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3-Arylamino-2*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxides as novel and selective CXCR2 antagonists

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Abstract—A series of 3-arylamino-2*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxides were prepared and shown to be novel and selective antagonists of the CXCR2 receptor. Synthesis, structure and activity relationships, selectivity, and some developability properties are described.

Interleukin-8 (IL-8, CXCL8) and related ELR+ (Glu[4]-Leu[5]-Arg[6]) containing 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, atherosclerosis, reperfusion injury, psoriasis, and asthma. Indeed, elevated plasma levels of IL-8 and GROα have been associated with these conditions in humans.[1] Two seven-transmembrane (7TM) G-protein coupled receptors (CXCR1 and CXCR2) have been identified, which are activated by IL-8. 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 and selective CXCR2 antagonists. This series was later expanded to include *N*,*N*′-diarylguanidines, *N*,*N*′-diarylcyanoguanidines,[7] and squaramides (3,4-diamino-1,2-dioxocyclobutenes)[8,9] (Fig. 1). In *N*,*N*′-diarylureas and their bioisosteres,[10] both left-hand side (LHS) and right-hand side (RHS) aryl moieties could freely rotate to adopt their optimal conformations when bound to the receptor. To examine if this free rotation was required for potent CXCR2 binding affinity, conformational restriction in the form of a six-membered ring (e.g., **4** in Fig. 1) was introduced. Herein, we describe the synthesis, initial structure and activity relationships (SAR), selectivity, and some developability properties of a novel series of 3-arylamino-2*H*-1,2,4-benzothiadiazin-5-ol-1,1-dioxides.

As shown in Scheme 1, the synthesis started with 2-methoxy-anilines **5** which were easily obtained from commercial sources or prepared from the corresponding

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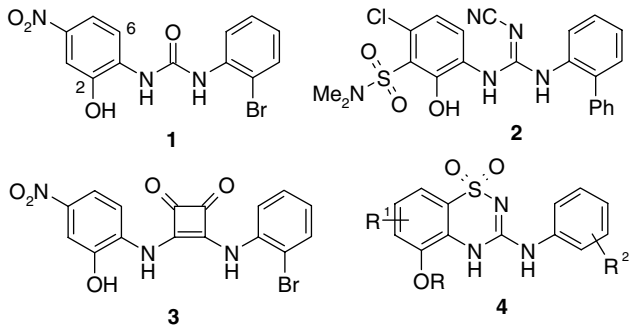


Figure 1. Examples of urea (**1**)-, cyanoquanidine (**2**)-, squaramide (**3**)-type CXCR2 antagonists and a new cyclic sulfonamide template (**4**).

nitro compounds through reduction.[11] Reaction of **5** with chlorosulfonylisocyanate led to substituted 5-methoxy-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-dioxides **6**.[12] The 3-oxo compounds (**6**) were converted into the corresponding amidoyl triflates **7**, which upon nucleophilic substitution by a variety of anilines gave the *N*-aryl-5-methoxy-2*H*-1,2,4-benzothiadiazin-3-amine 1,1-dioxides **8**.[13] Treatment of **8** with LiCl under conventional heating or microwave conditions produced the target compounds **9**.[14]

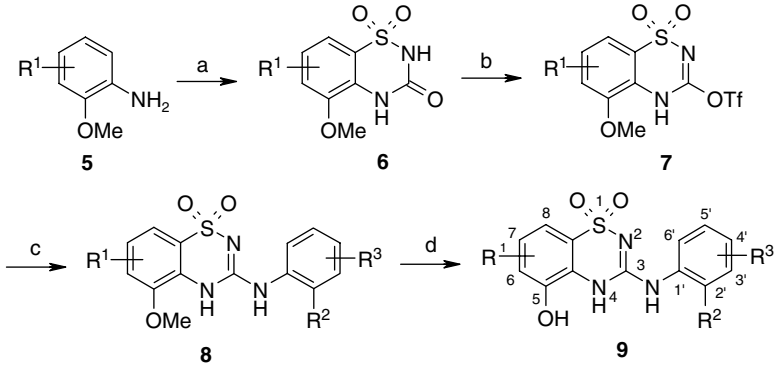
We first explored the SAR of the RHS moiety by preparing analogs containing anilines with different substituents (R^2 and R^3 in **9**). As shown in Table 1, the nature and location of these substituents were found to be important for CXCR2 binding affinities. For analogs with no or a halo substituent on 2′-position of aniline (**9a**–**9d**, **9w**, and **9ab**), CXCR2 binding affinity increased with the size of the substituents (H < F < Cl < Br). When shifting a halogen from the 2′-position (**9d**) to the 3′- or 4′-position (**9e** and **9f**, respectively), CXCR2 binding affinity of the corresponding analogs decreased dramatically. When introducing a second halogen on the 3′-position in addition to the one on the 2′-position, the CXCR2 binding affinity slightly increased (comparing **9g** with **9c**). However, a second halogen on the 4′-, 5′- or 6′-position decreased the CXCR2 binding affinity (comparing **9h**–**j** with **9d**). Among other substituents introduced at the 2′-position of the aniline, the phenoxy group stands out and binding IC_{50}s of the corresponding analogs, **9n** and **9aa**, are both 0.054 μM. While the binding IC_{50}s of analogs with methyl, phenyl, methoxy, trifluoromethyl, and trifluoromethoxy on the 2′-position of the aniline (**9k**–**p**, respectively) fell in the 0.2–1.0 μM range, nitrogen- and carbonyl-containing compounds were found to be much less active (**9r**) or completely inactive (**9q** and **9s**). Overall, the RHS SAR closely mirrors that of the diarylurea series.[9]

Table 1. CXCR2 receptor antagonist binding affinities of 3-arylamino-2*H*-1,2,4-benzothiadiazin-5-ol 1,1-dioxides (**9**)

Compound	R^1	R^2	R^3	Binding[a] IC_{50} (μM)
9a	7-Cl	H	H	2.4
9b	7-Cl	F	H	0.74
9c	7-Cl	Cl	H	0.092
9d	7-Cl	Br	H	0.064
9e	7-Cl	H	3′-Br	6.1
9f	7-Cl	H	4′-Br	5.1
9g	7-Cl	Cl	3′-F	0.064
9h	7-Cl	Br	4′-F	0.12
9i	7-Cl	Br	5′-F	3.9
9j	7-Cl	Br	6′-F	1.6
9k	7-Cl	Me	H	0.39
9l	7-Cl	Ph	H	0.23
9m	7-Cl	OMe	H	0.25
9n	7-Cl	OPh	H	0.054
9o	7-Cl	CF_3	H	0.83
9p	7-Cl	OCF_3	H	0.93
9q	7-Cl	(4′-Morpholinyl)	H	>30
9r	7-Cl	COMe	H	4.5
9s	7-Cl	CO_2H	H	>30
9t	7-H	Cl	H	5.2
9u	7-F	Br	H	0.36
9v	7-Br	Br	H	0.070
9w	7-NO_2	Br	H	0.026
9x	7-Me	Br	H	0.28
9y	8-F	Br	H	3.0
9z	8-Cl	Br	H	0.48
9aa	7-NO_2	OPh	H	0.054
9ab	7-NO_2	Cl	H	0.081

[a] CXCR2 binding assays were performed as described in Ref. 16.

Subsequently, the SAR of the LHS moiety was explored by changing substituents (R^1 in **9**) on the phenyl ring. As

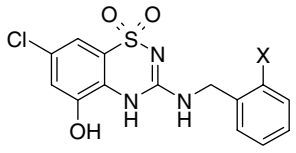


Scheme 1. Reagents and conditions: (a) ^{n}PrNO$_2$, ClSO$_2$NCO, AlCl$_3$ (R^1 = 7-Cl, 52%); (b) Tf$_2$O, Pyr, CH$_2$Cl$_2$ (R^1 = 7-Cl, 56%); (c) anilines, CH$_2$Cl$_2$ (R^1 = 7-Cl, R^2 = Cl, R^3 = H, 63%); (d) LiCl, DMSO, heating (R^1 = 7-Cl, R^2 = Cl, R^3 = H, 80%).

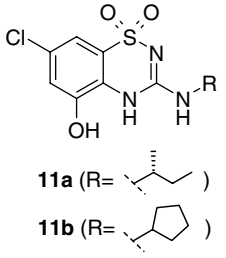
shown in Table 1, introduction of a substituent on the 7-position of LHS phenyl ring enhanced the CXCR2 binding affinity (comparing **9t** with **9c**). With regard to the 7-substituents (**9d**, **9u**–**9x**), the CXCR2 binding affinity increased in the following order: F, Me < Cl, Br < NO_2. This suggests that both electron withdrawing ability and size are important factors for substituents at this position. Analogs with substituent on the 8-position were found to be less potent than those with substituent on the 7-position (comparing **9y** with **9u** and **9z** with **9d**).[15]

Three of the 5-methoxy substituted intermediates **8g**, **8t**, and **8w** were also tested in order to examine the influence of the 5-hydroxy group on CXCR2 binding affinity. The IC_{50}s were >30, >30, and 12 μM, respectively, and therefore it seems quite clear that having the free phenol is of great importance to the receptor binding of this series which is entirely consistent with the findings obtained from the diarylurea series.[5,10] However, one aspect of the LHS SAR which appears to differ from the diarylurea series is the fact that the cyclic sulfonamide is attached to the aryl moiety in what is referred to as the 6-position in the diarylurea series (see Fig. 1). It has previously been shown that this position is highly intolerant of substitution,[5,9c] however, this may be due to the 6-substituent interfering with the co-planarity of the aryl ring with the urea moiety. In contrast, the sulfonamide ring in the present series enforces this co-planarity.

As an extension of exploration for RHS moiety, a few non-aniline analogs such as those with RHS benzylamines (**10a** and **10b**) and alkylamines (**11a** and **11b**) were prepared.[17] The CXCR2 binding IC_{50}s of **10a**, **10b**, **11a**, and **11b** were found to be 8.0, >30, 15, and 20 μM, respectively, which are much less potent than the aniline analogs.



10a (X=H)
10b (X=Cl)



11a (R=)
11b (R=)

In addition to the SAR explorations, a few exemplar compounds were further evaluated in a CXCR2 functional assay, selectivity and initial developability assays. As shown in Table 2, the compounds in the series exhibited good CXCR2 antagonist activity in a FLIPR assay[18] although the IC_{50}s dropped 5-fold or more, relative to the affinity obtained in the binding assay.

The compounds in Table 2 were tested in a CXCR1 binding assay and found to be at least 100-fold selective for CXCR2. A few exemplar compounds (e.g., **9aa**, **9ab**, and **9w**) were also evaluated against a panel of 19 other 7TM in vitro assays and found to be CXCR2 selective.

Table 2. CXCR2 FLIPR calcium mobilization activity, CXCR1 binding affinity, and aqueous solubility for exemplar compounds **9**

Compound	R^1	R^2	R^3	CXCR2 FLIPR[a] IC_{50}, (μM)	CXCR1 binding[b] IC_{50}, (μM)	Aqueous solubility[c] (μM)
9aa	NO_2	OPh	H	0.49	7.6	2
9w	NO_2	Br	H	0.60	3.2	233
9ab	NO_2	Cl	H	1.1	12	188
9n	Cl	OPh	H	0.32	20	<1
9d	Cl	Br	H	0.40	13	31
9c	Cl	Cl	H	0.52	24	68
9g	Cl	Cl	F	0.46	13	160

[a] CXCR2 FLIPR assays were performed as described in Ref. 18.
[b] CXCR1 binding assays were performed as described in Ref. 16.
[c] Aqueous solubility was measured in 0.05 M, pH 7.4, phosphate buffer containing 2% DMSO using standard HPLC based high throughput techniques.

For example, **9aa** showed CXCR2 selectivity over the following receptors (IC_{50}, μM): P2Y1, >25; PAI-1, 7.9; P38 Alpha, 25; Histamine H3, >3.2; 5HT1A, >3.2; 5HT1B, >3.2; 5HT1D, >3.2; 5HT6, >7.9; 5HT7, 25; Adrenergic Alpha 1A, 1.0; Histamine H1, >10; Adrenergic Beta 1, >25; Dopamine D2, >3.2; Dopamine D3, >3.2; 5HT4A, >25; 5HT2C, >10; Beta 2, >25; Adenosine A2a, 25; Adrenergic A1, >25.

The compounds in the series exhibited a range of aqueous solubilities (Table 2). While compounds with a RHS 2′-phenoxyaniline had poor solubility, other compounds with a 2′-chloro- or 2′-bromoaniline showed moderate to good aqueous solubility. In a CYP450 screen, compound **9ab** showed no inhibition for 4 major P450 isozymes (>25 μM for 1A2, 2C9, 2D6, 3A4) but some activity at 2C9 with IC_{50} of 0.6 μM. Compound **9ab** was also evaluated in in vivo rat PK studies (1.6 mg/kg iv and 3.2 mg/kg po). The compound demonstrated moderate clearance (Clb = 23 mL/min/kg) with $t_{1/2}$ of 2 h, volume of distribution (Vdss) of 0.53 L/kg, and bioavailability (F) of 5%. These preliminary developability data suggest that this series constitutes a reasonable starting point for further lead optimization.

In summary, we have designed and synthesized a series of cyclic sulfonamides as novel and selective CXCR2 antagonists. The initial SAR was explored, demonstrating that the nature and location of substituents on both RHS aniline ring and LHS phenol ring are important to CXCR2 binding affinity. Although the functional activity of the series appears to be somewhat lower, and a few developability issues were identified (e.g., 2C9 inhibition, %F), the series does appear to be an acceptable starting point for further lead optimization.

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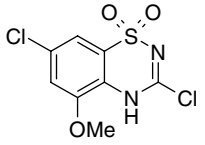

7a

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