Bioorganic & Medicinal Chemistry Letters 22 (2012) 1384–1387

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of 3-phenylsulfonylmethyl cyclohexylaminobenzamide-derived antagonists of CC chemokine receptor 2 (CCR2)

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ARTICLE INFO

Article history:
Received 6 August 2011
Revised 8 December 2011
Accepted 12 December 2011
Available online 16 December 2011

Keywords:
CCR2
Chemokine
GPCR
Antagonist

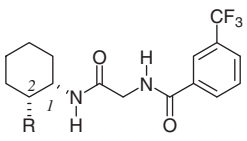
ABSTRACT

We report the synthesis of 3-phenylsulfonylmethyl cyclohexylaminobenzamides (**4**) as CCR2 inhibitors for the potential treatment of inflammatory diseases. Several of the compounds display nanomolar binding affinity for CCR2. The in vitro structure–activity relationships of **4** are described, and are also reconciled with those from the related 2-phenylsulfonylmethyl series.

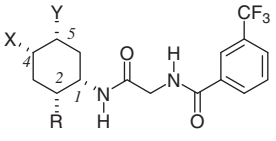
CC chemokine receptor 2 (CCR2) is a G protein-coupled receptor expressed primarily on monocytes—including so-called 'inflammatory monocytes'[1,2]—as well as T-cells, dendritic cells, and endothelial cells. CCR2 binds monocyte chemo-attractant proteins 1–4, a group of pro-inflammatory chemokines that mediate both cellular trafficking and activation. Notably, CCR2 plays a role in pre-clinical models of autoimmune, cardiovascular, and metabolic diseases.[3–6] Accordingly, several medicinal chemistry groups have pursued a strategy of blocking CCR2 interaction to blunt human disease.[7–9]

We previously reported on a series of 1,2-*cis* cyclohexylamine antagonists **1** (Fig. 1).[10] In a more recent publication, we demonstrated that the addition of an amine substituent to **1** significantly affects the compound's affinity for CCR2.[11] For example, the derivatives of (1,2,4-*cis*) **3** have shown improved CCR2 inhibitory activity relative to the analogs of both (1,2-*cis*) **1** and (1,2,5-*cis*) **2** (R = NHC(O)Ar, Fig. 1). The (1,2,4-*cis*) substitution scaffold **3** was also found to be potent in a series of sulfone antagonists ($R = CH_2SO_2Ar$, Fig. 1).[12] In order to probe more completely the structure–activity relationships (SAR) of the sulfone substituent, we investigated the movement of this group from C2 to C3 in the context of the previously optimized cyclohexyl-1,4-diamine scaffold. In this communication, we report the syntheses of the all *cis*- and alternating *trans*-(1,3,4)-scaffolds **4** and **5** (Fig. 1).

A general synthetic scheme for the preparation of racemic-(1,3,4)-*cis*-**4** is shown in Scheme 1. The tri-substituted 3-phenylsulfonylmethyl cyclohexylamino core **6** has previously been used in the preparation of CCR2 inhibitors and its synthesis has been reported.[13] Hydrogenation of azide **6** gave amine **7** which was then coupled to glycinamic acid **8**. The Boc group of **9** was removed by treating it with TFA followed by a reductive amination reaction to give antagonist **4b**. Further reductive amination of **4b** with formaldehyde provided **4c** in 90% yield. Racemic **4c** was then separated on a preparative chiral HPLC to give the enantiomerically pure compounds **4d** and **4e**, respectively.[14]

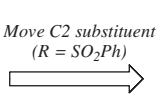


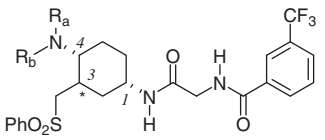
(1,2-*cis*) **1**



X = H, Y = NR_2 (*1,2,5-cis*) **2**

X = NR_2, Y = H (*1,2,4-cis*) **3**





all *cis*, (*1S,3S,4R*) **4**

(*1S,3R,4R*) **5**

Figure 1. Proposed targets of 3-phenylsulfonylmethyl cyclohexylamino benzamides **4** and **5**.

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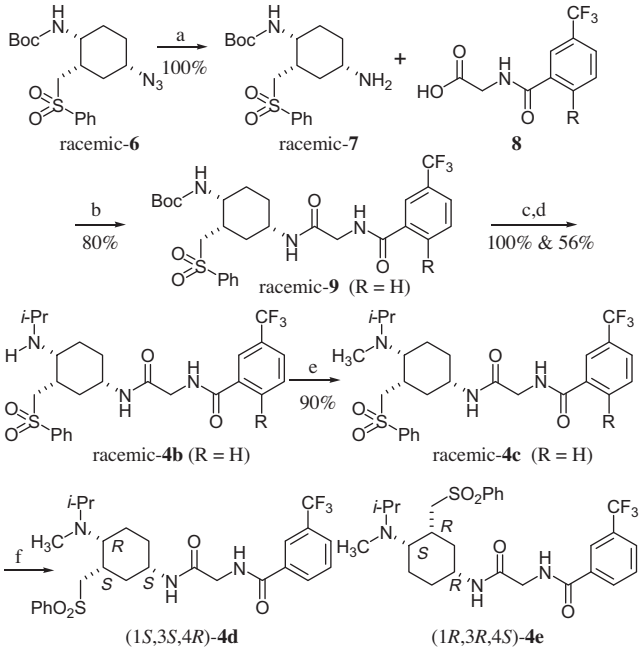
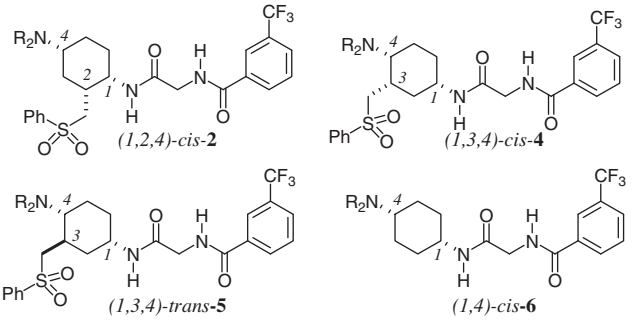
doi:10.1016/j.bmcl.2011.12.057



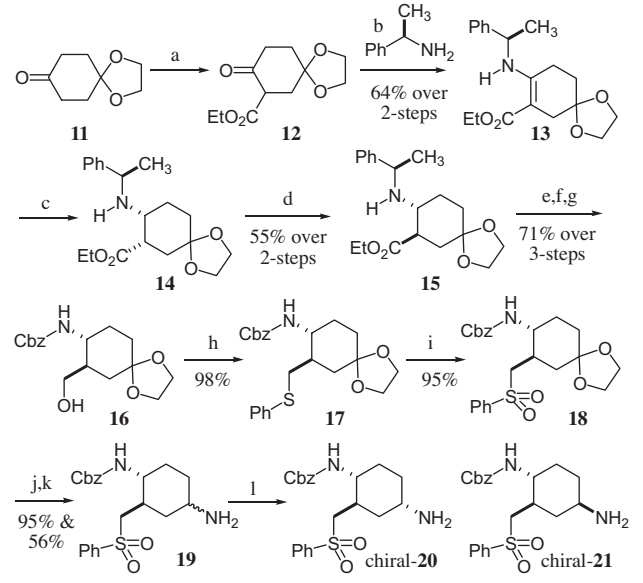
Scheme 1. Reagents and conditions: (a) H_2, Pd-C, EtOH; (b) HATU, *i*-prNEt, DMF; (c) TFA, CH_2Cl_2; (d) ^{i}PrCHO, $NaBH(OAc)_3$, CH_2Cl_2; (e) CH_2O, $NaBH(OAc)_3$, CH_2Cl_2; (f) chiral HPLC separation.

Table 1
CCR2 Binding IC_{50} values (nM) for cyclohexylglycinamides[a]



Compd	NR_2	**2**[b]	*cis*-**4**[b]	*trans*-**5**[b]	**6**
a	NH_2	37 ± 2	59 ± 11	3700	4100
b	*i*-PrNH	5.2 ± 2.3	12 ± 3	310 ± 100	570 ± 160
c	*i*-PrNMe	0.8 ± 0.5	89[c]	2900	72 ± 26

[a] Binding was performed using [^{125}I]MCP-1 and human peripheral blood mononuclear cells at RT. Data are typically reported as the mean of two determinations (±standard deviation). See Ref. 18.
[b] Compounds **2b**, **2c** and **5a–c** are homochiral as illustrated. Compounds **2a** and **4a–c** are racemic.
[c] Data generated using THP-1 cells instead of hPBMCs. Historical correlation with PBMC data was very tight (not shown).



Scheme 2. Reagents and conditions: (a) NaH, $EtOCO_2Et$, THF, reflux; (b) $Yb(OTf)_3$, benzene, reflux; (c) $NaBH(OAc)_3$, MeCN, $MeCO_2H$; (d) NaO_tBu, THF; (e) LAH, Et_2O; (f) H_2, 20% $Pd(OH)_2$/C, MeOH; (g) Cbz-Cl, K_2CO_3, CH_2Cl_2; (h) PhS-SPh, Bu_3P, CH_3CN; (i) m-CPBA, CH_2Cl_2; (j) HCl, acetone, reflux; (k) NH_3, $NaBH(OAc)_3$, dioxane; (l) HPLC separation using a phenomenex column with MeOH/H_2O mixture as the gradient.

To study the potential effect of stereochemical configuration on CCR2 binding activity, the alternating *trans*-isomer **5** was also desired. A synthetic scheme for the preparation of the key intermediate **20** is illustrated in Scheme 2. Acylation of **11** with diethyl carbonate provided ketoester **12** which was then exposed to (*R*)-1-phenylethanamine to form the corresponding enamine **13** in 64% yield over two steps. Diastereoselective reduction[15] of **13** with $NaBH(OAc)_3$ gave the *cis*-isomer **14**, which was subsequently isomerized, under basic conditions to the thermodynamically preferred *trans*-isomer **15** in 55% yield. Ester **15** was reduced to an alcohol, followed by a $Pd(OH)_2$/C-mediated hydrogenolysis to give the corresponding primary amine. The primary amine was then protected with a Cbz group to provide **16** in 71% yield over three steps. Thiolation of alcohol **16** with 1,2-diphenyldisulfide gave the phenylsulfide **17**, which was exposed to *m*-CPBA to provide the corresponding sulfone **18** in 95% yield. Upon removing the 1,3-dioxolane protecting group, the resulting ketone was treated with ammonia and sodium triacetoxy-borohydride to give a 1:1 diastereomeric mixture of amines **19**. This mixture was separated by preparative reverse phase HPLC to afford the single isomers **20** and **21**.[16] With intermediate **20** in hand, the final compounds **5a–c** were prepared by using the same reaction path as shown in Scheme 1, except that the Cbz group was removed by hydrogenolysis.

We compared the CCR2 binding affinities of (1,3,4)-trisubstituted cyclohexylamines **4** and **5** versus those of the historical (1,2,4)-trisubstitued series (Table 1).[17,18] Examination of the data reveals the following three SAR trends: (I) the primary amines **2a** and **4a** exhibit moderate and similar CCR2 potencies; (II) in the *cis*-(1,2,4)-series, the secondary amine is significantly less active than its tertiary counterpart (*cf.* **2b** and **2c**), whereas the secondary amine is superior to its tertiary counterpart in the *cis*-(1,3,4) series (*cf.* **4b** and **4c**); and (III) the alternating *trans*-(1,3,4) isomer is significantly less potent relative to its all *cis* counterpart (*cf.* **4a–c** and **5a–c**). Given that compounds **4a–c** were racemic, we could not distinguish between the relative contributions of the true *cis*-(1,3,4)-isomer and the pseudo *cis*-(1,4,5) isomer (see **4d** and **4e**, Scheme 1). After chiral HPLC separation.[14] and biological analysis, it was clear that the true *cis*-(1,3,4) isomer **4d** was markedly more potent, as it exhibited a binding IC_{50} value of 79 nM, whereas **4e** did not bind at concentrations up to and including 2,500 nM. Together with the data on **5a–c**, this result suggests that the placement of the phenylsulfonylmethyl appendage is critical, in both a regio- and stereochemical sense.

In order to probe further the role of the phenylsulfonylmethyl, we synthesized and tested the simple disubstituted *cis*-(1,4)

isomer **6**, which lacked this moiety (Table 1).[19] As expected, compounds **6a–b** were similar in potency to **5a–b** and clearly less potent than either **2a–b** or **4a–b** (Table 1). However, to our surprise, the simple disubstituted **6c** was more potent than **5c** and **4e**, and equipotent to **4d**. Thus, while regio- and stereodefined placement of the sulfone is important for CCR2 affinity, the substitution of the amine also needs to be matched to the sulfone regioisomer in order to achieve optimal affinity.

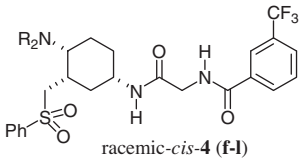
We turned to molecular modeling to rationalize the observations from Table 1.[20] The basis of the preferred stereochemistry (*cis*- vs alternating *trans*-) in the (1,3,4)-series becomes clear when one examines an overlay of the secondary amines **2b**, **4b**, and **5b** (Fig. 2A). As illustrated, it is possible to achieve good alignment of the key functionalities—isopropylamine, phenylsulfone, and glycinamide—between **2b** and **4b** because of the relative placement of the substituents on the cyclohexyl ring. The low energy conformation of **2b** places the glycinamide in an axial orientation, forcing the isopropylamine and phenylsulfone to be equatorial. In contrast, the isopropopylamine in **4b** is axial, with both the phenyl sulfone and gycinamide equatorial. The relative flipping of the cyclohexyl ring allows good alignment of **2b** and **4b**. However, it is not possible to achieve such overlap between all substituents of **2b** and **5b** in either conformation of the cyclohexyl ring, and the impact of the phenylsulfone on CCR2 potency is thus lost (*cf.* **5b** and **6b**, Table 1).

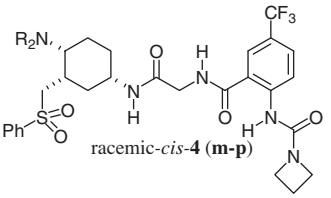
As expected, the basis of the interplay between the amine substitution and regiochemistry of the phenylsulfonylmethyl group is more subtle. In the (1,3,4)-regioisomers **4c** and **5c**, we propose that the proximity of the *methylene* off the phenylsulfone group to the *protonated tertiary amine* forces a projection of the amino NH toward this substituent in order to avoid an unfavorable steric interaction with the amino *N*-methyl group (Fig. 2B). In the case of **2c**, the methylene is distant enough to allow the amine to adopt the opposite stereochemistry, thereby exposing the conformation with the amino NH opposite the phenyl sulfone (Fig. 2B). We infer that this conformation is preferred in order to facilitate a better interaction with Glu291 of CCR2, a residue that has been shown to form a key contact with several different classes of CCR2 antagonists.[21] In summary, in the context of amine substitution, it is the regiochemistry of the phenyl-sulfonylmethyl (C2 vs C3) that is more important than its stereochemistry, consistent with the SAR data for C2, C3, and unsubstituted series **2**, **4**/**5**, and **6** (Table 1).

In order to extend our understanding of this series and optimize further its CCR2 affinity, we synthesized additional analogs of *cis*-(1,3,4) **4** (see Table 2). As in the *cis*-(1,2,4) series,[12] the *iso*-propyl group appeared to be the optimal amine substituent (*cf.* **4b** and **4f–4i**). As noted in Table 1, secondary amines were more typically potent than their tertiary counterparts (**4f–i** vs **4j–l**, Table 2). In addition, we observed that the incorporation of a urea moiety at the 2-position of the aromatic ring sometimes enhanced the CCR2 binding affinity (*cf.* Tables 1 and 2). Among these analogs, **4m** and **4o** were the most potent CCR2 antagonists, with binding IC_{50} values of 6.6 nM in THP-1 cells. In order to document selectivity over CCR5, a binding assay with MIP-1β was performed.[22] Compounds **4m** and **4o** displayed binding CCR5 IC_{50} values of 7,300 and 810 nM, respectively, indicating greater than 100-fold selectivity for binding CCR2 over CCR5. Finally, urea **4m** was also studied in a monocyte transmigration assay,[22] and exhibited IC_{50} values of 8 and 10 nM in the presence of 0.1% and 0.5% BSA, respectively.

A number of compounds were tested in the liver microsome stability assay (Table 3). The data reveal that both secondary and tertiary amines (**4b–4j**) are prone to oxidative de-alkylation reactions. The microsomal stability data is especially unfavorable for isopropyl amine and dimethyl amine-derived analogs (**4b** and **4j**). On the other hand, the primary amine derivatives (**4a** and **4m**) exhibit good microsomal stability in all three species (entries 1 and 6, Table 3). Unfortunately, when compound **4m** was dosed in

Table 2
CCR2 Binding IC_{50} values (nM) for phenylsulfonylmethyl benzamides **4f–4p**[a]





Entry	Substrate	NR_2	IC_{50} (nM)
1	**4b**	*i*-PrNH	12 ± 3
2	**4f**	MeNH	180 ± 12
3	**4g**	EtNH	27 ± 11
4	**4h**	*n*-PrNH	54 ± 11
5	**4i**	*c*-BuNH	34 ± 16
6	**4j**	NMe_2	130 ± 16
7	**4k**	*n*-PrNMe	370 ± 98
8	**4l**	*c*-$C_5H_{10}N$	340 ± 69
9	**4m**	NH_2	6.6 ± 3.6[b,c]
10	**4n**	EtNH	12[b]
11	**4o**	*i*-PrNH	6.6 ± 0.4[b]
12	**4p**	*i*-PrNMe	34[b]

[a] Except where indicated, binding was performed using [^{125}I]MCP-1 and human peripheral blood mononuclear cells at RT. Data are typically reported as the mean of two determinations (±standard deviation). See Ref. 18 for assay protocol.
[b] Data were generated using THP-1 cells. Historical correlation with PBMC data was good (not shown).
[c] A different lot of **4m** exhibited an IC_{50} of 1.4 nM in PBMC binding.

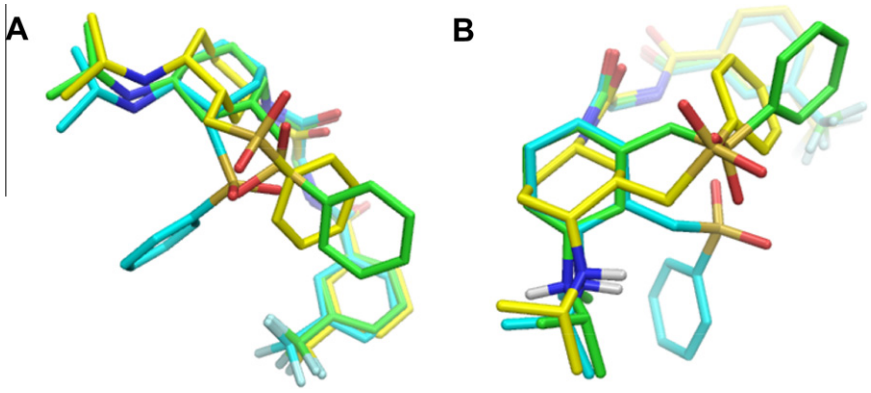


Figure 2. (A) Alignment of secondary amines **2b** (green), **4b** (yellow), and **5b** (cyan). (B) Alignment of the protonated forms of tertiary amines **2c** (green), **4c** (yellow) and **5c** (cyan). The amino proton for all compounds is shown in white; the N-Me is oriented away from the viewer. See discussion in text above.

Table 3
Metabolic stability assessed by liver microsomes[a]

Entry	Substrate (–NR_2)	Human	Rat	Mouse
1	**4a** (–NH_2)	100	57	96
2	**4b** (–NH^iPr)	4.4	0.9	9.6
3	**4g** (–NEtH)	24	23	25
4	**4h** (–NH_nPr)	11	8	28
5	**4j** (–NMe_2)	4.4	3.9	4.3
6	**4m** (–NH_2)	70	71	87

[a] Metabolic stability is defined as the percentage of parent compound remaining over 10 min of incubation time in the presence of human, rat, and mouse liver microsomes, respectively. The initial compound concentration was 0.5 μM.

mice by oral gavage, significant circulating levels were not detected. Thus, further optimization in this series is required in order to identify a compound with oral bioavailability.

In summary, we have found that 3-phenylsulfonylmethyl cyclohexylaminobenzamides are potent antagonists of CCR2. Our studies revealed a strong dependence on the regio- and stereochemical disposition of the phenylsulfonylmethyl group, along with an unexpected 'pairing' of the regiochemistry of this substituent with the substitution pattern of the exocyclic amine. Further studies on trisubstituted cyclohexanes as CCR2 antagonists will be reported in due course.

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