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1-Amido-1-phenyl-3-piperidinylbutanes – CCR5 antagonists for the treatment of HIV: Part 2

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ABSTRACT

Optimisation of a series of 4-piperidinyltriazoles led to the identification of compound **28a** which showed good whole cell antiviral activity, excellent selectivity over the hERG ion channel and complete oral absorption.

HIV is a global health problem which is estimated to have caused the deaths of 25 million people.[1] Decades of research have not removed the need for new treatments but have identified many potentia points of intervention that could be targeted. One essential step in the viral life-cycle is the infection of host cells. During the early phases of infection, this has been shown to occur mainly through the CCR5 receptor.[2] Further, CCR5 antagonists have been shown to inhibit cellular infection by R5-tropic HIV highlighting their potential to form part of the treatment of HIV.[3] Following the identification of maraviroc, we wished to identify additional CCR5 antagonists with the potential to maintain activity against resistant strains that may develop following long-term clinical use.[4]

We have previously described the identification of 3-piperidinylbutane **1** (Fig. 1).[5] The eutomer showed good activity in our cell fusion assay and excellent metabolic stability to human liver microsomes (HLM). Our knowledge of the SAR around maraviroc (**2**) led us to conclude that we should be able to increase potency by 10-fold whilst maintaining the metabolic advantage we had over the isolipophilic maraviroc.

Synthesis of 4-piperidinyl triazole **5** was achieved in four steps from 1-benzylpiperidin-4-amine (**3**) via reaction of acetohydrazide with the imidoyl chloride formed by treatment of **4** with phosphorus pentachloride to give **5** after removal of the *N*-benzyl-protecting group (Scheme 1). Ethyl 3-amino-3-phenyl-proprionate (**6**) was *N*-protected, converted to Weinreb amide **7**, and then treated with methyl Grignard reagent to afford ketone **8**. Reductive amination of **8** with **5** in the presence of titanium tetra isopropoxide gave **9** without control of the nascent stereocentre. Subsequent deprotection and acylation furnished **10** which were tested as ~1:1 mixtures of isomers (Table 1).

While ureas **10n** and **10o** and carbamate **10m** were not tolerated, potency could be enhanced through modification of the amidic group (**10a–l**). In particular, a significant advantage was observed when the substituent was cyclic with cyclopentyl and cyclohexyl analogues **10g** and **10h** showing sub-nanomolar activity in our cell fusion assay; however the attendant increase in lipophilicity had a detrimental effect upon metabolic stability. Attempts to reduce this metabolic vulnerability through inclusion of polarity at the 4-position of the cyclohexyl moiety resulted in significant potency loss (**10i–l**). This is in contrast to the SAR seen within the tropane series where polarity at the 4-position had been well-tolerated.[9] Geminal difluoro groups can occasionally be tolerated in lipophilic-favouring environments while also blocking CYP450-mediated metabolism.[10] Comparison of **1** and **10q**, and of **10h** and **10p** showed in both cases improved metabolic stability without potency loss.

Chromatographic separation of the diastereoisomers of **10p** and **10q** gave examples **11a–d** (Table 2).

We determined the absolute stereochemistry of **11c** through synthesis using the route shown in Scheme 2. Ketone **8** was reduced with sodium borohydride to diastereomers **12** and **13** which were readily separable by column chromatography. Absolute ste-

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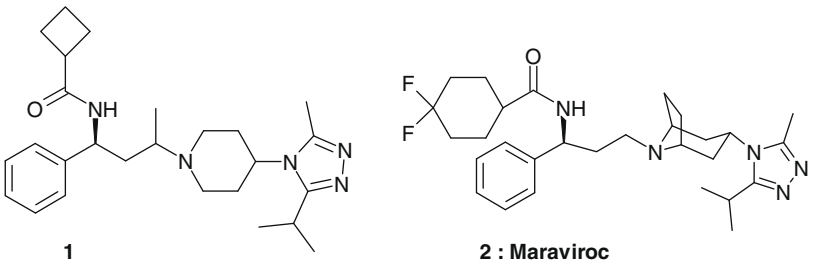
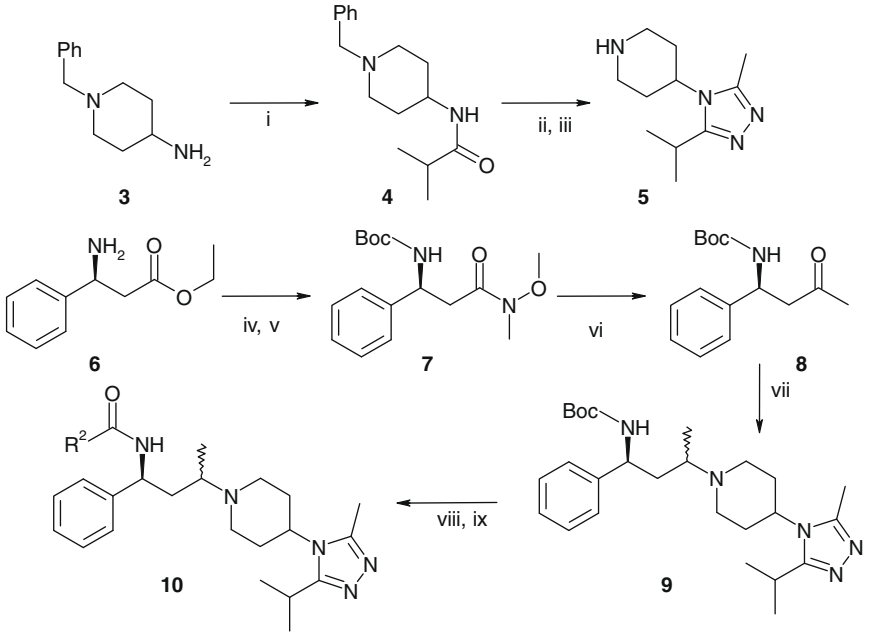


Figure 1. Comparison of maraviroc with α-methyl piperidine **1**.

reochemistries were determined by treatment of **12** and **13** with sodium hydride which resulted in cyclic carbamates **14** and **15**, respectively. nOe determinations of these cyclic carbamates enabled stereochemical assignment with reference to published values.[11] Direct conversion of **13** to the mesylate was low yielding which we believe resulted from competing intramolecular attack at the mesylate by the β-carbamate oxygen. This forced a change in protecting group to imine **16** which was successfully converted to the crude mesylate. This was undertaken at −40 °C with triethylamine added last to prevent formation of the corresponding chloride which would result in the scrambling of stereochemistry. Displacement of the mesylate by **5** followed by deprotection gave amine **17** which was converted to **11c** as before.

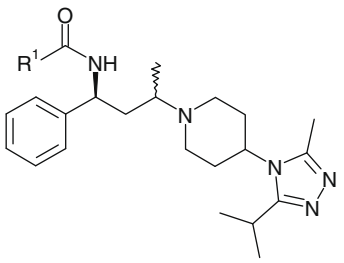
Compounds **11a** and **11c** showed good levels of metabolic stability and potency but the latter showed a superior window over activity at the hERG ion channel. Compound **11c** also showed excellent activity in a whole cell antiviral assay[8] (IC_{90} = 1.8 nM) comparable to maraviroc (IC_{90} = 2.8 nM). Good metabolic stability in rat and dog liver microsomal preparations was also demonstrated. However, a rat PK study gave a relatively high clearance of 92 ml/min/kg which in conjunction with a modest volume of distribution (V_d) of 10 L/kg resulted in a half-life of just 1.3 h. Oral bioavailability was limited to 6% which was ascribed to PGP-mediated efflux on the basis of an efflux ratio of 39 in MDCK cells. This showed little advantage over maraviroc which had shown a similar level of bioavailability in rat pharmacokinetic studies and a slightly shorter $t_{1/2}$ (0.9 h) as a result of a modest clearance (74 ml/min/kg) and V_d (6.5 L/kg).

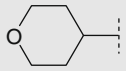
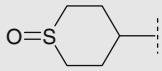
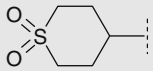
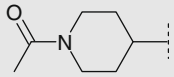
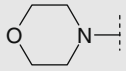
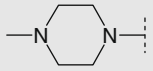
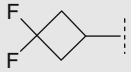
SAR from the tropane series suggested a potential solution to this poor oral absorption. We had noticed that isomeric triazoles **2** and **18** had shown significantly different biophysical properties (Table 3). In particular, the efflux ratio in caco-2 cells was improved with 1,2,4-triazole **18** over that seen with the 1,3,4-triazole **2** – a difference that remained when comparing isolipophilic analogues between the two series (data not shown).[9] Further, the disconnect between log*D* and c log*P* led us to determine pK_as which in turn demonstrated that **18** was considerably more basic than **2** – which has been ascribed to the interaction between the nitrogen lone pair and the triazole's dipole.[9] This analysis led us to conclude that the 1,2,4-triazoles could show an improved PK profile as a consequence of higher caco-2 flux and reduced metabolic clearance despite the increased lipophilicity.



Scheme 1. Preparation of 1-amido-1-phenyl-3-piperidinylbutanes. Reagents and conditions: (i) ^{t}BuCOCl, NaOH, Et_2O, rt, 96%; (ii) PCl_5, CH_2Cl_2, rt then $AcNHNH_2$ *t*-amyl alcohol then Δ toluene, 60%; (iii) NH_4CO_2H, $Pd(OH)_2$, EtOH, 60 °C, quant.; (iv) Boc_2O, Et_3N, THF, rt, quant.; (v) MeNHOMe.HCl, ^{i}PrMgCl, THF, 4 °C, 54%, (vi) MeMgBr, THF, 90%; (vii) Ti(O^iPr)$_4$, **5**, EtOH, rt, 18 h then $NaBH_3CN$, rt, 21%; (viii) HCl, EtOH, rt, quant.; (ix) **3a**–**k**, **3o** and **3p**—R^2O_2H, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, Et_3N, CH_2Cl_2, rt; **3l**–**n**—RCOCl, Et_3N, DMF, rt.

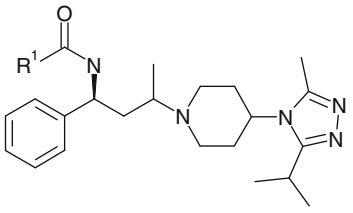
Table 1
1,3,4-Triazoles

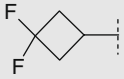
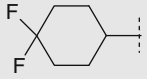


Compound	R^1	Fusion IC_{50}[a] (nM)	Log $D_{7.4}$	HLM Cl[b]
10a	Me	89.2		<8
10b	CF_3CH_2–	85.3		11.4
10c	$CF_3CH_2OCH_2$–	52.8		61.6
10d	cycloPrCH_2–	3.16	1.9	
10e	^{i}Pr	3.11	–	<8
10f	cycloPr	3.93		<8
10g	cyclopentyl	0.137		
10h	cyclohexyl	0.513	2.5	139
10i		164		10.8
10j		4.87		
10k		6.12	0.5	
10l		28.7		<8
10m	MeO	315	1.5	<7
10n		176	–	<8
10o		187		
10p[6]		0.84	2.0	19.0
10q[7]		0.38	2.0	<7

[a] For details of cell fusion assay, see Ref. 8.
[b] Units μl/min/mg microsomal protein.

Table 2
Enantiopure difluorocycloalkyl-1,3,4-triazoles



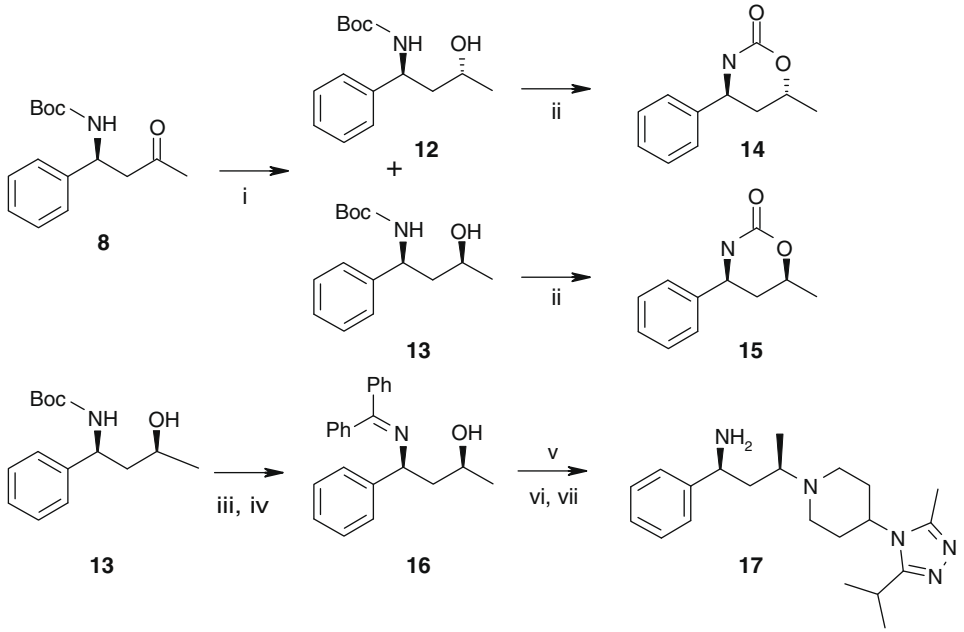
R^1				
	>			
Compound	**11a**	**11b**	**11c**	**11d**
α-Me Stereo	–	–	R	S
fusion IC_{50} (nM)	1.3 (n = 2)	62 (n = 4)	0.48 (n = 3)	49.9 (n = 4)
Log D	1.9	2.0	2.2	2.2
HLM	<7	16	12	<7
hERG[a] $IC_{50\ (\mu M)}$	2.3	2.1	>10	>10

[a] The concentration required to inhibit [^{3}H]dofetilide binding to hERG stably expressed on HEK-293 cells.

A small set of 1,2,4-triazoles were prepared with the difluorocyclohexyl amide moiety (Scheme 3).

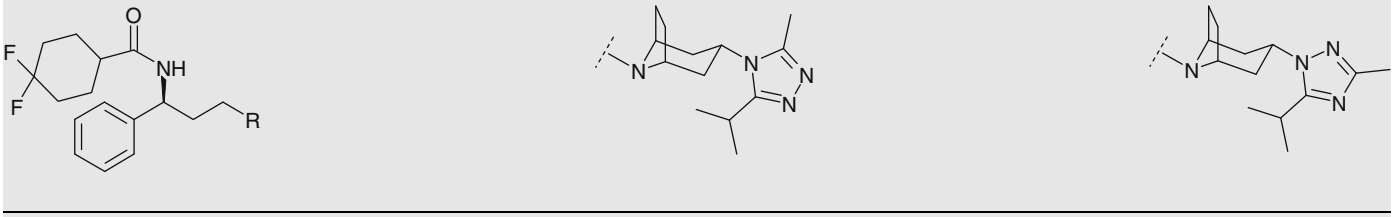
N-Benzylpiperidin-4-one **19** was reductively coupled to *N*-Boc-hydrazine and deprotected to yield 4-piperidinyl hydrazine **21** which was heated with aminoenamines **24** in the presence of acetic acid to yield 1,2,4-triazoles **26** following debenzylation. Reductive amination with *N*-Boc ketone **8** followed by deprotection and amide coupling gave targets **27**–**33** as a mixture of isomers at the α-methyl centre which were separated by column chromatography (Table 4).

Potency was reduced by 6-fold for 1,2,4-triazole **28a** over the isomeric 1,3,4-triazole **11c**. As expected, lipophilicity was increased but this did not impact upon the rate of microsomal clearance. In fact, **28a** shows remarkably little metabolic vulnerability illustrating the inherent stability of this chemotype despite a measured log D of 3 and a significant free fraction in human and rat plasma (fraction unbound = 0.15 and 0.24, respectively). Potency



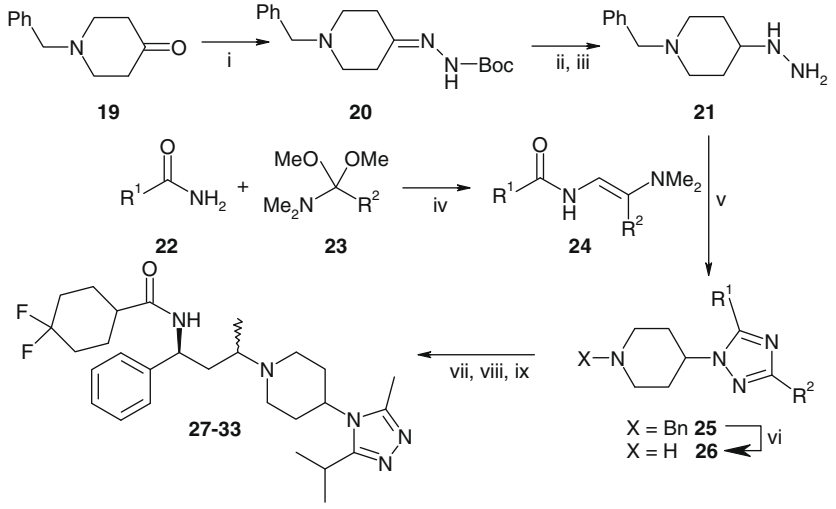
Scheme 2. Determination of absolute stereochemistry of **11c**. Reagents and conditions: (i) $NaBH_4$, EtOH, 70% anti:syn 2:1, chromatographic separation; (ii) NaH, THF, reflux; (iii) HCl, Et_2O; (iv) benzhydrylideneamine, CH_2Cl_2; (v) MsCl, CH_2Cl_2, −40 °C, then Et_3N; (vi) **5**, K_2CO_3, MeCN, reflux; (vii) HCl, CH_2Cl_2.

Table 3
A comparison of 1,2,4 and 1,3,4 triazoles



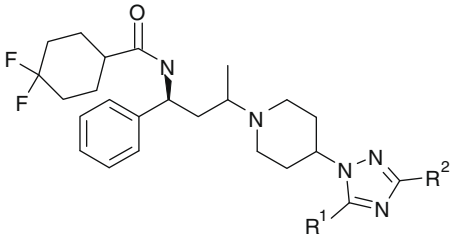
Compound	**2**	**18**
clog*P*	3.3	3.6
log*D*	1.9	2.7
Caco-2[a]	1/10	11/26
HLM Cl_{int}[b]	20	6
pK_a	7.7	8.4
Fusion IC_{50} (nM)	0.22	1.4

[a] Apical-to-basal/basal-to-apical.
[b] Units μl/min/mg microsomal protein.



Scheme 3. Preparation of 1,2,4-triazoles. Reagents and conditions: (i) $H_2NNHBoc$, AcOH, CH_2Cl_2, rt, 18 h, 95%; (ii) $NaBH_4$, CH_2Cl_2, AcOH, rt, 24 h, quant.; (iii) HCl, MeOH, rt, 12 h, quant.; (iv) toluene, 120 °C, 6 h, 80%; (v) AcOH, 90 °C, 3 h, 80%; (vi) $Pd(OH)_2$, NH_4CO_2H, EtOH, 60 °C, 3 h, 91%; (vii) $Ti(O^iPr)_4$, EtOH, **8**, rt, 16 h then $NaBH_3CN$, rt; (viii) HCl, EtOAc, rt, quant.; (ix) acid, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, Et_3N, CH_2Cl_2, rt.

Table 4
1,2,4-triazoles



Compound	R^1	R^2	Fusion IC_{50} (nM)	Log$D_{7.4}$	HLM Cl[a]
27a (**27b**)	Et	Me	12 (252)	2.5 (2.7)	7 (25)
28a (**28b**)	^{i}Pr	Me	3.08 (385)	3.0 (2.7)	15 (23)
29a (**29b**)	Me	Et	28 (549)	2.9 (2.8)	39 (166)
30a (**30b**)	Et	Et	15.7 (292)	3.3 (3.1)	40 (91)
31a (**31b**)	cycloPr	Me	15 (1000)	2.9 (2.9)	35 (99)
32a (**32b**)	CH_2OMe	Me	92.3 (4200)	2.6 (2.6)	13 (65)
33a (**33b**)	cycloBu	H	20.9 (302)	3.1 (3.0)	117 (>440)

Key: More active isomer (less active isomer).
[A] Units μl/min/mg microsomal protein.

was reduced for all other difluorocyclohexylamide analogues tested (**27a** and **29a**–**33a**). Compound **28a** showed the best balance of potency and metabolic stability together with an improved MDCK efflux ratio (A – B/B – A = 2) together and high flux (apical-basal $P_{apps} = 23 \times 10^{-6}$ cm/s). Whole cell antiviral activity was also good (IC_{90} = 2.6 nM) and little activity was seen against the hERG channel (IC_{50} > 10 μM). While **11c** showed no measureable inhibition against human CYP isoforms, modest activity against CYP3A4 was observed for **28a** (IC_{50} = 3.4 μM). No activity was observed for other isoforms (1A2, 2C9 or 2D6) up to 30 μM.

An oral rat PK study showed **28a** to be fully absorbed. This was a significant improvement over both **2** and **11c**. However, the half-life had not been extended as despite a reduction in clearance to 30 ml/min/kg, the volume of distribution dropped to 2.5 L/kg giving a $t_{1/2}$ of 1 h. This was unexpected given the rise in log*D* measured from **11c** to **28a**.

We have demonstrated with **28a** that within the α-methyl piperidine chemotype, it is possible to combine both maraviroc-like levels of antiviral activity with complete oral absorption. Replacement of the 1,2,4-triazole of **11c** with regioisomeric 1,3,4-triazole exhibited by **28a** led to an increase in lipophilicity that was not matched by a reduction in metabolic stability. While this change

significantly improved oral absorption, it had no impact upon the $t_{1/2}$ in rats giving a pharmacokinetic profile equivalent to maraviroc. These results have prompted further investigation into alternative heterocyclic systems which will be disclosed shortly.

Acknowledgements

We thank Manuel Perez for NMR analyses.

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