrome P450 metabolism risk (CYP_Risk), and toxicity risk (Tox_Risk). Figure 5 presents frequency distribution plots for MW, S+LogP, HBA, HBD, T_PSA (in Å^2), and Rotatable_Bonds of CX4338 analogues. The number of rotatable bonds represents the conformational space of a molecule. The conformational behaviors directly or indirectly affect pharmacodynamics as well as PK properties. Too many rotatable bonds (optimal value ≤7) are not desirable for a molecule to be drug-like.[25] Similar frequency plots of CXCR2 antagonists in clinical trials are presented in SI Figure 2. CX4338 analogues showed similar ADMET properties to CXCR2 antagonists in clinical trials

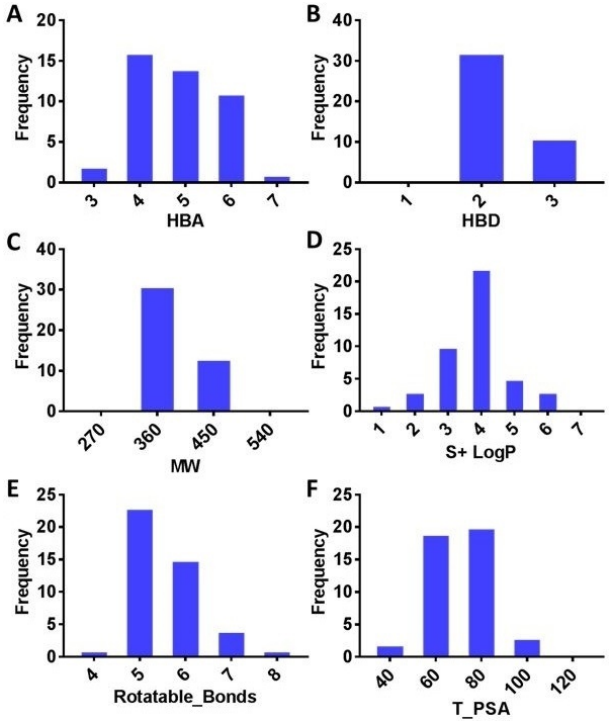


Figure 5. ADMET properties of CX4338 analogues. Frequency distribution plots for A) H-bond acceptors, B) H-bond donors, C) molecular weight, D) Log*P*, E) number of rotatable bonds, and F) topological polar surface area.

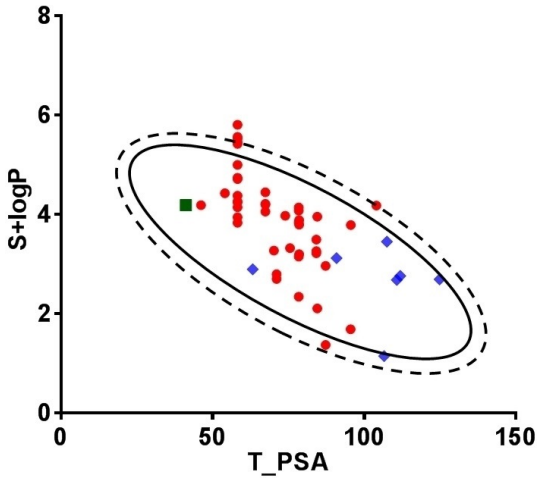


Figure 6. Topological polar surface area in Å^2 for each of CX4338 (green), its analogues (red), and CXCR2 antagonists in clinical trials (blue) is plotted against their corresponding calculated Log*P* values.

and obey Lipinski's rule of five. Figure 6 shows a T_PSA vs. S+ Log*P* plot (Egan plot), suggesting over 90% of the compounds are within the desirable range of T_PSA (<140) as well as S+ LogP (<5) values. The Egan plot represents a prediction of drug absorption and considers correlations between these two parameters to oral absorption. On the basis of the absorption model of Egan et al.,[26] the outer ellipse represents 99% confidence, whereas the inner ellipse is 95% confidence. T_PSA vs. S+logP plots for optimized analogues (red points) are shifted from CXC4338 (green point) toward 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-1-one derivatives were synthesized to select optimized ring substituents. Thirteen compounds showed IC_{50} values $<10\,\mu M$. We observed that both of the benzene rings as well as two NH groups are critical for potency. A trifluoromethylphenyl substituent on the carboxamide moiety and an α-benzylmethylamino moiety improve the activity. All active compounds show selectivity against CXCR2 over CXCR4 and do not exhibit significant cytotoxicity. Compounds **55** ($IC_{50}=2.5\pm0.6\,\mu M$) and **42** ($IC_{50}=2.5\pm0.9\,\mu M$) are new antagonists with desirable properties. In silico ADMET predicted properties suggest that lead optimization is reasonable according to an Egan plot, where properties of new analogues are improved toward the CXCR2 antagonists in clinical trials. Because this scaffold is new, our SAR studies provide valuable data for further optimization and of new CXCR2 antagonists 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 C_{18} 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 presented in the Supporting Information.

4-Chloro-6,6-dimethyl-2-oxo-*N*-phenyl-1,2,5,6-tetrahydropyridine-3-carboxamide 6: To 10 mL $POCl_3$ 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 three

Table 5. ADMET risk descriptors of CX4338 analogues and compounds in clinical trials.

Identifier	Absn_Risk[a]	ADMET_Risk[b]	CYP_Risk[c]	Tox_Risk[d]	RuleOf5[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
AZD-5069	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 five violation count. [f] Desired values are reported in the tutorial of the ADMET Predictor 8.0.[24]

times with EtOAc and 1 N aqueous sodium bicarbonate. The combined organics layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to afford the crude compound. The crude was purified by 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 CH_2Cl_2 (10 mL) was added triethylamine (0.074 mL, 0.538 mmol) and benzylamine (23 μL 0.215 mmol). The reaction mixture was stirred at room temperature for 3 h. Then, 10 mL 1 N aqueous HCl was added and the solution was extracted with EtOAc three times. The combined organics layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo and the crude was purified by 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 at reflux for 5 h. Then the mixture was cooled to 0 °C and was carefully added 1 N aqueous hydrochloric (50 mL). The resulting solid was collected by filtration, washed with water and diethyl ether 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. Ethanol (10 mL) 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 $POCl_3$ 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 anhydrous sodium acetate (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 **11** as a white solid (27 mg, 57% yield).

4-(Benzylamino)-2-oxo-*N*-phenyl-1,2-dihydroquinoline-3-carboxamide 2: To a solution of **11** (20 mg, 0.0692 mmol) in acetonitrile (10 mL) was added triethylamine (19 μL, 0.138 mmol) and benzylamine (8 μL, 0.0761 mmol). The reaction mixture was heated at reflux for 4 h. The reaction was quenched by 10 mL 1 N HCl solution and extracted with EA three times. The combined organics layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to afford the crude compound.

The crude was purified by silica gel chromatography to afford **2** (17 mg, 66% yield) as a white solid.

Ethyl-7-hydroxy-5-oxo-5*H*-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 at reflux for 5 h. Then solvent was removed under vacuum and the resulting brown solid was recrystallized with isopropanol to afford **13** as a yellow solid (1.44 g, 60% yield).

Ethyl-7-(benzylamino)-5-oxo-5*H*-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*-TsCl (158 mg, 0.833 mmol). The mixture was heated at reflux for 5 h. The mixture was cooled to room temperature and benzylamine was added (89 μL, 0.833 mmol). The solution was held at reflux for another 2 h. The reaction was quenched by 10 mL 1 N HCl solution and extracted with EA three times. The combined organics layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to afford the crude compound. The crude was purified by silica gel chromatography to afford **14** (189 mg, 69% yield) as a white solid.

7-(Benzylamino)-5-oxo-5*H*-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 1 N aqueous HCl was added and extracted with EA three times. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to afford **15** (32 mg, 70% yield).

7-(Benzylamino)-5-oxo-*N*-phenyl-5*H*-thiazolo[3,2-a]pyrimidine-6-carboxamide 3: To a solution of **15** (20 mg, 0.0664 mmol) in CH_2Cl_2 (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 1 N aqueous HCl was added and the solution was extracted with CH_2Cl_2 three times. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo and the crude was purified by 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 anhydrous sodium sulfate 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 twice with hot toluene. 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 1.00 g anhydrous sodium sulfate 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 twice with hot toluene. 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 crystals, 1.49 g, in 86% yield.

General procedure I: preparation of compounds 17–24, 27–29, 31–35, 37, 38, 40–42, 55, and 56

5-Imino-3,3-dimethylcyclohexanone (1 equiv) and 2 equiv 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 (5 mL) was added and stirred for 30 min and filtered to give pure compounds.

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*-(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)-*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 41: Compound **41** 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, 0.508 mmol) and one drop hydrochloric acid (36%). The reaction mixture was stirred at 78 °C for 3 h. The mixture was cooled to room temperature and adjusted to pH 8 with sodium carbonate solution (1 N). The solution was extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to afford the crude compound.

This crude was purified by 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**: 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 anhydrous sodium sulfate, filtered, and concentrated in vacuo to afford the crude compound. This crude was purified by 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 **27** (100 mg, 0.264 mmol) in anhydrous CH_2Cl_2 (20 mL) was stirred at −78 °C for 30 min, 1 M 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 CH_2Cl_2 three times. The combined organics layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to afford a clear oil. The crude was held at reflux in 1 M HCl ethanol solution for 2 h to dissociate the boron complex. Solvent was then removed and the crude was purified by silica gel chromatography to afford **26** (50 mg, 52% yield) as a white solid.

(*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.

General procedure II: preparation of isocyanates 67–70

A solution of triphosgene (0.35 equiv) in anhydrous toluene (10 mL) was added anilines (1 equiv) in anhydrous CH_2Cl_2 (10 mL) dropwise at 0 °C within 15 min. After addition, the reaction was held at reflux 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 by silica gel chromatography to afford **30** as a white solid (11 mg, 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%.

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 CH_2Cl_2 (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 (1.91 g, 16.50 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The reaction mixture was stirred at room temperature for 12 h. The reaction was quenched by 20 mL 1 N HCl solution and extracted with CH_2Cl_2 three times. The combined organics layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to afford the crude compound.

The crude was purified by 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 CH_2Cl_2 (20 mL) was added oxalyl chloride (3.30ml, 38.6 mmol) 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 1 N aqueous sodium bicarbonate three times. The combined organics layer was washed with brine, dried over anhydrous sodium sulfate, filtered, concentrated in vacuo to afford the crude compound. This crude was purified by 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 CH_2Cl_2 (10 mL) was added dropwise. The reaction mixture was heated at reflux for 12 h. The reaction was quenched by 20 mL 1 N HCl solution and extracted with CH_2Cl_2 three times. The combined organics layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to afford the crude compound. The crude was purified by 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 CH_2Cl_2 (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 1 N aqueous sodium bicarbonate three times. The combined organics layer was washed with brine, dried over anhydrous sodium sulfate, filtered, concentrated in vacuo to afford 105 mg 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 CH_2Cl_2 (10 mL) was added triethylamine (0.068 mL, 0.491 mmol) and benzylamine (21 μL, 0196 mmol). The reaction mixture was stirred at room temperature for 3 h. Then 10ml 1 N aqueous HCl was added and the solution was extracted with EtOAc three times. The combined organics layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo and the crude was purified by 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 CH_2Cl_2 (10ml) was added dropwise. The reaction mixture was heated at reflux for 12 h. The reaction was quenched by 20 mL 1 N HCl solution and extracted with CH_2Cl_2 three times. The combined organics layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to afford the crude compound. The crude was purified by 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 CH_2Cl_2 (10 mL) was added oxalyl chloride (0.335 mL, 3.92 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 1 N aqueous sodium bicarbonate three times. The combined organics layer was washed with brine, dried over anhydrous sodium sulfate, 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 CH_2Cl_2 (10 mL) was added triethylamine (0.076 mL, 0.549 mmol) and benzylamine (24 μL, 0.219 mmol). The reaction mixture was stirred at room temperature for 3 h. Then 10ml 1 N aqueous HCl was added and the solution was extracted with EtOAc three times. The combined organics layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo and the crude was purified by 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 1 N 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 CH_2Cl_2 (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 1 N aqueous HCl was added and the solution was extracted with CH_2Cl_2 three times. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo and the crude was purified by 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 CH_2Cl_2 (10 mL) 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 1 N aqueous HCl was added and the solution was extracted with CH_2Cl_2 three times. The combined organics layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo and the crude was purified by 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 1 N aqueous HCl was added. The solution was extracted with EtOAc three times. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude was purified

by 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 CH_2Cl_2 (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 1 N aqueous HCl was added and the solution was extracted with EtOAc three times. The combined organics layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo and the crude was purified by 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-((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-(((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-Hydroxybenzyl)amino)-4,4-dimethyl-6-oxo-*N*-phenylcyclohex-1-ene-1-carboxamide 44: A solution of **47** (50 mg, 0.132 mmol) in anhydrous CH_2Cl_2 (10 mL) was stirred at −78 °C for 30 min, 1 M 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. 20 mL water was slowly added to quench the reaction. The mixture was extracted with CH_2Cl_2 three times. The combined organics layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to afford a clear oil. The crude was held at reflux in 1 M HCl ethanol solution for 2 h to dissociate the boron complex. Solvent was then removed and the crude was purified by silica gel chromatography to afford **44** (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 (SimulationsPlus, Inc.).[24] 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% dialyzed FBS, zeocin (200 μg mL^{-1}), hygromycin (50 μg mL^{-1}), geneticin (100 μg 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 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 (β-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 464 nm. In the absence of β-lactamase, CCF4-AM will not be cleaved and excites at 409 nm and emits at

530 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 (11 000 per 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 Equations (1) and (2):

$$\text{Ratio} = \frac{\text{cleaved } (409/464)}{\text{uncleaved } (409/530)} \quad (1)$$

$$\%\ \text{Inhibition} = \left[1-\left(\frac{(\text{compound treated})-(\text{unstimulated control})}{(\text{IL-8/SDF stimulated})-(\text{unstimulated control})}\right)\right]\times 100 \quad (2)$$

MTT assay: Cell proliferation was assessed by standard MTT assay. Briefly, cells were seeded in 96-well plates (3000 cells per 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^{-1}) 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 Equation (3):

$$\%\ \text{Viable cells} = \left(\frac{(\text{OD of drug-treated sample } [-\text{blank}])}{(\text{OD of untreated sample } [-\text{blank}])}\right)\times 100 \quad (3)$$

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 continuous 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 three times. The colonies were imaged by iBRIGHT imaging system.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: ADMET · chemokine receptors · CXCR2 antagonists · structure–activity relationships

[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*, 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*, 335 – 342; c) C. Murdoch, P. N. Monk, A. Finn, *Cytokine* **1999**, *11*, 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*, 2882 – 2890.

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

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

[5] H. Ha, B. Debnath, N. Neamati, *Theranostics* **2017**, *7*, 1543 – 1588.

[6] a) V. M. Keatings, P. D. Collins, D. M. Scott, P. J. Barnes, *Am. J. Respir. Crit. Care Med.* **1996**, *153*, 530 – 534; b) K. Reich, V. Blaschke, C. Maurer, U. Lippert, C. Neumann, C. Garbe, P. Middel, G. Westphal, *J. Invest. Dermatol.* **2001**, *116*, 319 – 329; c) A. Kurdowska, J. M. Noble, I. S. Grant, C. R. Robertson, C. Haslett, S. C. Donnelly, *Crit. Care Med.* **2002**, *30*, 2335 – 2337; d) M. K. Schwarz, T. N. Wells, *Nat. Rev. Drug Discovery* **2002**, *1*, 347 – 358; e) C. Banks, A. Bateman, R. Payne, P. Johnson, N. Sheron, *J. Pathol.* **2003**, *199*, 28 – 35; f) K. M. Beeh, O. Kornmann, R. Buhl, S. V. Culpitt, M. A. Giembycz, P. J. Barnes, *CHEST J.* **2003**, *123*, 1240 – 1247; g) H. Erdem, S. Pay, M. Serdar, İ. Şimşek, A. Dinç, U. Muşabak, A. Pekel, M. Turan, *Rheumatol. Int.* **2005**, *26*, 162 – 167; h) F. Lally, E. Smith, A. Filer, M. A. Stone, J. S. Shaw, G. B. Nash, C. D. Buckley, G. E. Rainger, *Arthritis Rheum.* **2005**, *52*, 3460 – 3469; i) R. Chapman, J. Phillips, R. Hipkin, A. Curran, D. Lundell, J. Fine, *Pharmacol. Ther.* **2009**, *121*, 55 – 68; j) L. Donnelly, P. Barnes, *Drug Future* **2011**, *36*, 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*, 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*, 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*, 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*, 154; e) H. Katoh, D. Wang, T. Daikoku, H. Sun, S. K. Dey, R. N. DuBois, *Cancer Cell* **2013**, *24*, 631 – 644.

[8] J. Busch-Petersen, *Curr. Top. Med. Chem.* **2006**, *6*, 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*, 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*, 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*, 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. Cystic Fibrosis* **2013**, *12*, 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*, 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*, 1097 – 1103.

[15] R. Virtala, A. K. Ekman, L. Jansson, U. Westin, L.-O. Cardell, *Clin. Exp. Allergy* **2012**, *42*, 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*, 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*, 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*, 728.

[19] H. Ha, T. Bensman, H. Ho, P. M. Beringer, N. Neamati, *Br. J. Pharmacol.* **2014**, *171*, 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*, 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*, 1955 – 1963; b) M. Boyd, R. A. Neal, *Drug Metab. Dispos.* **1976**, *4*, 314 – 322.
[22] P. E. Hansen, F. Duus, S. Bolvig, T. S. Jagodzinski, *J. Mol. Struct.* **1996**, *378*, 45 – 59.
[23] a) B. Kuhn, P. Mohr, M. Stahl, *J. Med. Chem.* **2010**, *53*, 2601 – 2611; b) A. Alex, D. S. Millan, M. Perez, F. Wakenhut, G. A. Whitlock, *MedChemComm* **2011**, *2*, 669 – 674.
[24] ADMET Predictor 8.0, SimulationsPlus: http://www.simulations-plus.-com.
[25] G. Vistoli, A. Pedretti, B. Testa, *Drug Discovery Today* **2008**, *13*, 285 – 294.
[26] W. J. Egan, K. M. Merz, J. J. Baldwin, *J. Med. Chem.* **2000**, *43*, 3867 – 3877.

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