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Discovery of novel (*S*)-α-phenyl-γ-amino butanamide containing CCR5 antagonists via functionality inversion approach

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3*H*-[1,2,3]Triazolo[4,5-b]pyridine

ABSTRACT

By using functionality inversion approach, we identified a new scaffold containing (*S*)-α-phenyl-γ-amino butanamide as CCR5 antagonists derived from the 1,3-propanediamine carboxamide pharmacophore protocol. The (*2S*)-2-phenyl-4-(8-aza-bicyclo[3.2.1]octan-8-yl)-butanamide derivatives display significantly high potency to antagonize CCR5 receptor with nanomolar IC_{50} values.

Human immunodeficiency virus (HIV) is the causative pathogen of AIDS, a potentially fatal condition resulting from the failure of the immune system. The AIDS epidemic continues to be a significant global threat causing the deaths of 25 million people.[1] Although highly active antiretroviral therapy (HAART) has been successful in reducing the AIDS-related mortality rates dramatically, the rapid emergence of viral strains resistant to current clinical treatments and the dynamic nature of the HIV-1 genome demand a continued effort to identify alternative points of intervention in the viral life cycle. Targeting the viral entry process has become a new focus of research for the next generation of HIV antiretroviral therapies.[2]

CCR5 is an essential co-receptor for HIV-1 recognition and entry into $CD4^+$ macrophages and T-cells, but not essential for human functions.[3–5] Many pharmaceutical companies and academic institutions have been enthusiastically investigating potent antagonists against CCR5 as viral entry inhibitors,[6] and indeed several small-molecule CCR5 antagonists (Nifeviroc,[7] UK-427857,[8] GW-873140,[9] TAK 220[10] as shown in Fig. 1) are now being evaluated in clinical trials. Among them, the UK-427857 (Maraviroc) has become the first CCR5 antagonist approved by FDA for the treatment of HIV infection.[11]

Most of the active structures (represented in Fig. 1) share a common feature: a basic nitrogen atom which is believed to 'anchor' the ligand to the key residue E283 within the transmembrane region of the CCR5 receptor,[12] and a proximal lipophilic group, which likely lies in close contact with a tyrosine residue, Y108. Combining the structural features of the CCR5 binding pocket,[13] we proposed a three-domain pharmacophore model of propane-1,3-diamine skeleton flanked by two hydrophobic domains for CCR5 inhibition, from which we identified a new structure CCR5 inhibitor with sub-micromolar IC_{50} value (designated as TD0444, Fig. 2).[14] A similar six-feature pharmacophore model constructed via Catalyst was recently reported to accelerate the discovery of CCR5 antagonists.[15]

Since our hit compound (TD0444) is structurally related to Maraviroc, we were intrigued to derive novel scaffold by employing functionality inversion and bioisostere approach based on the three-feature pharmacophore model. We were particularly interested in the Maraviroc-like amide functionality, which has been shown to impart favorable properties on the Maraviroc series.[16] Taking this amide functionality as a breakthrough point, we designed a distinct template of (*2S*)-2-aryl-4-amino-butancarboxamide by inverting the amide functionality of hit compound TD0444 into carboxamide as a bioisostere, as depicted in Figure 2. Furthermore, the carboxamide group can be further transformed into carboxylic ester and ether functionality to derivatize structurally diverse skeletons.

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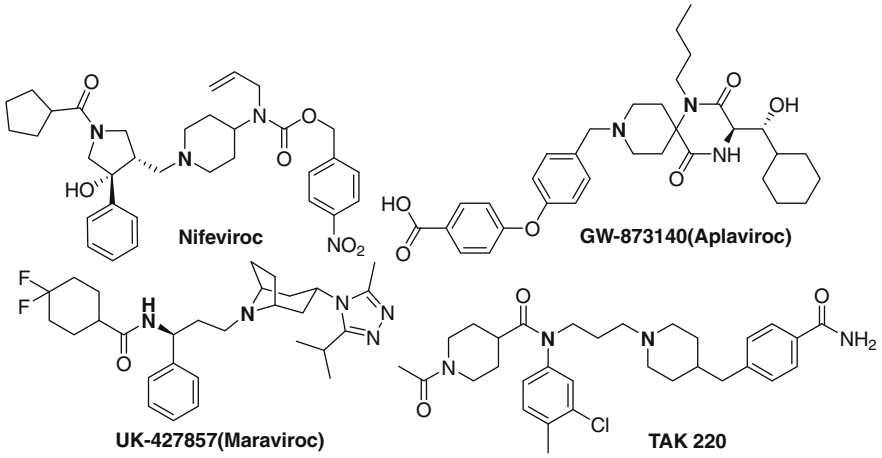


Figure 1. Representative structures of small-molecule CCR5 antagonists in clinic trials, sharing the common 1,3-propanediamine skeleton flanked by two hydrophobic domains.

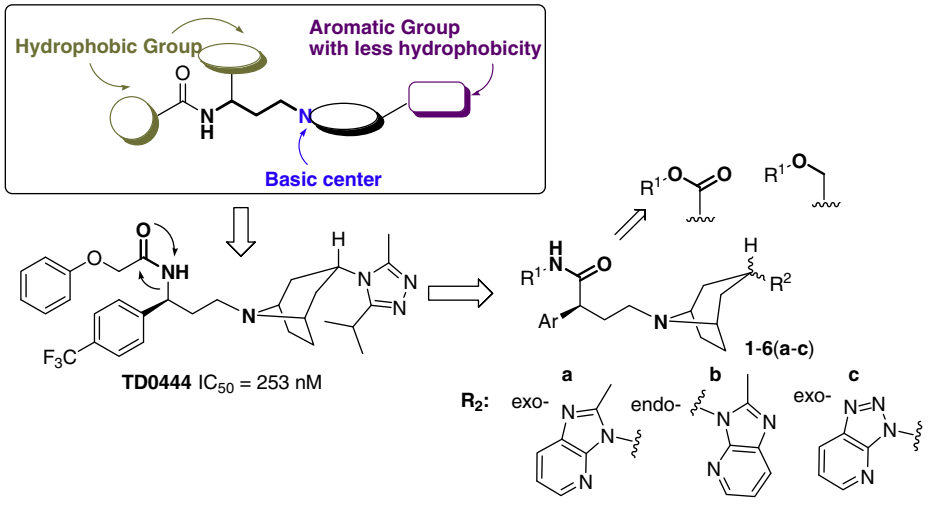


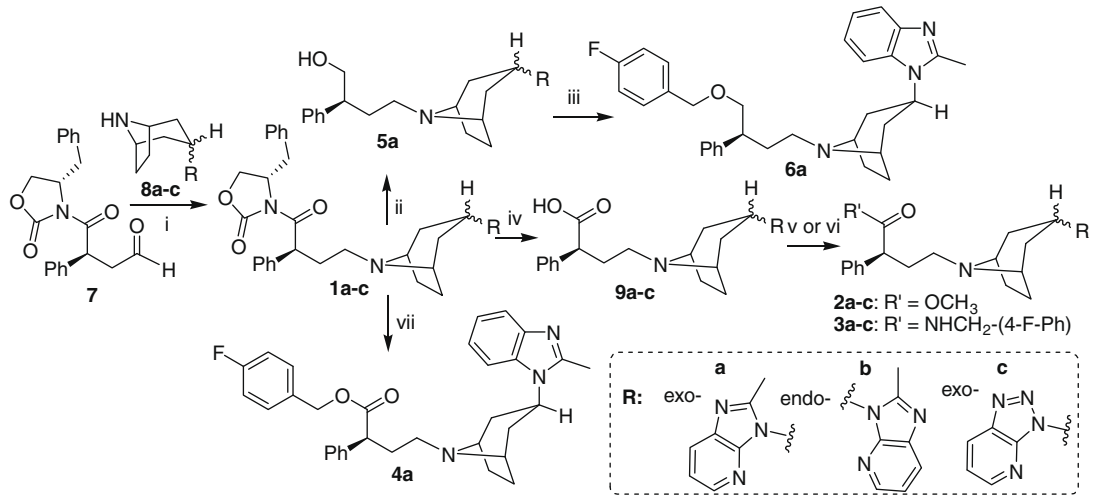
Figure 2. The design of (*2S*)-2-aryl-4-amino-butancarboxyl based CCR5 inhibitors by bioisosteric replacement strategy.

As shown in Figure 2, the substitution around the 4-tropane substituted-butancarboxamide skeleton was investigated. The *exo*- or *endo*-2-methyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl or *exo*-3*H*-[1,2,3]triazolo[4,5-*b*]pyridine-3-yl group was incorporated on the right hand side of the central tropane core. The substituted phenyl amide or ester or ether group was examined as hydrophobic group on the left hand side. As a comparison, some intermediate with polar groups on the left hand side was tested too. We hoped that the novel template with matched functionality substituents could ensure CCR5 potency, providing an orthogonal structure to the current CCR5 inhibitors.

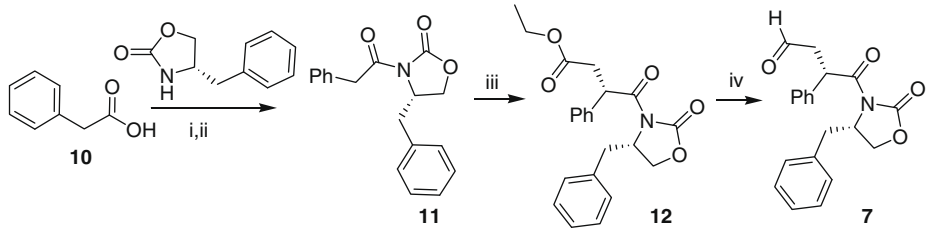
The synthesis of the α-aryl-γ-aminobutancarboxamide and its bioisosteric derivatives was achieved by employing convergent synthesis strategy. As depicted in Scheme 1, starting from the two building blocks (*S*)-4-((*S*)-4-benzyl-2-oxooxazolidin-3-yl)-4-oxo-3-phenylbutanal (**7**) and the heterocycle substituted tropane (**8a–c**), reductive amination proceeded in the presence of sodium triacetoxyborohydride and acetic acid to generate the central core structure (**1a–c**). Removal of the chiral auxiliary oxazolidinone via LAH reduction or nucleophilic replacement by (4-fluorophenyl)methanol or hydrolysis by lithium hydroxide/hydrogen peroxide produced corresponding alcohol (**5a**), ester (**4a**) or acid (**9a–c**). Further structural derivatization included the etherification of the alcohol by 4-fluorophenylmethyl bromide (**5a**→**6a**), the amidation of the acid by (4-fluorophenyl) methanamine in the presence of EDCI/HOAt/DIPEA (**9a–c**→**3a–c**), and the esterification of the acid by methanol in the solution of $SOCl_2$/MeOH (**9a–c**→**2a–c**).

For the synthesis of the chiral β-phenyl-β-carboxamide aldehyde (**7**), the Evans chiral induction protocol was employed as the key step.[17] As shown in Scheme 2, the 2-phenylacetic acid (**10**) was coupled with (*S*)-4-benzyloxazolidin-2-one to give the corresponding amide bearing a chiral auxiliary (**11**). The subsequent stereoselective alkylation in the presence of sodium bis(trimethylsilyl)amide afforded the (*S*)-ethyl 4-((*S*)-4-benzyl-2-oxooxazolidin-3-yl)-4-oxo-3-phenylbutanoate (**12**).[18] Because the (*S*)-4-benzyloxazolidin-2-one served as the carboxyl protective group as well for further transformation, we chose DIBAL-H reduction to convert the butanoate (**12**) into butanal (**7**) with the chiral auxiliary intact.

Before the synthesis of the heterocycle substituted tropane, the *exo*- and *endo*-tropanamine (compound **13a,b**) were prepared from 3-tropanone according to the literature method. The 3-*exo*-tropanamine (**13a**) was synthesized from 3-tropinone oxime by reduction with sodium in 1-propanol,[19] whereas 3-*endo*-tropanamine (**13b**) was obtained by a palladium-catalyzed reductive amination of 3-tropanone with ammonium formate in aqueous methanol.[20] Furthermore, the 3-*endo*-tropanamine (**13b**) was N-demethylated with ethyl chloroformate prior to the reductive amination, resulting in the *N*-ethoxycarbonyl protection.



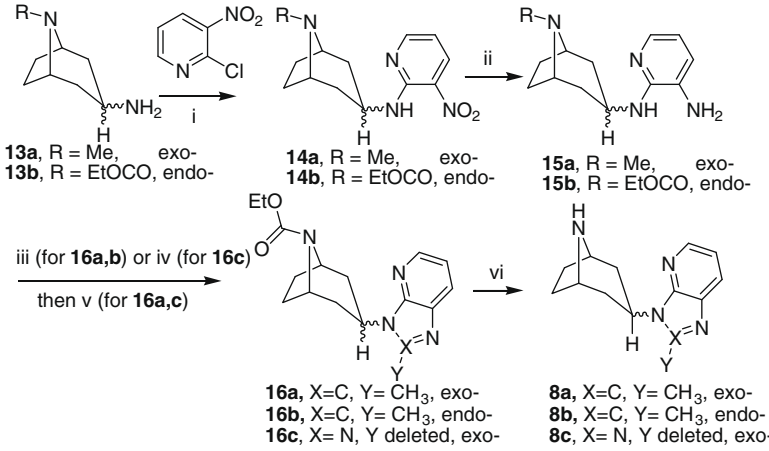
Scheme 1. Convergent synthesis of the tropane bridged α-phenyl-γ-aminobutanamide and its bioisosteric derivatives by employing reductive amination as the key step. Reagents and conditions: (i) $NaB(OAc)_3H$/HOAc, 1,2-dichloroethane, rt, 82–90%; (ii) $LiAlH_4$/THF, −78 °C, 1 h, 68–98%; (iii) NaH/DMF, 1-(bromomethyl)-4- fluorobenzene, rt, 85%; (iv) LiOH/H_2O_2, THF–H_2O (3:1/,v/v), rt, 10 h; (v) $SOCl_2$/CH_3OH, refluxing to rt, 97% (for **2a**–**c**); (vi) EDCI/HOAT/DIPEA, (4-fluorophenyl)methanamine, CH_2Cl_2, rt, two steps overall yield 64–75% (for **3a**–**c**); (vii) *n*-BuLi/THF, 4-fluorophenylmethanol, −78 to −50 °C, 10 h, 80%.



Scheme 2. Synthesis of (*S*)-4-oxo-3-phenylbutanal by using Evans reagent. Reagents and conditions: (i) $(COCl)_2$/CH_2Cl_2, rt, 3 h; (ii) *n*-BuLi/THF, −78 °C to 0 °C, 2 h, 91–95%; (iii) $NaN(SiMe_3)_2$/$BrCH_2COOEt$/THF, −78 °C, 3–5 h, 80–85%; (iv) DIBAl-H/CH_2Cl_2, −78 °C, 3–5 h, 60–80%.

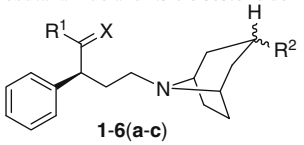
Then, treatment of the *exo*- or *endo*-tropanamine (compound **13a,b**) with 2-chloro-3-nitropyridine in dry acetonitrile followed by the Pd-catalyzed hydrogenation afforded the precursor pyridine-2,3-diamine substituted tropane (**15a,b**), as shown in Scheme 3. The 2-methyl-3*H*-imidazo[4,5-*b*]pyridine substituted tropane (**16a,b**) was directly formed in refluxing Ac_2O, while 3*H*-[1,2,3]triazolo[4,5-*b*]pyridine substituted tropane (**16c**) was prepared via diazotization in good yield.[21] Then, the *exo*-tropane derivatives were demethylated by reacting with ethyl chloroformate at this stage (**16a,c**). The subsequent deprotection in acidic conditions afforded the amine component (**8a–c**) for the final reductive amination, that is, *exo*- and *endo*-2-methyl-3*H*-imidazo[4,5-*b*]pyridine substituted tropanes and *exo*-3*H*-[1,2,3]triazolo[4,5-*b*]pyridine substituted tropane.

Based on the new template, a series of tropane substituted (*S*)-α-phenyl-γ-aminobutanamide and its bioisosteric derivatives were synthesized, with variation on the hydrophobic domains (Scheme 1). The heterocycles substituted on the tropane was

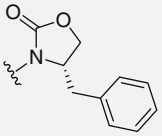
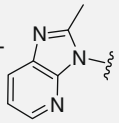
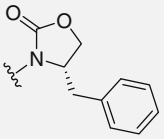
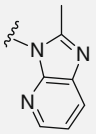
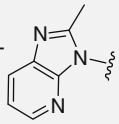
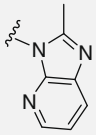
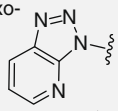
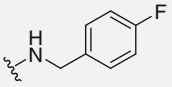
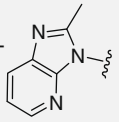
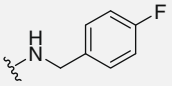
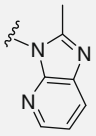
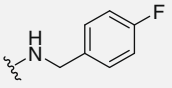
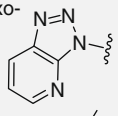
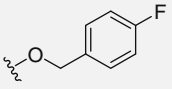
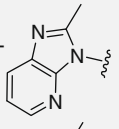
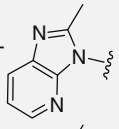
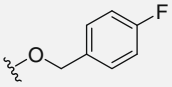
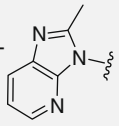


Scheme 3. Synthesis of the heterocycle substituted *exo*- and *endo*-tropane. Reagents and conditions: (i) K_2CO_3/CH_3CN, reflux, 24 h, 83–99%; (ii) 10% Pd–C/H_2 (1 atm), MeOH, 22 h, 90–94%; (iii) Ac_2O, 165 °C, 24 h, 85–95%; (iv) $NaNO_2$/HCl(aq), −10 °C 30 min to 10 °C, 1 h, 95%; (v) ethyl chloroformate/$CHCl_3$, K_2CO_3, reflux, 4 h, 40–80%; (vi) HCl, reflux, 10 h, 52–60%.

Table 1
CCR5 inhibition data of the tropane substituted (*S*)-α-phenyl-γ-aminobutanamide and its bioisosteric derivatives



1-6(a-c)

Compd	X	R^1	R^2	IC_{50}, nM or inhibition rate[a]
1a	O		exo- 	30% inhibition @ 300 nM
1b	O		endo- 	10% inhibition @ 300 nM
2a	O	–OCH_3	exo- 	22% inhibition @ 300 nM
2b	O	–OCH_3	endo- 	112
2c	O	–OCH_3	exo- 	>10 μM
3a	O		exo- 	14.4
3b	O		endo- 	30% inhibition @ 300 nM
3c	O		exo- 	11% inhibition @ 300 nM
4a	O		exo- 	19% inhibition @ 300 nM
5a	H	–OH	exo- 	NA[b]
6a	H		exo- 	42% inhibition @ 300 nM

[a] Inhibition of RANTES-stimulated [^{35}S]-GTPγS binding to CCR5-expressing CHO cell membranes.
[b] Inactive @ 10 μM

examined with *exo*- and *endo*-2-methyl-3*H*-imidazo[4,5-*b*]pyridine and *exo*-3*H*-[1,2,3]triazolo[4,5-*b*]pyridine, since the two heteroaromatic structures have been identified as an optimal fragment for CCR5 binding. The SAR investigation was focused on the carboxamide bioisosteres to identify an effective scaffold.

As shown in Table 1, these compounds were evaluated on the CCR5 inhibition. For the carboxamide bioisostere examined, com-

parison of the *exo*-2-methyl-3*H*-imidazo[4,5-*b*]pyridine substituted analogs (**2a, 3a, 4a, 5a** and **6a**) revealed that the 4-fluorobenzylbutamide motif provided a significant potency advantage versus butanoate (**2a**, **4a**) and butyl ether (**5a**), affording the most potent CCR5 inhibitor in this series (**3a**, IC_{50} = 14.4 nM). The polar butanol counterpart (**5a**) without hydrophobic group turned out inactive. This encouraging result proved the rationale of the functionality inversion approach in the design of novel CCR5 antagonists. In addition, the potency data of series **2a**–**c** and **3a**–**c** suggested a preference of 2-methyl-3*H*-imidazo[4,5-*b*]pyridine over 3*H*-[1,2,3]triazolo[4,5-*b*]pyridine as an optimal heterocycle motif, exemplified by the potent inhibitors **2b** (IC_{50} = 112 nM) and **3a** (IC_{50} = 14.4 nM). And in most cases, the *exo*-tropane (**1a**, **3a**) was more potent than the *endo*-tropane counterpart (**1b**, **3b**). This finding was consistent with the literature-reported activity difference between the *exo* and *endo*-tropane isomers.[16] According to the NMR-based conformational studies, the tropane in the *endo* series adopts a pseudo-boat conformation to minimize the 1,3-diaxial strain between the bulky heterocycle and the carbon bridge of the tropane, rather than the normally preferred chair conformation, as adopted by *exo* series.[16] Consequently, the 2-methyl-3*H*-imidazo[4,5-*b*]pyridine lies in approximately the same geometric position for both the *exo* (**1a**, **3a**) and *endo* (**1b**, **3b**) series, but the greater steric crowding for the *endo* isomer might result in a subtle conformational difference that is less well tolerated by the CCR5 receptor, leading to the deleterious effect.

In conclusion, by adopting the functionality inversion and bioisostere replacement strategy, a new scaffold of (*S*)-α-phenyl-γ-aminobutanamide was identified as potent CCR5 inhibitors. An efficient synthesis was developed to build the tropane substituted (*S*)-α-phenyl-γ-aminobutanamide and its bioisosteric derivatives, involving the Evans chiral induction protocol and the reductive amination reaction. The resulting (2*S*)-*N*-(4-fluorobenzyl)-4-(3-(2-methyl-1*H*-benzo[*d*]imidazol-1-yl)-8-aza-bicyclo[3.2.1]octan-8-yl)-2-phenylbutanamide displayed significantly high potency to antagonize CCR5 receptor with nanomolar IC_{50} values, affording a new scaffold for further development of CCR5 antagonists.

Acknowledgements

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