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Synthesis and structure–activity relationships of new disubstituted phenyl-containing 3,4-diamino-3-cyclobutene-1,2-diones as CXCR2 receptor antagonists

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Abstract—A series of 3,4- and 3,5-disubstituted phenyl-containing cyclobutenedione analogues were synthesized and evaluated as CXCR2 receptor antagonists. Variations in the disubstitution pattern of the phenyl ring afforded new compounds with potent CXCR2 binding affinity in the low nanomolar ranges. Moreover, two potent compounds **19** and **26** exhibited good oral pharmacokinetic profiles.

The chemokine receptor CXCR2, a seven-transmembrane G-protein-coupled receptor, was cloned and identified in the early 1990s.[1] Its natural ligands, interleukin-8 (IL-8), granulocyte chemotactic protein-2 (GCP-2), and other related CXC chemokines, bind with it to exert a number of pathophysiological effects such as attraction and accumulation of neutrophils toward the sites of inflammation.[2] CXCR2 mouse gene knockout studies show that there are elevated leukocytes and lymphocytes without apparent pathogenic consequences, indicating that CXCR2 is not required for normal physiology.[3] Increased levels of CXCR2 and its ligand IL-8 have been observed in humans with diseases such as arthritis, asthma, and chronic obstructive pulmonary disease (COPD).[4] This suggests that the CXCR2 receptor and IL-8 may play a pivotal role in these inflammatory disorders. Therefore, antagonists of CXCR2 receptor could be in principle used in the treatment of inflammatory and related diseases.

CXCR2 antagonists have indeed attracted much attention as targets for small-molecule drug discovery in the last decade.[5] Several structural classes have been identified to be potent inhibitors of the CXCR2 receptor (Fig. 1), including quinoxaline **1**,[6] triazolethiol **2**,[7] *N*,*N*′-diarylureas **3** and **4**,[8] cyanoguanidine **5**,[9] imidazolylpyrimidine **6**,[10] and diaminocyclobutenedione **7**.[11] Among these, CXCR2 antagonists **3** and **4**[8a] disclosed by GSK and antagonist **7**[11a] identified through our joint research program have been progressed into the clinical trials for COPD.

During the course of lead optimization leading to the discovery of compound **7**, it was observed that replacement of the 5-methylfuryl group in **7** with phenyl and 3-fluorophenyl in cyclobutenediones (**8** and **9**)[11a] also showed quite potent CXCR2 receptor affinity in the nanomolar ranges (Fig. 2). Based upon this observation, we decided to explore the impact of disubstitution of the phenyl ring on CXCR2 receptor binding and pharmacokinetic properties in hopes of finding a non-furanyl candidate with development potential. Herein we report the synthesis and preliminary structure–activity relationships of a series of 3,4- and 3,5-disubstituted phenyl-containing cyclobutenediones (**10**–**29**) as novel CXCR2 receptor antagonists.

Scheme 1 shows the synthesis of target compounds **26** and **27** from commercially available 3-bromo-5-fluorotoluene (**30**). Thus, lithiation of **30** with *n*-BuLi in

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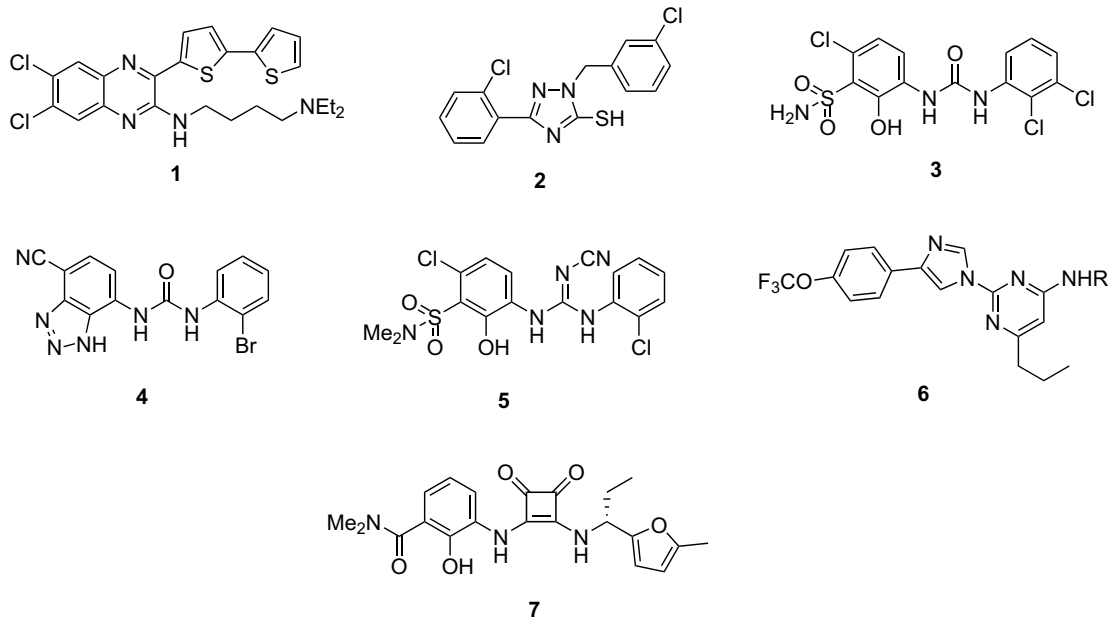


Figure 1. CXCR2 receptor antagonists.

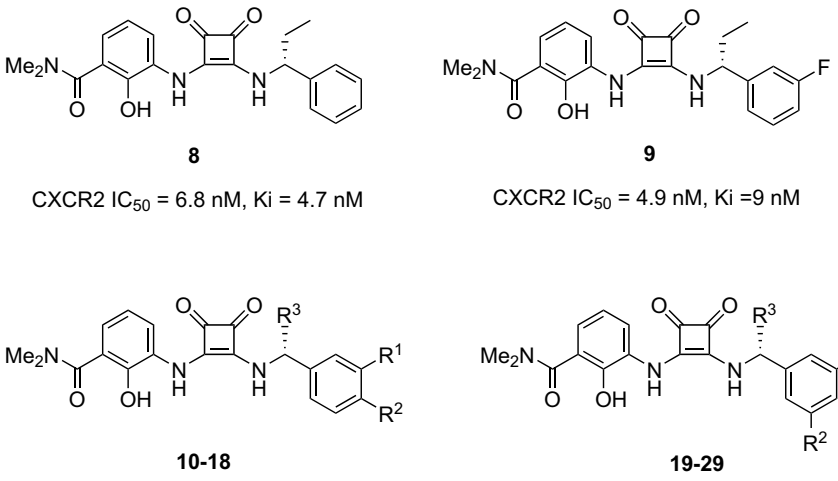
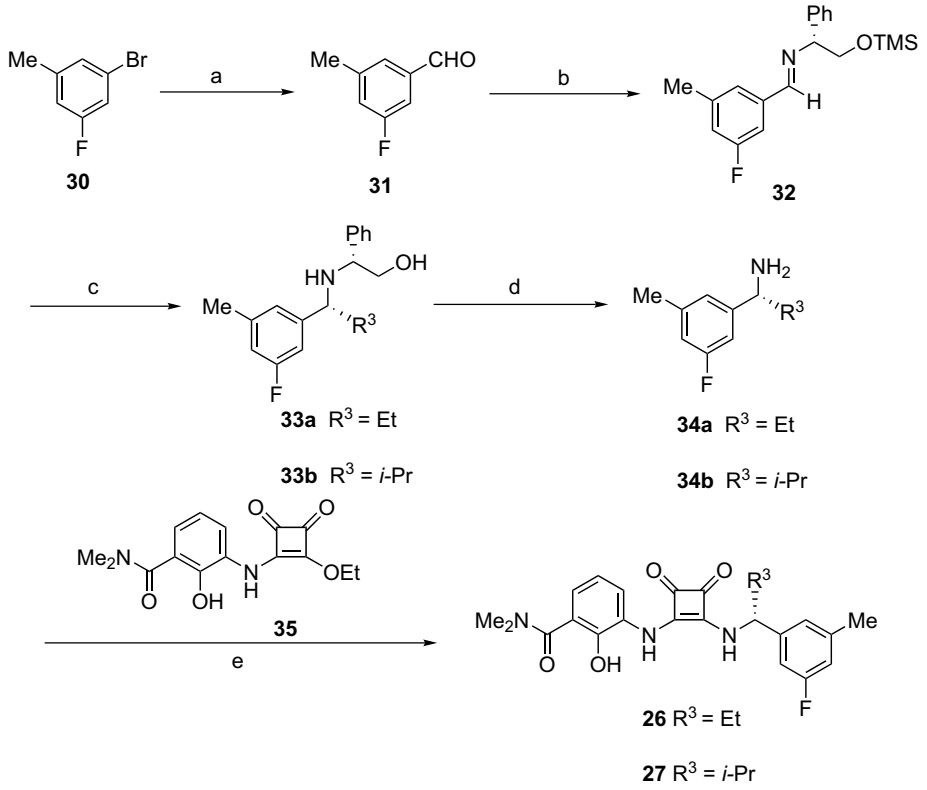


Figure 2. Cyclobutenedione CXCR2 receptor antagonists.

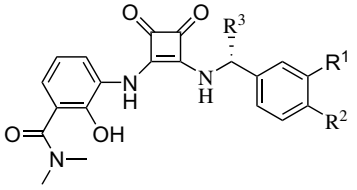
THF, followed by addition of DMF, readily furnished the desired aldehyde **31** in 66% yield. Treatment of **31** with (*R*)-(−)-2-phenylglycinol in the presence of $MgSO_4$ in THF and subsequent silylation with TMSCl gave rise to the protected imine **32**. Diastereoselective addition of ethylmagnesium chloride or isopropylmagnesium chloride to **32** in THF and desilylation of the corresponding adducts with 2.5 M H_2SO_4 solution provided the desired amino alcohols **33a** or **33b**.[12,13] Oxidative cleavage of **33a** and **33b** was accomplished with periodic acid in the presence of $MeNH_2$ in aqueous methanol to give the chiral amines **34a** and **34b**, respectively. Finally, reaction of **34a** and **34b** with the previously reported cyclobutenedione intermediate **35**[11a] afforded the target compounds **26** and **27**. Compounds **10**–**25** disclosed in Tables 1 and 2 were synthesized from the corresponding bromides or aldehydes in a similar manner as described in Scheme 1.

The target compound **28**, having a 3-cyano-5-methylphenyl moiety, was synthesized as outlined in Scheme 2. Sequential monolithiation of 3,5-dibromotoluene (**36**) with *t*-BuLi and formylation with DMF, followed by protection, provided the acetal **37**, which was subjected to another lithiation and treatment with DMF to give the monoprotected dialdehyde **38**. Reaction of **38** with (*R*)-(−)-2-phenylglycinol and subsequent silylation smoothly afforded the intermediate **39**. Diastereoselective nucleophilic addition of ethylmagnesium chloride to **39** was followed by removal of both the acetal and the silyl groups in the resulting adduct with 2.5 M H_2SO_4 solution to furnish the desired amino alcohol **40**.[12,13] Direct conversion of the formyl group in **40** to the cyano group was accomplished using ammonia and MnO_2 in the presence of $MgSO_4$ in THF[14] to give the desired nitrile **41** in 83% yield. Oxidative cleavage of **41** yielded the chiral amine **42**, which was reacted with the intermediate



Scheme 1. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C; then DMF; (b) (*R*)-(−)-2-phenylglycinol, $MgSO_4$, THF; then TMSCl, Et_3N, CH_2C1_2. (c) EtMgCl, THF, −20 °C, or *i*-PrMgCl, THF, −20 °C; then 2.5 M H_2SO_4; (d) H_5IO_6, $MeNH_2$, MeOH, H_2O, rt; (e) MeOH, DIEA, 65 °C, overnight.

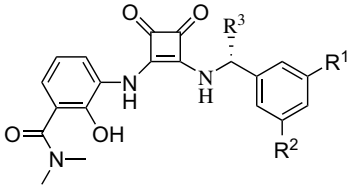
Table 1. CXCR2 binding data of 3,4-disubstitutedphenyl analogues



Compound	R^1	R^2	R^3	K_i[a] (nM)	Rat AUC[b] (μM h)
10	F	F	Et	28[c]	NT
11	F	OMe	Et	5.2	6.37
12	F	OMe	*i*-Pr	1.5	0.3
13	F	CF_3	Et	19.4	NT
14	F	CF_3	*i*-Pr	13.9	NT
15	Me	F	Et	6.1[c]	1.1
16	OMe	F	Et	11.9	NT
17	OMe	F	*i*-Pr	16.0	NT
18	CF_3	F	Et	38.5	NT

[a] Receptor binding was conducted as described in Ref. 16. Data are means of at least two independent determinations.
[b] Data were generated based on a 6-h study, po dosing (10 mg/kg), $n = 2$. NT, not tested.
[c] The tested compounds were racemic.

Table 2. CXCR2 binding data of 3,5-disubstituted phenyl analogues

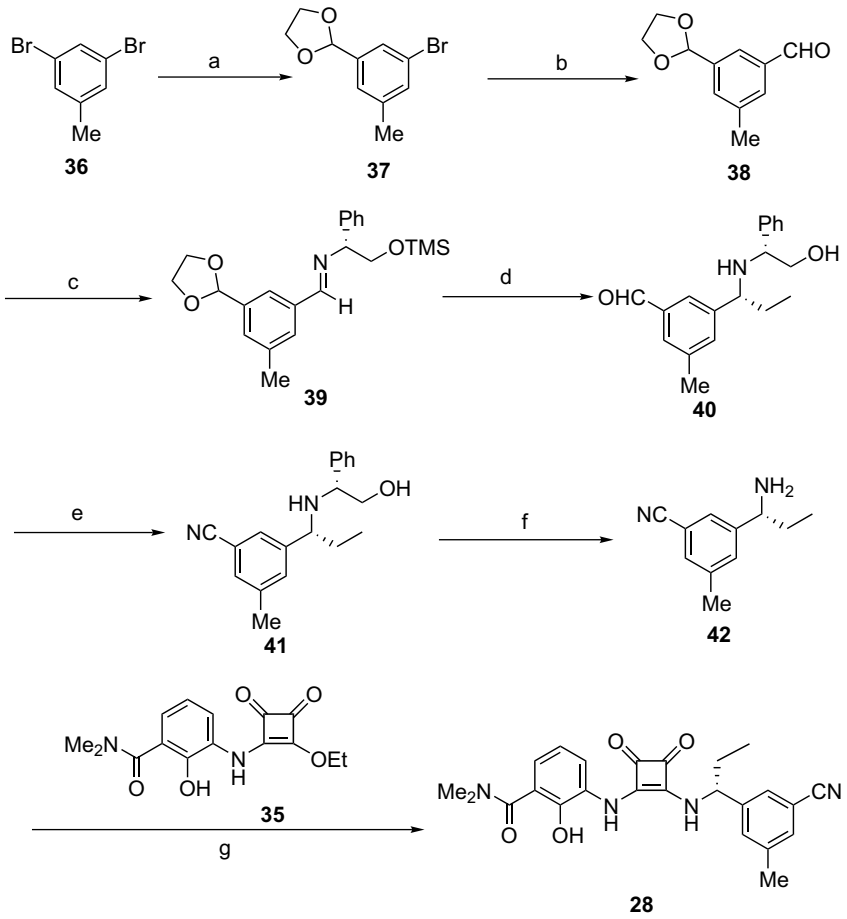


Compound	R^1	R^2	R^3	K_i[a] (nM)	Rat AUC[b] (μM h)
19	F	F	Et	1.7	18.52
20	F	F	*i*-Pr	1.9	1.28
21	F	F	cy-Pr	4.5	NT
22	F	CF_3	Et	7.9	NT
23	F	CF_3	*i*-Pr	13.3	NT
24	Me	CF_3	Et	11.8	NT
25	Me	CF_3	*i*-Pr	4.4	NT
26	F	Me	Et	2.8	3.52
27	F	Me	*i*-Pr	1.0	2.29
28	Me	CN	Et	1.9	NT
29	F	CN	Et	1.7	NT

[a] Receptor binding was conducted as described in Ref. 16. Data are means of at least two independent determinations.
[b] Data were generated based on a 6-h study, po dosing (10 mg/kg), $n = 2$. NT, not tested.

35[11a] to provide the target compound **28**. Compound **29** was synthesized in a similar fashion from 3-bromo-5-fluorobenzaldehyde.[15]

The CXCR2 binding activity was determined using a Ba/F3-hCXCR2 overexpressing membrane binding assay.[16] We first examined 3,4-disubstituted phenyl-containing cyclobutenedione derivatives and the results are summarized in Table 1. Attachment of the second fluorine atom at the C4 position of the phenyl ring (**10**) led to less binding potency as compared to compound **9**. Addition of methoxyl group at the C4 position moderately improved CXCR2 binding affinity (**11** and



Scheme 2. Reagents and conditions: (a) (i) *t*-BuLi, THF, −78 °C; then DMF; (ii) ethylene glycol, *p*-TsOH H_2O, C_6H_6, reflux; (b) *t*-BuLi, THF, −78 °C; then DMF; (c) (*R*)-(−)-2-phenylglycinol, $MgSO_4$, THF; then TMSCl, Et_3N, CH_2Cl_2; (d) EtMgCl, THF, −20 °C; then 2.5 M H_2SO_4; (e) $MgSO_4$, MnO_2, 2 M NH_3 in *i*-PrOH, THF; (f) H_5IO_6, $MeNH_2$, MeOH, H_2O, rt; (g) MeOH, DIEA, 65 °C, overnight.

12), whereas addition of the trifluoromethyl group at that position led to a threefold loss in CXCR2 affinity (**13** and **14**). When fluorine was switched to the C4 position of the phenyl ring, attaching methyl at the C3 position slightly improved CXCR2 affinity (**15**), while introduction of methoxyl or trifluoromethyl substituent at that position (**16**–**18**) markedly decreased the binding potency. The 3,4-disubstitution pattern did not provide a significant affinity increase from data presented in Table 1.

Next, we turned to assess the effect of 3,5-disubstitution of the phenyl ring on CXCR2 receptor binding activity. As shown in Table 2, the 3,5-difluorophenyl group-containing compound **19** displayed high binding affinity. Replacement of the ethyl group at the benzylic site of the right-side amine in **19** with isopropyl or cyclopropyl did not improve potency (**20** and **21**). Analogues **26** and **27** with a 3-fluoro-5-methylphenyl moiety showed excellent affinity. However, introduction of the trifluoromethyl group (**22** to **25**) decreased CXCR2 binding activity. Furthermore, the incorporation of the cyano and methyl groups or the cyano group and fluorine at the C3 and C5 positions of the phenyl ring (**28** and **29**) yielded potent affinity for CXCR2 receptor.

Pharmacokinetic studies have been conducted with selected compounds. As shown in Table 1, compound **12** displayed much lower oral exposure (AUC, 0.3 μM h) in rapid rat PK tests than the clinical trial compound **7** (AUC, 49.0 μM h).[11a] Table 3 lists some PK data for compounds **19**, **26**, and **7**. Both new compounds **19**

Table 3. Pharmacokinetic data of compounds **19**, **26** and **7**

Parameter	19		26		7	
	Rat	Monkey	Rat	Monkey	Rat	Monkey
Dose, po (mg/kg)	10	3	10	3	10	3
Oral bioavailability (%)	19.1		34.1		33	18
$t_{1/2}$ (h)	13		5.5		7.8	23.3
Mean residence time (h)	3.8		1.8		3.4	2
Oral AUC (0–24 h) (μM h)	8.8	3.1	18.1	5.6	22	2

and **26** exhibited comparable or better oral bioavailability and exposure (AUC) in full rat and rapid monkey PK tests as compared to **7**.

In summary, a series of 3,4- and 3,5-disubstituted phenyl-containing cyclobutenedione analogues have been synthesized and evaluated as CXCR2 receptor antagonists. Several new compounds have been identified to possess high CXCR2 binding affinity with the low nanomolar ranges. Two potent compounds **19** and **26** exhibited good oral pharmacokinetic profiles. Their further evaluation in animal pulmonary studies and other tests will be reported in due course.

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