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Comparison of *N*,*N*′-diarylsquaramides and *N*,*N*′-diarylureas as antagonists of the CXCR2 chemokine receptor

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Abstract—*N*,*N*′-diarylsquaramides were prepared and evaluated as antagonists of CXCR2. The compounds were found to be potent and selective antagonists of CXCR2. Significant differences in SAR was observed relative to the previously described *N*,*N*′-diarylurea series. As was the case in the *N*,*N*′-diarylurea series, placing sulfonamide substituent adjacent to the acidic phenol significantly reduced the clearance in rat pharmacokinetic studies.

Interleukin-8 (IL-8, CXCL8) and related CXC chemokines (ENA-78 (CXCL5), GCP-2 (CXCL6), GROα CXCL1), GROβ (CXCL2) and GROγ (CXCL3) play an important role in the trafficking of immune cells to sites of inflammation which is consistent with their potential involvement in pathophysiological processes such as arthritis, reperfusion injury and asthma. Indeed, elevated plasma levels of IL-8 and GROα have been associated with these conditions in humans.[1] Thus far, two seven transmembrane G-protein coupled receptors have been identified, which are activated by IL-8 (CXCR1 and CXCR2). CXCR1 binds IL-8 and GCP-2 with high affinity, while CXCR2 binds all of the above-mentioned ELR + chemokines with high affinity.[2] The potential therapeutic value for small-molecule antagonists of the IL-8 receptors is further supported by studies done with CXCR2 mouse gene knockouts which show elevated leukocytes and lymphocytes without apparent pathogenic consequences indicating that these receptors are not required for normal physiology.[3]

As previously disclosed,[4–6] a series of *N*,*N*′-diarylureas has been identified as potent, selective CXCR2 antagonists. This series was later expanded to include *N*,*N*′-diarylguanidines and *N*,*N*′-diarylcyanoguanidines (Fig. 1).[7] Squaramides (3,4-diamino-1,2-dioxocyclobutenes) have previously been described as bioisosteres of ureas and cyanoguanidines[8] and it was decided to incorporate this structural feature into the above-mentioned compound series.

Chemistry. Compounds to elucidate the basic SAR of the phenol-bearing left-hand side were prepared as

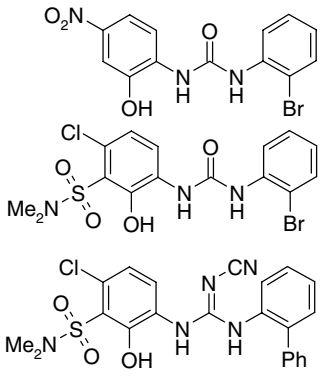

Figure 1. Examples of urea and cyanoguanidine-type CXCR2 antagonists.

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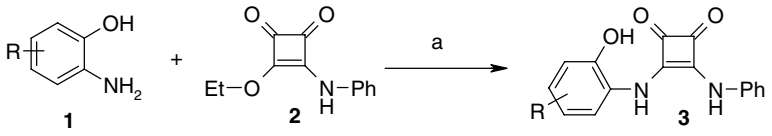
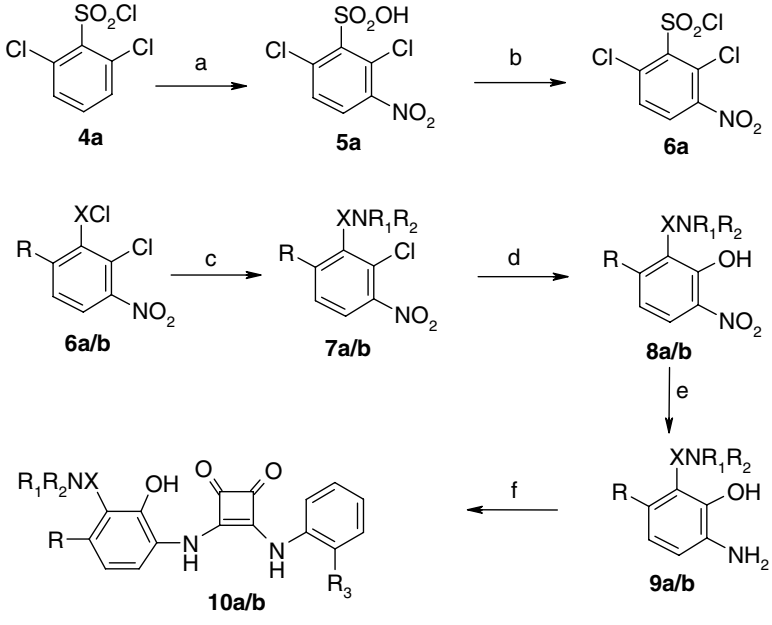


Scheme 1. Preparation of diarylsquaramides for basic SAR. Reagents: (a) $AlMe_3$, CH_2Cl_2.

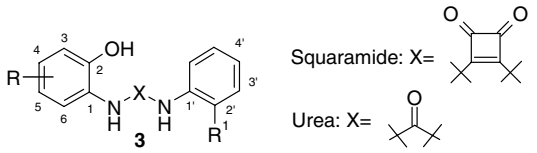
outlined in Scheme 1. Aminophenols **1**, either commercially available or obtained using standard methodology as previously described,[5] were treated with commercially available 3-(ethyloxy)-4-(phenylamino)-3-cyclobutene-1,2-dione **2** in the presence of trimethylaluminum to give the diarylsquaramides **3**. Sulfonamide and amide-containing diarylsquaramides were prepared as outlined in Scheme 2. For the sulfonamide-containing diarylsquaramides **10a**, the synthesis started from the commercially available 2,6-dichlorophenylsulfonyl chloride **4a** which was hydrolysed, selectively nitrated and re-chlorinated to yield the sulfonyl chloride **6a** which was reacted with a variety of amines to give sulfonamides **7a**. Selective displacement of the 2-chloro substituent with hydroxide produced the nitrophenols **8a** which were subsequently reduced to the aminophenols **9a**. Treatment with 3,4-dichlorocyclobut-3-ene-1,2-dione in THF at ambient temperature followed by exposure to a variety of anilines in DMF at 40 °C produced the diarylsquaramides **10a**. As for the amide-containing diarylsquaramides **10b**, the synthesis began with commercially available 2,6-dichloro-3-nitrobenzoic acid or 2-chloro-3-nitrobenzoic acid which following acid chloride formation under standard conditions (**6b**) were treated with a variety of amines to yield amides **7b**. Chloride displacement, reduction and squaramide formation were carried out under the same reaction conditions used for the sulfonamide containing diarylsquaramides.

The SAR of the diarylsquaramide series[9] is substantially different from that of the urea series as illustrated in Table 1. While both series require the presence of two urea/squaramide NHs as well as the phenolic group, the ureas are highly sensitive to the ionization state of the phenol[10] while the diarylsquaramides seem to be affected less. Thus, the squaramide **3a** ($R, R^1 = H$), which had a pK_a of 8.6[11] equal to 5.9% ionization under the assay conditions (pH 7.4), maintained reasonable affinity for the CXCR2 receptor with an IC_{50} of 235 nM. However, the corresponding urea (pK_a: 9.1; 2.0% ionization at pH 7.4) was a weak inhibitor of IL-8 binding with an $IC_{50} > 30$ μM. When an electron-withdrawing group such as nitro or cyano was added in the 3- or 4-position (entries **b**, **c**, **e** and **h**), the pK_a of the phenolic group was lowered which led to the compounds being predominantly ionized under the assay conditions (squaramide **3c**: pK_a: 7.09; %ionization, 67). For the diarylsquaramides, this resulted in an approx. 10-fold enhancement in receptor affinity while the effect on the corresponding ureas was a substantially greater increase of >100-fold. This suggests that while the establishment of a salt bridge between the phenoxide and the



Scheme 2. Preparation of sulfonamide and amide-containing diarylsquaramides **10**. Reagents and conditions: (a) i—LiOH, MeOH; ii—H_2SO_4, HNO_3, 85%; (b) i—KOH; ii—$POCl_3$, PCl_5; (c) HNR1R2, Et_3N, DCM, 74–89%; (d) NaH, H_2O, THF, 50–68%; (e) H_2, Pt/C, 65–85%; (f) i—3,4-dichlorocyclobut-3-ene-1,2-dione, THF, rt; ii—$ArNH_2$, DMF, 40 °C, 19–89%.

Table 1. CXCR2 receptor affinities of *N*,*N*′-diarylsquaramides and their corresponding ureas[a]



Compound **3**	R	R^1	Squaramide CXCR2[a,b,c] [IC_{50} (nM)]	Urea CXCR2[a,b] [IC_{50} (nM)]
a	H	H	235	>30,000
b	3-CN	H	32	276
c	4-CN	H	15	200
d	4-Me	H	205	25,000
e	4-NO_2	H	22	320
f	4-NO_2, 5-Cl	H	52	950
g	5-Me	H	308	>30,000
h	5-Cl	H	54	>30,000
i	6-Me	H	1720	>30,000
j	6-NO_2	H	358	>30,000
k	H	Br	51	>9000
l	4-CN	Br	36	25
m	4-NO_2	Br	37	22

[a] Binding assays were performed on Chinese hamster ovary (CHO) cell lines expressing either CXCR2. The CHO-CXCR2 membranes were prepared according to Kraft and Anderson.[12] All assays were performed in 96-well microtitre plates using radiolabelled [^{125}I]-IL-8 (human recombinant, concn: 0.23 nM). The binding results are expressed as a mean of three individual experiments.

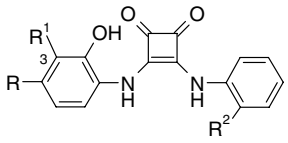
[b] All active compounds were at least 20-fold selective versus CXCR1.

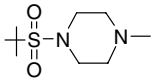
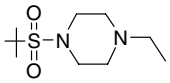
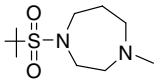
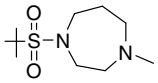
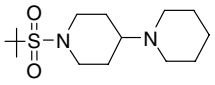
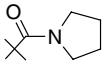
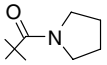
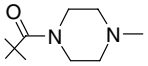
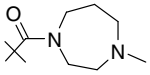
[c] The functional activities of the diarylsquaramides **3a**, **3c, 3e, 3f, 3h, 3k** and **3m** were tested in a calcium mobilization assay performed with human neutrophils using GROα as the ligand. The resulting IC_{50}'s were within 5-fold of the binding IC_{50}'s reported in this table.

receptor is very important in the urea series, the polar phenol/receptor interaction may be somewhat different in nature for the squaramide series. The SAR of the non-phenol-bearing phenyl ring was also quite different: whereas introduction of a 2-bromo substituent in the urea series led to a substantial increase (approx. 10-fold) in receptor affinity (entries **l** and **m**), the effect in the squaramide series appeared to be negligible. These differences are perhaps not surprising when you consider that the dioxocyclobutene core of the squaramides clearly is a larger group than the urea carbonyl, thus pushing the anilino moieties approximately 1 Å further apart. Hence, it is reasonable to expect that the two series will experience slightly different binding environments. The fact that both series are able to attain high-binding affinity may be seen as evidence that the receptor binding pocket is quite flexible. As was the case the urea and cyanoguanidines series,[7] the 3-position adjacent to the phenol is very tolerant to substitution and is an ideal location for the introduction of groups aimed at modulating physicochemical parameters such as solubility. The aqueous solubility of the diarylsquaramide template is inherently rather low and thus solubilizing sulfonamide and amide groups were introduced at this position. The results are summarized in Table 2. As was the case with the simple diarylsquaramides (Table 1), the nature of the substituents at the 4- and 2-positions appears to have a very limited effect on the CXCR2 potency of the amide and sulfonamide-substituted compounds **10**. In addition, the effect of these substituents on solubility is also rather limited. In the case of the 4-substituent, this is interesting since one might have expected that a higher degree of phenol ionization might result in higher solubility. One possible explanation would be that more acidic phenol also increases the lattice energy thus counteracting the solubilizing effect of the phenoxide ion. Regarding the 3-position substituent, the receptor was able to tolerate remarkably large groups such as the 4-piperidylpiperidinesulfonamide group (**10k**) without loss of receptor potency. With respect to solubility, amides appear to have slightly higher solubility than sulfonamides. Regarding the amide/sulfonamide substitution, methylhomopiperazine (**10i**, **10j** and **10p**) seems to be the substituent that most effectively solubilizes compounds of this type. The pharmacokinetic properties of several compounds were assessed in the rat. One interesting question was whether or not the presence of a 3-sulfonamide substituent would be required for suppression of rapid phenol glucuronidation leading to high clearance as was the case in the diarylurea series.[6] Certainly the sulfonamide-containing compounds (**10e**–**k**) appear to have low to very low clearance while non-sulfonamide-containing diarylsquaramides have higher clearance. It is important to note, however, that the clearance of the squaramide series as a whole appears to be lower than that of the diarylurea series. This, combined with the fact that the volumes of distribution observed with the diarylsquaramide series are very low, suggests that this series may be more protein bound than the diarylurea series. Some diarylsquaramides such as **10h**, however, do display a desirable pharmacokinetic profile with low clearance, not too low volume of distribution and acceptable oral bioavailability. Non-sulfonamide-containing diarylsquaramides by and large displayed poor oral bioavailability.

In summary, diarylsquaramides have been shown to be potent inhibitors of the CXCR2 receptor. The SAR of this series was found to be distinctly different from that of the closely related diarylurea series. While the volume

Table 2. C-3 sulfonamide and amide-substituted diarylsquaramides: CXCR2 affinity, solubility and pharmacokinetics



Compound **10**	R	R^1	R^2	CXCR2 [IC_{50} (nM)][a,b,c]	Aqueous solubility (μM)[b,d]	Rat PK (CL: mL/min/kg; Vdss: L/kg; %F)[b,e]
c	Cl	H	Br	32	3	—
d	CN	H	Br	15	14	15; 0.15; 1
e	Me	SO_2NMe_2	Me	15	10	4; 0.25; 83
f	Cl	$SO_2NH(CH_2)_2OMe$	H	8	73	0.6; 0.14; 23
g	F		Br	13	44	—
h	Cl		**n**-Pr	8	78	7; 0.89; 43
i	Cl		Br	10	138	4; 0.24; 23
j	Cl		Cl	20	160	4; 0.31; 58
k	Cl		H	18	7	1.2; 0.24; 5
l	Cl	$CONH_2$	H	14	88	—
m	H		CF_3		86	45; 0.66; 13
n	Cl		Br	28	143	—
o	H		H	27	121	—
p	H		H	44	178	15; 0.33; 0.6

[a] Binding assays were performed as described in Table 1.
[b] All active compounds were at least 20-fold selective versus CXCR1.
[c] Compounds **10g**, **10h**, **10o** and **10p** were tested as trifluoroacetic acid salts whereas compounds **10i**–**k** were tested as hydrochloric acid salts.
[d] Aqueous solubility was measured in 0.05 M, pH 7.4, phosphate buffer-containing 2% DMSO using standard high-throughput techniques.
[e] Pharmacokinetic experiments were conducted using an iv x po crossover design in male Sprague–Dawley rats ($n = 3$ per study). With the exception of compound **10h**, which was tested as a single compound, all compounds were tested in cassette experiments where four compounds are dosed simultaneously to each animal. Compounds were administered in an aqueous solution containing 1% DMSO and up to 20% hydroxypropyl-β-cyclodextrin at dosages of 4 and 8 μmol/kg for iv and po doses, respectively. Blood samples were obtained from a lateral tail vein and plasma analyzed for drug content using LC/MS/MS methodologies, with a lower limit of quantification of <10 ng/mL for each analyte.

of distribution generally was low in this series, some 3-sulfonamide-containing diarylsquaramides did display a desirable pharmacokinetic profile with low clearance and moderate to high oral bioavailability.

Acknowledgments

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