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Structure–Activity Relationship of novel phenylacetic CXCR1 inhibitors

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ABSTRACT

We reported recently the Structure–Activity Relationship (SAR) of a class of CXCL8 allosteric modulators. They invariably share a 2-arylpropionic moiety so far considered a key structural determinant of the biological activity. We show the results of recent SAR studies on a novel series of phenylacetic derivatives supported by a combined approach of mutagenesis experiments and conformational analysis. The results suggest novel insights on the fine role of the propionic/acetic chain in the modulation of CXCL8 receptors.

Chemokine receptors, belonging to the family of 7TM-GPCRs (seven-transmembrane G-protein-coupled receptors), are differentially expressed by a number of immune and nonimmune cell populations and mediate cell activities in response to soluble chemoattractant molecules called chemokines.[1–3] Chemokines and their receptors control the traffic of leucocytes and lymphocytes and a disregulation of the chemokines/chemokine receptors network has been implicated in a variety of diseases including RA (rheumatoid arthritis), COPD (chronic obstructive pulmonary disease), asthma, Alzheimer's disease, melanoma and psoriasis.[4–9]

Among chemotactic factors CXCL8/IL-8 is a key mediator of hPMN (human polymorphonuclear leucocyte) recruitment and activation.[10,11] CXCL8 activates CXCR1 (interleukin-8 receptor type 1) and CXCR2 (interleukin-8 receptor type 2) expressed on hPMN surface CXCL8 and its receptors are believed to play a pivotal role in several inflammatory diseases[12,13] thus CXCL8/CXCR1-CXCR2 are considered promising pharmacological targets. To date, only a limited number of SMW (Small Molecular Weight) CXCL8 inhibitors have been described.[14–16]

The unexpected finding that some NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) belonging to the family of 2-phenylpropionic acids, such as ketoprofen and ibuprofen, inhibited CXCL8-induced hPMN chemotaxis with a COX(cyclooxygenase)-independent mechanism,[17] encouraged us towards extensive medicinal chemistry studies aiming at the identification of novel CXCL8 inhibitors.

As a result, a huge number of 2-phenylpropionic acid derivatives was synthesized, including amide and acylsulfonamide derivatives, leading to potent and selective CXCL8 inhibitors. Within this class reparixin represents the clinical candidate currently under development in phase II clinical trials for the prevention of ischaemia/reperfusion injury during organ transplantation.[18] Specific mechanistic studies allowed us to demonstrate that reparixin and its analogues act as neutral allosteric inhibitors binding CXCR1 and CXCR2 in a well-defined pocket in the TM region bordered by the helices TM 1–3, 6 and 7.

In a previous Letter[17] we reported that the phenylacetic acid diclofenac (**1**) shares with ketoprofen and ibuprofen the property to inhibit CXCL8-induced hPMN chemotaxis. By contrast, subsequent SAR studies clearly evidenced the crucial role of the propionic chain in determining the biological activity of the class that is invariably lost in the related phenylacetic analogues.

In this work, we examined a novel class of CXCL8 inhibitors belonging to the class of diclofenac analogues to clarify the mechanism of action and SAR of these atypical phenylacetic CXCL8 inhibitors. SAR studies herein reported provide further insights into the specific determinants involved in the fine tuning of allosteric modulation of CXCL8 receptors.

Compounds **1** and **22**–**27** are commercially available whereas the synthetic methods for the preparation of the test compounds **10**–**19**[19–22] and **21**[23] were previously published. Compounds **2**–**9** were prepared according to Scheme 1. Intermediate **1a** was obtained by N-acylation of commercial 2,6-dichlorophenylamine with 2-chloropropanoyl chloride followed by a classical

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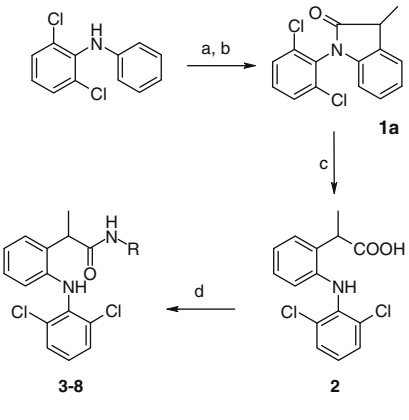


Compds	R
3	H
4	CH_3SO_2
5	$CH_2CH_2N(CH_3)_2$
6	CH_2CH_2OH
7	(2,6-diCH_3)Ph
8	$C(CH_3)_3$
9	$CH_2CH_2N^+(CH_3)_3I^-$

Scheme 1. Synthesis of Compounds **2**–**9**. Reagents and conditions: (a) 2-chloropropanoyl chloride, reflux; (b) $AlCl_3$, 160 °C, 85%; (c) 2 N NaOH, EtOH, reflux, 98%; (d) RNH_2, CDI, $^iPr_2NEt$, CH_2Cl_2, room temperature, 45–75%; (e) CH_3I, THF, room temperature, 75%.

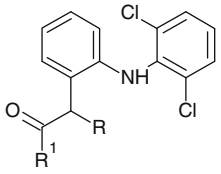
Friedel–Crafts internal alkylation of the aromatic ring in 85% overall yield. The ring opening reaction in ethanolic sodium hydroxide allowed us to obtain in almost quantitative yield the carboxylic acid **2**. It was transformed into compounds **3**–**8** by reaction with the appropriate sulfonamide or amine by classical coupling procedures. Treatment of **5** with iodomethane afforded the quaternary ammonium iodide **9**. Compound **20** was prepared following the same procedure of **4**, but starting from [2-(2-fluorophenoxy)phenyl]acetic acid **18**.

As previously mentioned, the observation that several 3- and 4-substituted phenylpropionic acids, as well as their amide derivatives, inhibited the biological activity of CXCL8 whereas the corresponding phenylacetic derivatives were completely inactive (IC_{50} >10 μM), led to the conclusion that the methyl group is crucial for receptor binding. Nevertheless, receptor binding studies, molecular modelling techniques and receptor point mutagenesis, did not provide clear structural information on specific interactions engaged by the methyl group in the binding pocket. Since diclofenac (**1**) is the only phenylacetic acid so far described able to inhibit CXCL8-induced hPMN chemotaxis,[17] a set of analogues was synthesized (Table 1) to derive additional information on the SAR of this class. With the aim of investigating whether a methyl group is compatible with the biological activity, the phenylpropionic analogue **2** was synthesized and tested in the hPMNs chemotaxis assay. Compounds **1** and **2** exhibited a comparable potency (IC_{50} = 8 nM and 10 nM, respectively), thus suggesting a possible correlation with the class of the phenylpropionic allosteric inhibitors. This hypothesis was further supported by the observation that identical substitutions of the carboxylic group are tolerated in the two classes. In fact, primary amide **3**, acylmethanesulfonamide **4**, ω-aminoalkylamide **5** as well as ethoxyethanolamide **6** derived from **2** exhibited comparable activity as the parent carboxylic acid (Table 1).

In strict analogy with reported SAR[14] the introduction of bulky aliphatic or aromatic amide groups significantly reduced the potency of the inhibitors (**7** and **8**). Correspondingly, the conversion of the tertiary amine of the ω-aminoalkylamide **5** in the quaternary ammonium salt **9** resulted in a significant loss of potency (IC_{50} >1000 nM). Thus, in this set of 2-arylamino substituted phenylacetic derivatives, the reported SAR are intriguingly superimposable to those of phenylpropionic acids demonstrating that the methyl group was not essential for the biological activity.

In our previous work[14] we highlighted that aliphatic or aromatic substituents in the 2-position strongly compromise the affinity with the receptor pocket. To further investigate SAR, a series of diclofenac analogues differently substituted on the second aromatic ring was synthesized and tested in the chemotaxis assay (Table 2). Several observations can be derived: an additional methyl group (**10**) on the aromatic ring is tolerated in the binding pocket, although leading to a potency decrease

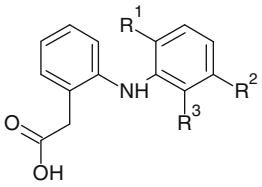
Table 1
Effect of diclofenac derivatives on CXCL8-induced hPMN chemotaxis



Compds	R	R^1	CXCL8 IC_{50}[a] (nM)
1	H	OH	8
2	CH_3	OH	10
3	CH_3	NH_2	12
4	CH_3	$NHSO_2CH_3$	13
5	CH_3	$NH(CH_2)_3N(CH_3)_2$	25
6	CH_3	$NH(CH_2)_2OH$	20
7	CH_3	NHPh[2,6-$(CH_3)_2$]	600
8	CH_3	$NHC(CH_3)_3$	650
9	CH_3	$NH(CH_2)_3N^+(CH_3)_3$ I^-	>1000

[a] Values are means of three or more experiments, std. dev. are <20% of the IC_{50} values.

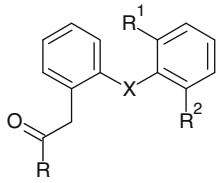
Table 2
Effect of diclofenac derivatives on CXCL8-induced hPMN chemotaxis



Compds	R^1	R^2	R^3	CXCL8 IC_{50}[a] (nM)
10	Cl	CH_3	Cl	500
11	Cl	Cl	H	25
12	Cl	H	H	400
13	F	H	H	9
14	H	H	H	>1000
15	CH_3	H	H	>1000
16	F	H	F	>1000

[a] Values are means of three or more experiments, std. dev. are <20% of the IC_{50} values.

Table 3
Effect of phenylacetic derivatives on CXCL8-induced hPMN chemotaxis



Compds	X	R	R^1	R^2	CXCL8 IC_{50}[a] (nM)
17	O	OH	H	H	>1000
18	O	OH	F	H	13
19	O	OH	Cl	H	500
20	O	$NHSO_2CH_3$	F	H	50
21	CH_2	OH	Cl	Cl	>1000

[a] Values are means of three or more experiments, std. dev. are <20% of the IC_{50} values.

(IC_{50} = 500 nM), the position of the two chlorine atoms (**11**) does not significantly influence the biological activity (IC_{50} = 25 nM); while the monochloro derivative **12** shows a lower potency (IC_{50} = 400 nM). The marked activity of the 2-fluoro derivative **13** (IC_{50} = 9 nM) would suggest that a strong EWG is mandatory for the biological activity. In agreement with this hypothesis, the unsubstituted and the 2-methyl substituted analogue **14** and **15** did not show significant activity (IC_{50} >1000 nM for both). Yet, the lack of activity of the 2,6-difluoro compound **16** (IC_{50} >1000 nM) would suggest the existence of a threshold for the positive effect of EW substituents.

A similar trend was observed also within the series of arylethers (**17**–**20**) (Table 3), thus excluding a direct involvement of the amino group in donor hydrogen bond inter or intramolecular interactions.

Consistent with the discussed effect of EWGs, the replacement of the heteroatom by a methylene group (**21**), resulted in a significant reduction of the inhibitory potency (IC_{50} >1000 nM).

Sustained by the evident SAR correlations, we attempted to derive a hypothesis for the binding mode of **1** in CXCR1, using the reparixin binding model as a template. Compound **1** was manually docked in the CXCR1 binding pocket and subjected to molecular dynamics calculations.[24,25] Interestingly, the results (Fig. 1) show that **1** binds CXCR1 in a conformation that strictly resembles the complex COX-2/**1** (pdb code: 1PXX; http://www.pdb.org). In the derived model the carboxylic acid establishes a double ionic interaction with $Lys99_{2.64}$ and $Glu291_{7.39}$ residues, respectively, as observed for 2-phenylpropionic acids. The chlorinated aromatic ring seems to be involved in a direct electrostatic interaction with the phenolic moiety of $Tyr46_{1.39}$ that behaves as hydrogen bond donor. The proposed model nicely fits with the observed SAR inasmuch as the OH-π interaction is clearly favoured by EWG. In addition, **1** establishes hydrophobic interactions with the lipophilic side chains of $Val42_{1.35}$, $Ile43_{1.36}$, $Val113_{3.28}$, $Ile283_{7.31}$ and $Leu287_{7.35}$ residues. As in the complex COX-2/**1**, the complex is stabilized by the intramolecular hydrogen bond between the amino and the carboxylic acid groups.

To test the computational hypothesis, **1** was tested in the chemotaxis assay using L1.2 cells transiently transfected with wild-type CXCR1 or mutated Lys99Ala CXCR1, respectively (Fig. 2). The CXCR1 mutant did not significantly differ from wild-type CXCR1 transfectant in terms of receptor expression levels, ligand binding properties and chemotactic response (data not shown). Compound **1** significantly inhibited (IC_{50} = 12 nM) the wild-type CXCR1/L1.2 transfectant migration induced by CXCL8 (10 nM) while the efficacy of **1** was completely lost in Lys99Ala CXCR1/L1.2 (IC_{50} >10 μM).

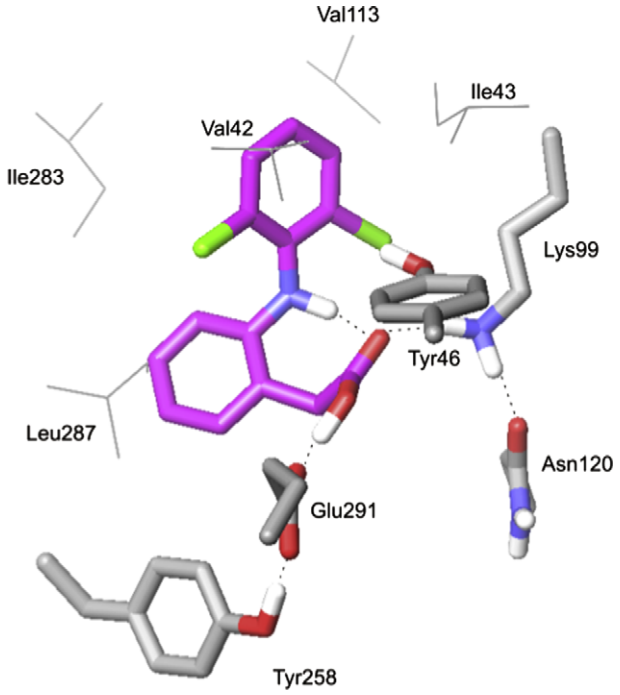


Figure 1. Predicted binding mode of **1** in CXCR1. Compound **1** and polar residues are shown as bold sticks. All the carbons of **1** are shown in magenta whereas both intramolecular and intermolecular hydrogen bonds are depicted as black dashed lines. The torsional angles found in the complex (χ = +70°) and by ab initio calculation (χ = +80°) are close to the conformation extracted by crystallographic analysis of the **1**/COX-2 complex (χ = +65°).

These data support the binding model hypothesis but do not yet provide a clarification for the different role of the methyl group that is mandatory in the series of 3- and 4-substituted phenylpropionic inhibitors[26] and optional for the 2-substituted phenylacetic acids.

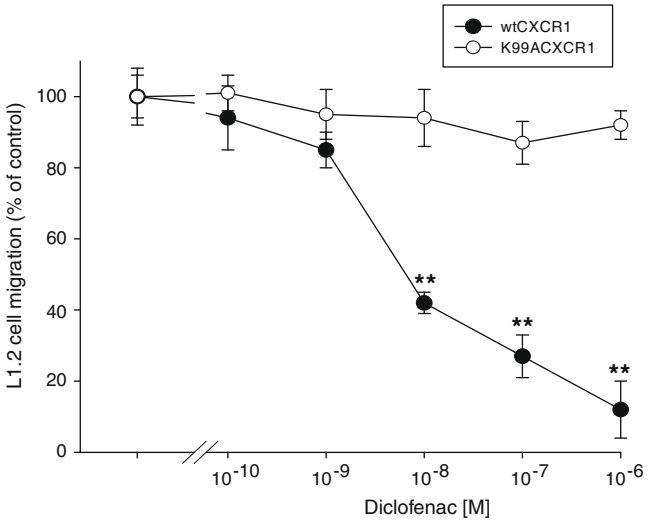


Figure 2. Migration of L1.2 transfectants expressing wild-type CXCR1 (●) or Lys99Ala CXCR1 (○) was induced by 10 nM CXCL8 in the presence or absence of increasing concentrations of **1**, as indicated. Results are expressed as percent of migration considering 100% cell migration in the absence of **1** (±SD) at least in three independent experiments: (**) P <0.01 versus cell migration in the absence of **1** (Mann–Whitney U-test). Spontaneous migration was 1000 ± 500 cells/well; CXCL8-induced cell migration was 55,700 ± 2100 cells/well and 53,250 ± 2900 cells/well for wtCXCR1 and Lys99AlaCXCR1 transfectants, respectively.

As compared to 3- and 4-substituted phenylacetic acids, this series of diclofenac analogues is characterized by a limited conformational flexibility due to the presence of bulky aromatic groups at the 2-position. Starting from this consideration, we focused our attention on the internal flexibility of the inhibitors. In particular, assuming the bound inhibitor as a conformationally confined system, an attractive approach consists in evaluating the reduction of internal fluctuation upon binding. This approach, previously followed in medicinal chemistry studies[27,28] allows to relate, within the assumption of a similar enthalpic behaviour, the affinity of an inhibitor with the loss of entropy associated to the binding of the rigid inhibitor to the receptor. Following this line of investigation, a conformational energy scanning around the C1–C2 torsional angle χ (Fig. 3) was carried out on a small set of active and inactive phenylacetic and phenylpropionic derivatives (Table 4), by using ab initio computations at the Hartree-Fock (HF) level of theory by means of 6–31 g(d) basis set. By comparison of the energy trend of the two pairs of compounds **22**–**25** (Fig. 3; panels a–b) it is noteworthy that the introduction of the methyl group significantly increases the rotational barrier around the C1–C2 bond and that the energy barrier value is markedly influenced by the nature of the substituent of the aromatic ring (ΔE_{max} 3-benzoyl >4-isobutyl). As compared to the biologically inactive phenylacetic acids **23** and **24** that exhibit a free rotor behaviour, the related phenylpropionic analogues **22** and **25** show a higher rotation barrier between the accessible minima. This general trend is confirmed also for the pair of 2-substituted derivatives **1** and **2** (Fig. 3, panel c), whose torsional energy profiles significantly differ from those of the phenylacetic acids **23** and **24**, showing deep minima (at $\chi = \pm 80°$ for **1**) separated by a relatively high energy barrier (>40 KJ/mol for **1**).

This limited dataset seems to support the initial hypothesis that, within the class of phenylacetic acids, a correlation could exist between the biological activity and the loss of entropy associated to receptor binding.

To further investigate this hypothesis, we tested compounds **26** and **27** (Table 4), methylated analogues of two phenylacetic acids found inactive in the CXCL8-chemotaxis assay (data not shown). The insertion of the *ortho*-methyl group on the heteroaromatic ring, despite the small size, resulted in a significant increase of the rotational barrier between the accessible minima (Fig. 3, panel d) and, was paralleled by a marked increase of the inhibitory potency (Table 4).

Based on these speculations, a reasonable interpretation could be that the methyl group of phenylpropionic acids does not contribute to the receptor binding by direct favourable interactions

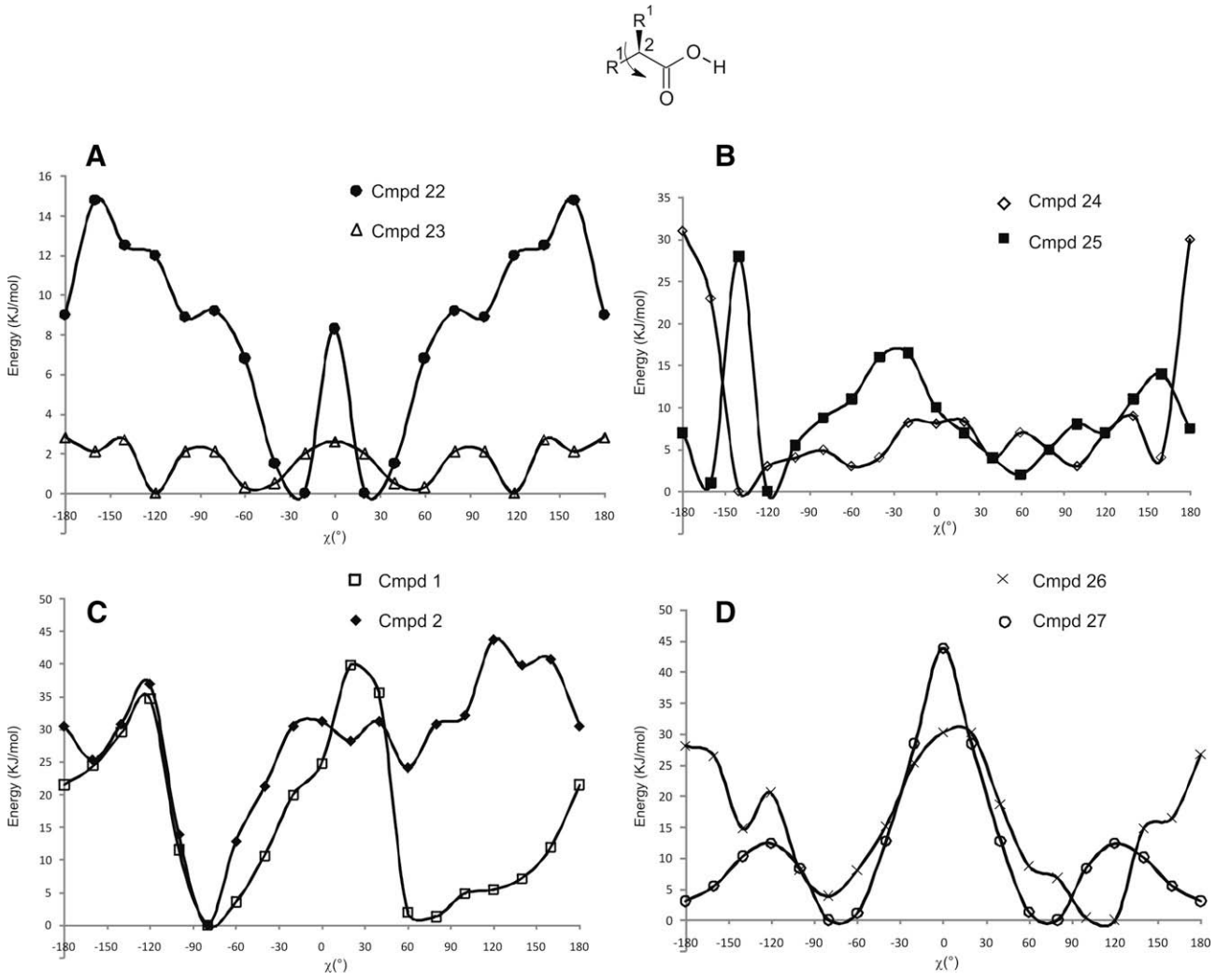
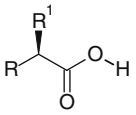
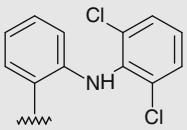
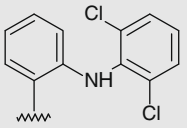
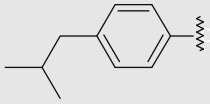
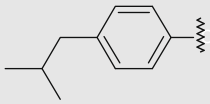
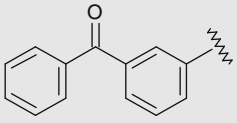
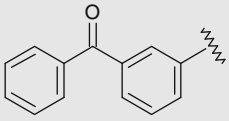
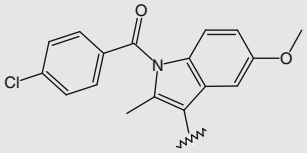
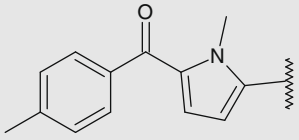


Figure 3. Conformational energies versus the variation of C1–C2 torsional angle χ for the couple of compounds **22–23** (A), **24–25** (B), **1–2** (C) and **26–27** (D) shown in Table 4. The scanning of the potential energy was performed around the torsional angle with an incremental step of 20°. Each point on each curve was obtained by mechanical minimizations by freezing the investigated dihedral angle.

Table 4
CXCL8-induced hPMN chemotaxis inhibitory activity of phenylacetic and their corresponding phenylpropionic derivatives



Compds	R	R^1	CXCL8 IC_{50}[a] (nM)
1		H	8
2		CH_3	10
22		CH_3	50
23		H	>1000
24		H	>1000
25		CH_3	20
26		H	50
27		H	90

[a] Values are means of three or more experiments, std. dev. are <20% of the IC_{50} values.

but plays a determinant role in stabilizing a rotational conformer constraining the inhibitors in a minimum close to the receptor binding conformation.

In conclusion, the above results support the concept that the unfavourable entropy variation associated with receptor binding could be a key factor in the SAR of this class. The data here discussed, even if not conclusive, put forward the importance of combining different experimental and theoretical approaches (molecular modelling, site-directed mutagenesis and specific ab initio computational studies) for the interpretation of complex SAR results.

Acknowledgements

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Supplementary data

Supplementary data (experimental and computational procedures) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.027.

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