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Design, synthesis, and evaluation of novel 3-amino-4-hydrazine-cyclobut-3-ene-1,2-diones as potent and selective CXCR2 chemokine receptor antagonists

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ABSTRACT

We describe herein a novel series of 3-amino-4-hydrazine-cyclobut-3-ene-1,2-diones as potent and selective inhibitors against the CXCR2 chemokine receptor and IL-8-mediated chemotaxis of a CXCR2-expressing cell line. Furthermore, these alkyl-hydrazine series inhibitors such as **5b** demonstrated acceptable metabolic stability when incubated in human and rat microsomes.

Owing to the relevance of IL-8 (CXCL8) and related chemokines in a wide range of inflammatory diseases such as arthritis, asthma, and COPD, the search for small-molecule antagonists for CXCR2 has attracted a lot of attention within the past two decades.[1,2] As a result of these efforts, many structurally diverse CXCR2 antagonists have been identified, which include the bis-aryl urea series such as **1**,[3–6] the 3,4-diamino-cyclobut-3-ene-1,2-dione series such as **2**a and **2b**,[7–9] the thiazolopyrimidine series **3**,[10–12] and the 3,4-diamino-substituted 1,2,5-thiadiazole series **4**[13–15] as shown in Figure 1. A careful analysis on SAR trends reported for **Series 2** antagonists[7–9] suggested the possibility of replacing the alkyl amine moiety as installed for **2a** or **2b** with a hydrazine linkage as seen for **Series 5** (see Fig. 1). The rationale for incorporation of hydrazine moiety in **Series 5** antagonists was further supported by the observation that such linkage was incorporated into HIV protease inhibitors by Reddy et al.,[16] Human Rhino Viruses (HRV) 3C protease inhibitors by Kati et al.,[17] HCV protease inhibitors by Bailey et al.,[18] as well as SARS 3CL protease inhibitors by Anand et al.[19] Furthermore, a recently FDA approved HIV protease inhibitor Reyataz with hydrazine linkage incorporated as subunit further validates our design concept for **Series 5** antagonists[20] (see Fig. 2).

With the intention to validate our hypothesis, we designed a number of *N*-alkyl, both electron rich and deficient *N*-aryl, and *N*-acyl substituted hydrazines as the initial target compounds (**5a**–**j**) as shown in Figure 3. In this communication, we describe the design, synthesis, and SAR trend observed with the novel hydrazine linkage bearing cyclobutene diones **5** as CXCR2 antagonists. The most promising compound identified within this series thus far, **5b** showed good receptor binding potency, functional activity, and excellent selectivity against CXCR1, and acceptable metabolic stability, thus rendering itself as a new lead compound for further optimization.

Chemical synthesis: The syntheses of all target compounds were accomplished according to the chemistries depicted in Scheme 1 through **6**. It is worthwhile to mention that many attempts to prepare these seemingly related target analogs (**5a**–**j**) via a convergent route such as that shown in Scheme 1 were not successful (e.g., for **5d**–**f**). Consequently, multiple routes were exploited for the preparation of various hydrazine bearing cyclobutene diones derivatives **5**.

The syntheses of compounds **5a** and **5f** were completed according to Scheme 1. Towards this end, coupling of the known

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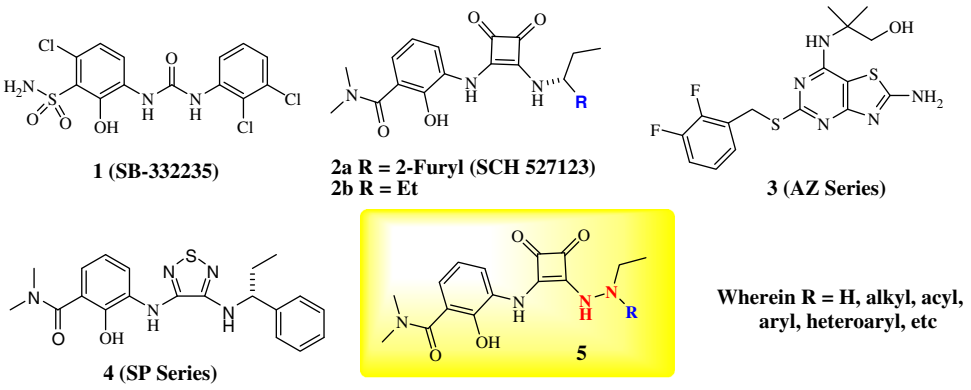


Figure 1. Representative CXCR2 antagonists.

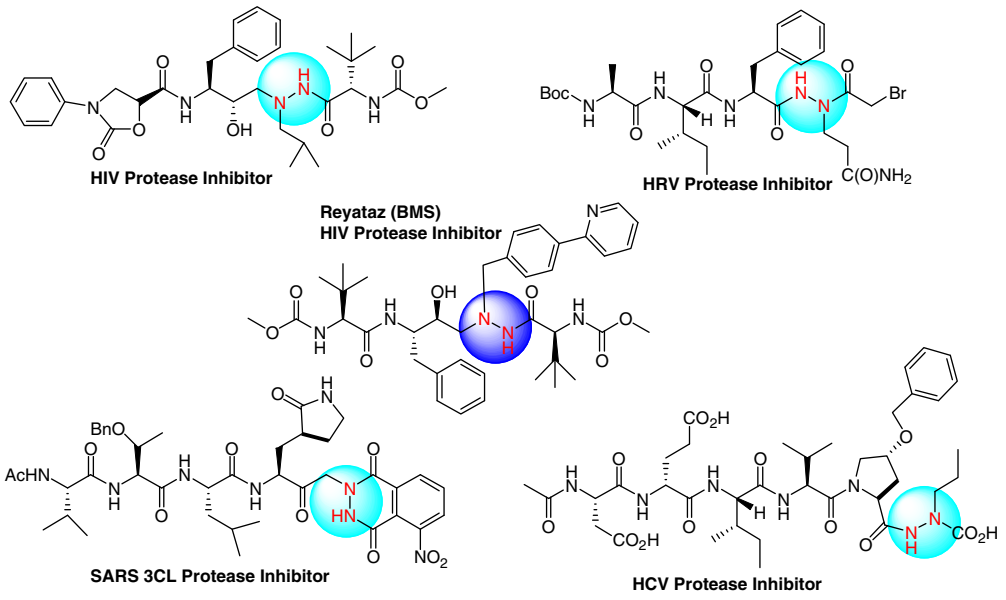


Figure 2. Selected examples of hydrazine bearing viral protease inhibitors.

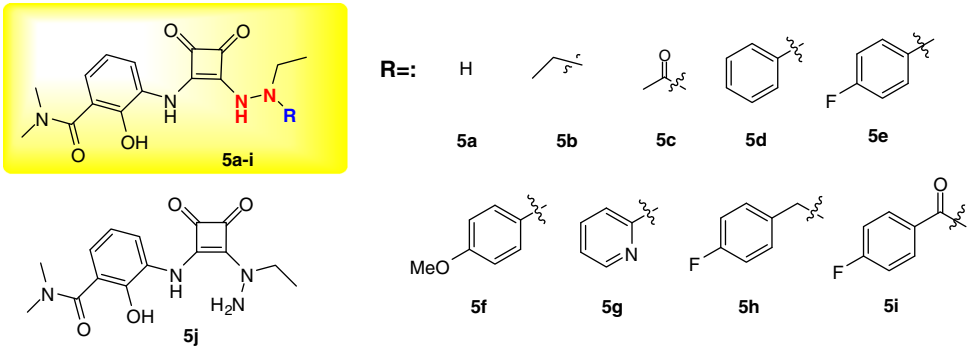


Figure 3. Detailed target list for series **5** antagonists.

substituted aniline **6**[8] with diethyl squarate **7** in EtOH afforded the expected adduct **8** (70%), which was converted to free hydrazine bearing derivative **9** (94%) upon treatment with hydrazine-hydrate. Compound **9** was further converted to the mono-*N*-ethyl analog **5a** via a reductive amination reaction in low yield. Subsequent N-alkylation of **5a** with para-fluorobenzyl bromide thus yielded **5h** in 30% yield.

As shown in Scheme 2, the synthesis of **5b** was accomplished in 65% yield via condensation of the intermediate **8** (see Scheme 1) with *N,N*-di-ethyl hydrazine **13**, which was in turn prepared from Boc-hydrazine via a two-step sequence consisting of N-alkylation and Boc-deprotection.

Scheme 3 shows the synthetic route utilized for the preparation of three *N*-aryl bearing analogs **5d**, **5e**, and **5f**. In this event, various

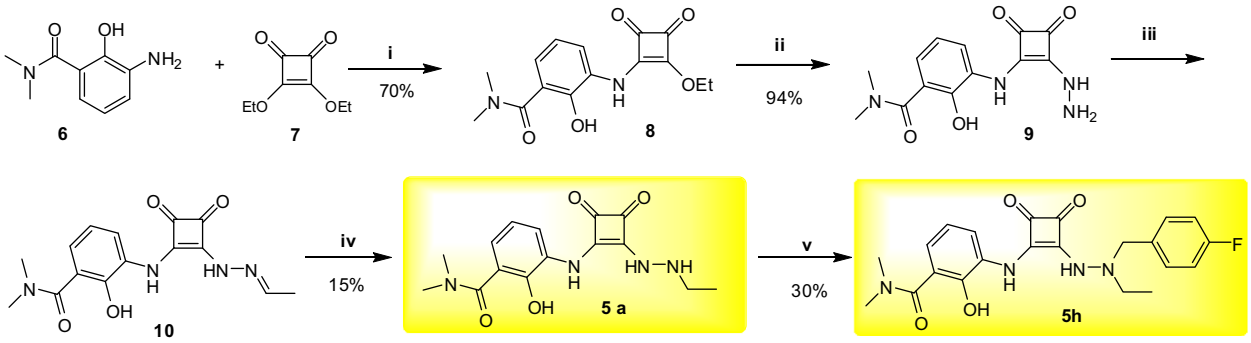
arylamines **15d–f** were firstly converted to their respective *N*-aryl-*N*-alkyl amines **16d–f** according to the protocol of Sajiki,[21] which were then transformed into their corresponding *N*-nitroso derivatives **17d–f** via an N-nitrosation reaction.[22] **17d–f** were further converted to the requisite *N*-aryl-*N*-ethyl hydrazines **18d–f** upon a Zn mediated reduction.[23] Treatment of the intermediate **8** with **18d–f** thus afforded the desired final products **5d–f** in moderate yields.

The synthesis of *N*-pyrido bearing analog **5g** was achieved as described in Scheme 4. Reaction of 2-fluoropyridine **19** with *N*-ethyl hydrazine afforded the desired adduct **20**, which was condensed with the already described intermediate **8** to yield the desired *N*-pyrido derivative **5g** in moderate yield.

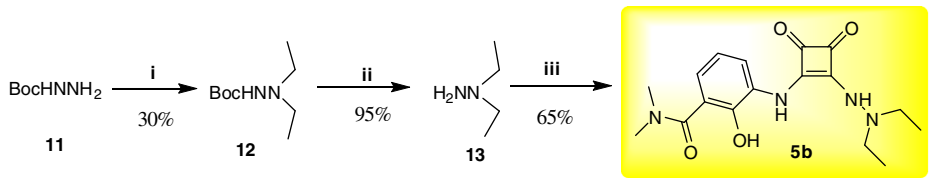
The preparation of two N-acylated analogs is depicted in Scheme 5. In this case, *N*-Boc hydrazine **21** was converted to its mono-alkylated product **23** via a reductive amination reaction through imine intermediate **22** in good yield. It is worthwhile to point out that direct base mediated N-alkylation (e.g., NaH/EtI) would produce N-di-alkylated compound as the main product. Subsequent treatment of **23** with either of two acyl chlorides provided **24c** (98%) and **24i** (80%), which were further reacted with HCl in ether to afford the *N*-ethyl-*N*-acyl hydrazines **25c** and **25i** in excellent yields. Final condensation of **25c** and **25i** with **8** afforded the desired analogs **5c** and **5i**, albeit in low yield.

As highlighted in Scheme 6, the synthesis of the mono-*N*-ethyl derivative **5j** began with coupling of two known intermediates, namely *N*-ethyl-*N*′-Boc hydrazine **23** and **8**. The expected product **26** was obtained in 70% yield, which was then treated with TFA to provide the desired analog **5j** in 85% yield.

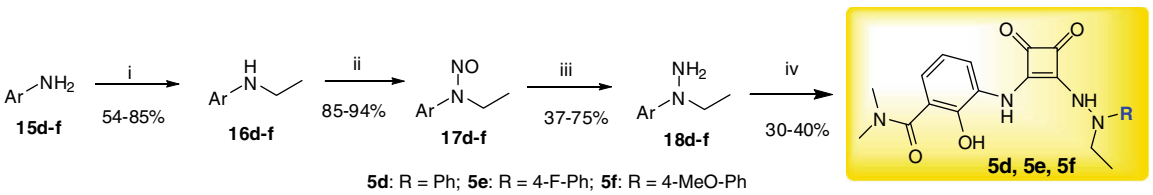
After the compounds synthesized, their biological activities were evaluated. The novel series of CXCR2 antagonists described herein (**5a–j**) were evaluated for their binding affinity against the CXCR2 receptor according to a literature protocol with minor modification.[8,24] Promising compounds emerging from this evaluation



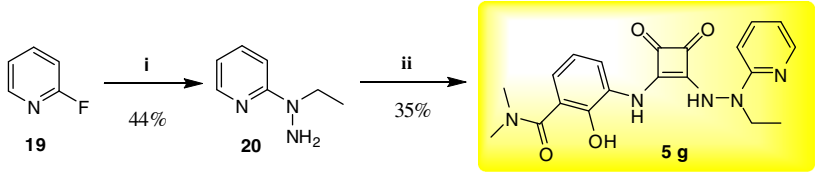
Scheme 1. Reagent and conditions: (i) EtOH, rt,12 h; (ii) EtOH, hydrzine hydrate, rt, 8 h; (iii) acetaldehyde, MeOH, rt 4 h; (iv)BH3·THF, MeOH, 0 °C to rt; (v) DMF, K_2CO_3, 4-fluorobenzyl bromide, rt, 3 h.



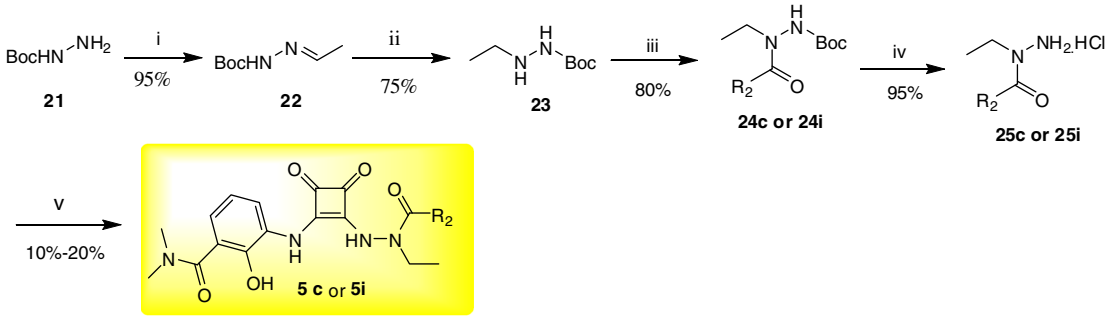
Scheme 2. Reagent and conditions: (i) $NaBH_3CN$, CH_3CHO, EtOH, rt, 5 h, (ii)HCl/Et_2O, rt, 2 h; (iii) compound **8**, EtOH, rt, 12 h.



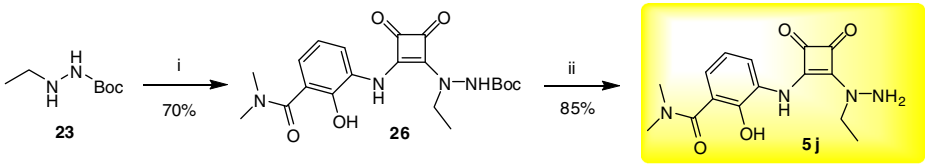
Scheme 3. Reagent and conditions: (i) MeCN, Pd/C, MeOH, rt 16 h; (ii) HCl, ice, $NaNO_2$ 0–5 °C; (iii) Zn, AcOH, 5–10 °C; (iv) compound **8**, EtOH, rt, 16 h.



Scheme 4. Reagent and conditions: (i) $EtNHNH_2$, DIEA, 150 °C, microwave 30 min.; (ii) compound **8**, EtOH, K_2CO_3, 40 °C, 12 h.



Scheme 5. Reagent and conditions: (i) CH_3CHO, toluene, 50 °C, 1 h; (ii) DIBAL-H, THF, −30 to 40 °C,4 h; (iii) AcCl or 4-fluorobenzoyl chloride, CH_2Cl_2, pyridine, 4 h; (iv)HCl/Et_2O, rt 1 h; (v) compound **8**, EtOH, K_2CO_3, rt, 12 h.



Scheme 6. Reagent and conditions: (i) compound **8**, EtOH, K_2CO_3, rt 12 h; (ii)TFA, CH_2CL_2, rt,4 h.

were further evaluated for selectivity against the CXCR1 receptor and for functional activity.[8] Benchmark compound **2b** was included as a positive control. The literature reported data on **2a** (SCH527123) is also included for comparison.[8] The testing results are highlighted in Table 1.

Benchmark compound performance: As shown in Table 1, the positive control **2b** showed CXCR2 inhibitory potency (IC_{50}) of 15 nM and 60-fold selectivity against CXCR1 according to Dwyer et al.[8] The same compound exhibited CXCR2 binding affinity (K_i) of 16 nM and 90-fold selectivity versus CXCR1 in our assays [MK]. It is worthwhile to point out that the furyl bearing version of **2b**, analog **2a** (SCH527123) was reported to be fourfold more potent (IC_{50} = 3.8 nM) against CXCR2 and yet less selective (sevenfold) against CXCR1 in comparison to **2b**.[8]

SAR trend observed in CXCR2 binding assay: When evaluated for CXCR2 binding affinity, the newly designed diethyl-hydrazine antagonist **5b** (a close analog of **2b** having one additional nitrogen in place of a carbon) exhibited K_i value of 120 nM. Replacement of one ethyl from **5b** with an acetyl moiety led to **5c**, which was found to be threefold less potent than **5b** (K_i = 320 nM) in the binding assay. When three closely related hydrazine bearing analogs were tested in the CXCR2 binding inhibition assay, **5b** was found to be 2- or threefold more potent than **5c** or **5a** (with one ethyl group replaced by a hydrogen), respectively.[24] It should be pointed out herein that **5j** shown in Scheme 6 (the *N*-ethyl isomer of **5a**) was found totally inactive in the binding assay. This SAR trend found within hydrazine series is in good agreement with the literature report on the Schering CXCR2 antagonist series **2a** and **2b**.[7–9]

On the other hand, incorporation of a series of ethylaryl hydrazine moieties into **5b** resulted in compounds **5d–g**. It is noted that **5d** and **5g** retained the similar binding affinity (K_i = 110–130 nM) for CXCR2 as that found with **5b** (K_i = 120 nM). Furthermore, since the pyridyl moiety presented in **5g** should promote aqueous solubility relative to the phenyl bearing antagonist **5d**, thus the physical chemical properties (e.g., solubility, membrane permeability, and even tissue distribution, etc.) of **5d** and **5g** could be quite different, which brings in added advantage of this series compound.

Further inspection of the binding data obtained for **5e** and **5f** reveals that whilst installment of an electron donating group (OMe for **5f**) on the phenyl ring of **5d** had minimal effect on CXCR2 binding (K_i = 180 nM), addition of an electron withdrawing group (F for **5e**) on the same phenyl ring in **5d** resulted in fourfold reduction in CXCR2 binding affinity (K_i = 540 nM). Comparative evaluation of CXCR2 binding affinity of *p*-F-Ph bearing analog **5e** (K_i = 540 nM) and its *p*-F-Bn counterpart **5h** (K_i = 7.2 μM) indicated a sharp 13-fold drop in binding potency for the latter. Surprisingly, replacement of the benzyl linker in **5h** with its corresponding benzoyl moiety led to **5i**, which displayed close to 30-fold enhanced binding potency (K_i = 260 nM) relative to **5h**. Taken together all of the SAR data obtained with the ethylaryl hydrazine series compounds, compounds **5d** and **5g** were the most promising CXCR2 antagonists synthesized thus far (Table 1).

SAR trend observed in CXCR-1 selectivity assay: Encouraged by their CXCR2 binding affinities, promising novel hydrazine bearing antagonists **5b** and **5d–g** were tested in a CXCR1 binding assay. To our satisfaction, the diethyl hydrazine analog **5b** demonstrated 80-fold selectivity against CXCR1. This level of selectivity is quite comparable to that exhibited by **2b** (60–90-fold) (Table 1).

As also shown in Table 1, when tested in the CXCR1 selectivity assay, a series of ethylaryl hydrazine analogs **5d**, **5f**, and **5g** showed about 40-fold selectivity. In addition, whilst *p*-F-Ph analog **5e** showed >20-fold selectivity, its related analog **5i** exhibited 2x improved selectivity (>46-fold).

Table 1
Effects of **5a–i** on CXCR2 and CXCR-1 binding inhibition and CXCR2 in CHO cells

Compd	CXCR-2 K_i/[IC_{50}] (μM)	CXCR-1 K_i/[IC_{50}] (μM)	CXCR-2 [6] functional IC_{50} (μM)
2a	[0.0038] Ref. 8	[0.026] Ref. 8	//
2b	*[0.015] Ref. 8*	*[0.91] Ref. 8*	//
	0.016	*1.5*	*0.012 (n = 5)*
5a	[5.49] Ref. 24	//	//
5b	0.12 (*n* = 4)	9.7 (*n* = 3)	0.046 (*n* = 5)
	[1.86] Ref. 24	//	//
5c	[3.29] Ref. 24	//	//
	0.32 (*n* = 4)	>12 (*n* = 3)	1.25 (*n* = 3)
5d	0.11 (*n* = 3)	4.1 (*n* = 3)	0.054 (*n* = 5)
5e	0.55 (*n* = 3)	>12 (*n* = 3)	0.84 (*n* = 4)
5f	0.18 (*n* = 3)	6.5 (*n* = 3)	0.10 (*n* = 5)
5g	0.13 (*n* = 3)	5.2 (*n* = 3)	0.075 (*n* = 5)
5h	7.2 (*n* = 3)	>12 (*n* = 3)	6.34 (*n* = 5)
5i	0.26 (*n* = 3)	>12 (*n* = 3)	0.84 (*n* = 4)

SAR trend observed in functional assay: When tested in a human neutrophil chemotaxis assay, compound **5b** exhibited functional inhibitory activity with an IC_{50} value of 46 nM. The *N*-ethyl-*N*-Ac incorporated analog of **5c** showed much weaker potency with an IC_{50} value close to 1.25 μM. The positive control **2b** demonstrated the good potency with IC_{50} value of 15 nM.

To our satisfaction, three ethylaryl hydrazine bearing analogs **5d**, **5f**, and **5g** also exhibited impressive potencies (IC_{50}) ranging from 54 to 100 nM, which are almost equal potent to that demonstrated by **5b**. It is also worthwhile to mention that the functional activities detected with these *N*-ethylaryl hydrazine analogs tracks well with the receptor binding potencies obtained (Table 1).

In accordance with the reduced CXCR2 binding affinities, compounds **5e** and **5i** exhibited about 10-fold weaker functional activity than **5g** or **5f** with IC_{50} value of 840 nM. The least potent analog 5 h displayed weakest functional activity (IC_{50} = 6.34 μM).

In vitro microsomal stability evaluation—in light of the demonstrated promising binding affinity for the CXCR2 receptor and good potency in functional assay, three newly designed hydrazine containing antagonists **5b**, **5d**, and **5g** along with two positive controls **2a** and **2b** were tested for metabolic stability upon incubation with human liver microsome at 37 °C for 30 min (with 1 μM final concentration for each compound) according to the protocol reported by Merritt et al.[7] All test compounds including **5b**, **5d**, and **5g** exhibited good stability with >50% of drug remaining after incubation at 37 °C for 120 min ($T_{1/2}$ >2 h). Furthermore, compound **5b** showed good stability against rat microsome with a $T_{1/2}$ value of 84 min.

In this communication, we have reported our preliminary data on the discovery of a novel series of 3-amino-4-hydrazine-cyclobut-3-ene-1,2-dione containing CXCR2 antagonists including the bis-*N*-ethyl bearing analog **5b**. This compound was shown to possess potent CXCR2 binding affinity (K_i = 120 nM), adequate CXCR1 selectivity (80-fold), functional activity (IC_{50} = 46 nM) against IL-8-mediated chemotaxis in a Chinese hamster ovary (CHO) cell line (CXCR2 expressing line) as well as acceptable rat and human microsomal stability. In addition, replacement of the bis-*N*-ethyl moiety in **5b** with ***N***-ethyl-*N*-aryl hydrazines led to **5d** and **5g**, each of which displayed good CXCR2 binding affinity (K_i = 110 or 130 nM) and acceptable human microsomal stability ($T_{1/2}$ >120 min). It is conceivable that further modification of either bis-*N*-alkyl or *N*-alkyl-*N*-aryl hydrazine moieties could yield more potent and selective CXCR2 antagonists. The results of this research will be reported in due time.

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24. WX's Binding assay conditions:*Reagent*: wheatgerm-agglutinin coated SPA beads and [^{125}I]IL-8 were from GE Healthcare (Piscataway, NJ), receptor membranes CXCR2 and CXCR1 were from Chemicon (Temecula, CA). HEPES was from Sigma (St Louis, MO). $MgCl_2$ and KOH were from SCRC (Shanghai, China). DMSO was from Sigma–Aldrich (Steinheim, Germany).*Method*: for each 200 μL reaction, a working mixture of receptor over-expressing membranes (0.020 μg/μL CXCR2 or 0.040 μg/μL CXCR1) and 2 mg/mL wheatgerm-agglutinin coated SPA beads was prepared in assay buffer. The assay buffer was 25 mM HEPES, 3 mM MgCl2, pH 7.4. This mixture was incubated on ice for 5 min. A 0.040 nM 125I labeled IL-8 stock solution was prepared in the assay buffer. Test compounds were serially diluted by half-log concentration in DMSO. The above solutions were added to a 96 well plate (Perking Elmer) in the following sequence: 45 μL assay buffer and 5 μL test compound or DMSO, 100 μL of membranes and SPA bead mixture and 50 μL [125I]IL-8 solution stock solution. The assay plates were incubated for 2 h at room temperature, keeping from light. Binding was detected using a Perking Elmer-Wallace Microbeta 1450 liquid scintillation counter. The data was analyzed using SigmaPlot (Systat Software Inc., San Jose, CA).