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Discovery of a potent, selective and orally bioavailable 3,9-diazaspiro[5.5]undeca-2-one CCR5 antagonist

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ABSTRACT

Replacement of the cyclic carbamate in our previously disclosed 1-oxa-3,9-diazaspiro[5.5]undecan-2-one template led to the discovery of two novel series of 3,9-diazaspiro[5.5]undecane and undeca-2-one CCR5 antagonists. The synthesis, SAR, and antiviral activities of these two series are described. One compound (**32**) was found to have attractive combination of antiviral potency, selectivity, and pharmacokinetic profile. The asymmetric synthesis of **32** was also accomplished and both enantiomers were equally potent.

The development of new agents for the treatment of HIV-1/AIDS remains a necessity, particularly due to the ongoing problem of resistance development to current therapies.[1] Viral entry inhibitors are a validated target for controlling HIV-1 infection, particularly blockade of the CCR5 receptor, which serves as one of the major viral binding sites.[2,3] Efforts at Roche have led to the discovery of a series of potent CCR5 antagonists represented by structure **1,** featuring a 1-oxa-3,9-diazaspiro[5.5]undecan-2-one template (Fig. 1).[4] In a continuation of our work, two new templates were proposed. Inspired by this proposal, we developed a convenient synthesis of 9-benzyl-1-butyl-3,9-diazaspiro[5.5]undecane **4** and undecan-2-one **5** via Michael addition of the lithium enolate **2** to the tetrasubstituted olefin acceptor **3** (Scheme 1).[5] Herein, we report novel and potent CCR5 antagonists featuring these new templates.

Two routes were developed for the synthesis of 3,9-diazaspiro[5.5]undecane **4**-based analogs (Scheme 2). Coupling of **4** with tetrahydropyran-4-carboxylic acid gave amide **6**, which was then debenzylated and subjected to a Strecker reaction promoted by $Ti(O\text{-}iPr)_4$ to give nitrile **7**. Treatment of **7** with excess methyl magnesium bromide (5–8 equiv) at room temperature led to the expected displacement of the cyanide by methyl, as well as an unexpected cleavage of the amide bond to yield amine **8**. Reaction of **8** with a variety of sulfonyl, sulfamyl, and acid chlorides afforded intermediate **9**, which was converted to analogs **10**–**23** (Tables 1 and 2) via Boc deprotection and coupling. Alternatively, starting from chiral amine **24,** Boc protection, debenzylation and Strecker reaction of **25** with *N*-Cbz-4-piperidone gave versatile intermediate **26**, which was further converted to analogs **27** and **28** (Tables 1 and 2).

Synthesis of 5-butyl-3,9-diazaspiro[5.5]undeca-2-one **5**-based analogs is outlined in Scheme 3. Alkylation of **5** with alkyl bromide or tosylate was achieved with NaH and DMF at elevated temperature (100 °C). However, large excesses of bromide and tosylate (>5 equiv) were needed to achieve reasonable conversion. To our

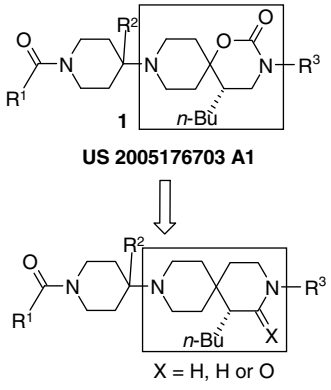

Figure 1.

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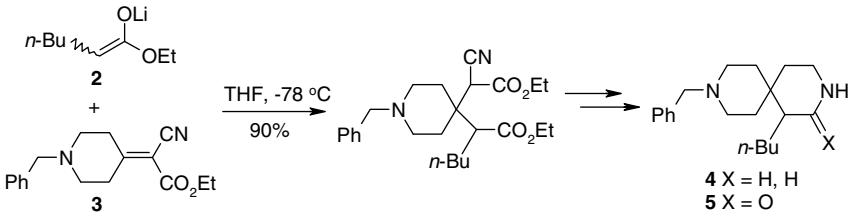
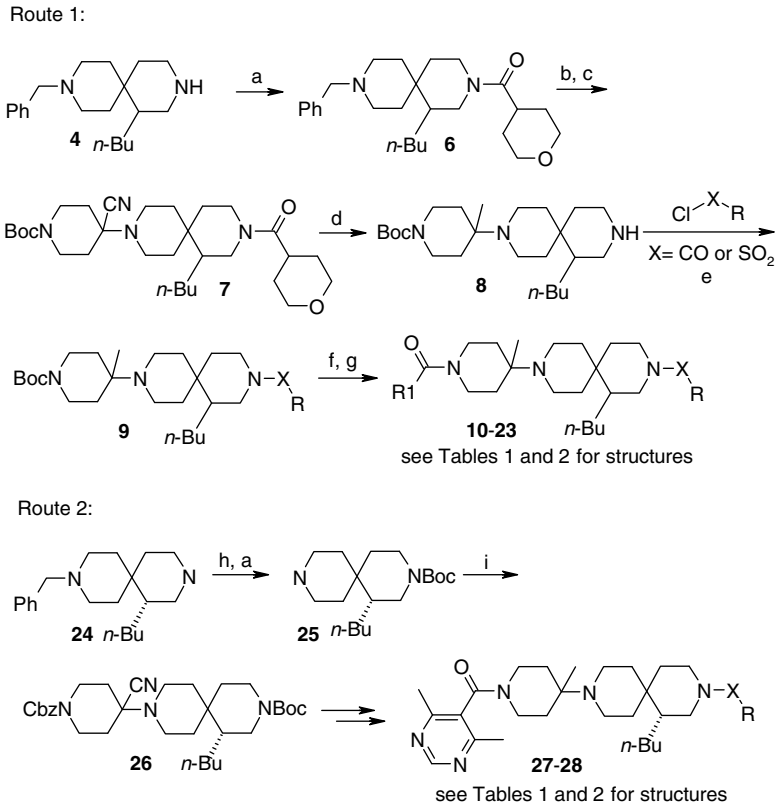


Scheme 1.



Scheme 2. Reagents and conditions: (a) EDCI, HOBT, DIPEA, tetrahydropyran-4-carboxylic acid, DCM, rt, 71%; (b) Pd/C, EtOH, H_2 (60 psi); (c) *N*-Boc-4-piperidone, Ti(O-*i*Pr)$_4$, DCE; then Et_2AlCN; (d) CH_3MgBr, THF, rt, 58% over three steps; (e) triethyl amine, DCM; (f) TFA, DCM, rt; (g) EDCI, HOBT, DIPEA, acid, DCM, rt; (h) Boc_2O, iPr_2EtN, DCM; (i) *N*-Cbz-4-piperidone, Ti(O-*i*Pr)$_4$, DCE; then Et_2AlCN.

delight, under phase transfer conditions (solid NaOH, K_2CO_3, tetrabutylammonium bromide (TBAB), 2 equiv of alkyl bromide or tosylate, toluene, 90 °C), the alkylation occurred smoothly to give intermediate **29**, which was transformed to analogs **30**–**37** (Table 3) following procedures outlined in Scheme 2.

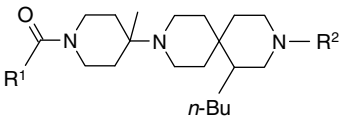
The requisite alkyl tosylates or bromides that were utilized in Scheme 2 were available commercially or prepared according to Scheme 3. Reduction of 4-methoxycyclohexyl carboxylic acid with $NaBH_4$ and BF_3 etherate in THF gave alcohol **38**, which was tosylated to give isomers **39** and **40**. Separation of **39** and **40** was achieved by flash column chromatography on silica gel (Scheme 4).

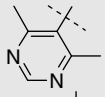
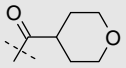
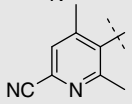
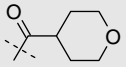
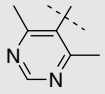
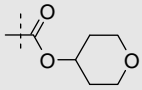
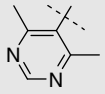
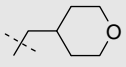
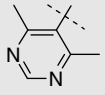
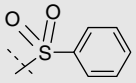
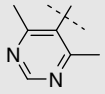
The synthesized compounds were screened for CCR5 antagonism activity by using radiolabelled RANTES binding assay and CCR5-mediated antiviral activities employing a cell–cell fusion (CCF) assay.[6,7] Our initial structure–activity relationship (SAR) studies on the 1-oxa-3,9-diazaspiro[5.5]undecan-2-one series suggested that a hindered amide (R^1, Table 1) with restricted rotation was optimal for antiviral activity, therefore, only a few selected R^1 groups were employed throughout our study and emphasis was placed on finding the optimal R^2 group.

For the 3,9-diazaspiro[5.5]undecane **4**-based analogs, the choice of types of R^2 group was important for antiviral activity (Table 1). For example, compound **10** with an amide on the right-hand nitrogen was inactive. Replacing the left-hand 4,6-dimethyl-5-pyrimidinecarboxyamide in **10** with 6-cyano-2,4-dimethyl-3-pyridinecarboxyamide in **11** reinstalled some of the activity ($CCFIC_{50}$ = 170 nM). In addition, carbamate **12** and amine **13** were also inactive. On the other hand, benzenesulfonamide **14** and urea **27** were quite potent ($CCFIC_{50}$ < 1 nM and =4 nM, respectively). While both **14** and **27** had promising potency in CCF, screening in our definitive antiviral assay[7] showed **14** to have superior potency ($CCFIC_{50}$s: 1.6 nM for **14** and 53 nM for **27**).

Further profiling of **14** identified its weakness—high clearance in in vitro human liver microsomal assay (Table 2). Replacement of the phenyl group with 2-pyridyl (compound **15**) did improve the microsomal stability without much loss in potency ($CCFIC_{50}$ = 4 nM). However, the clearance (310 μl/min/mg) was still considered to be high. The methyl sulfonamide **16** was stable, but unfortunately, a 100-fold loss in potency was observed compared to **14**. A bulkier cyclopropyl group (compound **17**) increased po-

Table 1
RANTES binding and cell–cell fusion activity of diazaspiroundecane analogs.



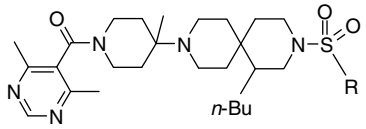
Compound	R^1	R^2	IC_{50} (nM)	
			RANTES	CCF[a]
10			>500	>5000
11			217	170
12			349	>5000
13			>500	>5000
14			19	<1
27[b]			35	4

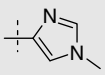
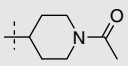
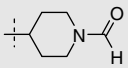
[a] Values are means of two experiments.
[b] (*R*)-Enantiomer.

tency ($CCFIC_{50}$ = 7 nM), however, at the expense of microsomal stability. Saturated heterocycles, such as 4-substituted tetrahydropyran (compound **19**), morpholine (compound **21**), and 4-substituted piperidines (compounds **22** and **23**), are tolerated. To our disappointment, none of those analogs provided desirable combination of potency and microsomal stability.

Parallel investigation of 3,9-diazaspiro[5.5]undeca-2-one series proved to be quite successful (Table 3). Compound **30** retained CCR5 antiviral potency comparable to its 1-oxa-3,9-diazaspiro[5.5]undecan-2-one counterpart (structure **1**, Fig. 1). This confirms our earlier hypothesis that the cyclic carbamate moiety in **1** served as a linker to place all the substituents at the proper orientation. Replacement of the 4-tetrahydropyran with a *para*-fluorophenyl (compound **31**) led to a substantial loss of potency ($CCFIC_{50}$ = 100 nM). On the other hand, the tetrahydropyran moiety can be replaced by a cyclohexyl group with 1,4-trans substitution (compounds **32**–**34**). Both ethers (methyl and ethyl) and the polar hydroxyl group are tolerated. In addition, the tetrahydropyran can also be replaced with 4-substituted piperidine (compound **35**) with comparable activity. However, the difluoroethyl piperidine analog (compound **36**) led to reduced activity.

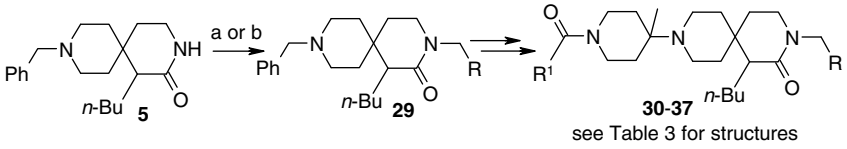
Table 2
RANTES binding, cell–cell fusion activity and HLM of diazaspiroundecane sulfonamide series.



Compound	R	IC_{50} (nM)		HLM[b] (μl/min/mg)
		RANTES	CCF[a]	
14		19	<1	773
15		18	4	310
16		25	100	7
17		22	7	100
18		60	7	207
19		22	6	280
20		27	7	96
21		11	1	276
22		78	10	70
23		64	14	60
28[c]		19	3	53

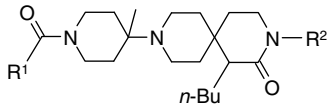
[a] Values are means of two experiments.
[b] Human liver microsomal clearance.
[c] (*R*)-Enantiomer.

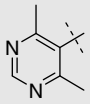
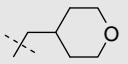
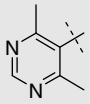
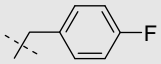
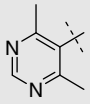
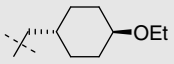
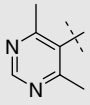
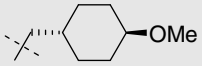
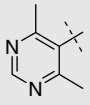
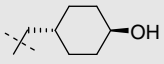
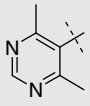
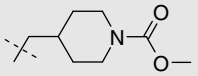
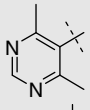
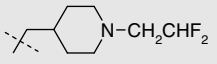
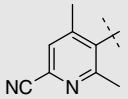
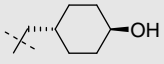
We also investigated modification of the butyl side chain (Table 4). Replacement of the butyl with either propoxy (compound **41**) or phenyl (compound **42**) led to a substantial loss of activity. This is in agreement with our CCR5 homology model where the butyl group binds in a narrow hydrophobic pocket within CCR5 receptor.[8]



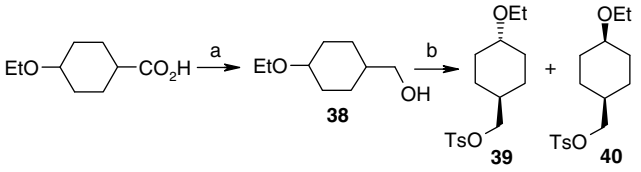
Scheme 3. Reagents and conditions: (a) NaH, alkyl bromide or tosylate, DMF 100 °C; (b) solid NaOH, TBAB, K_2CO_3, toluene, 90 °C.

Table 3
RANTES binding and cell–cell fusion activity of diazaspiroundecane series.



Compound	R^1	R^2	IC_{50} (nM)	
			RANTES	CCF[a]
30			30	4
31			19	100
32			30	5
33			37	6
34			76	10
35			60	8
36			70	26
37			31	<2.5

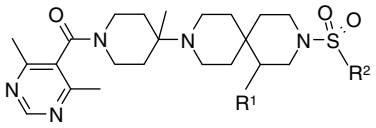
[a] Values are means of two experiments.

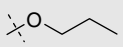


Scheme 4. Reagents and conditions: (a) $NaBH_4$, BF_3 etherate, THF, reflux, 90%; (b) *p*-toluenesulfonyl chloride, pyridine, rt, 7% of **39** and 7% of **40**.

Several compounds from the 3,9-diazaspiro[5.5]udecan-2-one series were selected for pharmacokinetic evaluation in rat. Among them, **32** displayed attractive pharmacokinetic properties as shown in Table 5. It has 66% bioavailability and an half life of 4.19 h (PO), which compared favorably with other clinically advanced CCR5 antagonists.[9] Compound **32** was also quite selective against other chemokine receptors: it inhibits RANTES binding to CCR5 with an IC_{50} value of 24 nM but shows no significant inhibition for CCR1, CCR2, CCR3, CCR4, CCR6, and CXCR4 (all with IC_{50} > 50μM). In addition, **32** showed no significant CYP inhibition against major subtypes such as 3A4, 1A2, 2C9, 2C19, and 3D6. Furthermore, **32** had no significant hERG inhibition activity with an in vitro IC_{20} value of 3 μM at 37 °C.

Table 4
Effect of side-chain modification.



Compound	R^1	R^2	IC_{50} (nM)	
			RANTES	CCF[a]
41			80	400
42			104	1900

[a] Values are means of two experiments.

Table 5
Pharmacokinetic properties of **32** in rat.

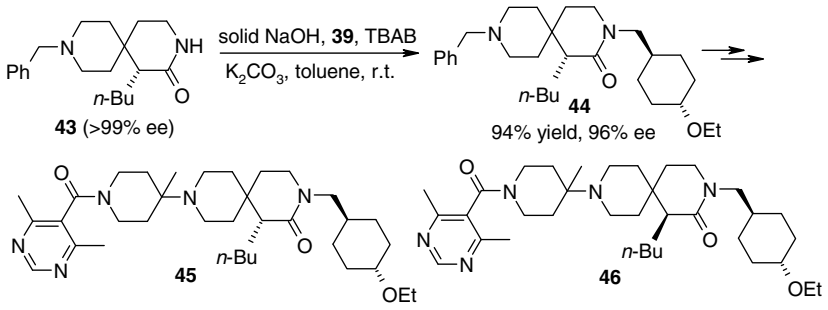
Parameter	IV (0.5 mg/kg)[a]	PO (2 mg/kg)[a]
T_{max} (h)	–	1.00
C_{max} (ng/mL)	–	203
$T_{1/2}$ (h)	3.80	4.19
MRT (h)	2.65	4.33
AUC (ng h/mL)	334	910
CL (mL/min/kg)	24.1	–
V_{dss} (L/kg)	4.71	–
%*F*	–	66

[a] Values are means of two experiments.

Encouraged by the rat PK and selectivity data for **32**, we pursued an asymmetric synthesis (Scheme 5). One of the key steps was alkylation of the chiral template **43**[10] (99% ee) with tosylate **39**. Surprisingly, under the previously employed phase transfer conditions (solid NaOH, tetrabutylammonium bromide, K_2CO_3, toluene, 90 °C), the desired product **44** was obtained in merely 50% ee. After extensive optimization, it was found that running the reaction at room temperature for 2 d gave **44** in 94% yield and 96% ee, which was further enriched to >99% ee by chiral HPLC purification.[11] Compound **44** was then converted to (*R*)-enantiomer **45** following procedures outlined in Scheme 1. The (*S*)-enantiomer **46** was prepared in a same fashion.

Compounds **32**, **45** and **46** were evaluated in peripheral blood mononuclear cell (PBMC) viral replication assay with 10% FBS using an R5-tropic HIV-1_{Ba-L} strain. The results were shown in Table 6. Interestingly, both enantiomers of **32** displayed similar activities with IC_{90} values of 4 and 3 nM for **45** and **46,** respectively. This result is in sharp contrast to our earlier series represented by general structure **1**, in which only the (*S*)-enantiomer is active. Efforts to explain this discrepancy by invoking our homology model[8] are inconclusive due to its low resolution.

In conclusion, replacement of the spirocyclic carbamate in our 1-oxa-3,9-diazaspiro[5.5]undecan-2-one series led to the discovery of two novel series of CCR5 antagonists. While the 3,9-diazaspiro[5.5]undecane series provided potent compounds in CCF assay, its further development was hampered by high in vitro human microsomal clearance. On the other hand, 3,9-diazaspiro[5.5]undeca-2-one series also yielded potent analogs. Analog **32** showed an attractive combination of antiviral potency, selectiv-



Scheme 5.

Table 6
Peripheral blood mononuclear cell (PBMC) viral replication assay results for compounds **32**, **45** and **46**.

Compound	Chirality	PBMC assay[a]	
		IC_{50} (nM)	IC_{90} (nM)
32	Racemic	1.4	7.5
45	*R*	0.8	4
46	*S*	0.5	3

[a] Values are means of two experiments.

ity, and a favorable pharmacokinetic profile. In addition, asymmetric synthesis of **32** was accomplished and both enantiomers were equally potent in PBMC assay with IC_{90} values of 4 and 3 nM, respectively.

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10. For an asymmetric synthesis of **43**, see Ref. 5.
11. Chiral HPLC condition: chiralpak AD column, 95/5 hexane/EtOH as solvent at 1.4 mL/min. Compound **44** has a retention time of 9.29 min.