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Discovery of a small molecule inhibitor through interference with the gp120–CD4 interaction

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ABSTRACT

A series of piperazine derivatives were designed and synthesised as gp120–CD4 inhibitors. SAR studies led to the discovery of potent inhibitors in a cell based anti viral assay represented by compounds **9** and **28**. The rat pharmacokinetic and antiviral profiles of selected compounds are also presented.

Human immunodeficiency virus (HIV-1) infection remains a major medical problem with approximately 33 million people worldwide living with the disease, together with 2.5 million people newly infected with HIV in 2007.[1] Despite advances in combination therapies and the approval of 29 marketed drugs that cover 5 main classes (nucleotide/nucleoside, non nucleotide reverse transcriptase inhibitors, protease inhibitors, entry inhibitors and integrase inhibitors), emergence of drug resistant viral strains,[2] and tolerance/toxicity[3] remain major issues in the treatment of HIV infection. As a consequence, the demand for new classes of anti-retroviral drugs that target different mechanisms remains high.

The interaction of HIV-Env, the gp160 envelope protein comprising gp120–gp41, with CD4 and a co-receptor triggers a cascade of events that results in the insertion of gp41 into the host cell membrane and the cell becoming infected. Compounds interfering with gp120/co-receptor interactions, for example, maraviroc (Selz-entry), via blocking of the CCR5 co-receptor, have shown strong antiviral efficacy in patients infected with CCR5-tropic (R5) virus.[4] Additionally, the first clinically approved entry inhibitor, Enfuvirtide, (Fuzeon) blocks HIV-1 entry into the host cell by binding to the gp41 subunit of the HIV-Env glycoprotein and has been shown to reduce viral load in the clinic.[5] Targeting the first stage of the fusion process that is, the gp120–CD4 interaction presents an alternative, attractive anti-retroviral approach. Targeting such an early stage of the viral cycle has the advantage of being able to inhibit CCR5-tropic (R5), CXCR4-tropic (X4) and dual-tropic (DM) viruses.[6]

Bristol-Myers Squibb has developed a series of small molecule gp120–CD4 inhibitors based on an *alpha* keto amide structure (Fig. 1) which specifically inhibit HIV entry.[7]

These compounds exhibit potent antiviral activity in cell culture. Additionally, it has been shown that BMS378806 binds directly to gp120, and is active against a range of lab adapted strains of HIV-1, irrespective of chemokine receptor tropism.[8–10] BMS488043 has been evaluated in healthy volunteers where it was shown to be safe, tolerable and adequate pharmacokinetic profile when administered over a fourteen day period.[11] Additionally, BMS488043 has been evaluated in a multi-dose study (800 mg or 1800 mg *bid*) in HIV-1 infected patient where the preliminary results established mean maximal changes in plasma HIV-1 RNA of up to -1.2 $\log_{10}$,[8–10] thus establishing proof of concept for blocking the gp120–CD4 interaction as being clinically efficacious.

In this Letter we would like to disclose some of our recent findings towards identifying an orally bioavailable gp120–CD4 inhibitor as a novel class of anti-HIV medicines which would also be complementary with other classes of anti-retroviral drugs.

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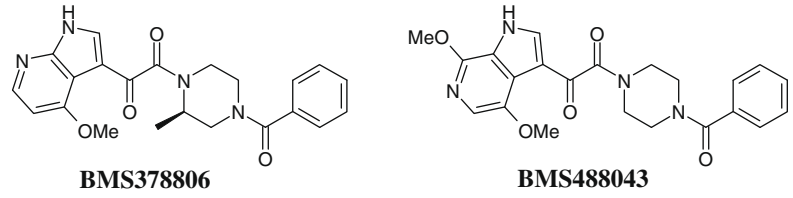
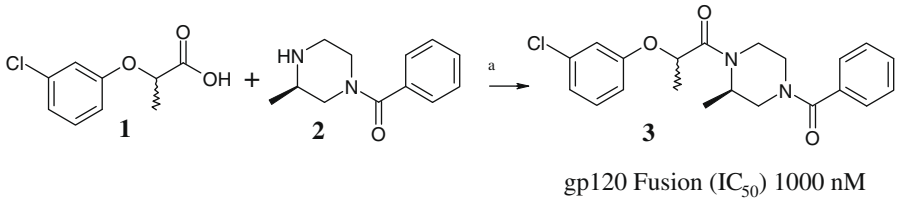


Figure 1.



Scheme 1. Reagents and conditions: (a) *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluroniumhexafluorophosphate, NEt_3, DCM, rt, 24 h, 65%.

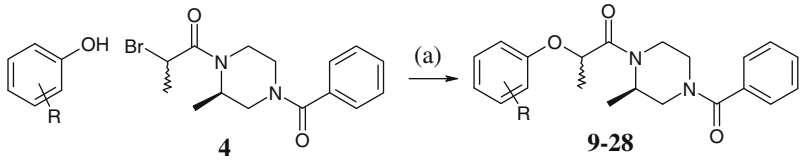
Our overall target was to identify an orally bioavailable gp120–CD4 inhibitor possessing pharmacokinetic properties commensurate with a low dose (<300 mg *bid*) regimen. At this dose, the C_{min} for this compound should be in excess of the in vitro IC_{90} for the majority of primary clinical isolates.[1]

As part of a programme seeking novel inhibitors of the gp120–CD4 interaction, our work focused initially on the above ketoamides where we looked to replace the potentially reactive and metabolically vulnerable ketone. As a consequence of plate based coupling of acids to **2** to make a range of analogues designed to replace the keto acid, it led to the identification of **3** (Scheme 1). This compound is relatively lipophilic and possessed moderate activity (IC_{50} of 3 μM) in our high throughput cell–cell fusion assay.[12] Our choice of **3** for further optimisation in our hit-to-lead programme was driven by the fact that the template is amenable to parallel chemistry. Hence, the goal of the initial hit-to-lead study was to rapidly optimise this hit by tuning the physiochemical properties by incorporating small polar groups into lead **3** as a strategy to increase the polarity whilst keeping the molecular weight low according to Lipinsky's rule of five.[13] This would maximise our chances to achieve our desired profile of an orally bioavailable potent gp120–CD4 inhibitor.

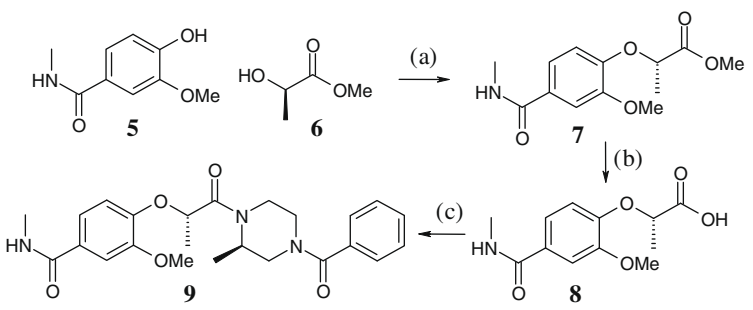
All compounds **9–28** were prepared as mixtures of diastereomers by the general method shown in Scheme 2. The versatile common intermediate **4**[14] was prepared in one step from the coupling of 2-bromopropionylchloride with 2*R*-4-benzoyl-2-methylpiperazine.[15]

Single diastereomers were synthesised by the general method exemplified in Scheme 3 for compound **9**. Reaction of *R*-methyl lactate **6** and the appropriate phenol **5** in THF under standard Mitsunobu conditions afforded the aryl ether **7** in good general yield. Alkaline hydrolysis of the methyl ester to furnish the carboxylic acid **8** followed by a carbodiimide mediated condensation with 2*R*-4-benzoyl-2-methyl-piperazine **2** afforded the enantiomerically pure **9** in Scheme 3.

From the initial set of analogues prepared, some intriguing SAR emerged (Table 1). Most notably, polar substituents on the phenyl ring were relatively well tolerated with only modest erosion in

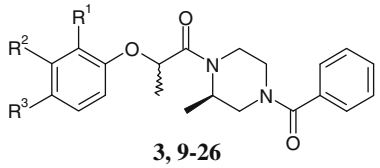


Scheme 2. Reagents and conditions: (a) Cs_2CO_3, acetone, rt, 24 h, 40–75%.



Scheme 3. Reagents and conditions: (a) Triphenylphosphine, diisopropylazodicarboxylate, THF, 5 °C, rt, 2 h; (b) LiOH, MeOH/THF, rt, 2 h, 75% over two steps; (c) 1-*N*-hydroxybenzotriazole, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl, triethylamine, rt, 17 h, 72%.

Table 1
SAR Summary: in vitro inhibition gp120 (JRFL) mediated cell–cell fusion,[a] *c*log *P* calculations,[b] log $D_{7.4}$ and in vitro metabolic stability[c]



3, 9-26

Compd	R^1	R^2	R^3	gp120[a] IC_{50} (μM)	*c*log *P*[b]	Log *D*	HLM[c] Clint (μl/min/mg)
3	H	Cl	H	1	4.2	ND	ND
10	CONHMe	H	H	>25	2.3	1.5	23
11	H	CONHMe	H	1.4	2.4	1.3	<8
12	H	H	CONHMe	2.6	2.4	1.1	<8
13	$CONMe_2$	H	H	>25	2.1	1.1	31
14	H	$CONMe_2$	H	>25	2.1	1	12
15	H	H	$CONMe_2$	>25	2.1	0.9	<8
16	Me	Me	H	0.1	4.3	ND	158
17	Me	CONHMe	H	12	2.5	1.2	<8
18	OMe	CONHMe	H	>10	2	1	<8
19	Me		H	0.075	3.1	2.1	141
20	H		H	0.035	3.7	2.3	61
21	H	Me	CONHMe	1	2.53	1.1	<8
22	Me	H	CONHMe	2.9	2.9	1.8	<8
23	H	OMe	CONHMe	2.7	2.3	ND	ND
9[d]	OMe	H	CONHMe	0.017	2.1	1	<8
24[e]	OMe	H	CONHMe	>2	2.1	ND	ND
25	OEt	H	CONHMe	>2	2.6	ND	ND
26	F	H	CONHMe	2	2.5	1.4	<8

ND denotes not determined.
[a] The concentration required to inhibit gp120 (JRFL) mediated cell–cell fusion.[12]
[b] Biobyte Corporation, 201 W. 4th St., #204, Claremont CA 91711-4707, *c*LOG *P* version 4.3.
[c] Human Liver Microsomes, minimum measurable in vitro intrinsic clearance was 8 μl/min/mg protein.
[d] Single *S*,*R* diastereomer.
[e] Single *R*,*R* diastereomer.

potency as exemplified by the carboxamides **11** and **12,** but significant drop in lipophilicity. Tertiary amide substitution **13**, **14** and **15** and the *ortho* secondary amide in **10** were not well tolerated. Introduction of modestly lipophilic substituents on the phenyl ring **16** proved to be advantageous affording improvements in potency over the starting 3-chlorophenyl group in **2**. Unfortunately, these changes resulted in increased metabolic vulnerability of **16** and precluded further advancement of the compound.

The strategy of introducing polar substituents at the *meta* position while incorporating the favourable substitution of **16** met with limited overall success (**17**–**20**). Analogues **17** and **18**, whilst achieving improved levels of in vitro metabolic stability, displayed disappointing potency. The oxadiazole **19** and pyrazole **20** gave highly encouraging levels of potency however, the increased Log*D* of these compounds resulted in unacceptably high metabolic rates in human liver microsomal incubations. For this series large increases in metabolic instability were observed with compounds having *c*log*P* >2.5 and Log*D* >1.5, so efforts were made to keep lipophilicty below these values in further analogues. We next turned our attention to the synthesis of *para* secondary amide analogues (**9**, **21–26**). This strategy proved to be highly rewarding with the identification of amide **9**. A homochiral synthesis of the two diastereoisomers (**9** and **24)** unambiguously established that the activity resided with the *S*, *R* diastereomer **9** (Table 1).

As shown in Table 1 the *para* carboxamide series is characterised by some very subtle SAR, introduction of the 3-methyl and the 2-methyl substituent **21** and **22**, respectively, resulted in no significant improvement in potency relative to the parent carboxamide. Previous SAR suggested that methyl substituents in the *ortho* and *meta* position were advantageous for potency.

Replacement of the *ortho*-methyl in **23** for a methoxy (**9)** resulted in a dramatic 100 fold improvement in potency as well as approximately a unit reduction in overall lipophilicity relative to **22**.

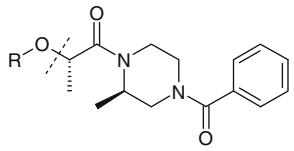
Encouraged by the latter data a number of substituents at the *ortho* position were investigated as exemplified by **25–26**. Disappointingly, these latter derivatives (**25**, **26**) resulted in a precipitous loss in potency.

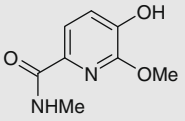
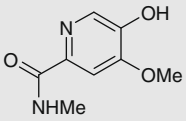
With the identification of amide **9** as our most promising lead, we sought to further improve the physicochemical properties of the series through the introduction of a ring nitrogen (Table 2). A series of pyridyl analogues based around **9** were synthesised (**27**, **28**). The pyridyl derivative **27** showed a significant drop off in potency in our assay, however, compound **28** retained a high level of potency and excellent metabolic stability.

Based on their overall in vitro profiles **9** and **28** were progressed for full pharmacokinetic (Table 3) and antiviral assay[15] profiling. Encouragingly, **9** demonstrated moderate oral bioavailability and excellent anti viral activity with an IC_{50} of 3 nM. Excitingly, the full pharmacokinetic profile of **28** showed the compound to posses improved clearance and oral bioavailability and excellent antiviral activity of IC_{50} 2 nM. Projected human pharmacokinetics suggested that **28** would exhibit an oral bioavailability of greater than 90% and a predicted half life of around 5 h.

The potency of **28** against a panel of viral strains is listed in Table 4. The compound shows encouraging antiviral potency

Table 2
SAR Summary: in vitro inhibition gp120 (JRFL) mediated cell–cell fusion,[a] clog *P* calculations,[b] log $D_{7.4}$ and in vitro metabolic stability[c]



Compd	ROH	gp120[a] IC_{50} (μM)	clog *P*[b]	Log *D*	HLM[c] Clint (μl/min/mg)
27		0.257	2.3	ND	ND
28		0.011	2.2	0.7	<8

ND denotes not determined.
[a] The concentration required to inhibit gp160 (JRFL) mediated cell–cell fusion.[12]
[b] Biobyte Corporation, 201 W. 4th St., #204, Claremont CA 91711-4707, *CLOGP* version 4.3.
[c] Human Liver Microsomes, minimum measurable in vitro intrinsic clearance was 8 μl/min/mg protein.

Table 3
Pharmacokinetic (iv 1 mg/kg, *po* 1 mg/kg ($n = 2$)), in vitro metabolic stability, physiochemical and potency data associated with compounds **9** and **28**

	9	**28**
Cl[a] (mL/min/kg)	75	18
Vd[b] (L/kg)	4.3	1.5
ppb[c] (%)	19	31
$t_{1/2}$ (h)	0.7	1
F[d] (%)	48	65
RLM[e] $t_{1/2}$ (min)	>120	>120
AV[f] IC_{50} (nM)	3	2

[a] Clearance.
[b] Volume of distribution.
[c] Plasma protein binding.
[d] Bioavailability.
[e] Rat Liver Microsomes, maximum measurable half life ($t_{1/2}$) was 120 min.
[f] The concentration required to inhibit viral entry of lab adapted HIV NL4-3 in HeLa-P4 cells to 50%.[16] All compounds had negligible cytotoxicity with CC_{50} >10 μM.

Table 4
HIV-1 Inhibitory data against lab adapted (LA) and primary isolate (PI) strains tested in HeLa-P4 reporter cell line or in human PBMCs for compound **28**

Virus	Tropism	Cells	Geo. Mean IC_{50} (nM)	*n*
NL4-3 (LA)	X4	HeLa-P4	2	3
IIIB (LA)	X4	PBMCs	10	3
BaL (LA)	R5	PBMCs	25	6
BR92004 (PI)	R5	PBMCs	6	3
BR92023 (PI)	R5	PBMCs	0.6	2

against a number of lab adapted strains and primary isolates assayed in human PBMCs (peripheral blood mononuclear cells). Additionally, compound **28** showed potent antiviral activity against a panel of 25 clade B isolates[17] where 70% of these viruses were inhibited at an IC_{90} below C_{min} of 500 nM.

Modelling of the predicted human pharmacokinetics of **28** ($t_{1/2}$ 5 h, F 90% and Vd 1 L/kg) suggests that the C_{min} of 500 nM could be achieved with a twice daily dose of 200 mg, hence, enabling good antiviral coverage against many relevant isolates. Based on these data and further profiling, **28** was selected for clinical development as a broad spectrum gp120–CD4 inhibitor for the treatment of HIV.

In summary, a series of 2*R*-methyl-piperazinylbenzamide derivatives were successfully designed and efficiently synthesised, incorporating polar functionality as a strategy to improve overall metabolic stability. These neutral low molecular weight inhibitors that interfere with the interaction of gp120 to CD4 displayed very good cell based efficacy against a broad spectrum of viral strains. Overall analogue **28** displayed the most attractive profile and was found to possess good rat pharmacokinetics commensurate with its physicochemical properties.

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16. Antiviral assays: activity against NL4-3 virus in HeLa-P4 cells was determined as follows: Diluted compound was added to wells of a sterile 96-well flat-bottomed black tissue culture plate with clear base and lid (Costar). HeLa-P4 cells were mixed with NL4-3 virus and added to the wells at a concentration of 10^5 cells/mL in a volume of 90 μL. The plates were incubated for 5 days at 37 °C in a 5% CO_2 humidified incubator, then virus infection was quantified using the FluorAce β-galactosidase reporter assay kit (Bio-Rad) according to the manufacturer's instructions.
17. Antiviral assay was carried out by Monogram Biosciences using their Phenosense assay.