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Camphor sulfonamide derivatives as novel, potent and selective CXCR3 antagonists

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ABSTRACT

A series of *N*-arylpiperazine camphor sulfonamides was discovered as novel CXCR3 antagonists. The synthesis, structure–activity relationships, and optimization of the initial hit that resulted in the identification of potent and selective CXCR3 antagonists are described.

CXCR3 is a chemokine receptor belonging to the superfamily of seven transmembrane spanning G-protein-coupled receptors (GPCRs) and is primarily expressed on activated T-cells with a Th1 phenotype.[1,2] CXCR3 binds to three natural chemokine ligands, Mig (CXCL9), IP-10 (CXCL10) and I-TAC (CXCL11), which are believed to play a key role in directing activated T-cells to the sites of inflammation. Blockade of CXCR3 activation may provide potential therapeutic benefits in the treatment of inflammatory diseases. Studies in animal models and human patients have suggested a role for CXCR3 in multiple sclerosis,[3] arthritis,[4] IBD,[5] asthma,[6] COPD[7] and transplant rejection.[8] As such, CXCR3 has become an attractive target for the development of anti-inflammatory agents. Several small molecule CXCR3 antagonists have been reported in the literature.[9,10] Herein, we describe the identification, synthesis, structure–activity relationships (SARs), selectivity, animal ortholog activity and some development properties of a novel series of camphor sulfonamides as CXCR3 antagonists.

High throughput screening (HTS) of our compound collection using a fluorometric imaging plate reader (FLIPR) assay (which measures inhibition of hIP-10 induced Ca^{2+} flux in CHO cells expressing human recombinant CXCR3 receptor)[11] led to the identification of

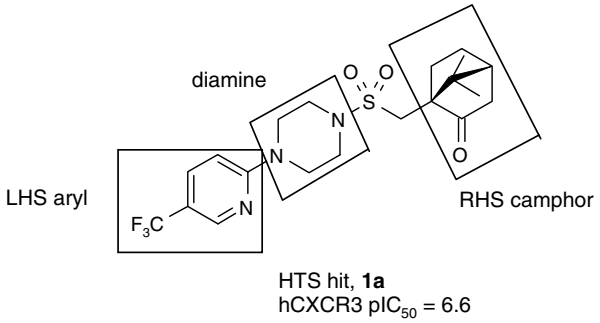

Figure 1. Structure of HTS hit **1a**.

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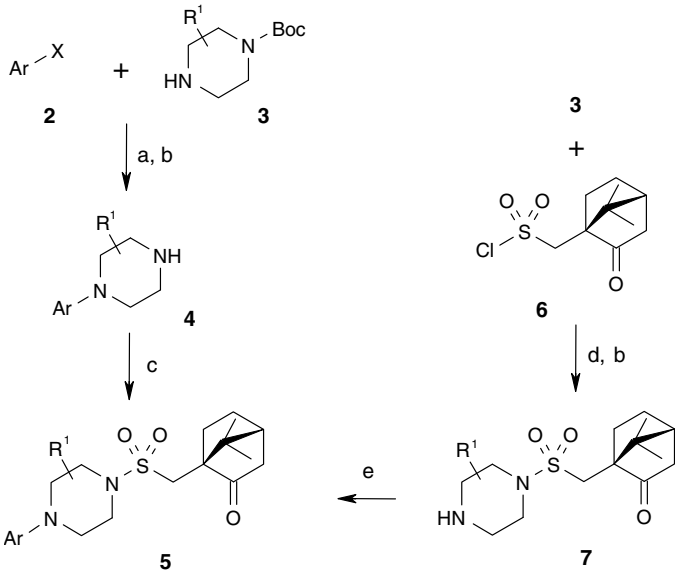


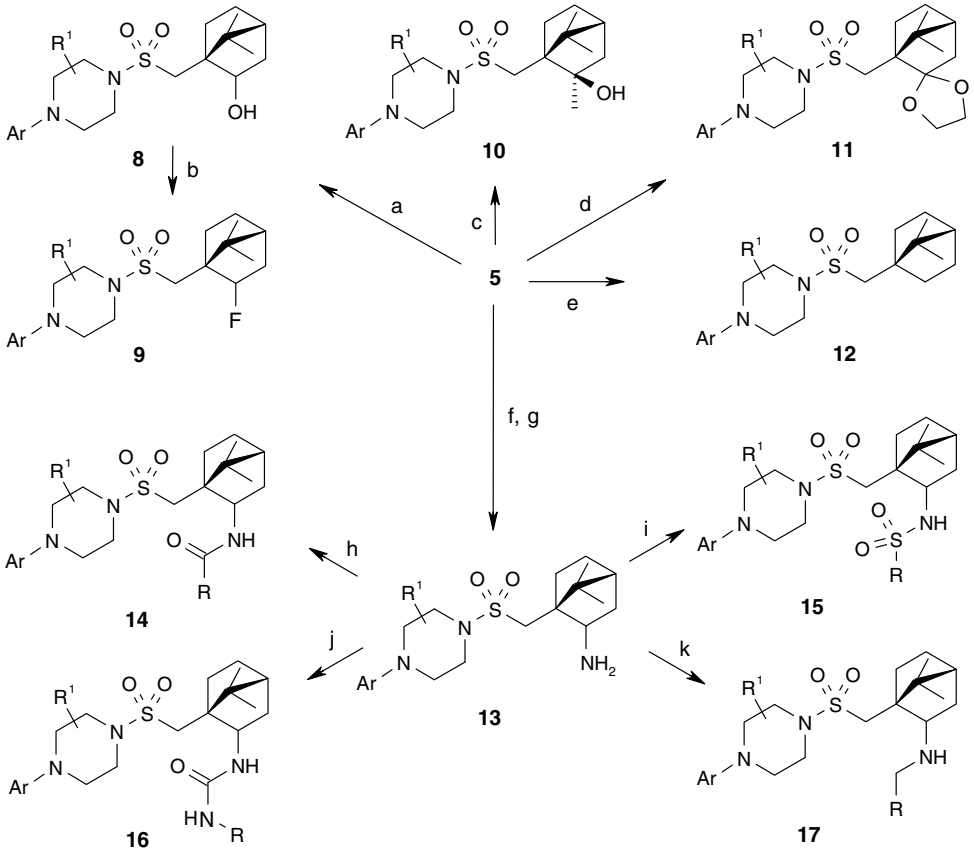
Scheme 1. Reagents and conditions: (a) K_2CO_3, TBAI, DMSO or DIEA, DMSO, heat; (b) 20% TFA in DCM, rt; (c) (1*S*,2*R*)-camphor-derived sulfonylchloride (**6**), DIEA, DCM, rt.; (d) DIEA, DCM, rt; (e) Ar-X (**2**), DIEA, DMSO, heat.

1a as a CXCR3 antagonist hit with a pIC_{50} of 6.6 (Fig. 1). This FLIPR assay was later used as the primary assay to support SAR work.

In order to explore the SAR around the three key regions (left-hand side (LHS) aryl, central diamine and right-hand side (RHS) camphor) of this chemical series, a general synthetic route was developed (Scheme 1).[12] Aryl halides (X = Cl or F) **2** were treated with Boc-protected diamines **3** to produce Boc-protected *N*-aryldiamines, which upon deprotection led to *N*-aryldiamines **4**.[13] Sulfonylation of **4** with (1*S*,4*R*)-camphor-derived sulfonylchlorides such as **6** afforded *N*-aryldiamine sulfonamides **5**. In an alternative route to make **5**, Boc-protected diamines **3** were reacted with (1*S*,4*R*)-camphor-derived sulfonylchloride **6** to give sulfonamides **7** after deprotection.[13] Nucleophilic aromatic substitution of **7** with aryl halides **2** yielded *N*-aryldiamine sulfonamides **5**.

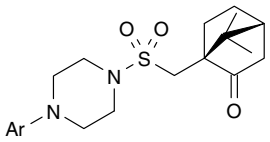
Various reactions at the carbonyl group on the camphor portion of **5** led to a series of camphor-derived analogs illustrated in Scheme 2. Ketones **5** underwent reduction to produce alcohols **8**, which upon treatment with DAST provided fluorides **9**. Grignard addition to **5** formed tertiary alcohols **10**[14] and ketal formation produced compounds **11**. Wolff–Kishner reduction of ketones **5** under microwave conditions produced saturated analogs **12**.[15] Reaction of ketones **5** with hydroxyamine produced ketone oximes, which upon reduction yielded primary amines **13**, which in turn underwent amide formation, sulfonylation, urea formation, and reductive alkylation to afford amides **14**, sulfonamides **15**, ureas **16** and secondary amines **17**, respectively.

We first explored the LHS aryl moiety in the hit **1a** while keeping the camphor moiety and diamine constant (Table 1). It was observed that the position and nature of a substituent on the 2-pyridinyl ring is important for CXCR3 potency. Repositioning the trifluoromethyl group from the 5-position (**1a**) to the 3-, 4- or 6-position (**5a–5c**) on the 2-pyridinyl ring led to a dramatic decline in CXCR3 potency. Replacing the trifluoromethyl with a bromo substituent reduced CXCR3 potency (**5d**) while other



Scheme 2. Reagents and conditions: (a) $NaBH_4$, EtOH, rt; (b) DAST, DCM, rt; (c) MeMgBr, THF, rt; (d) $(CH_2)_2(OH)_2$, toluene, reflux; (e) $NH_2NH_2{\cdot}H_2O$, bis(ethylene glycol), K_2CO_3, microwave, 200 °C; (f) $NH_2OH{\cdot}HCl$, TEA, EtOH, reflux; (g) NH_4OAc, $NaCNBH_3$, $TiCl_3$ (15% in HCl), 0 °C to rt; (h) RCOCl, PS-DIEA, DCM, rt; (i) RSO_2Cl, DIEA, DCM, rt; (j) RNCO, THF, rt; (k) RCHO, MP-borohydride, THF, rt.

Table 1
SAR of LHS aryl moiety



Compound	Ar	hCXCR3 pIC_{50}(FLIPR)[a]
1a	5-(Trifluoromethyl)-2-pyridinyl	6.6
5a	3-(Trifluoromethyl)-2-pyridinyl	<4.5
5b	4-(Trifluoromethyl)-2-pyridinyl	4.6
5c	6-(Trifluoromethyl)-2-pyridinyl	4.6
5d	5-Bromo-2-pyridinyl	5.8
5e	2-Pyridinyl	<4.5
5f	5-Methyl-2-pyridinyl	<4.5
5g	5-Nitro-2-pyridinyl	<4.5
5h	5-Cyano-2-pyridinyl	<4.5
5i	5-Acetyl-2-pyridinyl	<4.5
5j	3-Nitro-5-(trifluoromethyl)-2-Pyridinyl	5.5
5k	3-Fluoro-5-(trifluoromethyl)-2-pyridinyl	6.8
5l	3-Chloro-5-(trifluoromethyl)-2-pyridinyl	6.4
5m	6-Chloro-5-(trifluoromethyl)-2-pyridinyl	6.6
5n	3,6-Dichloro-5-(trifluoromethyl)-2-pyridinyl	6.5
5o	4-(Trifluoromethyl)phenyl	<4.5
5p	2-Fluoro-4-(trifluoromethyl)phenyl	5.7
5q	5-(Trifluoromethyl)-2-pyrimidinyl	6.5
5r	5-Bromo-2-pyrimidinyl	6.2
5s	6-(Trifluoromethyl)-3-pyridazinyl	5.3
5t	3-Isoquinolinyl	4.8
5u	1,3-Benzothiazol-2-yl	<4.9

[a] The FLIPR results are expressed as a mean of two or more individual experiments; pIC_{50} was calculated from IC_{50} using formula, $pIC_{50} = -\log(IC_{50})$.

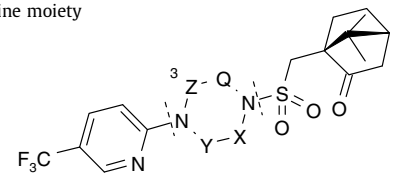
replacements such as a hydrogen, methyl, nitro, cyano, or acetyl at the 5-position of the 2-pyridinyl ring produced inactive analogs (**5e**–**5i**). With a trifluoromethyl group at the 5-position, additional substituents at the 3- and/or 6-position of the 2-pyridinyl ring were well tolerated (**5j**–**5n**). For example, compound **5k** with a 3-fluoro-5-(trifluoromethyl)-2-pyridinyl LHS provided a CXCR3 pIC_{50} of 6.8. Replacement of the pyridine ring with a phenyl ring resulted in a decrease in CXCR3 activity of at least one log unit (compare **5o** to **1a** and **5p** to **5k**). CXCR3 potency was maintained, however, when the pyridine ring was replaced with a pyrimidine ring (compare **5q** to **1a** and **5r** to **5d**) but decreased when replaced with a pyridazine ring (**5s** vs **1a**). Other heteroaryl analogs, such as **5t** and **5u,** showed little CXCR3 activity.

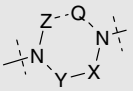
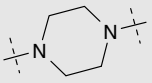
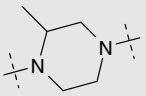
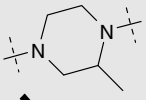
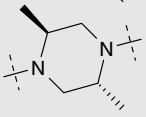
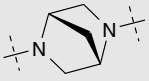
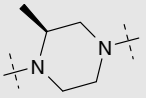
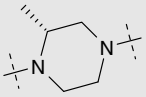
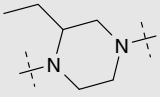
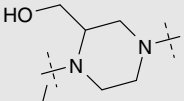
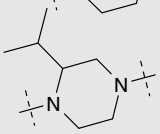
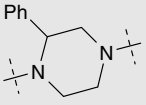
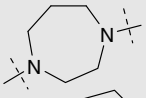
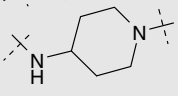
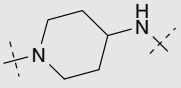
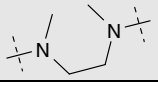
We next explored the central diamine region of the hit **1a** while keeping the camphor moiety and LHS aryl constant (Table 2). A methyl group on the piperazine ring was found to be tolerated with a slight improvement in potency when the methyl group was at the 3-position (**5A**) rather than the 2-position (**5B**).[16] Installing two methyl groups at the 2- and 5-positions of the piperazine ring (**5C**)[16] attenuated CXCR3 potency and introducing a methylene bridge (**5D**) completely abolished CXCR3 potency.

When examining optically pure methyl piperazines, we found that the 3-(*S*)-methyl analog (**5E**) was more potent than the 3-(*R*)-methyl analog (**5F**). Increasing the size of the substituent on the piperazine ring decreased the CXCR3 potency. While 3-ethyl (**5G**) and 3-hydroxymethyl (**5H**) maintained CXCR3 potency, the more hindered 3-isopropyl (**5I**) and 3-phenyl (**5J**) analogs abolished the CXCR3 potency.[16] Compared to piperazine, a homopiperazine (**5K**) decreased CXCR3 potency. Other cyclic diamines (**5L** and **5M)** and an acyclic diamine (**5N**) showed little CXCR3 activity.

The camphor portion of the hit **1a** was subsequently explored (Table 3). Stereochemistry of the camphor plays an important role in CXCR3 potency. The *S*-isomer **1a** [(1*S*,4*R*)-7,7-dimethyl-bicyclo[2.2.1]heptan-2-one] was found to be more potent than the

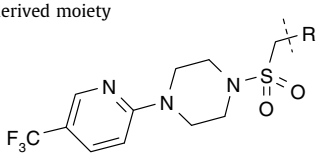
Table 2
SAR of the diamine moiety

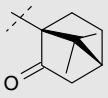
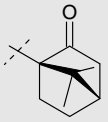
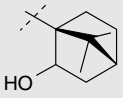
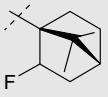
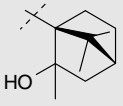
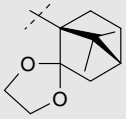
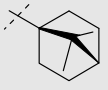
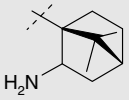
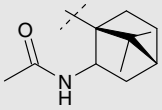
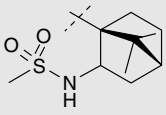
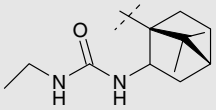
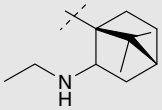


Compound		hCXCR3 pIC_{50} (FLIPR)[a]
1a		6.6
5A		6.5
5B		6.2
5C		5.2
5D		<4.5
5E		6.8
5F		6.3
5G		6.2
5H		6.4
5I		<4.5
5J		<4.5
5K		5.2
5L		4.5
5M		4.8
5N		<4.5

[a] The FLIPR results are expressed as a mean of two or more individual experiments; pIC_{50} was calculated from IC_{50} using formula, $pIC_{50} = -\log(IC_{50})$.

Table 3
SAR of the camphor-derived moiety

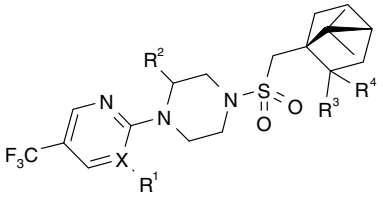


Compound	R	hCXCR3 pIC_{50} (FLIPR)[a]
1a		6.6
1b		5.8
8a		6.8
9a		6.1
10a		6.7
11a		6.7
12a		6.1
13a		6.1
14a		5.7
15a		6.2
16a		6.1
17a		5.1

[a] The FLIPR results are expressed as a mean of two or more individual experiments; pIC_{50} was calculated from IC_{50} using formula, $pIC_{50} = -\log(IC_{50})$.

R-isomer **1b** [(1*R*,4*R*)-7,7-dimethyl-bicyclo[2.2.1]heptan-2-one]. In general, the derivatives obtained from modification of the ketones **1a** and **1b** were well tolerated. Alcohol **8a**[17] was found to be slightly more potent than its corresponding ketone (**1a**). The tertiary alcohol **10a** and the cyclic ketal **11a** maintained CXCR3 potency while derivatives with smaller substituents such as fluorine (**9a**)[17] or hydrogen (**12a**) decreased CXCR3 potency. The amine **13a**[17] and its derivatives (**14a**–**17a**)[17] showed lower CXCR3 potency.

Table 4
Data of some combination compounds



Compound	X	R^1	R^2	R^3/R^4	hCXCR3 pIC_{50} (FLIPR)[a]	mCXCR3 pIC_{50} (FLIPR)[a]
1a	C	H	H	=O	6.6	5.9
5k	C	F	H	=O	6.8	6.1
5E	C	H	(*S*)—Me	=O	6.8	5.6
18a	C	F	(*S*)—Me	=O	7.1	6.3
18b	C	F	(*R*)—Me	=O	6.3	–
18c	C	F	(*S*)—CH_2OH	=O	6.5	5.2
18d	C	H	(*S*)—Me	(±)—OH	6.8	6.2
18e	C	H	(*S*)—Me	(*S*)—OH	7.5	6.7
18f	C	H	(*S*)—Me	(*R*)—OH	6.5	6.2
18g	C	F	(*S*)—Me	(±)—OH	7.3	6.8
18h	C	F	(*S*)—Me	(*S*)—OH	7.5	6.7
18i	C	F	(*S*)—Me	(*R*)—OH	7.2	5.8
18j	C	F	(*S*)—Me	—OC_2H_5O—	7.4	5.8
18k	N	—	(*S*)—Me	=O	6.9	6.0

[a] The FLIPR results are expressed as a mean of two or more individual experiments; pIC_{50} was calculated from IC_{50} using formula, $pIC_{50} = -\log(IC_{50})$.

Having explored the three regions in the hit **1a** and identified the potency-improving moieties in each region, some combination compounds which incorporated the best substituents in each area were prepared (Table 4). It is worth noting that for (1*S*,4*R*)-camphor alcohols, two diastereomers were isolated from each initial mixture (e.g., **18e** and **18f** from **18d**, **18h** and **18i** from **18g**).[18] The *S*-isomer [(1*S*,2*S*,4*R*)-7,7-dimethyl-bicyclo[2.2.1]heptan-2-ol] was generally found to be more potent than the *R*-isomer [(1*S*,2*R*,4*R*)-7,7-dimethyl-bicyclo[2.2.1]heptan-2-ol]. As shown in Table 4, in most cases an improvement in CXCR3 potency was observed when combining potency-enhancing groups identified from each region. For example, compound **18a** with both a 3-fluoro on the pyridine ring (**5k**, $pIC_{50} = 6.8$) and a (*S*)-3-methyl on the piperazine ring (**5E**, $pIC_{50} = 6.8$) showed a CXCR3 FLIPR pIC_{50} of 7.1. Compound **18h** with a 3-fluoro on the pyridine ring, a (*S*)-3-methyl on the piperazine ring and a (*S*)-2-hydroxyl on the camphor moiety showed a CXCR3 FLIPR pIC_{50} of 7.5.

The compounds in this series were found to be reversible CXCR3 antagonists that are competitive with hIP-10. For example, compounds **1a**, **18a** and **18h** showed CXCR3 FLIPR pA_2 values of 6.9, 7.6 and 7.7, respectively.[19] Some camphor sulfonamides were also tested in a mouse CXCR3 FLIPR assay[20] and found to be active but with lower potency as compared to human CXCR3 (Table 4). For example, compounds **1a**, **18a** and **18h** showed mouse CXCR3 FLIPR pIC_{50}s of 5.9, 6.3 and 6.7, respectively.

Key compounds in the series were evaluated in selectivity, hERG, CYP450 inhibition, and in vivo PK studies. With regards to selectivity, compound **18a**, for example, was found to be highly selective in a CEREP screen of 50 receptors, transporters and ion channels (<23% of inhibition at 1 μM against all 50 targets in the panel) and showed at least 100-fold selectivity versus a number of 7TM receptors including chemokine receptors (5HT1A, 5HT1B, 5HT1D, 5HT2A, 5HT2C, 5HT6, 5HT7, H1, H3, Adrenergic Alpha 1A, Adrenergic Alpha 1B, Adrenergic Beta 2, Adenosine A1, Adenosine A2a, D2, D3, CXCR2, CXCR4, CXCR1). Compound **18a** did not

show a hERG liability (binding IC_{50} > 63 μM). In a CYP450 screen, the ketone **18a** showed some inhibition for two major P450 isozymes [IC_{50} = 0.16 μM for 1A2 and 0.32 μM for 3A4 (red)]. However, little 1A2 and 3A4 inhibition was observed for the more potent alcohol analog **18h** [IC_{50}s: 1A2, > 25 μM; 2C9, 4.0 μM; 2C19, 4.0 μM; 2D6, > 25 μM; 3A4(red), > 25 μM; 3A4(green), 13.6 μM]. The compounds in the series generally exhibited good artificial membrane permeability (e.g., 352 nm/s for **18a**) but low aqueous solubility (e.g., 0.004 mg/mL for **18a**) unless a polar group was introduced to the molecule. For example, the compound with (*S*)-3-hydroxymethyl group on the piperazine ring (**18c**) had a solubility of 0.04 mg/mL and permeability of 560 nm/s. The pharmacokinetic properties for compound **18a** were determined in rat, dosed 1.1 mg/kg iv and 2.0 mg/kg po. This compound demonstrated high clearance (Clb = 108 mL/min/kg) with a half-life of 0.5 h, volume of distribution (Vdss) of 2.2 L/kg and oral bioavailability (F) of 8%. The preliminary developability data suggest that this series constitutes a reasonable starting point for further lead optimization.

In summary, exploration of a novel series of camphor sulfonamides identified via HTS led to the discovery of hIP-10 competitive and reversible CXCR3 antagonists such as **18a** and **18h** with excellent functional activity, cross-species activity and selectivity. The further optimization of this series (e.g., camphor replacement) to improve PK will be the subject of future publications.

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15. The methyl Grignard addition to **5** formed the tertiary alcohol **10** as a single diastereomer.
16. Compounds **5A–C** (*trans*-2,5-dimethyl piperazine was used for the preparation of **5C**) and **5G–J** were tested as a 1:1 mixtures of diastereomers.
17. Camphor alcohols **8a**, **18d**, **18g** were prepared and tested as mixtures of diastereomers with ratios of ~5.6:1 favoring the *R*-isomer (determined by ^1H NMR); Compound **9a** was prepared from the corresponding alcohol **8a**, so the ratio of the two diastereomes of **9a** was estimated to be ~1:5.6 favoring the *S*-isomer, assuming a complete inversion of configuration in a simple S_N2 reaction; Compounds **13a–17a** were tested as mixtures of diastereomers with ratios of ~2.6:1, favoring the *R*-isomer. The individual diastereomers were not isolated and characterized except for **18d** and **18g** (see Ref. 18).
18. (a) Chiral HPLC was employed to separate two diastereomers from each mixture of camphor alcohols (conditions for separating **18e** and **18f** from **18d**: Agilent OA LC using Phenomenex Luna eluting with 50–90% CH_3CN in H_2O with 0.1% FTA; SFC conditions for separating **18h** and **18i** from **18g**: 30 mm AD, 140 bar, 40 °C, 75 g/min CO_2, 13 mL/min MeOH, UV @ 250 nm); (b) Stereochemistry of **18e**, **18f**, **18h** and **18i** was assigned by Vibrational Circular Dichroism (VCD) analysis.
19. Concentration response curves of human IP-10 induced Ca^{2+} mobilization were generated in the presence of a single 30 min pre-treated concentration of antagonist, [B] (1 μM), or vehicle alone. The EC_{50}, the concentration of human IP-10 to elicit 50% of the maximal response induced by human IP-10 for a given treatment condition, was calculated. A dose ratio (DR) was calculated as the ratio of the EC_{50} for compound pre-treated human IP-10 curves over the EC_{50} for vehicle treated human IP-10 curves. pA2 serves as an empirical measure of potency and is generated for a single concentration of antagonist using the following formula: pA2 = log(DR-1) − log[B]. The FLIPR results are expressed as a mean of two or more individual experiments.
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