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Discovery of orally available spirodiketopiperazine-based CCR5 antagonists

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ABSTRACT

Using the previously reported novel spirodiketopiperazine scaffold, the design and synthesis of orally available CCR5 antagonists was undertaken. Compounds possessing a carboxylic acid function in the appropriate position showed improved oral exposure (AUC) relative to the initial chemical leads without reduction in the antagonist activity. The optimized compound **40** was found to show potent anti-HIV activity. Full details of structure–activity relationship (SAR) study are presented.

1. Introduction

Despite a tremendous effort to develop antiretroviral therapy, millions of people in the world are still suffering from Acquired Immune Deficiency Syndrome (AIDS).[1] Although several classes of antiretroviral medications are currently available, challenges remain with anti-HIV therapies due to a gradual spread of drug-resistant strains, severe side effects, and expensive therapeutic costs among others. Hence there is a growing need for novel treatments for AIDS patients who are treatment-experienced and have become resistant to one or multiple classes of antiretroviral agents.[2] These issues require new anti-HIV drugs to have a different mode of action from conventional drugs. CCR5 antagonists work by inhibiting HIV entry into target cells and are one of the most promising approaches to treat AIDS because of their ability to inhibit a previously untargeted step in the HIV-1 replication cycle.[3] Maraviroc is currently the only approved CCR5 antagonist on the market.[4]

We previously reported the discovery of spirodiketopiperazine derivatives **1a** and **1b** (Fig. 1), as structurally novel CCR5 antagonist leads generated from a combinatorial library targeting GPCRs.[5,7] However, it was unfortunate that both **1a** and **1b** showed poor oral exposure (AUC) in rodents (Table 7). Based on the analysis of their PK data, large clearance (CL) and tissue distribution values (V_{ss}) were assumed to be one of the plausible reasons for poor AUC. Herein we report full details of the discovery of a new chemical lead 40 with an improved PK profile.

2. Results and discussion

2.1. Chemistry

As shown in Schemes 1 and 2, compounds **1a**, **1b**, **2**–**13**, **15**–**18**, **21**–**23**, **25**, **26**, and **28** were efficiently synthesized using solid phase synthesis.[5] The Ugi four-component condensation using the Rink-isonitrile resin is outlined in Scheme 1a. A mixture of 1-*N*-allyloxycarbonyl piperidone **41a**, an optional alkyl amine **42**, an optional *N*-Boc-amino acid **43**, and the Rink-isonitrile resin **44** in THF/MeOH (1:1) was shaken at 65 °C to afford **45**. Palladium catalyzed removal of the *N*-allyloxycarbonyl moiety of **45** was followed by reductive amination with an optional aryl aldehyde to produce **46**. Removal of the Boc protecting group of **46** followed by heating under acidic conditions resulted in cyclization accompanied by removal of the supporting-resin to afford spirodiketopiperazines **1a**, **1b**, **2**–**13**, **15**–**18**, **23**, **25**, **26**, and **28**. Compound **14** was prepared by alkaline hydrolysis of **15**. 2-Butynylamine **42f** was prepared from 2-butynyl alcohol as described in Scheme 1b. O-Methanesulfonylation of **47** followed by the substitution reaction with potassium phthalimide

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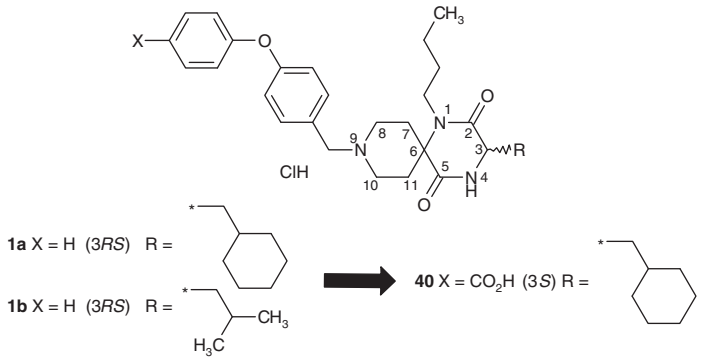
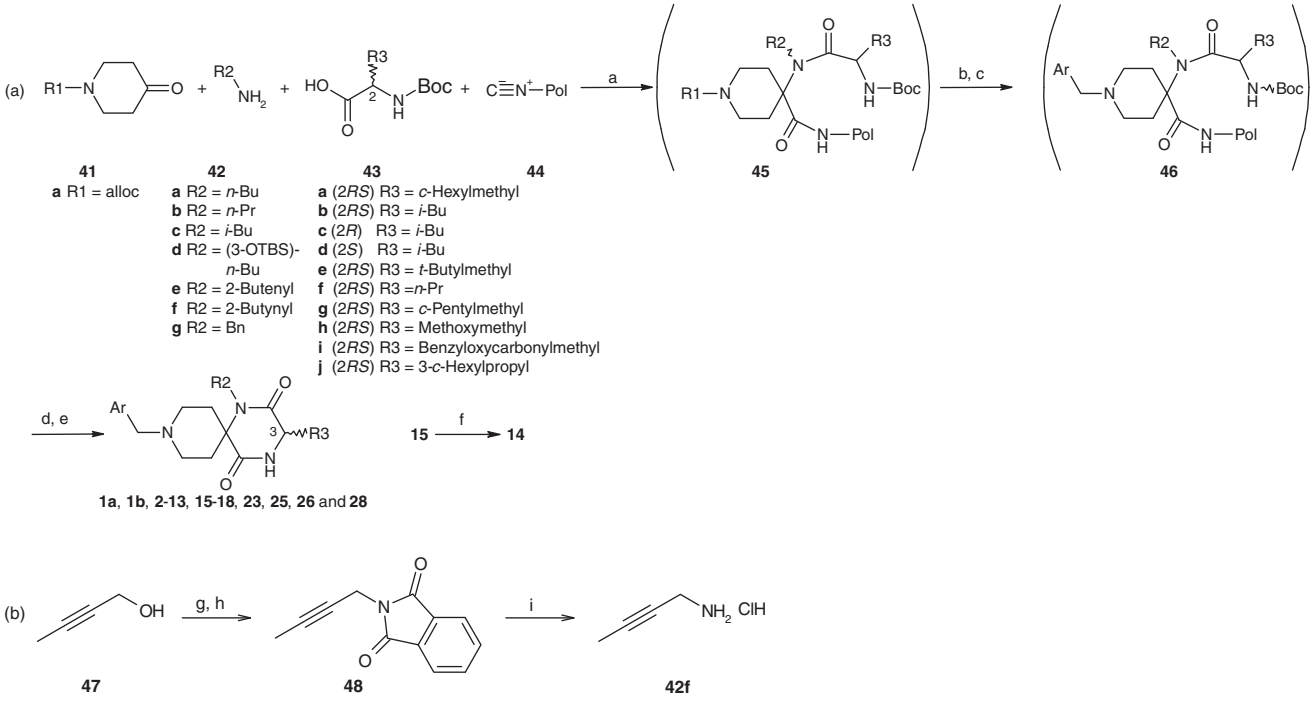
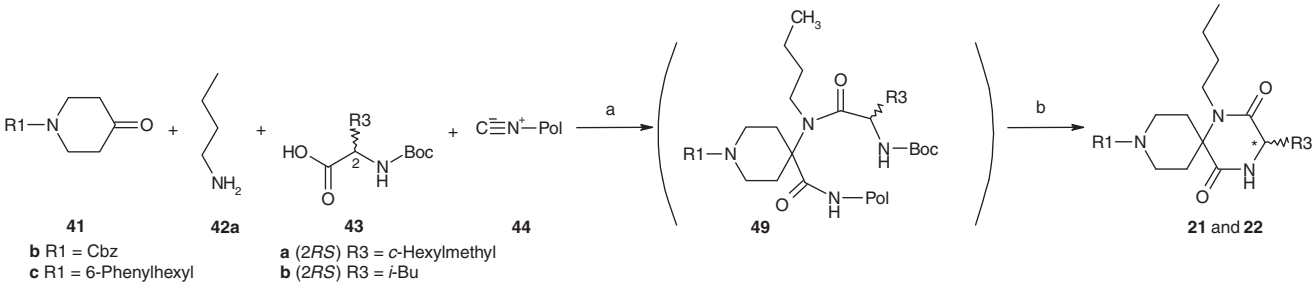


Figure 1. Molecular design of orally active chemical leads.



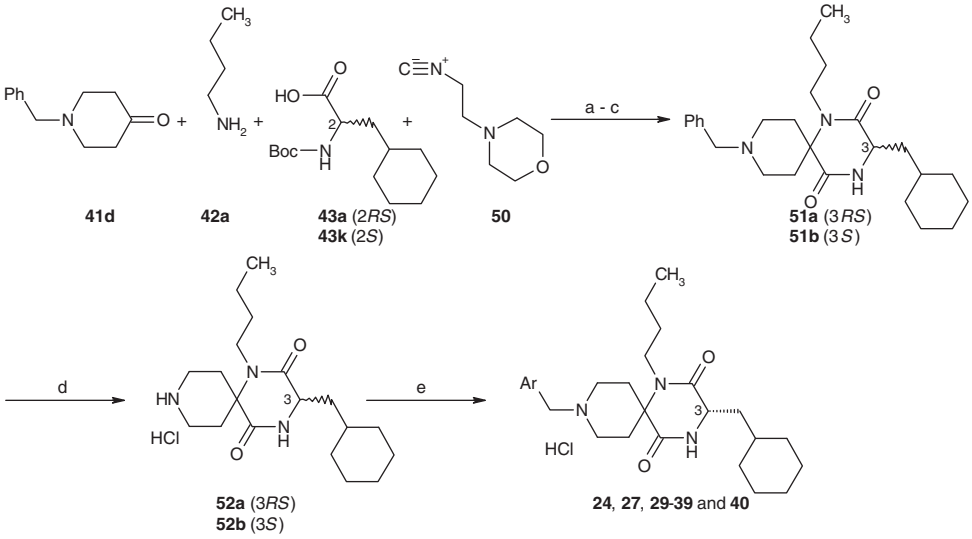
Scheme 1. Solid phase synthesis of spirodiketopiperazines **1a**, **1b**, **2**–**18**, **23**, **25**, **26**, and **28**. Reagents and conditions: (a) THF/MeOH (1:1), 65 °C; (b) $Pd(PPh_3)_4$, Bu_3SnH, AcOH/CH_2Cl_2, rt; (c) Ar-CHO, $NaBH(OAc)_3$, AcOH/DMF; (d) TFA/CH_2Cl_2 (1:1); (e) AcOH/toluene, 90 °C and then 4 N HCl/AcOEt; (f) 2 N NaOH, MeOH; (g) MsCl, Et_3N, CH_2Cl_2; (h) potassium phthalimide, DMF, 70 °C; (i) $NH_2NH_2{\cdot}H_2O$, EtOH, then concd HCl, reflux.



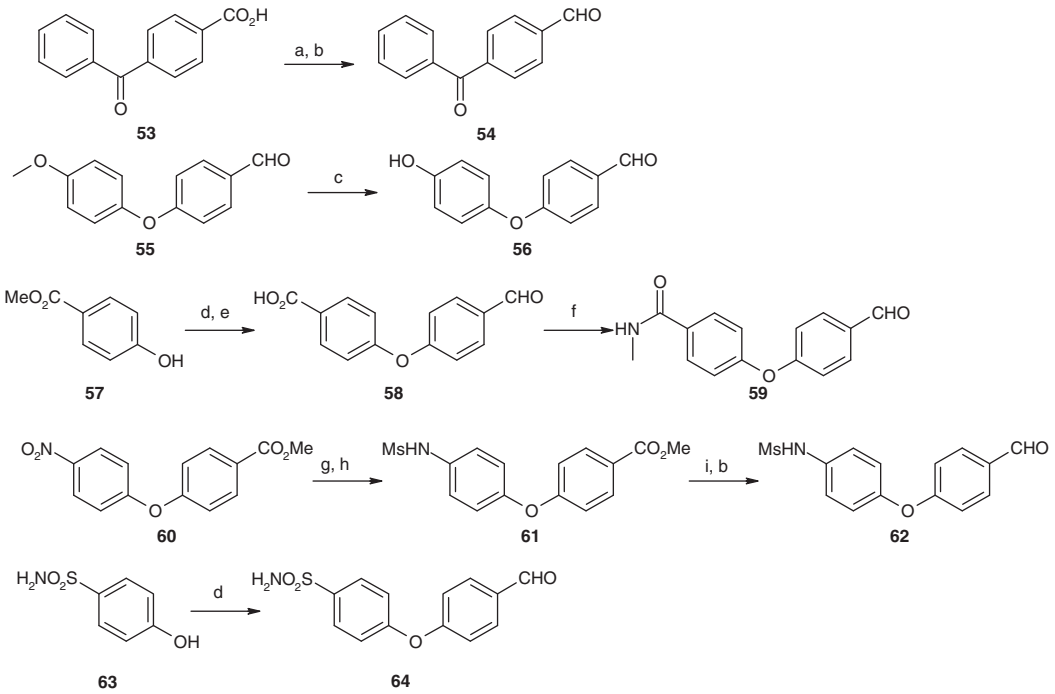
Scheme 2. Synthesis of **21** and **22**. Reagents and conditions: (a) THF/MeOH (1:1), 65 °C; (b) TFA/CH_2Cl_2 (1:1); (c) AcOH/toluene, 90 °C and then 4 N HCl/AcOEt.

afforded **48**, deprotection of which with hydrazine hydrate produced **42f** in good yield. As described in Scheme 2, compounds **21** and **22** were also synthesized from 1-Cbz-piperidone **41b** and 1-(6-phenylhexyl)-4-piperidone **41c**, respectively, by the solid phase synthesis.

Solution phase synthesis of compounds **24**, **27**, and **29**–**40** was carried out by reductive alkylation of **52a** and **52b** with an optional aryl aldehyde, respectively, as described in Scheme 3. Compounds **52a** and **52b** were prepared by solution phase synthesis. The Ugi



Scheme 3. Solution phase synthesis of spirodiketopiperazines, **24**, **27**, and **29**–**40**. Reagents and conditions: (a) MeOH, 55 °C; (b) concd HCl, 55 °C; (c) AcOH/toluene, 80 °C; (d) H_2, $Pd(OH)_2/C$, EtOH, 50 °C, then 4 N HCl/AcOEt (50–90% in four steps); (e) Ar-CHO, $NaBH(OAc)_3$, AcOH, DMF and then 4 N HCl/AcOEt (50–90%).



Scheme 4. Synthesis of aryl aldehydes **54**, **56**, **58**, **59**, **62**, and **64**. Reagents and conditions: (a) *i*-BuOCOCl, Et_3N, THF then $NaBH_4$, H_2O; (b) MnO_2, DME; (c) BBr_3, CH_2Cl_2; (d) 4-fluorobenzaldehyde, K_2CO_3, DMA, 150 °C; (e) NaOH, MeOH; (f) EDC, HOBt, $MeNH_2$, DMF; (g) H_2, 5% Pd/C, AcOEt; (h) MsCl, pyridine, THF; (i) DIBAL, THF.

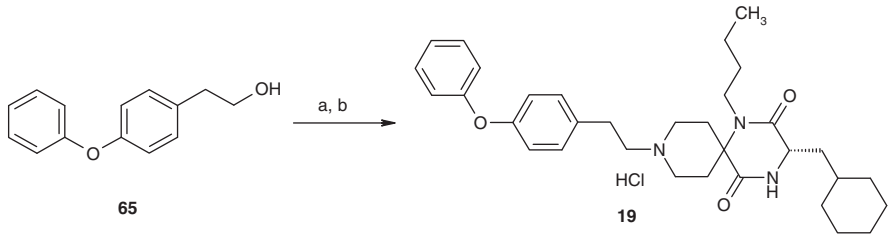
four-component condensation of 1-benzyl-4-piperidone **41d**, *n*-butylamine **42a**, *N*-Boc-1-cyclohexylalanine **43a** and 2-(4-morpholinyl)-ethylisonitrile **50** afforded spirodiketopiperazine **51a**. Catalytic hydrogenation of **51a** followed by the acid treatment provided **52a** as a hydrochloride salt. Compound **52b** was prepared by the same procedures as described above using **43k** instead of **43a** as one of the starting materials. Reductive alkylation of **52b** with an optional aldehyde produced **24**, **27**, and **29**–**40**, respectively. Commercially unavailable aryl aldehydes **54**, **56**, **58**, **59**, **62**, and **64** were prepared as outlined in Scheme 4.

Synthesis of 9-*N*-(4-phenoxy)phenylethyl analog **19** is described in Scheme 5. 9-N-Alkylation of **52b** with polymer-supported 4-phenoxyphenylethyl tosylate, which was prepared by O-tosylation of 4-phenoxyphenylethanol **65** with polystyrene-supported tosyl chloride in pyridine, followed by the treatment with hydrogen chloride afforded **19**.

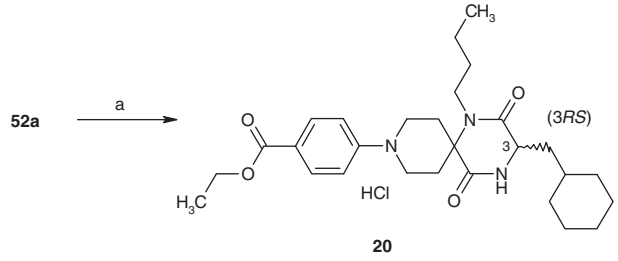
As described in Scheme 6. 9-*N*-phenyl analog **20** was prepared by the substitution reaction of ethyl 4-fluorobenzoate with **52a** in the presence of potassium carbonate in DMSO followed by the acid treatment.

2.2. CCR5 antagonist activity

Compounds listed in Tables 1–6 were evaluated for their inhibitory activities against calcium mobilization of human CCR5

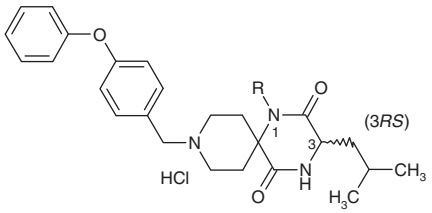


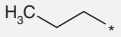
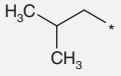
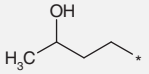
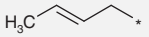
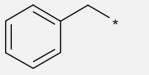
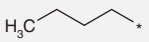
Scheme 5. Synthesis of **19**. Reagents and conditions: (a) polystyrene-supported tosyl chloride, pyridine, CH_2Cl_2; (b) **52b**, *i*-Pr_2NEt, MeCN and then 4 N HCl/AcOEt (48% in two steps).



Scheme 6. Synthesis of **20**. Reagents and conditions: ethyl 4-fluorobenzoate, K_2CO_3, DMSO and then 4 N HCl/AcOEt (27%).

Table 1
Effect of the 1-N-substituent on activity profiles



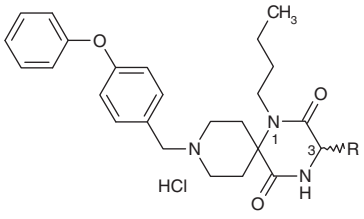
Compds	R	IC_{50} (nM) Ca assay	$T_{1/2}$ in rat liver microsomes[a] (min)
2		500	NT[b]
3		330	15
4		>3000	12
5		69	14
6		54	15
7		300	NT[b]
1b		94	12

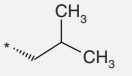
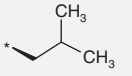
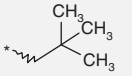
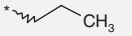
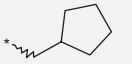
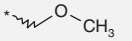
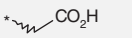
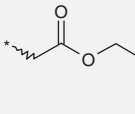
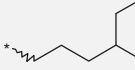
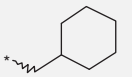
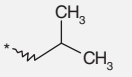
[a] The data show $T_{1/2}$ after incubating with the 0.2 mg/mL rat liver microsomes.
[b] NT: not tested.

overexpressed CHO cells (hCCR5/CHO) stimulated with MIP-1α and with IC_{50} values as the mean of two experiments.[6]

As described in our previous paper,[5] the 1,4,9-triazaspiro[5.5]undecane-2,5-dione (spirodiketopiperazine) is an attractive scaffold to use to identify small molecule ligands of GPCRs because of the following three reasons. (1) This spirodiketopiperazine is a simple heterocyclic scaffold in which diversity can be introduced at up to four positions, and can be prepared from readily available α-amino acids. (2) Very few compounds with this spirodiketopiperazine scaffold have been reported at the starting point of this project. (3) The predicted 3D structure of spirodiketopiperazine suggested that utilization of three substituents on this template might have a similar orientation to three side chains on the type I β-turn structure of the protein.

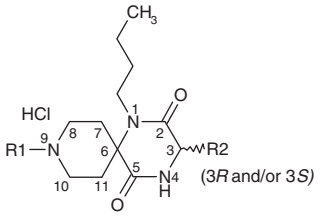
Table 2
Effect of the 3-substituent on activity profiles

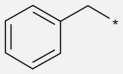
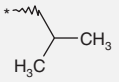
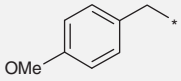
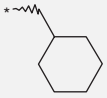
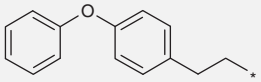
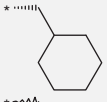
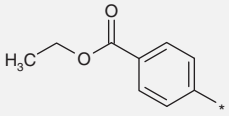
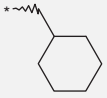
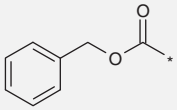
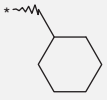
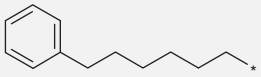
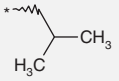
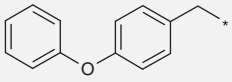
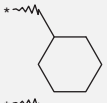
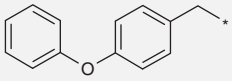
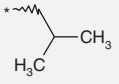


Compds	R	C3 Configuration (*R* and/or *S*)	IC_{50} (nM) Ca assay	$T_{1/2}$ in rat liver microsomes[a] (min)
8		(3*S*)	84	7.1
9		(3*R*)	130	NT[b]
10		(3*RS*)	79	12
11		(3*RS*)	50	22
12		(3*RS*)	52	20
13		(3*RS*)	320	16
14		(3RS)	>3000	NT[b]
15		(3*RS*)	590	NT[b]
16		(3*RS*)	>3000	23
1a		(3*RS*)	28	24
1b		(3*RS*)	94	12

[a] The data show $T_{1/2}$ after incubating with the 0.2 mg/mL rat liver microsomes.
[b] NT: not tested.

Table 3
Effect of the 9-N-substituent on activity profiles



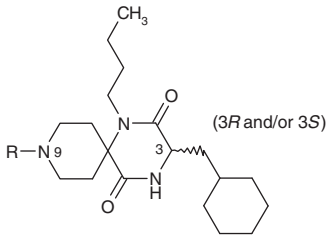
Compds	R1	R2	C3 Configuration (3*R* and/or 3*S*)	IC_{50} (nM) Ca assay	$T_{1/2}$ in rat liver microsomes[a] (min)	Calculated pK_a[b]
17			(3*RS*)	900	NT[c]	7.54
18			(3*RS*)	120	8.3	7.53
19			(3*S*)	>3000	NT[c]	7.51
20			(3*RS*)	>3000	NT[c]	4.12
21			(3*RS*)	3000	NT[c]	2.52
22			(3*RS*)	270	NT[c]	8.24
1a			(3*RS*)	28	24	7.15
1b			(3*RS*)	94	12	7.18

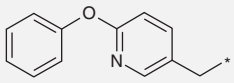
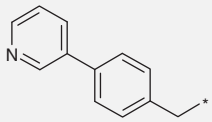
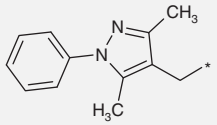
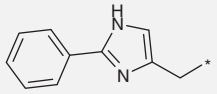
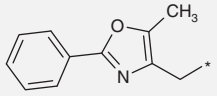
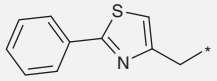
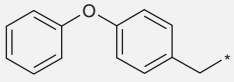
[a] The data show $T_{1/2}$ after incubating with the 0.2 mg/mL rat liver microsomes.
[b] Calculated by ADMET predictor (ver. 4.0).
[c] NT: not tested.

Compounds **1a** and **1b** blocked the infectivity and replication of laboratory and clinical strains of HIV as well as highly drug-resistant HIV variants with minimal cytotoxicity.[6a] Despite their promising activity profiles, both **1a** and **1b** had very poor AUCs after oral dosing in rat. Pharmacokinetic analysis after iv dosing indicated that they exhibited rapid clearance (CL) and a large distribution volume (V_{ss}). A large clearance value (CL) after iv dosing suggests that they are metabolically unstable which may explain their poor AUC and bioavailability (BA). Moreover, the large distribution volume (V_{ss}) of analogs **1a** and **1b** after iv dosing is unfavorable as anti-HIV-1 drugs exert their effects in the blood. Thus, our synthetic effort was focused on increasing oral exposure (AUC), reduction of in vivo clearance (CL) and distribution volume (V_{ss}). The optimization of chemical leads **1a** and **1b** was initiated by evaluating each of the diversity sites as described in Tables 1–6.

Optimization of the 1-*N*-alkyl residue was carried out with the aim of further increasing the in vitro activities and metabolic stability (Table 1). Replacement of the *n*-butyl residue of **1b** with *n*-propyl, *i*-butyl, 3-hydroxybutyl, 2-butenyl, 2-butynyl and benzyl residues afforded **2**–**7**, respectively. 1-*N*-(*n*-Propyl) analog **2** exhibited less potent activity relative to **1b**. Analog **3** possessing a branched 1-*N*-alkyl residue, showed less potent activity relative to **1b**. 1-*N*-(3-Hydroxybutyl) analog **4** had a remarkable reduction in its antagonist activity. Thus, a hydrophilic OH group was found to be unacceptable at this site. Compounds **5** and **6** possessing 1-*N*-(2-butenyl) and 1-*N*-(2-butynyl) residues, showed nearly equipotent activity as **1b**. 1-*N*-Benzyl analog **7** showed significantly reduced potency. As a result, shortening and branching of the 1-*N*-(*n*-butyl) residue as illustrated by **2** and **3** negatively impacted the activity. After substantial chemical modification, lipophilic groups such as *n*-butyl, 2-butenyl and 2-butynyl residues, which are relatively linear and have a limited length (C4), were found to be the most optimal 1-N-substituent. The stability in rat liver microsomes of compounds **1b** and **3**–**6** was investigated to estimate their metabolic stability. All the analogs tested were not as metabolically stable as **1b**. Although this hydrophobic 1-N-residue was considered to be one of sites that were

Table 4
Effect of the 9-N-substituent with heteroaryl moieties on activity profiles



Compds	R	C3 Configuration (3*R* and/or 3*S*)	IC_{50} (nM) Ca assay	Stability in rat liver microsomes[a] remaining%	*c*Log *P*[b]
23		(3*RS*)	21	10	6.16
24		(3*S*)	71	29	6.16
25		(3*RS*)	35	23	6.05
26		(3*RS*)	160	NT[c]	5.49
27		(3*S*)	450	NT[c]	5.55
28		(3*RS*)	570	NT[c]	6.00
1a		(3*RS*)	28	33	7.66

[a] The data show the remaining% 15 min after incubating with the 0.5 mg/mL rat liver microsomes.
[b] Calculated by ADMET predictor (ver. 4.0).
[c] NT: not tested.

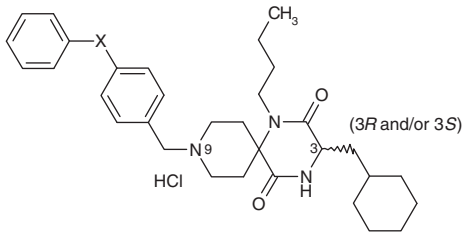
metabolically vulnerable, it seemed to be difficult to improve metabolic stability only by chemical modification of this moiety.

To identify an optimal C3-substituent, synthesis and evaluation of analogs **8**–**16** was carried out using the easily available optically active *S*-form or racemic *RS*-form as the starting amino acids. This was possible because there were no significant differences in antagonist activities between the enantiomers **8** (3*S*) and **9** (3*R*), both of which showed nearly equipotent activities relative to **1b**. Analogs **10**–**12** possessing lipophilic C3-substituents exhibited equipotent activities relative to **1b**. Analogs **13**–**15** possessing relatively hydrophilic substituents such as methoxymethyl, carboxymethyl, and benzyloxycarbonylmethyl tended to show reduced activities. Cyclohexylethyl analog **16**, which is a C2 homolog of **1a**, exhibited a remarkable decrease in its activity. Based on these results, only lipophilic C3-substituents are acceptable for the receptor pocket as illustrated by **1a**, **1b** and **8**–**16** while their length is strictly limited as illustrated by the result of **16.** Analogs **8**–**12**, which showed nearly equipotent activities relative to **1a** and **1b**, did not show any improvement in stability in liver microsomes as shown in Table 2.

Further structural optimization of the 9-N-substituent was explored to provide additional increases in activity and metabolic stability. Results are summarized in Tables 3–5. To identify the optimal structure of the 9-N-substituent on the piperidine moiety of the lead structures **1a** and **1b**, compounds listed in Table 3 were synthesized and evaluated. To confirm the structural requirement of the 9-*N*-arylalkyl moiety, compounds **17** to **22** were synthesized and evaluated. 9-*N*-Phenylmethyl analogs **17** and **18** tended to show reduced activities relative to **1b** and **1a**, respectively. Replacement of the 9-*N*-{(4-phenoxy)phenylmethyl} residue with 9-*N*-{(4-phenoxy)phenylethyl} afforded **19** with a remarkable reduction in activity. Based on this result, the 9-*N*-phenylmethyl moiety was found to be required for the antagonist activity. The less basic aniline type analog **20** (calculated pK_a 4.12) and non-basic analog **21** (calculated pK_a 2.52) also showed remarkable reduction in activity. Recovery of the antagonist activity for 9-*N*-{6-(phenyl)hexyl} analog **22** relative to that of **17** strongly suggested another interaction site with the receptor. As a result, the 9-*N*-{(4-phenoxy)phenylmethyl} moiety was concluded to be the optimal 9-N-substituent.

The effects of the 9-N-substitution with heteroarylmethyl moieties on the activity profiles were investigated. Results are summarized in Table 4. Analogs **23**–**28** possess lower *c*Log *P* values relative to **1a**. 9-*N*-{2-(phenoxy)pyridine-5-ylmethyl} analog **23** exhibited nearly equipotent in vitro activities relative to **1a** although it showed less stability in rat liver microsomes. Accordingly, the

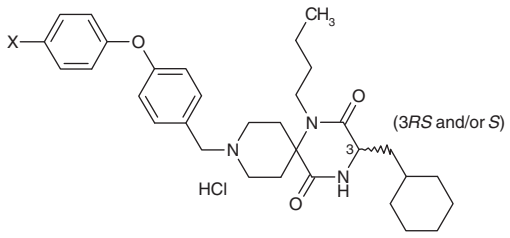
Table 5
Effect of chemical modification of the linker X on activity profiles



Compds	X	C3 Configuration (3*R* and/or 3*S*)	IC_{50} (nM) Ca assay	Stability in rat liver microsomes[a] remaining%
29		(3*S*)	170	38
30		(3*S*)	86	19
31		(3*S*)	81	23
32		(3*S*)	57	29
1a		(3*RS*)	28	33

[a] The data show the remaining% 15 min after incubating with the 0.5 mg/mL rat liver microsomes.

Table 6
Effect of the *p*-substituent of the biphenyl ether residue on activity profiles



Compds	X	C3 Configuration (3*R* and/or 3*S*)	IC_{50} (nM) Ca assay	Stability in rat liver microsomes[a] remaining%	*c*Log *P*[b]
33	F–	(3*S*)	92	53	7.80
34	Me–	(3*S*)	79	48	8.16
35	MeO–	(3*S*)	130	34	7.58
36	HO–	(3*S*)	42	22	6.99
37	MeHNC(O)–	(3*S*)	33	4	6.38
38	$MeSO_2HN$–	(3*S*)	40	15	6.47
39	H_2NO_2S–	(3*S*)	28	24	5.82
40	HO_2C–	(3*S*)	13	22	5.13
1a	H	(3*RS*)	28	33	7.66

[a] The data show the remaining% 15 min after incubating with the 0.5 mg/mL rat liver microsomes.
[b] Calculated by ADMET predictor (ver. 4.0).

benzylic phenyl of **1a** could be replaced by a more hydrophilic pyridine moiety without loss of the in vitro activities. 9-*N*-{4-(pyrid-3-yl)phenylmethyl} analog **24** exhibited slightly less potent activity relative to **1a**. Five-membered heteroarylmethyl analogs **25**–**28** were also synthesized and evaluated. Unexpectedly, 9-*N*-(3,5-dimethyl-1-phenylpyrazol-4-ylmethyl) analog **25** was found to show agonist-like activity in the calcium assay. 9-*N*-(2-Phenylimidazol-4-ylmethyl) analog **26**, 9-*N*-(2-phenyl-5-methyloxazole-4-ylmethyl) analog **27**, and 9-*N*-(2-phenyl-4-thiazolylmethyl) analog **28** exhibited weaker antagonist activity than **1a**. Unfortunately, their stabilities in rat liver microsomes were not improved using this approach.

As described in Table 5, the SAR of the linker X which connects the two phenyl moieties of the 9-*N*-{4-phenoxyphenylmethyl} moiety of **1a** was investigated. Results are summarized in Table 5. Replacement of the ether oxygen of **1a** with a sulfur atom afforded the corresponding sulfide analog **29** with less potent activity and an increased stability in rat liver microsomes. The corresponding sulfone analog **30** exhibited less potent activity relative to **1a**. Replacement of the ether oxygen of **1a** with a carbonyl provided **31** which also had slightly less potent activity relative to **1a**. Replacement of the ether oxygen of **1a** with an amide moiety afforded **32** with slightly less potent activity. Accordingly, an ether oxygen was selected as the best linker to connect the two phenyls of the 9-*N*-{4-(phenoxy)phenylmethyl} residue.

The structure activity relationship (SAR) study described in Tables 3–5 strongly suggests that concurrent conversion of the activity and stability in rat liver microsomes by chemical modification of the 9-N-substituent from 9-*N*-[4-phenoxy]phenylmethyl] moiety is difficult. Accordingly, stepwise optimizations of this cite which had one of the most promising activity profiles was attempted to improve its PK profiles as described in Tables 6 and 7.

The effects of a *p*-substitution at the predicted metabolic site[8] of the terminal phenyl moiety of **1a** on the stability in rat liver microsomes were investigated. As shown in Table 6, introduction of *p*-methyl and *p*-methoxy groups as electron-donating substituents into the terminal phenyl moiety of **1a** afforded 4-(*p*-methylphenoxy)phenylmethyl and 4-(*p*-methoxyphenoxy)phenylmethyl analogs **34** and **35**, respectively. Both had a tendency to be slightly less potent relative to **1a**, while demethylation of **35** afforded the analog **36** which had slightly more potent activity relative to the corresponding methoxy analog **35**. Introduction of a *p*-fluoro group as an electron-withdrawing group instead of utilizing the electron-donating *p*-methyl and *p*-methoxy groups afforded **33** with slightly less potent activity relative to **1a**. Introduction of *N*-methyl aminocarbonyl, methanesulfonylamino, aminosulfonyl and hydroxycarbonyl groups as the electron-withdrawing and hydrophilic *p*-substituent afforded **37**, **38**, **39**, and **40**, respectively, with nearly equipotent in vitro activities. The stability of these test compounds in the rat liver microsomes was investigated but in vitro data did not indicate a significant improvement in their metabolic stability. However, analogs possessing hydrophilic substituents are predicted to have the potential of improved physicochemical properties such as solubility. PK studies of analogs **36**, **37**, and **40**, which possess relatively good in vitro potency and hydrophilic substituents such as hydroxyl, *N*-methyl aminocarbonyl and hydroxycarbonyl residues, were carried out.[9]

2.3. Pharmacokinetics

PK data obtained after single-dose oral administration of the initial chemical leads **1a**, **1b** and the representative compounds **36**, **37**, **40** to rats, are presented in Table 7. As described previously, the initial leads **1a** and **1b** showed very poor bioavailability (1.9% and 1.3%, respectively). Other PK values such as the maximum plasma concentration (C_{max}), plasma elimination half-life ($T_{1/2}$) and AUC were also very poor. The probable reasons for such poor PK values for **1a** and **1b** were the large clearance (CL = 113 and 137 mL/min/kg, respectively) and the large distribution volumes (V_{ss} = 2542 and 2349 mL/kg, respectively) which are unfavorable for compounds such as anti-HIV drugs which require transport in the blood to achieve efficacy.

Table 7
Pharmacokinetic data for **1a**, **1b**, **36**, **37**, and **40** in rat ($n = 3$)

Compds	30 mg/kg, po				3 mg/kg, iv			
	C_{max}[a] (ng/mL)	$T_{1/2}$ (min)	AUC[a] (ng h/mL)	BA (%)	AUC (ng)	$T_{1/2}$ (min)	CL (mL/min/mL)	V_{ss} (mL/kg)
1a	16.7	103	74.4	1.9	400	19.9	113	2542
1b	100	75.7	290	1.3	372	13	137	2349
36	100	205	195	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
37	1900	72.7	5453	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
4	7200	48.4	10,532	34.1	3091	11.1	16	145

[a] C_{max} and AUC are normalized to a dose of 30 mg/kg.
[b] ND: not determined.

With the expectation of improved PK profiles, analogs **36**, **37**, and **40** possessing hydrophilic substituents were investigated for their pharmacokinetics. *N*-Methyl carboxy amide analog **37** tended to show improved PK values in its C_{max} and oral AUC relative to **1a** and **1b**, while the phenol analog **36** did not. Interestingly, benzoic acid analog **40** showed significantly improved PK values in its C_{max} (7200 ng/mL), oral exposure (AUC = 10,532 ng h/mL) and bioavailability (BA = 34%) after oral dosing. The marked reduction in clearance (CL = 16 mL/min/kg) and distribution volume (V_{ss} = 145 mL/kg) after iv dosing was considered to be the most plausible reason for the improved AUC and BA. Additionally, remarkable improvement of solubility and Caco-2 permeability of **40** relative to **1a** and **1b** was estimated to be other possible reasons as shown in Table 8. The marked reduction of the CL of **40** strongly suggested in vivo metabolic stabilization, though in vitro studies did not indicate a significant improvement in metabolic stability.

2.4. Anti-HIV activity

The anti-HIV activities of compounds **39** and **40**, both potent CCR5 antagonists, were evaluated in the p24 assay. Results are shown in Table 9 and compared with **1b**. Compounds **39** and **40** were found to show more potent anti-HIV activity in the p24 assay relative to **1b**. Especially **40**, which has the most favorable PK profiles among the compounds tested, potently inhibited not only the replication of laboratory and primary (R5) HIV-1 strains but also that of various multidrug-resistant monocyte/macrophage tropic (R5) HIV-1 strains.[6] Compound **40** was inactive against T cell tropic (X4) HIV-1. These results support the hypothesis that spirodiketopiperazines such as **1b**, **39**, and **40** possess potent anti-HIV activities through their antagonist effects on CCR5.

3. Conclusions

In conclusion, starting with the initial hit compounds **1a** and **1b**, which showed unfavorable PK profiles, we identified **40** which showed significant improvement in its bioavailability (BA) and oral exposure (AUC) without reduction of its antagonist activity by introducing a carboxylic acid function into the *p*-position of the terminal phenyl moiety. Although the role of the carboxylic acid function is still unclear, significant reduction of CL and V_{ss} was considered to be the most plausible reason for the increased C_{max}, AUC and BA of **40**. Its improved physicochemical properties, through the increase of hydrophilicity, were thought to contribute to the improved C_{max}, AUC and BA after oral dosing. *N*-Methyl aminocarbonyl analog **37** also tended to show improved PK values including C_{max} and AUC after its oral dosing. As such, introduction of hydrophilic hydroxycarbonyl and *N*-methyl aminocarbonyl functions into the *p*-position of the terminal phenoxy moiety was found to be effective not only to block the predicted metabolic deactivation but also to improve PK profiles. Compound **40** showed more potent activity than **1b**, one of the initial hit compounds, in the p24 assay (the BAL strain of HIV[6]). Further optimization of **40** to improve its activity and PK profile to provide CCR5 antagonists suitable for clinical use is ongoing.

4. Experimental

4.1. Chemistry

4.1.1. General methods

Analytical samples were homogeneous as confirmed by thin layer chromatography (TLC), and afforded spectroscopic results consistent with the assigned structures. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Varian Gemini-200 or a MERCURY-300 spectrometer with tetramethylsilane as an internal standard. The chemical shift values δ are reported in ppm and coupling constants (J) in Hertz (Hz). Fast atom bombardment (FAB) and electron ionization (EI) mass spectra were obtained with a JEOL JMS-700 spectrometer. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectra were obtained on Perceptive Voyager Elete. Atmospheric pressure chemical ionization (APCI) mass spectra were determined by Hitachi M-1200H spectrometer. IR spectra were measured on a JASCO FTIR-430 spectrometer. Elemental analyses were performed with a Perkin–ElmerPE2400 series II CHNS/O Analyzer and were only indicated as the elements within ±0.4% of the theoretical values unless otherwise noted. Column chromatography was carried out on silica gel [Merck Silica Gel 60 (0.063–0.200 mm), Fuji Silysia BW235 or Fuji Silysia FL60D]. TLC was performed on silica gel (Merck TLC, Silica Gel 60 F_{254}).

4.1.2. A typical procedure for the Ugi four-component condensation using Rink-isonitrile resin. (1a, 1b, 2–13, 15–18, 23, 25, 26, and 28)

4.1.2.1. 1-Butyl-3-(cyclohexylmethyl)-9-(4-phenoxybenzyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (1a). The Rink-isonitrile resin **44** (0.45 mmol/g, 500 mg, 0.23 mmol) was washed with THF/MeOH (1:1, 4 mL × 2). To a suspension of the resin in THF/MeOH (1:1, 4 mL) were added *N*-allyloxycarbonyl-4-piperidone **41a** (206 mg, 1.13 mmol), *n*-butylamine **42a** (82 mg, 1.13 mmol) and *N*-Boc-*dl*-cyclohexylalanine **43a** (305 mg, 1.13 mmol). The mixture was shaken for 16 h at 65 °C. After cooling to room temperature, the resin was collected by filtration and successively washed with THF/MeOH (1:1, 4 mL × 3) and then CH_2Cl_2 (4 mL × 3). To a suspension of the resin in CH_2Cl_2 (4 mL) were successively added acetic acid (135 mg, 2.25 mmol), tetrakis(triphenylphosphine)palladium(0) (52.0 mg, 0.045 mmol) and tributyl tin hydride (327 mg, 1.13 mmol). The mixture was shaken for 4 h at room temperature. The resin was collected by filtration and successively washed with CH_2Cl_2 (4 mL × 4) and DMF (4 mL × 4). To a suspension of the resin in 1% acetic acid in DMF (4 mL) were successively added 4-phenoxybenzaldehyde (223 mg, 1.13 mmol) and then sodium triacetoxyborohydride (238 mg, 1.13 mmol). The mixture was

shaken for 6 h at room temperature. The resin was collected by filtration and successively washed with MeOH (4 mL × 2), DMF (4 mL × 4), and then CH_2Cl_2 (4 mL × 4). The resin was suspended in 25%TFA in CH_2Cl_2 (4 mL) at 0 °C. The mixture was allowed to warm up to room temperature and then stirred for 30 min. The resin was collected by filtration and rinsed with CH_2Cl_2 (4 mL × 3), toluene (4 mL × 3), and 1.25 M acetic acid in toluene (4 mL). The resin was suspended in 1.25 M acetic acid in toluene (4 mL). The suspension was shaken for 24 h at 90 °C. After cooling to room temperature, the resin was collected by filtration and washed with $CHCl_3$/MeOH (1:1, 4 mL). The combined filtrate and washings were evaporated. The resulting residue was purified by column chromatography on silica gel with a gradient of AcOEt/MeOH (from 1:0 to 10:1). The resulting residue after evaporation was treated with 4 N HCl in AcOEt and then washed with Et_2O to afford the title compound **1a** (23 mg, 70% yield) as a white powder. TLC R_f 0.73 ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (200 MHz, CD_3OD) δ 7.74–7.56 (m, 1H), 7.53 (d, J = 8.8 Hz, 2H), 7.40 (m, 2H), 7.18 (m, 1H), 7.10–7.00 (m, 3H), 4.33 (s, 2H), 4.04 (dd, J = 7.4, 4.8 Hz, 1H), 3.80 (m, 2H), 3.60–3.35 (m, 4H), 2.43 (m, 2H), 2.17 (m, 2H), 1.90–1.60 (m, 7H), 1.60–1.45 (m, 2H), 1.45–1.30 (m, 2H), 1.30–1.15 (m, 4H), 1.10–0.80 (m, 5H); IR (KBr) 3434, 3210, 3064, 2926, 2851, 2664, 2558, 1672, 1590, 1509, 1489, 1418, 1373, 1241, 1173, 1118, 1072, 1048 cm^{-1}; MS (FAB, Pos) m/z 518 (M+H)$^+$; Elemental Anal. Calcd for $C_{32}H_{43}N_3O_3$·HCl·0.5H_2O: C, 68.25; H, 8.05; N, 7.46. Found: C, 68.23; H, 7.88; N, 6.77.

4.1.2.2. 1-Butyl-3-isobutyl-9-(4-phenoxybenzyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (1b). The title compound was synthesized in 48% yield according to the same procedures as described for the preparation of **1a** using *N*-Boc-*dl*-leucine for **43b**. TLC R_f 0.63 ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (200 MHz, CD_3OD) δ 7.54 (d, J = 8.8 Hz, 2H), 7.40 (m, 2H), 7.18 (m, 1H), 7.11–7.00 (m, 4H), 4.33 (s, 2H), 4.01 (dd, J = 7.6, 4.8 Hz, 1H), 3.80 (m, 2H), 3.60–3.35 (m, 4H), 2.43 (m, 2H), 2.18 (m, 2H), 1.80 (m, 1H), 1.70 (m, 1H), 1.54 (m, 2H), 1.37 (m, 3H), 1.00–0.90 (m, 9H); IR (KBr) 3440, 3221, 3066, 2957, 2871, 2559, 1673, 1590, 1509, 1489, 1419, 1371, 1329, 1242, 1172 cm^{-1}; MS (FAB. Pos., Glycerin+*m*-NBA) m/z 478 (M+H)$^+$, 183 (base peak); Elemental Anal. Calcd for $C_{29}H_{39}N_3O_3$·HCl·H_2O: C, 65.46; H, 7.96; N, 7.90. Found: C, 65.67; H, 7.89; N, 7.83.

4.1.2.3. 3-Isobutyl-9-(4-phenoxybenzyl)-1-propyl-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (2). The title compound was synthesized in 87% yield according to the same procedures as described for the preparation of **1a** using *n*-propylamine **42b** and *N*-Boc-*dl*-leucine **43b**. TLC R_f 0.61 ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (200 MHz, CD_3OD) δ 7.55 (m, 2H), 7.40 (m, 2H), 7.18 (m, 1H), 7.05 (m, 4H), 4.33 (s, 2H), 4.01 (dd, J = 7.6, 4.8 Hz, 1H), 3.79 (m, 2H), 3.60–3.30 (m, 4H), 2.46 (m, 2H), 2.17 (m, 2H), 1.95–1.40 (m, 5H), 0.94 (m, 9H); IR (KBr) 3439, 3220, 3066, 2959, 2872, 2663, 2561, 1672, 1590, 1509, 1489, 1418, 1370, 1330, 1241, 1200, 1172, 1072 cm^{-1}; MS (APCI, Pos) m/z 464 (M+H)$^+$; Elemental Anal. Calcd for $C_{28}H_{37}N_3O_3$·HCl·H_2O: C, 64.59; H, 7.74; N, 8.07. Found: C, 64.38; H, 7.67; N, 8.07.

4.1.2.4. 1,3-Diisobutyl-9-(4-phenoxybenzyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (3). The title compound was synthesized in 62% yield according to the same procedures as described for the preparation of **1a** using *i*-butylamine **42c** and *N*-Boc-*dl*-leucine **43b**. TLC R_f 0.50 ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.50 (d, J = 8.7 Hz, 2H), 7.39 (dd, J = 8.7, 7.5 Hz, 2H), 7.17 (t, J = 7.5 Hz, 1H), 7.13–7.04 (m, 4H), 4.28 (s, 2H), 4.04 (dd, J = 8.1, 4.2 Hz, 1H), 3.81–3.54 (m, 2H), 3.52–3.21 (m, 4H), 2.46–2.11 (m, 4H), 2.00–1.57 (m, 4H), 0.94 (d, J = 6.3 Hz, 6H), 0.90 (d, J = 6.3 Hz, 3H), 0.90 (d, J = 6.3 Hz, 3H); IR (KBr) 2958, 2516, 1676, 1590, 1510, 1489, 1410, 1242, 1199, 1172, 1097, 1071 cm^{-1}; MS (APCI, Pos) m/z 478 (M+H)$^+$; Elemental Anal. Calcd for $C_{29}H_{39}N_3O_3$·HCl: C, 67.75; H, 7.84; N, 8.17. Found: C, 67.35; H, 7.91; N, 8.06.

4.1.2.5. 1-(3-Hydroxybutyl)-3-isobutyl-9-(4-phenoxybenzyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (4). The title compound was synthesized in 31% yield according to the same procedures as described for the preparation of **1a** using 3-*t*-butyldimethylsilylhydroxybutylamine **42d** and *N*-Boc-*dl*-leucine **43b**. TLC R_f 0.49 ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (300 MHz, CD3OD) δ 7.54 (d, J = 8.5 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.18 (t, J = 7.5 Hz, 1H), 7.04 (m, 4H), 4.33 (s, 2H), 4.02 (m, 1H), 3.80 (m, 3H), 3.51 (m, 4H), 2.46 (m, 2H), 2.19 (m, 2H), 1.85–1.57 (m, 5H), 1.17 (d, J = 6.0 Hz, 3H), 0.94 (d, J = 9.0 Hz, 6H); IR (KBr) 3405, 2960, 1675, 1590, 1510, 1489, 1421, 1242, 1172, 1048 cm^{-1}; MS (APCI, Pos) m/z 494 (M+H)$^+$; HRMS Calcd 494.3019, Obsd 494.3025.

4.1.2.6. 1-[(2*E*)-2-Butenyl]-3-isobutyl-9-(4-phenoxybenzyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (5). The title compound was synthesized in 29% yield according to the same procedures as described for the preparation of **1a** using 2-butenylamine **42e** and *N*-Boc-*dl*-leucine **43b**. TLC R_f 0.32 ($CHCl_3$/MeOH, 20:1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.52 (d, J = 8.7 Hz, 2H), 7.44–7.35 (m, 2H), 7.22–7.14 (m, 1H), 7.06 (d, J = 8.7 Hz, 2H), 7.10–7.00 (m, 2H), 5.75–5.60 (m, 1H), 5.52–5.38 (m, 1H), 4.33 (s, 2H), 4.15–3.93 (m, 2H), 4.03 (dd, J = 7.8, 4.5 Hz, 1H), 3.88–3.66 (m, 2H), 3.55–3.42 (m, 2H), 2.52–2.35 (m, 2H), 2.28–2.08 (m, 2H), 1.90–1.57 (m, 3H), 1.65 (dd, J = 6.3, 1.5 Hz, 3H), 0.95 (d, J = 6.6 Hz, 3H), 0.94 (d, J = 6.6 Hz, 3H); IR (KBr) 2955, 2512, 1673, 1590, 1509, 1490, 1417, 1241 cm^{-1}; MS (APCI, Pos) m/z 476 (M+H)$^+$; Elemental Anal. Calcd for $C_{29}H_{37}N_3O_3$·HCl: C, 68.02; H, 7.48; N, 8.21.Found: C, 66.1; H, 7.51; N, 8.00.

4.1.2.7. 1-(2-Butynyl)-3-isobutyl-9-(4-phenoxybenzyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (6). The title compound was synthesized in 23% yield according to the same procedures as described for the preparation of **1a** using 2-butynylamine hydrochloride **42f** and *N*-Boc-*dl*-leucine **43b**. TLC R_f 0.70 ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.51 (d, J = 8.7 Hz, 2H), 7.39 (dd, J = 8.7, 7.2 Hz, 2H), 7.18 (t, J = 7.2 Hz, 1H), 7.09–7.00 (m, 4H), 4.33 (br s, 2H), 4.28–4.10 (m, 2H), 4.05

Table 8
In vitro pharmacokinetic data for **1a**, **1b**, **36**, **37**, and **40**

Compds	Caco-2 papp (×10^{-6} cm/s)		Solubility (μM)	*c*Log *P*[a]
	A to B	B to A		
1a	0.15	0.27	<5	7.66
1b	1.51	2.23	14	6.46
36	0.33	0.14	<5	6.99
37	3.51	3.91	6	6.38
40	11.0	16.8	29	5.13

[a] Calculated by ADMET predictor (ver. 4.0).

Table 9
Anti-HIV activity of representative compounds

Compds	Mean IC_{50} + SD (nM)	
	Anti-HIV-1 activity in p24 assay HV-1_{Ba-L} (R5)	CCR5 Ca assay
1b	160 ± 40	94
39	10	28
40	39 ± 34.4	13
Zidovudine[a]	7 ± 4	—
Nelfinavir[b]	12 ± 8	—

[a] Zidovudine is a reverse transcriptase inhibitor.
[b] Nerfinavir is a HIV-1 protease inhibitor.

(dd, J = 7.8, 4.5 Hz, 1H), 3.86–3.70 (m, 2H), 3.56–3.43 (m, 2H), 2.59–2.40 (m, 2H), 2.34–2.15 (m, 2H), 1.89–1.57 (m, 6H), 0.94 (d, J = 6.6 Hz, 3H), 0.93 (d, J = 6.6 Hz, 3H); IR (KBr) 3432, 2956, 1675, 1590, 1509, 1489, 1413, 1242, 1173 cm^{-1}; MS (APCI, Pos) m/z 474 $(M+H)^+$; HRMS Calcd 474.2757, Obsd 474.2753.

4.1.2.8. 1-Benzyl-3-isobutyl-9-(4-phenoxybenzyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (7). The title compound was synthesized in 50% yield according to the same procedures as described for the preparation of **1a** using benzylamine **42g** and *N*-Boc-*dl*-leucine **43b**. TLC R_f 0.66 ($CHCl_3$/MeOH, 10:1); ^1H NMR (200 MHz, CD_3OD) δ 7.50 (d, J = 8.4 Hz, 2H), 7.45–7.12 (m, 8H), 7.10–6.98 (m, 4H), 4.82 (m, 2H), 4.29 (s, 2H), 4.18 (dd, J = 8.0, 4.6 Hz, 1H), 3.73 (m, 2H), 3.42 (m, 2H), 2.65–2.30 (m, 2H), 2.20–2.05 (m, 2H), 2.00–1.60 (m, 3H), 0.98 (d, J = 6.2 Hz, 6H); IR (KBr) 3405, 3194, 3063, 2954, 2871, 2661, 2508, 2462, 1681, 1614, 1589, 1511, 1488, 1470, 1455, 1412, 1361, 1331, 1307, 1240, 1200, 1176, 1155, 1130, 1069 cm^{-1}; MS (MALDI, Pos) m/z 512 $(M+H)^+$; Elemental Anal. Calcd for $C_{32}H_{37}N_3O_3{\cdot}Cl{\cdot}H$: C, 70.12; H, 6.99; N, 7.67. Found: C, 69.64; H, 7.03; N, 7.63.

4.1.2.9. (3*S*)-1-Butyl-3-isobutyl-9-(4-phenoxybenzyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (8). The title compound was synthesized in 13% yield according to the same procedures as described for the preparation of **1a** using *N*-Boc-*l*-leucine **43c**. $[\alpha]_D^{22}$ −2.11 (*c* 0.95, MeOH);TLC R_f 0.29 ($CHCl_3$/MeOH, 10:1); ^1H NMR (300 MHz, CD_3OD) δ 7.54 (d, J = 8.7 Hz, 2H), 7.42–7.36 (m, 2H), 7.18 (m, 1H), 7.05 (d, J = 8.7 Hz, 2H), 7.05–7.02 (m, 2H), 4.33 (s, 2H), 3.98 (dd, J = 8.1, 4.5 Hz, 1H), 3.86–3.72 (m, 2H), 3.53–3.37 (m, 4H), 2.47–2.36 (m, 2H), 2.24–2.12 (m, 2H), 1.80–1.30 (m, 7H), 0.95 (t, J = 7.2 Hz, 3H), 0.95 (d, J = 6.3 Hz, 3H), 0.93 (d, J = 6.3 Hz, 3H); IR (KBr) 3445, 2956, 2565, 1676, 1590, 1509, 1489, 1418, 1329, 1242, 1172, 1073, 1049 cm^{-1}; MS (APCI, Pos) m/z 478 $(M+H)^+$; HRMS Calcd 478.307, Obsd 478.3069.

4.1.2.10. (3*R*)-1-Butyl-3-isobutyl-9-(4-phenoxybenzyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (9). The title compound was synthesized in 14% yield according to the same procedures as described for the preparation of **1a** using *N*-Boc-*d*-leucine **43d**. $[\alpha]_D^{22}$ +2.25 (*c* 1.15, MeOH); TLC R_f 0.29 ($CHCl_3$/MeOH, 10:1); ^1H NMR (300 MHz, CD_3OD) δ 7.54 (d, J = 8.7 Hz, 2H), 7.42–7.36 (m, 2H), 7.18 (m, 1H), 7.05 (d, J = 8.7 Hz, 2H), 7.05–7.02 (m, 2H), 4.32 (s, 2H), 4.01 (dd, J = 7.8, 4.8 Hz, 1H), 3.85–3.72 (m, 2H), 3.50–3.39 (m, 4H), 2.52–2.38 (m, 2H), 2.24–2.11 (m, 2H), 1.84–1.20 (m, 7H), 0.95 (t, J = 7.2 Hz, 3H), 0.95 (d, J = 6.3 Hz, 3H), 0.93 (d, J = 6.3 Hz, 3H); IR (KBr) 3444, 2957, 2565, 1676, 1590, 1509, 1489, 1418, 1242, 1172 cm^{-1}; MS (APCI, Pos) m/z 478 $(M+H)^+$; HRMS Calcd 478.307, Obsd 478.3069.

4.1.2.11. (3*S*)-1-Butyl-3-neopentyl-9-(4-phenoxybenzyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (10). The title compound was synthesized in 51% yield according to the same procedures as described for the preparation of **1a** using *N*-Boc-*dl*-*t*-butylalanine **43e**. TLC R_f 0.52 ($CHCl_3$/MeOH, 20:1); ^1H NMR (300 MHz, CD_3OD) δ 7.52 (d, J = 9.0 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.18 (t, J = 7.5 Hz, 1H), 7.04 (m, 4H), 4.33 (s, 2H), 4.01 (dd, J = 7.2, 3.3 Hz, 1H), 3.82 (m, 1H), 3.71 (m, 1H), 3.50 (m, 2H), 3.43 (m, 2H), 2.38 (m, 2H), 2.24 (m, 2H), 2.00 (dd, J = 14.0, 3.3 Hz, 1H), 1.55 (dd, J = 14.0, 7.2 Hz, 1H), 1.50 (m, 2H), 1.36 (m, 2H), 0.99 (s, 9H), 0.95 (t, J = 7.0 Hz, 3H); IR (KBr) 2957, 2506, 1678, 1590, 1510, 1489, 1419, 1370, 1285, 1243, 1174, 1115 cm^{-1}; MS (APCI, Pos) m/z 492 $(M+H)^+$; HRMS Calcd 492.3226, Obsd 492.3226.

4.1.2.12. 1-Butyl-9-(4-phenoxybenzyl)-3-propyl-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (11). The title compound was synthesized in 52% yield according to the same procedures as described for the preparation of **1a** using *N*-Boc-*dl*-norvaline **43f**. TLC R_f 0.36 ($CHCl_3$/MeOH, 10:1); ^1H NMR (300 MHz, CD_3OD) δ 7.51 (d, J = 8.7 Hz, 2H), 7.39 (dd, J = 8.7, 7.5 Hz, 2H), 7.18 (t, J = 7.5 Hz, 1H), 7.10–7.00 (m, 4H), 4.33 (s, 2H), 4.04 (dd, J = 5.7, 4.5 Hz, 1H), 3.93–3.66 (m, 2H), 3.55–3.31 (m, 4H), 2.47–2.09 (m, 4H), 1.92–1.68 (m, 2H), 1.61–1.21 (m, 6H), 1.01–0.90 (m, 6H); IR (KBr) 3436, 2958, 2872, 2550, 1673, 1590, 1509, 1489, 1419, 1243, 1172, 1071, 954, 873, 787, 693 cm^{-1}; MS (APCI, Pos) m/z 464 $(M+H)^+$; HRMS Calcd 464.2913, Obsd 464.2915.

4.1.2.13. 1-Butyl-3-(cyclopentylmethyl)-9-(4-phenoxybenzyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (12). The title compound was synthesized in 53% yield according to the same procedures as described for the preparation of **1a** using *N*-Boc-cyclopnetyl-*dl*-alanine **43g**. TLC R_f 0.66 ($CHCl_3$/MeOH, 10:1); ^1H NMR (300 MHz, CD_3OD) δ 7.52 (d, J = 8.5 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.18 (t, J = 7.5 Hz, 1H), 7.05 (m, 4H), 4.34 (s, 2H), 4.00 (t, J = 6.0 Hz, 1H), 3.82 (m, 2H), 3.49 (m, 2H), 3.39 (m, 2H), 2.37 (m, 2H), 2.17 (m, 2H), 1.96 (m, 1H), 1.81 (m, 4H), 1.58 (m, 6H), 1.38 (m, 2H), 1.17 (m, 2H), 0.95 (t, J = 7.0 Hz, 3H); IR (KBr) 3433, 3199, 2953, 2870, 2499, 1681, 1589, 1510, 1488, 1419, 1373, 1336, 1240, 1199, 1174, 1114, 1070, 1049 cm^{-1}; MS (APCI, Pos) m/z 504 $(M+H)^+$; HRMS Calcd 504.3226, Obsd 504.3231.

4.1.2.14. 1-Butyl-3-(methoxymethyl)-9-(4-phenoxybenzyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (13). The title compound was synthesized in 59% yield according to the same procedures as described for the preparation of **1a** using *N*-Boc-*O*-methyl-*dl*-serine **43h**. TLC R_f 0.48 ($CHCl_3$/MeOH, 10:1); ^1H NMR (300 MHz, CD_3OD) δ 7.51 (d, J = 8.7 Hz, 2H), 7.39 (dd, J = 8.7, 7.2 Hz, 2H), 7.17 (t, J = 7.2 Hz, 1H), 7.09–6.99 (m, 4H), 4.30 (s, 2H), 4.07 (t, J = 3.0 Hz, 1H), 3.91 (m, 1H), 3.77 (dd, J = 9.0, 3.0 Hz, 1H), 3.67 (m, 1H), 3.58–3.39 (m, 4H), 3.31 (s, 3H), 3.26 (m, 1H), 2.48–2.13 (m, 4H), 1.65 (m, 1H), 1.53–1.28 (m, 3H), 0.95 (t, J = 7.5 Hz, 3H); IR (KBr) 3424, 2931, 2551, 1662, 1590, 1509, 1489, 1423, 1371, 1242, 1198, 1116, 1075 cm^{-1}; MS (APCI, Pos) m/z 466 $(M+H)^+$; Elemental Anal. Calcd for $C_{27}H_{35}N_3O_4{\cdot}Cl{\cdot}H$: C, 64.59; H, 7.23; N, 8.37. Found: C, 63.45; H, 7.12; N, 8.21.

4.1.2.15. Benzyl [1-butyl-2,5-dioxo-9-(4-phenoxybenzyl)-1,4,9-triazaspiro[5.5]undec-3-yl]acetate hydrochloride (15). The title compound was synthesized in 36% yield according to the same procedures as described for the preparation of **1a** using *N*-Boc-*dl*-aspartic acid β-benzyl ester **43i**. TLC R_f 0.74 ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, CD_3OD) δ 7.52 (d, J = 7.0 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.33 (m, 5H), 7.18 (t, J = 7.5 Hz, 1H), 7.05 (m, 4H), 5.12 (s, 2H), 4.33 (s, 2H), 4.31 (m, 1H), 3.88 (m, 1H), 3.66 (m, 1H), 3.50–3.35 (m, 4H), 3.08 (dd, J = 17.7, 4.8 Hz, 1H), 2.86 (dd, J = 17.7, 3.0 Hz, 1H), 2.34 (m, 2H), 2.25 (m, 2H), 1.50 (m, 2H), 1.36 (m, 2H), 0.94 (t, J = 7.5 Hz, 3H); IR (KBr) 3735, 3412, 1736, 1675, 1489, 1423, 1242, 1175 cm^{-1}; MS (APCI, Pos) m/z 570 $(M+H)^+$; HRMS Calcd 570.2968, Obsd 570.297.

4.1.2.16. 1-Butyl-3-(3-cyclohexylpropyl)-9-(4-phenoxybenzyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (16). The title compound was synthesized in 39% yield according to the same procedures as described for the preparation of **1a** using *N*-Boc-*dl*-3-cyclohexylpropylalanine **43j**. TLC R_f 0.76 ($CHCl_3$/MeOH, 10:1); ^1H NMR (300 MHz, CD_3OD) δ 7.53–7.49 (m, 2H), 7.42–7.36 (m, 2H), 7.18 (m, 1H), 7.10–7.02 (m, 4H), 4.32 (s, 2H), 4.04 (t, J = 4.8 Hz, 1H), 3.87 (m, 1H), 3.71 (m, 1H), 3.56–3.40 (m, 3H), 3.35 (m, 1H), 2.48–2.12 (m, 4H), 1.86–1.10 (m, 19H), 0.95 (t, J = 7.5 Hz, 3H), 0.95 (m, 2H); IR (KBr) 2923, 2564, 1679, 1591, 1510, 1490, 1245, 1172 cm^{-1}; MS (APCI, Pos) m/z 546 $(M+H)^+$; HRMS Calcd 546.3696, Obsd 546.3703.

4.1.2.17. 9-Benzyl-1-butyl-3-isobutyl-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (17). The title compound was synthesized in 59% yield according to the same procedures as described for the preparation of **1a** using *N*-Boc-*dl*-leucine **43b** and benzaldehyde for 4-phenoxybenzaldehyde. TLC R_f 0.54 ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.64–7.44 (m, 5H), 4.36 (s, 2H), 4.01 (dd, J = 7.8, 4.8 Hz, H), 3.77 (m, 2H), 3.55–3.35 (m, 4H), 2.60–2.30 (m, 2H), 2.17 (m, 2H), 1.95–1.75 (m, 1H), 1.75–1.60 (m, 2H), 1.60–1.45 (m, 2H), 1.45–1.20 (m, 2H), 1.10–0.80 (m, 9H); IR (KBr) 3435, 3230, 2957, 2871, 2505, 2454, 1680, 1647, 1459, 1413, 1370, 1326, 1147 cm^{-1}; MS (MALDI, Pos) *m/z* 386 (M+H)$^+$, 91; Elemental Anal. Calcd for $C_{23}H_{35}N_3O_2$·HCl: C, 65.46; H, 8.6; N, 9.96. Found: C, 65.09; H, 8.63; N, 9.88.

4.1.2.18. 1-Butyl-3-(cyclohexylmethyl)-9-(4-methoxybenzyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dionehydrochloride (18). The title compound was synthesized in 59% yield according to the same procedures as described for the preparation of **1a** using 4-methoxybenzaldehyde for 4-phenoxybenzaldehyde. TLC R_f 0.63 ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (200 MHz, CD_3OD) δ 7.47 (d, J = 8.8 Hz, 2H), 7.03 (d, J = 8.8 Hz, 2H), 4.29 (s, 2H), 4.04 (dd, J = 7.6, 4.8 Hz, 1H), 3.83 (s, 3H), 3.74 (m, 2H), 3.55–3.35 (m, 4H), 2.41 (m, 2H), 2.15 (m, 2H), 1.85–1.55 (m, 7H), 1.55–1.42 (m, 3H), 1.42–1.30 (m, 3H), 1.30–1.10 (m, 2H), 1.08–0.80 (m, 5H); IR (KBr) 3436, 3221, 2926, 2851, 2666, 2560, 2362, 1672, 1613, 1585, 1517, 1448, 1419, 1373, 1305, 1255, 1182, 1031 cm^{-1}; MS (FAB, Pos) *m/z* 456 (M+H)$^+$, 121; Elemental Anal. Calcd for $C_{27}H_{41}N_3O_3$·HCl·1.5H_2O: C, 62.47; H, 8.74; N, 8.09. Found: C, 62.59; H, 8.35; N, 7.90.

4.1.2.19. 1-Butyl-3-(cyclohexylmethyl)-9-[(6-phenoxypyridin-3-yl)methyl]-1,4,9-triazaspiro[5.5]undecane-2,5-dione dihydrochloride (23). The title compound was synthesized in 15% yield according to the same procedures as described for **1a** using 6-phenoxynicotinaldehyde for 4-phenoxybenzaldehyde. TLC R_f 0.67 ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.31 (s, 1H), 8.07 (d, J = 8.3 Hz, 1H), 7.44 (t, J = 7.5 Hz, 2H), 7.26 (t, J = 7.5 Hz, 1H), 7.14 (d, J = 7.5 Hz, 2H), 7.06 (d, J = 8.3 Hz, 1H), 4.39 (s, 2H), 4.04 (dd, J = 7.8, 4.6 Hz, 1H), 3.90–3. 76 (m, 2H), 3.52–3.38 (m, 4H), 2.58–2.36 (m, 2H), 2.25–2.11 (m, 2H), 1.80–1.42 (m, 10H), 1.42–1.17 (m, 5H), 1.05–0.85 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H); IR (KBr) 3449, 2926, 2564, 1671, 1479, 1260, 1200 cm^{-1}; MS (APCI, Pos) *m/z* 519 (M+H)$^+$; HRMS Calcd 519.3335, Obsd 519.3337.

4.1.2.20. 1-Butyl-3-(cyclohexylmethyl)-9-[(3,5-dimethyl-1-phenyl-1*H*-pyrazol-4-yl)methyl]-1,4,9-triazaspiro[5.5]undecane-2,5-dione dihydrochloride (25). The title compound was synthesized in 33% yield according to the same procedures as described for the preparation of **1a** using 3,5-dimethyl-1-phenylpyrazole-4-carboxaldehyde for 4-phenoxybenzaldehyde. TLC R_f 0.35 ($CHCl_3$/MeOH, 20:1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.63–7.48 (m, 5H), 4.33 (s, 2H), 4.05 (dd, J = 7.8, 4.5 Hz, 1H), 3.95–3.74 (m, 2H), 3.67–3.56 (m, 2H), 3.48 (m, 2H), 2.72–2.58 (m, 2H), 2.45 (s, 3H), 2.41 (s, 3H), 2.30–2.07 (m, 2H), 1.84–1.10 (m, 15 H), 1.02–0.92 (m, 2H), 0.96 (t, J = 7.2 Hz, 3H); IR (KBr) 3426, 2926, 1670, 1421 cm^{-1}; MS (APCI, Pos) *m/z* 520 (M+H)$^+$; HRMS Calcd 520.3652, Obsd 520.3651.

4.1.2.21. 1-Butyl-3-(cyclohexylmethyl)-9-[(2-phenyl-1*H*-imidazol-4-yl)methyl]-1,4,9-triazaspiro[5.5]undecane-2,5-dione dihydrochloride (26). The title compound was synthesized in 77% yield according to the same procedures as described for the preparation of **1a** using 2-phenyl-1*H*-imidazole-4-carboxaldehyde for 4-phenoxybenzaldehyde. TLC R_f 0.25 ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (200 MHz, CD_3OD) δ 8.04–7.92 (m, 3H), 7.74–7.62 (m, 3H), 4.58 (s, 2H), 4.05 (dd, J = 7.4, 4.8 Hz, 1H), 3.88 (m, 2H), 3.65 (m, 2H), 3.50 (m, 2H), 2.68 (m, 2H), 2.19 (m, 2H), 1.90–1.60 (m, 6H), 1.60–1.45 (m, 3H), 1.45–1.30 (m, 3H), 1.30–1.10 (m, 3H), 1.10–0.80 (m, 5H); IR (KBr) 3410, 2927, 2854, 2699, 2574, 1781, 1671, 1644, 1554, 1448, 1421, 1373, 1349, 1308, 1257, 1178, 1096, 1052, 1001 cm^{-1}; MS (FAB, Pos) *m/z* 492 (M+H)$^+$, 336; Elemental Anal. Calcd for $C_{29}H_{41}N_5O_2$·2HCl: C, 61.69; H, 7.68; N, 12.4. Found: C, 55.71; H, 7.32; N, 10.71.

4.1.2.22. 1-Butyl-3-(cyclohexylmethyl)-9-[(2-phenyl-1,3-thiazol-4-yl)methyl]-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (28). The title compound was synthesized in 51% yield according to the same procedures as described for the preparation of **1a** using 2-phenyl-1,3-thiazole-4-carboxaldehyde for 4-(3-pyridyl)benzaldehyde. TLC R_f 0.62 ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.03–8.00 (m, 2H), 7.87 (s, 1H), 7.52–7.49 (m, 3H), 4.54 (s, 2H), 4.04 (dd, J = 7.6, 4.8 Hz, 1H), 4.04–3.87 (m, 2H), 3.70–3.58 (m, 2H), 3.51–3.39 (m, 2H), 2.58–2.38 (m, 2H), 2.26–2.13 (m, 2H), 1.7 8–1.43 (m, 9H), 1.40–1.15 (m, 6H), 1.10–0.90 (m, 5H); IR (KBr) 3426, 3298, 3190, 3128, 3086, 2991, 2955, 2927, 2854, 2653, 2512, 2452, 1678, 1645, 1481, 1462, 1450, 1415, 1371, 1351, 1336, 1314, 1146, 1003 cm^{-1}; MS (APCI, Pos) *m/z* 509 (M+H)$^+$; HRMS Calcd 509.295, Obsd 509.295.

4.1.3. [1-Butyl-2,5-dioxo-9-(4-phenoxybenzyl)-1,4,9-triazaspiro[5.5]undec-3-yl]acetic acid hydrochloride (14)

To a stirred solution of the compound **15** (173 mg, 0.303 mmol) in MeOH (5 mL) was added 2 M NaOH (2 mL). After being stirred for 3 h at room temperature, the reaction mixture was acidified with 2 M HCl and then extracted with AcOEt. The organic layer was washed with water, brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was triturated with Et_2O to yield the title compound (127 mg, 56%) as a white powder. TLC R_f 0.51 ($CHCl_3$/MeOH/AcOH, 20:4:1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.55–7.53 (m, 2H), 7.42–7.36 (m, 2H), 7.20–7.15 (m, 1H), 7.07–7.02 (m, 4H), 4.33 (s, 2H), 4.27 (t, J = 4.5 Hz, 1H), 3.96–3.90 (m, 1H), 3.72–3.66 (m, 1H), 3.54–3.38 (m, 4H), 2.97 (dd, J = 18.0, 4.8 Hz, 1H), 2.79 (dd, J = 18.0, 4.8 Hz, 1H), 2.50–2.36 (m, 3H), 2.27–2.16 (m, 1H), 1.62–1.48 (m, 2H), 1.41–1.30 (m, 2H), 0.94 (t, J = 7.2 Hz, 3H); IR (KBr) 3485, 3214, 2960, 2717, 2579, 1664, 1589, 1419, 1241, 1076, 1051, 1005 cm^{-1}; MS (APCI, Neg) *m/z* 478 (M-H)$^-$; HRMS Calcd 480.2498, Obsd 480.2499.

4.1.4. Benzyl 1-butyl-3-(cyclohexylmethyl)-2,5-dioxo-1,4,9-triazaspiro[5.5]undecane-9-carboxylate (21)

To a suspension of the Rink-isonitrile resin **44** (0.75 mmol/g, 10.0 g, 7.5 mmol) resin in THF/MeOH (1:1, 200 mL) were added *N*-benzyloxycarbonyl-4-piperidone **41b** (5.24 g, 22.5 mmol), *n*-butylamine **42a** (2.22 mL, 22.5 mmol) and *N*-Boc-*dl*-cyclohexylalanine **43a** (6.50 g, 22.5 mmol). The mixture was shaken for 16 h at 65 °C. After cooling to room temperature, the resin was collected by filtration and successively washed with THF (80 mL × 3), MeOH (80 mL × 3) and then CH_2Cl_2 (80 mL × 3). The resin was treated with 50% trifluoroacetic in CH_2Cl_2 (100 mL). The mixture was shaken for 30 min at room temperature. The resin was collected by filtration and successively washed with CH_2Cl_2 (70 mL × 4) and toluene (100 mL). The resin was suspended in 1.25 M acetic acid in toluene (100 mL). The suspension was shaken for 18 h at 90 °C. After cooling to room temperature, the resin was collected by filtration and washed with $CHCl_3$/MeOH (1:1, 100 mL × 3). The combined filtrate and washings were evaporated. The resulting residue was purified by column chromatography on silica gel with a gradient of $CHCl_3$/MeOH (40:1) to afford the title compound **21** (209 mg, 5.9% yield) as a white powder. TLC R_f 0.46 ($CHCl_3$/MeOH, 20:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.29 (m, 5H), 5.98 (br s, 1H), 5.15 (s, 2H), 4.14 (br s, 2H), 4.00 (m, 1H), 3.65 (br s, 1H), 3.43 (br s, 1H), 3.26 (m, 2H), 2.03–1.81 (m, 4H), 1.80–1.60 (m, 5H), 1.60–1.10 (m, 10H), 1.10–0.85 (m, 5H); IR (KBr) 3449, 2925,

2852, 1675, 1418, 1352, 1281, 1239, 1179, 1153, 1105, 1018 cm^{-1}; MS (APCI, Pos) *m/z* 470 $(M+H)^+$.

4.1.5. 1-Butyl-3-isobutyl-9-(6-phenylhexyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (22)

The Rink-isonitrile resin (0.45 mmol/g, 500 mg, 0.225 mmol) was washed with THF/MeOH (1:1) (4 mL × 2). To a suspension of the resin in THF/MeOH (1:1) (4 mL) were successively added 1-(6-phenylhexyl)-4-piperidone **41c** (292 mg, 1.125 mmol), *n*-butylamine **42a** (82 mg, 1.125 mmol), and *N*-Boc-*dl*-leucine **43b** (260 mg, 1.125 mmol). The mixture was shaken for 16 h at 65 °C. After cooling to room temperature, the mixture was filtrated. The collected resin was washed with THF/MeOH (1:1) (4 mL × 3), and CH_2Cl_2 (4 mL × 3). The resin was then added 25% TFA in CH_2Cl_2 (4 mL) at 0 °C. The mixture was allowed up to room temperature, and then stirred for 30 min. After filtration, the resin was washed with CH_2Cl_2 (4 mL × 3), toluene (4 mL × 3), and 1.25 M acetic acid in toluene (4 mL). The suspension of the resin in 1.25 M acetic acid in toluene was agitated for 24 h at 90 °C. After cooling to room temperature, the mixture was filtrated. The resin was washed with $CHCl_3$/MeOH (1:1) (4 mL × 2). The filtrate and washings were concentrated under reduced pressure. The residue was purified by column chromatography over silica gel with a gradient of AcOEt/MeOH from 1:0 to 10:1 to give the title compound (35% yield) as a white powder. TLC R_f 0.62 ($CHCl_3$/MeOH, 10:1); ^1H NMR (200 MHz, CD_3OD) δ 7.30–7.06 (m, 5H), 4.02 (dd, J = 7.8, 4.8 Hz, 1H), 3.70 (m, 2H), 3.56 (m, 2H), 3.43 (m, 2H), 3.11 (m, 2H), 2.63 (t, J = 7.8 Hz, 2H), 2.46 (m, 2H), 2.18 (m, 2H), 1.95–1.50 (m, 9H), 1.50–1.25 (m, 6H), 0.97 (m, 9H); IR (KBr) 3447, 3199, 2934, 2869, 2663, 2502, 2440, 1673, 1455, 1418, 1372, 1329, 1152, 1086, 1003 cm^{-1}; MS (MALDI, Pos) *m/z* 456 $(M+H)^+$; Elemental Anal. Calcd for $C_{28}H_{45}N_3O_2 \cdot HCl \cdot 0.4H_2O$: C, 67.35; H, 9.45; N, 8.41. Found: C, 67.67; H, 9.39; N, 8.42.

4.1.6. A typical procedure for the solution phase Ugi four-component condensation

4.1.6.1. (3*S*)-9-Benzyl-1-butyl-3-cyclohexylmethyl-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (52b). To a stirred solution of 1-benzyl-4-piperidone **41d** (49 g, 260 mmol), *n*-butylamine **42a** (19 g, 260 mmol) and *N*-Boc-*l*-cyclohexylalanine **43k** (80 g, 260 mmol) in MeOH (1.3 L) was added 2-(4-morpholinyl)-ethylisonitrile **50** (36 g, 260 mmol). After being stirred at 55 °C overnight, the reaction mixture was treated with concd HCl (260 L) with cooling. The reaction mixture was stirred at 55 °C for another 2 h, evaporated, treated with Na_2CO_3 and extracted with AcOEt. The combined organic layers were washed with brine, dried (Na_2SO_4) and evaporated to give deprotected Ugi product as a yellow oil, which was dissolved in AcOH/toluene (1.25 M, 1.3 L) and stirred at 80 °C for 1 h. The reaction mixture was cooled to room temperature, diluted with AcOEt and washed twice with a small amount of water. The organic layer was washed with aqueous $NaHCO_3$, brine, dried (Na_2SO_4) and evaporated to afford *N*-benzylpiperidinodiketopiperazine **51b** as an oil (91.3 g). This compound was used to next step without further purification. Debenzylation was carried out by the catalytic hydrogenation at an atmospheric pressure of the resulting oily product **51b** (91.3 g, 210 mmol) in EtOH (1.2 L) in the presence of 20% $Pd(OH)_2/C$ (15 g) for 3 h at 50 °C. Catalyst was removed by filtration through a pad of Celite. The filtrate was treated with 4 N HCl in AcOEt (130 mL) and evaporated. The resulting powder was washed with *t*-butyl methyl ether to afford **52b** as a white powder (70 g, 89% yield in four steps). $[\alpha]_D^{18}$ −37.5 (*c* 1.04, MeOH); TLC R_f 0.08 ($CHCl_3$/MeOH/AcOH, 9:1:0.1); ^1H NMR (CD_3OD) δ 4.05 (dd, J = 7.8, 4.8 Hz, 1H), 3.84–3.68 (m, 2H), 3.46–3.34 (m, 4H), 2.40–2.04 (m, 4H), 1.83–1.46 (m, 10H), 1.39 (sextet, J = 7.5 Hz, 2H), 1.33–1.15 (m, 3H), 1.05–0.86 (m, 2H), 0.97 (t, J = 7.2 Hz, 3H).

4.1.6.2. (3*RS*)-9-Benzyl-1-butyl-3-cyclohexylmethyl-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (52a). Compound **52a** was prepared from *N*-Boc-*dl*-cyclohexylalanine **43a** according to the same procedure as described for the preparation of **52b.**

TLC R_f 0.65 ($CHCl_3$/MeOH/NH_3 aq, 20:5:1); ^1H NMR (CD_3OD) δ 4.00 (dd, J = 7.8, 4.5 Hz, 1H), 3.46–3.24 (m, 4H), 3.03–2.92 (m, 4H), 2.08–1.08 (m, 19H), 1.05–0.84 (m, 5H).

4.1.7. General procedure for the preparation of 1-butyl-3-(cyclohexylmethyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione 9-*N*-aryl analogs

4.1.7.1. (3*S*)-1-Butyl-3-(cyclohexylmethyl)-9-[(6-phenylpyridin-3-yl)methyl]-1,4,9-triazaspiro[5.5]undecane-2,5-dione dihydrochloride (24). To a stirred solution of **52b** (100 mg, 0.27 mol), 4-(3-pyridyl)benzaldehyde (59 mg, 0.32 mmol) and 1 drop of acetic acid in DMF (2 mL) was added sodium triacetoxyborohydride (114 mg, 0.54 mmol). After being stirred overnight, the reaction mixture was evaporated. The resulting residue was purified by column chromatography on silica gel (AcOEt/MeOH from 1:0 to 10:1) and treated with 4 N HCl in AcOEt (2 mL) to give the title compound in 50% yield. TLC R_f 0.50 ($CHCl_3$/MeOH, 10:1); ^1H NMR (300 MHz, CD_3OD) δ 9.14 (m, 1H), 8.75 (m, 1H), 8.36 (m, 1H), 8.02–7.99 (m, 2H), 7.68–7.62 (m, 3H), 4.63 (s, 2H), 4.05 (dd, J = 7.5, 4.5 Hz, 1H), 4.02–3.94 (m, 2H), 3.64–3.42 (m, 4H), 2.72–2.56 (m, 2H), 2.25–2.06 (m, 2H), 1.80–1.10 (m, 15H), 1.00–0.86 (m, 5H); IR (KBr) 3408, 3017, 2925, 2852, 2648, 2494, 2426, 1681, 1666, 1636, 1605, 1452, 1427, 1387, 1374, 1347, 1331, 1314, 1276 cm^{-1}; MS (APCI, Pos) *m/z* 503 $(M+H)^+$; HRMS Calcd 503.3386, Obsd 503.3394.

4.1.7.2. (3*S*)-1-Butyl-3-(cyclohexylmethyl)-9-[(5-methyl-2-phenyl-1,3-oxazol-4-yl)methyl]-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (27). The title compound was synthesized in 76% yield according to the same procedures as described for the preparation of **24** using 5-methyl-2-phenyl-1,3-oxazole-4-carboxaldehyde for 4-(3-pyridyl)benzaldehyde. TLC R_f 0.48 ($CHCl_3$/MeOH, 10:1); ^1H NMR (300 MHz, CD_3OD) δ 8.04–8.00 (m, 2H), 7.51–7.49 (m, 3H), 4.34 (s, 2H), 4.04 (dd, J = 7.8, 4.8 Hz, 1H), 3.98–3.82 (m, 2H), 3.70–3.60 (m, 2H), 3.44–3.38 (m, 2H), 2.52 (s, 3H), 2.50–2.36 (m, 2H), 2.28–2.12 (m, 2H), 1.80–1.12 (m, 15H), 1.00–0.86 (m, 5H); IR (KBr) 3407, 3182, 3131, 3084, 2956, 2926, 2855, 2658, 2554, 2443, 1681, 1664, 1652, 1559, 1485, 1471, 1450, 1413, 1372, 1337, 1313, 1287, 1146, 1096, 1082, 1068, 1050 cm^{-1}; MS (APCI, Pos) *m/z* 507 $(M+H)^+$; Elemental Anal. Calcd for $C_{30}H_{42}N_4O_3 \cdot HCl$: C, 66.34; H, 7.98; N, 10.32. Found: C, 66.06; H, 8.17; N, 10.05.

4.1.7.3. (3*S*)-1-Butyl-3-(cyclohexylmethyl)-9-[4-(phenylsulfanyl)benzyl]-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (29). The title compound was synthesized in 49% yield according to the same procedures as described for the preparation of **24** using 4-phenylsulfanyl-benzaldehyde for 4-(3-pyridyl)benzaldehyde. TLC R_f 0.74 ($CHCl_3$/MeOH, 10:1); ^1H NMR (300 MHz, CD_3OD) δ 7.50–7.37 (m, 7H), 7.29 (d, J = 8.4 Hz, 2H), 4.31 (s, 2H), 4.03 (dd, J = 7.5, 7.8 Hz, 1H), 3.84–3.70 (m, 2H), 3.50–3.32 (m, 4H), 2.56–2.38 (m, 2H), 2.24–2.05 (m, 2H), 1.81–1.06 (m, 15H), 1.02–0.84 (m, 5H); IR (KBr) 3425, 3207, 3073, 2957, 2924, 2872, 2850, 2656, 2549, 2436, 1679, 1647, 1601, 1582, 1494, 1472, 1445, 1419, 1373, 1340, 1333, 1312, 1288, 1270, 1146, 1116, 1096, 1081, 1049 cm^{-1}; MS (APCI, Pos) *m/z* 534 $(M+H)^+$; HRMS Calcd 534.3154, Obsd 534.3159.

4.1.7.4. (3*S*)-1-Butyl-3-(cyclohexylmethyl)-9-[4-(phenylsulfonyl)benzyl]-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (30). The title compound was synthesized in 57% yield according

to the same procedures as described for the preparation of **24** using 4-(benzenesulfonyl)benzaldehyde for 4-(3-pyridyl)benzaldehyde. TLC R_f 0.77 (AcOEt/MeOH, 9:1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.08 (d, J = 8.4 Hz, 2H), 8.02–7.96 (m, 2H), 7.80 (d, J = 8.4 Hz, 2H), 7.70–7.55 (m, 3H), 4.43 (s, 2H), 4.02 (dd, J = 7.8, 4.8 Hz, 1H), 3.89–3.73 (m, 2H), 3.49–3.34 (m, 4H), 2.48–2.33 (m, 2H), 2.23–2.04 (m, 2H), 1.82–1.14 (m, 15H), 1.03–0.85 (m, 5H); IR (KBr) 3362, 3202, 3065, 2925, 2851, 2516, 2420, 1672, 1476, 1469, 1447, 1416, 1372, 1346, 1309, 1156, 1106, 1071 cm^{-1}; MS (APCI, Pos) m/z 566 (M+H)$^+$; HRMS Calcd 566.3053, Obsd 566.3055.

4.1.7.5. (3*S*)-9-(4-Benzoylbenzyl)-1-butyl-3-(cyclohexylmethyl)-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (31). The title compound was synthesized in 43% yield according to the same procedures as described for the preparation of **24** using 4-benzoylbenzaldehyde for 4-(3-pyridyl)benzaldehyde. TLC R_f 0.68 ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.87 (d, J = 8.4 Hz, 2H), 7.82–7.74 (m, 4H), 7.67 (t, J = 8.4 Hz, 1H), 7.57–7.51 (m, 2H), 4.48 (s, 2H), 4.04 (dd, J = 7.8, 4.8 Hz, 1H), 3.84–3.78 (m, 2H), 3.58–3.38 (m, 4H), 2.58–2.40 (m, 2H), 2.30–2.10 (m, 2H), 1.82–1.14 (m, 15H), 1.02–0.86 (m, 5H); IR (KBr) 3434, 3370, 3209, 3061, 2925, 2851, 2659, 2517, 2422, 1660, 1612, 1598, 1577, 1469, 1447, 1418, 1372, 1347, 1317, 1278, 1179, 1148, 1115, 1097, 1074 cm^{-1}; MS (APCI, Pos) m/z 530 (M+H)$^+$; HRMS Calcd 530.3383, Obsd 530.3375.

4.1.7.6. 4-{[(3*S*)-1-Butyl-3-(cyclohexylmethyl)-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl]methyl}-*N*-phenylbenzamide hydrochloride (32). The title compound was synthesized in 38% yield according to the same procedures as described for the preparation of **24** using 4-(phenylcarbamoyl)benzaldehyde for 4-(3-pyridyl)benzaldehyde. TLC R_f 0.25 ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.07 (d, J = 8.1 Hz, 2H), 7.73–7.67 (m, 2H), 7.71 (d, J = 8.1 Hz, 2H), 7.38 (t, J = 7.5 Hz, 2H), 7.17 (t, J = 7.5 Hz, 1H), 4.45 (s, 2H), 4.05 (dd, J = 7.8, 4.8 Hz, 1H), 3.92–3.72 (m, 2H), 3.58–3.36 (m, 4H), 2.50–2.08 (m, 4H), 1.84–1.08 (m, 15H), 0.96 (t, J = 7.8 Hz, 3H), 0.96 (m, 2H); IR (KBr) 3362, 3250, 3061, 2925, 2851, 2662, 2552, 2424, 1669, 1599, 1575, 1540, 1509, 1498, 1493, 1469, 1442, 1420, 1372, 1321, 1261, 1147, 1113, 1098, 1076 cm^{-1}; MS (APCI, Pos) m/z 545 (M+H)$^+$; HRMS Calcd 545.3492, Obsd 545.3499.

4.1.7.7. (3*S*)-1-Butyl-3-(cyclohexylmethyl)-9-[4-(4-fluorophenoxy)benzyl]-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (33). The title compound was synthesized in 87% yield according to the same manner as described for the preparation of **24** using 4-(4-fluorophenoxy)benzaldehyde for 4-(3-pyridyl)benzaldehyde. TLC R_f 0.53 ($CHCl_3$/MeOH, 20:1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.53 (d, J = 8.7 Hz, 2H), 7.18–7.00 (m, 6H), 4.33 (s, 2H), 4.04 (dd, J = 7.5, 4.5 Hz, 1H), 3.87–3.69 (m, 2H), 3.55–3.32 (m, 4H), 2.52–2.32 (m, 2H), 2.28–2.08 (m, 2H), 1.83–1.12 (m, 15H), 1.06–0.83 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H); IR (KBr) 3432, 3194, 3134, 3071, 2923, 2852, 2656, 2553, 2426, 1678, 1655, 1648, 1614, 1498, 1473, 1446, 1420, 1375, 1332, 1314, 1250, 1214, 1192, 1173, 1146 cm^{-1}; MS (APCI, Pos) m/z 536 (M+H)$^+$; HRMS Calcd 536.3288, Obsd 536.3287.

4.1.7.8. (3*S*)-1-Butyl-3-(cyclohexylmethyl)-9-[4-(4-methylphenoxy)benzyl]-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (34). The title compound was synthesized in 58% yield according to the same procedures as described for the preparation of **24** using 4-(4-methylphenoxy)benzaldehyde for 4-(3-pyridyl)benzaldehyde. TLC R_f 0.71 (AcOEt); ^{1}H NMR (300 MHz, CD_3OD) δ 7.50 (d, J = 8.7 Hz, 2H), 7.19 (d, J = 8.7 Hz, 2H), 7.02 (d, J = 8.7 Hz, 2H), 6.92 (d, J = 8.7 Hz, 2H), 4.32 (s, 2H), 4.04 (dd, J = 7.5, 4.5 Hz, 1H), 3.87–3.69 (m, 2H), 3.55–3.42 (m, 2H), 3.42–3.34 (m, 2H), 2.49–2.30 (m, 2H), 2.33 (s, 3H), 2.30–2.08 (m, 2H), 1.82–1.10 (m, 15H), 1.05–0.85 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H); IR (KBr) 3368, 3206, 3066, 3033, 2925, 2851, 2661, 2514, 2427, 1670, 1657, 1604, 1502, 1478, 1467, 1449, 1420, 1366, 1347, 1331, 1315, 1243, 1210, 1172, 1113, 1101 cm^{-1}; MS (APCI, Pos) m/z 532 (M+H)$^+$; HRMS Calcd 532.3539, Obsd 532.3541.

4.1.7.9. (3*S*)-1-Butyl-3-(cyclohexylmethyl)-9-[4-(4-methoxyphenoxy)benzyl]-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (35). The title compound was synthesized in 70% yield according to the same procedures as described for the preparation of **24** using 4-(4-methoxyphenoxy)benzaldehyde for 4-(3-pyridyl)benzaldehyde. TLC R_f 0.67 (AcOEt); ^{1}H NMR (300 MHz, CD_3OD) δ 7.49 (d, J = 8.4 Hz, 2H), 7.02–6.92 (m, 6H), 4.31 (s, 2H), 4.03 (dd, J = 7.5, 4.5 Hz, 1H), 3.86–3.69 (m, 2H), 3.79 (s, 3H), 3.54–3.30 (m, 4H), 2.50–2.30 (m, 2H), 2.28–2.06 (m, 2H), 1.83–1.10 (m, 15H), 1.05–0.83 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H); IR (KBr) 3417, 3204, 3069, 2925, 2851, 2656, 2496, 2426, 1681, 1615, 1500, 1475, 1464, 1447, 1420, 1374, 1345, 1315, 1300, 1230, 1199, 1173, 1113, 1098, 1035 cm^{-1}; MS (APCI, Pos) m/z 548 (M+H)$^+$; HRMS Calcd 548.3488, Obsd 548.3481.

4.1.7.10. (3*S*)-1-Butyl-3-(cyclohexylmethyl)-9-[4-(4-hydroxyphenoxy) benzyl]-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (36). The title compound was synthesized in 45% yield according to the same procedures as described for the preparation of **24** using 4-(4-hydroxyphenoxy)benzaldehyde for 4-(3-pyridyl)benzaldehyde. TLC R_f 0.54 ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.47 (d, J = 8.4 Hz, 2H), 6.97 (d, J = 8.4 Hz, 2H), 6.88 (d, J = 9.0 Hz, 2H), 6.80 (d, J = 9.0 Hz, 2H), 4.30 (s, 2H), 4.03 (dd, J = 7.5, 4.5 Hz, 1H), 3.83–3.72 (m, 2H), 3.49–3.34 (m, 4H), 2.38 (m, 2H), 2.23–2.10 (m, 2H), 1.78–1.16 (m, 15H), 1.02–0.92 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H); IR (KBr) 3363, 3199, 2924, 2851, 2670, 2566, 1674, 1638, 1503, 1468, 1448, 1420, 1373, 1346, 1315, 1227, 1196, 1172 cm^{-1}; MS (APCI, Pos) m/z 534 (M+H)$^+$; HRMS Calcd 534.3332, Obsd 534.3333.

4.1.7.11. 4-(4-{[(3*S*)-1-Butyl-3-(cyclohexylmethyl)-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl]methyl}phenoxy)-*N*-methylbenzamide hydrochloride (37). The title compound was synthesized in 68% yield according to the same procedures as described for the preparation of **24** using 4-(4-*N*-methylaminocarbonylphenoxy)benzaldehyde for 4-(3-pyridyl)benzaldehyde. TLC R_f 0.25 (AcOEt/MeOH, 10:1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.85 (d, J = 8.7 Hz, 2H), 7.62 (d, J = 8.7 Hz, 2H), 7.15 (d, J = 8.7 Hz, 2H), 7.08 (d, J = 8.7 Hz, 2H), 4.37 (s, 2H), 4.05 (dd, J = 7.5, 4.5 Hz, 1H), 3.90–3.68 (m, 2H), 3.58–3.36 (m, 4H), 2.92 (s, 3H), 2.58–2.36 (m, 2H), 2.28–2.06 (m, 2H), 1.84–1.10 (m, 15H), 1.06–0.84 (m, 2H), 0.96 (t, J = 7.2 Hz, 3H); IR (KBr) 3263, 2927, 2547, 1674, 1600, 1499, 1417, 1314, 1245, 1175, 1112, 1049, 1006 cm^{-1}; MS (APCI, Pos) m/z 575 (M+H)$^+$; Elemental Anal. Calcd for $C_{34}H_{46}N_4O_4$·HCl: C, 66.81; H, 7.75; N, 9.17. Found: C, 64.8; H, 7.98; N, 8.92.

4.1.7.12. *N*-[4-(4-{[(3*S*)-1-Butyl-3-(cyclohexylmethyl)-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl]methyl}phenoxy)phenyl]methanesulfonamide hydrochloride (38). The title compound was synthesized in 47% yield according to the same procedures as described for the preparation of **24** using 4-(4-methansulfonylaminophenoxy)benzaldehyde for 4-(3-pyridyl)benzaldehyde. TLC R_f 0.42 ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (300 MHz, CD_3OD) δ 7.53 (d, J = 9.0 Hz, 2H), 7.29 (d, J = 9.0 Hz, 2H), 7.08–7.00 (m, 4H), 4.33 (s, 2H), 4.03 (dd, J = 7.5, 4.8 Hz, 1H), 3.85–3.72 (m, 2H), 3.54–3.36 (m, 4H), 2.95 (s, 3H), 2.48–2.34 (m, 2H), 2.25–2.08 (m, 2H), 1.80–1.14 (m, 15H), 0.98–0.88 (m, 5H); IR (KBr) 3361, 3237, 3108, 3057, 2925, 2851, 2663, 2580, 1677, 1632, 1502, 1477, 1449, 1420, 1402, 1389, 1330, 1302, 1276, 1254, 1248, 1218, 1171,

1150, 1109 cm^{-1}; MS (APCI, Pos) *m/z* 611 $(M+H)^+$; HRMS Calcd. 611.3267, Obsd 611.3267.

4.1.7.13. 4-(4-{[(3*S*)-1-Butyl-3-(cyclohexylmethyl)-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl]methyl}phenoxy)benzenesulfonamide hydrochloride (39). The title compound was synthesized in 40% yield according to the same procedures as described for the preparation of **24** using 4-(4-aminosulfonylphenoxy)benzaldehyde for 4-(3-pyridyl)benzaldehyde. TLC R_f 0.33 ($CHCl_3$/MeOH, 10:1); ^1H NMR (300 MHz, DMSO-d_6) δ (300 MHz,) 11.03 (br s, 1H), 8.42 (br s, 1H), 7.82 (d, *J* = 8.7 Hz, 2H), 7.71 (d, *J* = 8.7 Hz, 2H), 7.33 (br s, 2H), 7.16 (d, *J* = 8.7 Hz, 4H), 4.38–4.23 (m, 2H), 3.91 (m, 1H), 3.61–3.23 (m, 6H), 2.58–2.30 (m, 2H), 2.18–1.91 (m, 2H), 1.76–1.00 (m, 15H), 0.98–0.71 (m, 5H); MS (APCI, Pos) *m/z* (APCI, Pos) 597 $(M+H)^+$.

4.1.7.14. 4-(4-{[(3*S*)-1-Butyl-3-(cyclohexylmethyl)-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl]methyl}phenoxy)benzoic acid hydrochloride (40). The title compound was synthesized in 60% yield according to the same procedures as described for the preparation of **24** using 4-(4-formylphenoxy)benzoic acid for 4-(3-pyridyl)benzaldehyde. $[\alpha]_D^{25}$ −27.7 (*c* 1.03, MeOH); TLC R_f 0.37 ($CHCl_3$/MeOH, 10:1); ^1H NMR (300 MHz, CD_3OD) δ 8.03 (d, *J* = 8.7 Hz, 2H), 7.63 (d, *J* = 8.7 Hz, 2H), 7.16 (d, *J* = 8.7 Hz, 2H), 7.07 (d, *J* = 8.7 Hz, 2H), 4.37 (s, 2H), 4.04 (dd, *J* = 7.5, 4.5 Hz, 1H), 3.90–3.70 (m, 2H), 3.56–3.35 (m, 4H), 2.59–2.38 (m, 2H), 2.27–2.05 (m, 2H), 1.83–1.08 (m, 15H), 1.05–0.83 (m, 2H), 0.95 (t, *J* = 7.2 Hz, 3H); IR (KBr) 2925, 1673, 1598, 1502, 1417, 1243, 1160 cm^{-1}; MS (APCI, Pos) *m/z* 562 $(M+H)^+$; Elemental Anal. Calcd for $C_{33}H_{43}N_3O_5$·HCl: C, 66.26; H, 7.41; N, 7.02. Found: C, 64.8; H, 7.64; N, 6.98.

4.1.8. (3*S*)-1-Butyl-3-(cyclohexylmethyl)-9-[2-(4-phenoxyphenyl)ethyl]-1,4,9-triazaspiro[5.5]undecane-2,5-dione hydrochloride (19)

A solution of 4-phenoxyphenethyl alcohol **65** (214 mg, 1 mmol) in pyridine/CH_2Cl_2 (1:1, 4 mL) was added to chlorosulfonated polystyrene resin (305 mg, 0.5 mmol). The mixture was shaken for 5 h at room temperature. The resin was collected by filtration and successively washed with CH_2Cl_2 (4 mL), DMF (4 mL), DMF/H_2O (3:1, 4 mL), THF (4 mL), CH_2Cl_2 (4 mL) and CH_3CN (4 mL). To a suspension of the resin in MeCN (5 mL) were added *N*,*N*-diisopropylamine (271 mg, 2.1 mmol) and then compound **52b** (112 mg, 0.3 mmol). The mixture was shaken for 18 h at 70 °C. After cooling to room temperature, the resin was collected by filtration and washed with MeCN. The combined filtrate and washings were evaporated. The resulting residue was purified by column chromatography on silica gel AcOEt/MeOH (from 1:0 to 10:1). The resulting residue after evaporation was treated with 4 N HCl in AcOEt (2 mL) and then washed with Et_2O to afford **19** (81 mg, 48% yield) as a powder. TLC R_f 0.54 (AcOEt/MeOH, 10:1); ^1H NMR (300 MHz, CD_3OD) δ 7.37–7.29 (m, 4H), 7.11(t, *J* = 7.2 Hz, 1H), 6.97–6.95 (m, 4H), 4.06 (d, *J* = 7.5, 4.5 Hz, 1H), 3.88–3.77 (m, 2H), 3.65 (m, 2H), 3.46–3.36 (m, 4H), 3.13–3.07 (m, 2H), 2.48 (m, 2H), 2.28–2.14 (m, 2H), 1.80–1.21(m, 15H), 0.98 (t, *J* = 7.0 Hz, 3H), 0.99–0.91 (m, 2H); IR (KBr) 3364, 3195, 3065, 2924, 2851, 2661, 2525, 2421, 1671, 1589, 1508, 1489, 1470, 1449, 1418, 1373, 1347, 1333, 1317, 1238, 1200, 1169, 1148, 1073 cm^{-1}; MS (APCI, Pos) *m/z* 532 $(M+H)^+$; HRMS Calcd 532.3539, Obsd 532.3536.

4.1.9. Ethyl 4-[1-butyl-3-(cyclohexylmethyl)-2,5-dioxo-1,4,9-triazaspiro[5.5]undec-9-yl]benzoate hydrochloride (20)

To a solution of compound **52a** (186 mg, 0.501 mmol) in MeCN (2.5 mL) were added ethyl 4-fluorobenzoate (164 mg, 0.975 mmol) and then K_2CO_3 (141 mg, 1.02 mmol). After being stirred for 12 h at 100 °C, the reaction mixture was treated with DMSO (0.5 mL). Stirring was continued for another 12 h at 140 °C. The reaction mixture was cooled to room temperature, diluted with H_2O and extracted with *t*-butyl methyl ether. The combined organic layers were washed with brine, dried ($MgSO_4$) and evaporated. The resulting residue was purified by column chromatography on silica gel (hexane/AcOEt, from 4:1 to 3:1). The resulting residue after evaporation was treated with 4 N HCl in AcOEt (2 mL) and washed with *t*-butyl methyl ether to afford the title compound **20** (67 mg, 26% yield). TLC R_f 0.27 (hexane/AcOEt, 2:1); ^1H NMR (300 MHz, CD_3OD) δ 8.13 (d, *J* = 8.7 Hz, 2H), 7.59 (d, *J* = 8.7 Hz, 2H), 4.37 (q, *J* = 7.2 Hz, 2H), 4.31–4.15 (m, 2H), 4.07 (dd, *J* = 7.5, 4.5 Hz, 1H), 3.85–3.75 (m, 2H), 3.47–3.38 (m, 2H), 2.67–2.50 (m, 2H), 2.30–2.12 (m, 2H), 1.85–1.46 (m, 10H), 1.44–1.19 (m, 5H), 1.38 (t, *J* = 7.2 Hz, 3H), 1.05–0.88 (m, 2H), 0.95 (t, *J* = 7.2 Hz, 3H); IR (KBr) 3214, 3085, 2959, 2927, 2851, 2630, 2463, 2401, 1726, 1675, 1658, 1608, 1469, 1459, 1448, 1417, 1407, 1374, 1315, 1307, 1277, 1188, 1177, 1146, 1109, 1020, 1006 cm^{-1}; MS (APCI, Pos) *m/z* 484 $(M+H)^+$; HRMS Calcd 484.3175, Obsd 484.317.

4.1.10. 2-Butynylamine hydrochloride (42f)

4.1.10.1. 2-Butynylphthalimide (48). To a stirred solution of 2-butynylalchol **47** (5,0 g, 71.3 mmol) in triethylamine (14.9 mL 107 mmol) and CH_2Cl_2 (210 mL) was slowly added methanesulfonyl chloride (6.1 mL, 78.5 mmol) at 0 °C under argon atmosphere. After being stirred for 1 h, the reaction mixture was quenched with water and extracted with AcOEt. The organic layer was washed with 1 N HCl, saturated $NaHCO_3$ aq, brine, dried over Na_2SO_4, and evaporated. To a stirred solution of the resulting residue in DMF (80 mL) was added potassium phthalimide (11.4 g, 61.5 mmol). After being stirred for 1.5 h at 70 °C, the reaction mixture was poured into water (400 mL) and extracted with AcOEt. Precipitates were removed by filtration and washed with diethyl ether. The filtrate was extracted with AcOEt, and the organic layer was washed with 1 N NaOH and brine, dried over Na_2SO_4 and evaporated. The resulting solid was triturated with diethyl ether and dried in vacuo to yield **48** (10.4 g, 73% in two steps). TLC R_f 0.56 (hexane/AcOEt, 2:1); ^1H NMR (200 MHz, DMSO-d_6) δ 7.90–7.77 (m, 4H), 4.30 (q, *J* = 2.4 Hz, 2H), 1.74 (t, *J* = 2.4 Hz, 3H).

4.1.10.2. 2-Butynylamine hydrochloride (42f). To a stirred suspension of **48** (10.3 g, 51.7 mmol) in EtOH (600 mL) was added hydrazine hydrate (7.6 mL, 155 mmol). After being stirred for 1.5 h at 100 °C, the mixture was cooled to room temperature, quenched with concd HCl (25 mL) and evaporated. The resulting residue was treated with 5 N NaOH and extracted with CH_2Cl_2 repeatedly. The combined organic layers were washed with brine, dried over Na_2SO_4. After the addition of 4 N HCl/AcOEt (20 mL), the solution was evaporated. The resulting solid was washed with diethyl ether to yield **42f** (5.25 g, 96%). TLC R_f 0.23 ($CHCl_3$/MeOH/AcOH, 20:4:1); ^1H NMR (200 MHz, DMSO-d_6) δ 8.70–8.15 (br, 3H), 3.62 (br, 2H), 1.83 (t, *J* = 2.4 Hz, 3H).

4.1.11. 4-Benzoylbenzaldehyde (54)

To a stirred solution of 4-benzoylbenzencalboxylic acid **53** (590 mg, 2.61 mmol) in triethylamine (0.44 mL, 3.13 mmol) and THF (7 mL) was added *i*-butylchloroformate (0.40 mL, 3.13 mmol) at −78 °C. After being stirred at room temperature for 30 min, triethylamine hydrochloride salt was removed by filtration. The filtrate was added to the suspension of sodium borohydride (296 mg, 7.83 mmol) in water at 0 °C. After being stirred at room temperature for overnight, the mixture was concentrated. The resulting residue was extracted with AcOEt. The organic layer was washed with water, brine, dried over Na_2SO_4 and evaporated. To a stirred solution of the resulting residue in DME (20 mL) was added MnO_2 (682 mg, 7.84 mmol). After being stirred at 100 °C for overnight, additional MnO_2 (2.02 g, 23.2 mmol) was added to the solution. The reaction mixture was stirred at 110 °C for 4 h

and then cooled to room temperature. MnO_2 was removed by filtration through the pad of Celite, and the filtrate was evaporated. The resulting residue was purified by column chromatography on silica gel to yield **54** (277 mg, 51%). TLC R_f 0.35 (hexane/AcOH, 4:1); ^{1}H NMR (300 MHz, CD_3OD) δ 10.14 (s, 1H), 7.94–7.92 (m, 2H), 7.83–7.80 (m, 2H), 7.67–7.61 (m, 1H), 7.54–7.49 (m, 2H).

4.1.12. 4-(4-Hydroxyphenoxy)benzaldehyde (56)

To a stirred solution of **55** (3.69 g, 16.1 mmol) in CH_2Cl_2 (80 mL) was slowly added 1.0 M boron tribromide/CH_2Cl_2 solution (35 mL, 0.35 mmol) at 0 °C. After being stirred for 2 h, the reaction mixture was quenched with water and extracted with diethyl ether. The organic layer was washed with water, brine, dried over Na_2SO_4 and evaporated. The resulting residue was purified by column chromatography on silica gel to yield the title compound **56** (3.06 g, 86%). TLC R_f 0.28 (hexane/AcOEt, 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.90 (s, 1H), 7.83 (d, J = 9.0 Hz, 2H), 7.01 (d, J = 9.0 Hz, 2H), 6.98 (d, J = 9.0 Hz, 2H), 6.89 (d, J = 9.0 Hz, 2H), 5.47 (br s, 1H). MS (APCI, Pos) 257 $(M+H)^+$.

4.1.13. 4-(4-Formylphenoxy)benzoic acid (58)

A mixture of methyl 4-hydroxybezoate **57** (25.2 g, 16.5 mmol), 4-fluorobenzaldehyde (18.6 g, 14.9 mmol) and K_2CO_3 in DMF (150 mL) was stirring under reflux for 2 h. After being cooled to room temperature, the reaction mixture was quenched with water and extracted with AcOEt. The organic layer was washed with water, brine, dried over $MgSO_4$ and evaporated. The resulting solid was triturated with *n*-hexane and dried to yield the methyl ester of **58** (27.8 g, 72%). To a stirred solution of the methyl ester in methanol (400 mL) was added 2 N NaOH (108 mL, 54 mmol). After being stirred for 2 h at 50 °C, the reaction mixture was cooled to room temperature and quenched with 2 M HCl (110 mL) and water (200 mL). The precipitates were collected by filtration and dried to yield **58** (23.4 g, 89%). TLC R_f 0.20 (hexane/AcOEt, 1:1); ^{1}H NMR δ (300 MHz, $CDCl_3$) 9.98 (s, 1H), 8.16 (d, J = 8.7 Hz, 2H), 7.92 (d, J = 8.7 Hz, 2H), 7.17 (d, J = 8.7 Hz, 2H), 7.13 (d, J = 8.7 Hz, 2H).

4.1.14. 4-(4-*N*-Methylaminocarbonylphenoxy)benzaldehyde (59)

To a stirred solution of compound **58** (9.8 g, 40.5 mmol) in DMF were added HOBt (6.56 g, 48.6 mmol), EDC hydrochloride (9.32 g, 48.6 mmol) and a solution of methylamine in THF (2.0 M, 41 mL, 81 mmol). After being stirred for 1.5 h, the reaction mixture was poured into water (500 mL), acidified with 2 N HCl (10 mL) and extracted with AcOEt. The organic layer was washed with saturated $NaHCO_3$ aq, brine, dried over Na_2SO_4, and evaporated. The resulting solid was triturated with *t*-butyl methyl ether to yield **59** (9.23 g, 89%). TLC R_f 0.55 ($CHCl_3$/MeOH = 10:1); ^{1}H NMR δ (300 MHz, $CDCl_3$) 9.95 (s, 1H), 7.89 (d, J = 8.7 Hz, 2H), 7.81 (d, J = 9.0 Hz, 2H), 7.13–7.10 (m, 4H), 3.03 (d, J = 4.8 Hz, 3H); MS (APCI, Pos) 256 $(M+H)^+$.

4.1.15. 4-(4-Methansulfonylaminophenoxy)benzaldehyde (62)

4.1.15.1. Methyl-4-(4-methansulfonylaminophenoxy)benzoate (61). A suspension of **60** (25.5 g, 93.5 mmol) and 5% Pd–C (1.3 g) in AcOEt (300 mL) was stirred overnight under hydrogen atmosphere. The reaction mixture was filtered through a pad of Celite and the filtrate was evaporated. To the resulting residue in THF (300 mL) and pyridine (22.4 mL, 277 mmol) was added methanesulfonyl chloride (10.7 mL, 139 mmol) at 0 °C. After being stirred for overnight at room temperature, the reaction mixture was quenched with water and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated. The resulting solid was triturated with *t*-butyl methyl ether to yield **61** (29.0 g, 98%). TLC R_f 0.5 (hexane/AcOEt, 1:1); ^{1}H NMR (300 MHz, CD_3OD) δ 8.01 (d, J = 8.7 Hz, 2H), 7.26 (d, J = 9.0 Hz, 2H), 7.26 (s, 1H), 7.05 (d, J = 9.0 Hz, 2H), 6.98 (d, J = 8.7 Hz, 2H), 3.91 (s, 3H), 3.04 (s, 3H).

4.1.15.2. 4-(4-Methansulfonylaminophenoxy)benzaldehyde (62). To a stirred solution of **61** (29 g, 90.3 mmol) in THF (300 mL) was added 1.0 M diisobutyl aluminum hydride/*n*-hexane solution (270 mL, 270 mmol) at 0 °C. After being stirred for 2 h, the reaction mixture was treated with additional diisobutyl aluminum hydride (1.0 M, 90 mL, 90 mmol) and stirred for 20 min at 0 °C. The reaction mixture was quenched with saturated Na_2SO_4 aq and the resulting precipitates were removed by filtration. The filtrate was evaporated and the resulting residue was dissolved in CH_2Cl_2/DME (150:150). MnO_2 (40 g, 482 mmol) was added to the solution The reaction mixture was stirred for overnight and then filtered through a pad of Celite and the filtrate was concentrated in vacuo to yield **62** (26.0 g, 99%). TLC R_f 0.46 (hexane/AcOEt); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.92 (s, 1H), 7.85 (d, J = 8.7 Hz, 2H), 7.28 (d, J = 8.7 Hz, 2H), 7.25 (s, 1H), 7.10–7.05 (m, 4H), 3.05 (s, 3H).

4.1.16. 4-(4-Aminosulfonylphenoxy)benzaldehyde (64)

A suspension of **63** (4.33 g, 25 mmol), 4-fluorobenzaldehyde (2.68 mL, 25 mmol), and K_2CO_3 (6.9 g, 50 mmol) in DMA (20 mL) was stirring under reflux for 2 h. After being cooled to room temperature, the mixture was quenched with water and extracted with AcOEt. The organic layer was washed with water, brine, dried over Na_2SO_4 and evaporated. The resulting solid was recrystallized from AcOEt/hexane to yield the title compound **64** (1.22 g, 18%). TLC R_f ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (300 MHz, CD_3OD) δ 9.93 (s, 1H), 7.96 (d, J = 8.7 Hz, 2H), 7.95 (d, J = 8.7 Hz, 2H), 7.21 (d, J = 8.7 Hz, 2H), 7.19 (d, J = 8.7 Hz, 2H); MS (APCI, Neg) 276 $(M-H)^-$.

4.2. Biology

4.2.1. Stability study of liver microsomes

The test substance (5 μL:10 mmol/L in DMSO) was diluted with 195 μL of 50% acetonitrile in water to make a 250 μmol/L solution of the test substance.

Phosphate buffer (0.1 mol/L, 245 μL) containing 0.2 mg/mL or 0.5 mg/mL rat liver microsomes and NADPH-Co-factor* was added to a reaction container, pre-warmed to 37 °C in a water bath, and incubated for 5 min. The reaction was initiated with the addition of 5 μL of the test substance solution (in 0.975% acetonitrile with 0.05% DMSO, final concentration of 5 μmol/L). A 20 μL aliquot was taken from the mixture immediately after the start of the reaction, and transferred to 180 μL of acetonitrile containing the internal standard (warfarin) to terminate the reaction. A 20 μL aliquot of the mixture was stirred with 180 μL of 50% acetonitrile on a plate with a filter for deproteinization, and filtered by suction. The filtrate was used as the standard sample.

After a 15 min incubation of the above mixture, a 20 μL aliquot was transferred to 180 μL of acetonitrile containing the internal standard (warfarin) to terminate the reaction. A 20 μL aliquot of the mixture was stirred with 180 μL of 50% acetonitrile on a plate with a filter for deproteinization, and filter by suction. The filtrate was used as the reaction sample.

*NADPH-Co-factor: Dilute solutions A and B in the NADPH-regenerating system (BD-Bioscience) 20- and 100-fold, respectively, with 0.1 mol/L phosphate buffer ($NADP^+$ 2.6 mmol/L).

4.2.1.1. Determination of test substance concentration and data processing. One microliter of aliquot was injected into an LC–MS/MS system. The percent residue (%) is calculated by dividing the peak area ratio (i.e., test substance/I.S.) for the reaction sample by the peak area ratio for the standard sample and multiplying by 100.

4.2.2. Primary screening of solubility

The test substance (10 mmol/L DMSO) was diluted with acetonitrile. Acetonitrile containing the internal standard (warfarin) was added to prepare samples for calibration curves at 0.1, 0.4, and 2 μmol/L.

The test substance (5 μL:10 mmol/L DMSO) was added to 495 μL of JP Solution 2. The solution was stirred at room temperature for 5 h, and transferred onto a filter plate, and filtered by centrifugation at 3000 rpm, 24 °C for 15 min. The filtrate (20 μL) was diluted with acetonitrile, and additional acetonitrile containing the internal standard to prepare a sample solution.

The sample solution (1 μL) was injected into an LC–MS/MS system for quantification (range: 0.1–2 μmol/L). The solubility was determined as 50 times of the observed value. The solubility was reported as <5 μmol/L or 100 μmol/L if the observed value is outside the range.

4.2.3. Caco-2 membrane permeability assay

Caco-2 cells were grown on a 12-well Costar Transwell plate (with a collagen-coated fine porous polycarbonate membrane) until a confluent monolayer is formed. Buffer for permeability assay was prepared using 10 mM HEPES and Hank's balanced salt solution containing 15 mM glucose with the pH being adjusted at 7.3–7.5. A test substance solution was prepared at 10 mM the assay buffer. The test substance solution was added to the apical side of Caco-2 cell monolayer at a final concentration of 10 μM, and then the plate was incubated in a humidified incubator (5% CO_2, 37 °C). Two hours later, 200- and 50 μL aliquots were taken from the receiver chambers. The measurements should be performed in duplicate. The concentration of test substance in the samples was determined by LC–MS/MS.

The apparent permeability coefficient (Papp) was calculated according to the following equation:

$$\mathrm{Papp} = (\mathrm{dCr}/\mathrm{dt}) \times \mathrm{Vr}/(A \times C_0)$$

dCr/dt: slope of compound accumulation in the receiver compartment over time (μM/s)

Vr: volume of the receiver compartment (cm^3)

A: area of the cell monolayer (1.13 cm^2 for 12-well transwell plate)

C_0: initial normality of the buffer (μM)

4.2.4. Pharmacokinetic (PK) studies

Single dose pharmacokinetics was studied in rats. Formulation for intravenous injection was prepared using SWI (sterile water for injection) containing 30% HP-β-CD (w/v). Formulation for oral dosing was prepared using SWI containing 1% sucrose fatty acid ester (w/v). Test compounds (3 mg/kg) were dosed intravenously to the fasted male rats (n = 3). Test compounds (30 mg/kg) were dosed orally to the fasted male rats (n = 3). After dosing, blood samples (250 μL) were collected from the jugular vein using a heparinized syringe at the selected time points (iv: pre-dosing, 2, 5, 15, and 30 min; po: 1, 2, 4, 6, and 8 h, respectively). The blood samples were ice-chilled and then centrifuged at 12,000 rpm for 2 min at room temperature to obtain plasma, which was preserved at −70 °C in a freezer. The AUC, C_{max}, T_{max}, $T_{1/2}$, V_{ss}, and CL were obtained by measuring the time course of the plasma concentration of the test compounds. Bioavailability (BA) was calculated according to the following equation:

$$\mathrm{BA}\ (\%) = (\mathrm{AUCpo}/\mathrm{Dpo})/(\mathrm{AUCiv}/\mathrm{Div}) \times 100$$

References and notes

1. Hoffmann, C.; Mulcahy, F. Overview of Antiretroviral Agents. In *HIV Medicine 2006*; Hoffmann, C., Rockstroh, J. K., Kamps, B. S., Eds.; Flying Publisher (FlyingPublisher.com): Paris, 2006; pp 94.
2. (a) Schwarz, M. K.; Wells, T. N. C. *Nat. Rev. Drug Disc.* **2002**, *1*, 347; (b) Gerard, C.; Rollins, B. J. *Nat. Immunol.* **2001**, *2*, 108; (c) Pirotte, B.; Tullio, P.; Nguyen, Q. A.; Somwes, F.; Fraikin, P.; Florence, X.; Wahl, P.; Hansen, J. B.; Lebrun, P. *J. Med. Chem.* **2010**, *53*, 147.
3. (a) Feng, Y.; Broder, C. C.; Kennedy, P. E.; Berger, E. A. *Science* **1996**, *272*, 872; (b) Deng, H. K.; Liu, R.; Ellmeier, W.; Choe, S.; Unutmaz, D.; Burkhart, M.; Marzio, P. D.; Marmon, S.; Sutton, R. E.; Hill, C. M.; Davis, C. B.; Peiper, S. C.; Schall, T. J.; Littman, D. R.; Landau, N. R. *Nature* **1996**, *381*, 661; (c) Dragic, T.; Litwin, V.; Allaway, G. P.; Martin, S. R.; Huang, Y.; Nagashima, K. A.; Cayanan, C.; Maddon, P. J.; Koup, R. A.; Moore, J. P.; Paxton, W. A. *Nature* **1996**, *381*, 667; (d) Alkhatib, G.; Combadiere, C.; Broder, C. C.; Feng, Y.; Kennedy, P. E.; Murphy, P. M.; Berger, E. A. *Science* **1996**, *272*, 1955; (e) Berger, E. A.; Murphy, P. M.; Farber, J. M. *Annu. Rev. Immunol.* **1999**, *17*, 657; (f) Caldwell, D. J.; Evans, J. D. *Expert Opin. Pharmacother.* **2008**, *9*, 3231.
4. (a) Leonard, J. T.; Roy, K. *Curr. Med. Chem.* **2006**, *13*, 91; (b) Price, D. A.; Armour, D.; Groot, M.; Leishman, D.; Napier, C.; Perros, M.; Stammen, B. L.; Wood, A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4633.
5. Habashita, H.; Kokubo, M.; Hamano, S.; Hamanaka, N.; Toda, M.; Shibayama, S.; Tada, H.; Sagawa, K.; Fukushima, D.; Maeda, K.; Mitsuya, H. *J. Med. Chem.* **2006**, *49*, 4140.
6. (a) Maeda, K.; Yoshimura, K.; Shibayama, S.; Habashita, H.; Tada, H.; Sagawa, K.; Miyakawa, T.; Aoki, M.; Fukushima, D.; Mitsuya, H. *J. Biol. Chem.* **2001**, *276*, 35194; (b) Maeda, K.; Nakata, H.; Koh, Y.; Miyakawa, T.; Ogata, H.; Takaoka, Y.; Shibayama, S.; Sagawa, K.; Fukushima, D.; Moravek, J.; Koyanagi, Y.; Mitsuya, H. *J. Virol.* **2004**, *78*, 8654.
7. (a) Nishizawa, R.; Nishiyama, T.; Hisaichi, K.; Matsunaga, N.; Minamoto, C.; Habashita, H.; Takaoka, Y.; Toda, M.; Shibayama, S.; Tada, H.; Sagawa, K.; Fukushima, D.; Maeda, K.; Mitsuya, H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 727; (b) Nishizawa, R.; Nishiyama, T.; Hisaichi, K.; Hirai, K.; Habashita, H.; Takaoka, Y.; Tada, H.; Sagawa, K.; Shibayama, S.; Maeda, K.; Mitsuya, H.; Nakai, H.; Fukushima, D.; Toda, M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 763.
8. Kalfutkar, A. S.; Gardner, I.; Obach, R. S.; Shaffer, C. L.; Callegari, E.; Henne, K. R.; Mutlib, A. E.; Dalvie, D. K.; Lee, J. S.; Nakai, Y.; O'Donnell, J. P.; Boer, J.; Harriman, S. P. *Curr. Drug Metab.* **2005**, *6*, 161.
9. Further PK studies of **38** and **39** were not carried out because of the poor AUC after their oral cassette dosing.