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Design and optimisation of potent gp120-CD4 inhibitors

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ABSTRACT

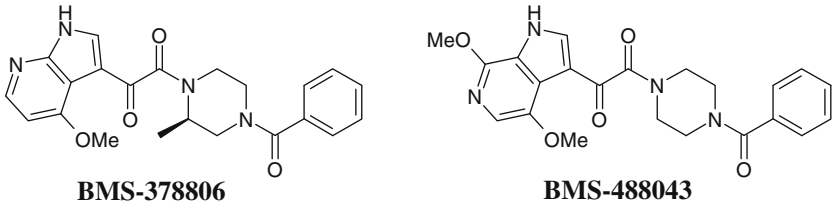
The synthesis and structure–activity relationship of a series of novel gp120-CD4 inhibitors are described. Pharmacokinetic studies and antiviral spectrum assessment of lead compounds led to the identification of compound **36**, a potent gp120-CD4 inhibitor which exhibited antiviral potency across a spectrum of 25 clade B isolates.

Infection with human immunodeficiency virus (HIV) affects millions of people worldwide.[1] Despite the introduction of highly active antiretroviral therapy (HAART) regimes using combination therapies which target one of the essential viral proteins, there is still a need for new therapeutic agents with improved dosing regimes that are better tolerated with a reduced side effect burden.[2]

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.[3] These compounds exhibit potent antiviral activity in cell culture and BMS-488043 has demonstrated the ability to reduce viral loads in man.[4–6]

Trimeris has also reported a series of small molecule gp120-CD4 inhibitors based on the modifications of the *alpha* keto amide with an isosteric sulfonamide group.[7]

As part of our research efforts to identify new therapeutic agents for the treatment of HIV, we recently reported the discovery of compound **1**,[8] the prototype of a series of novel HIV-1 entry inhibitors that bind directly to the viral gp120 envelope protein and prevent the interaction between gp120 and host CD4 receptors on T cells, the very first step of the viral entry process. Compound **1** has an exciting profile with a 200 mg bid dose predicted to give a C_{min} of 500 nM, covering 70% of clade B viruses at IC_{90}.[9] The compound is active against both -R5, -X4 and dual tropic viruses and has an estimated half-life in human of 5 h. The present work



BMS-378806 BMS-488043

Figure 1. gp120-CD4 inhibitors reported by BMS.

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E-mail address: thien.tran@pfizer.com (T.-D. Tran).

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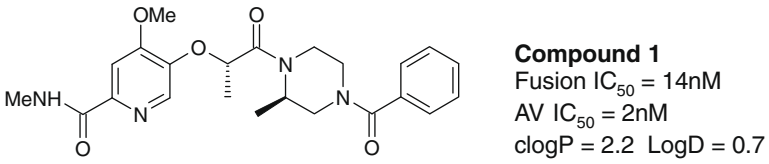
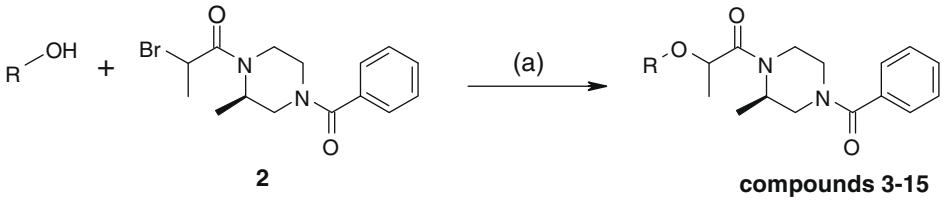


Figure 2. Profile of compound **1**.



Scheme 1. Reagents and conditions: (a) Cs_2CO_3, acetone, rt, 24 h, *y* = 60–90%.

describes our attempts to identify a back-up with improved profile in terms of antiviral spectrum and/or pharmacokinetic properties over compound **1** (Fig. 2).

Our goals were to identify a more potent compound with comparable pharmacokinetic profile to compound **1** to lower the dose

Table 1
gp160 fusion inhibitory activity[a] and antiviral activity[b] of piperazine analogues

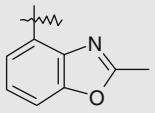
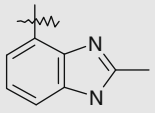
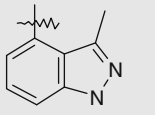
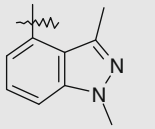
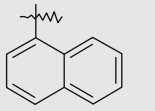
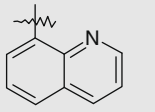
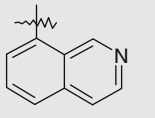
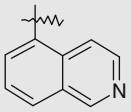
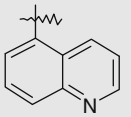
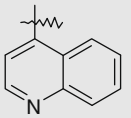
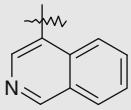
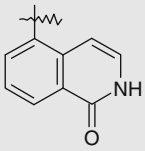
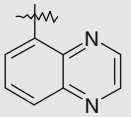
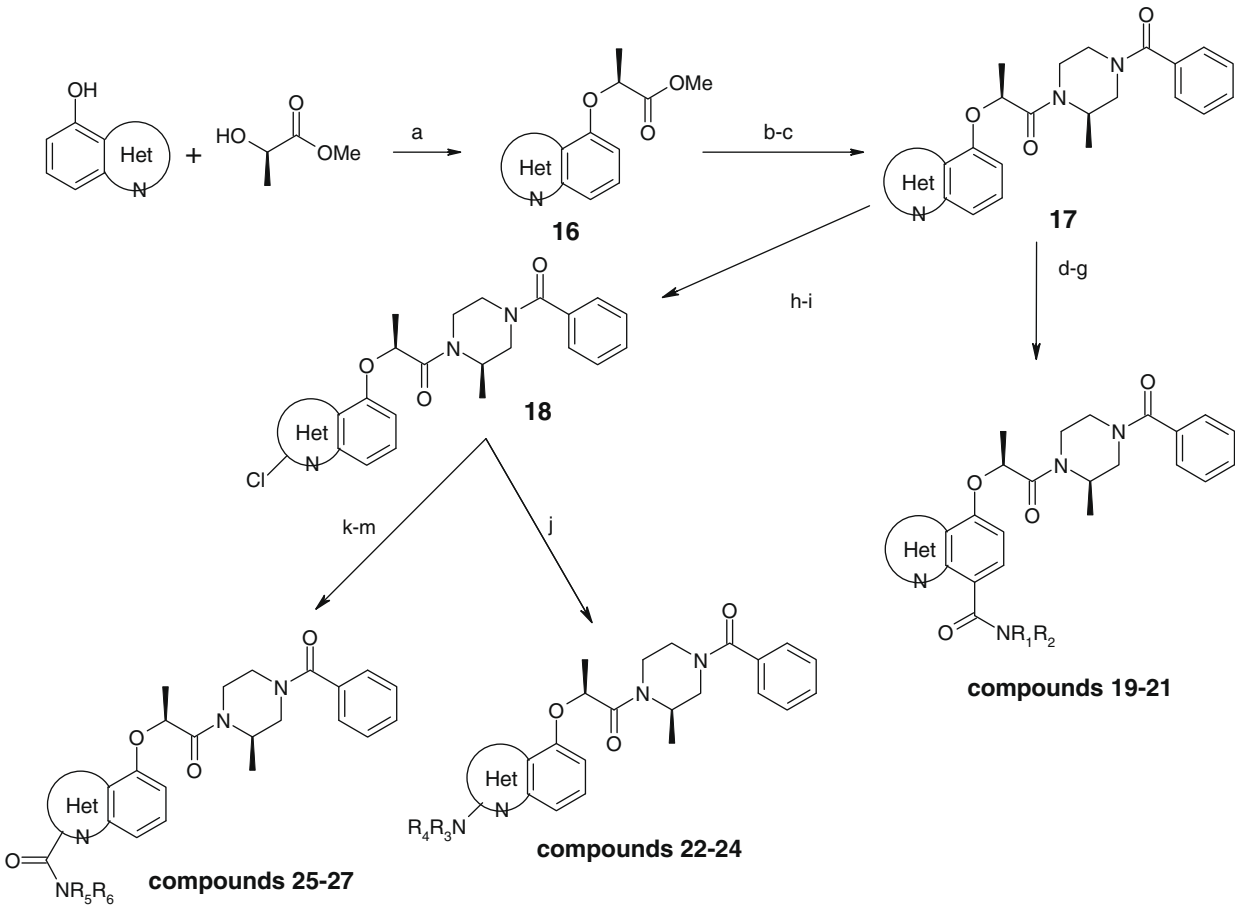
Compound	R	Fusion IC_{50} (nM)	Anti-viral IC_{50} (nM)	clog *P*[d]	MW
3		2040	ND[c]	3.28	407
4		834	ND	3.44	406
5		16	36	3.54	406
6		15	79	3.34	420
7		123	1070	4.54	402
8		433	449	3.63	403
9		575	2040	3.42	403

Table 1 (*continued*)

Compound	R	Fusion IC_{50} (nM)	Anti-viral IC_{50} (nM)	clog *P*[d]	MW
10		103	492	3.42	403
11		99	271	3.63	403
12		7430	ND	3.75	403
13		9370	ND	3.54	403
14		68	1050	2.31	419
15		493	ND	2.98	404

[a,b] See Refs. 5 and 6 for complete details of assay conditions.
[c] ND = not determined.
[d] Biobyte Corporation, 201 W. 4th St.,#204, Claremont CA 91711-4707, CLOGP version 4.3,

prediction and to search for significantly structurally-differentiated leads to give a different antiviral spectrum to compound **1**. Our work focused initially on finding a novel expression of the methoxy pyridine amide moiety as this fragment could be easily replaced from a synthetic point of view. A range of polar bicyclic analogues were prepared to mimic the methoxy substitution pattern of the pyridine template. Test compounds were conveniently



Scheme 2. Reagents and conditions: (a) DIAD, PPh_3, THF, rt, o/n, $y \sim 70\%$; (b) NaOH, dioxane, 55 °C, 3 h, $y \sim 80\%$; (c) HBTU, triethylamine, 2-(*R*)-4-benzoyl-2-methyl-piperazine, DCM, DMF, rt, o/n, $y = 40–90\%$; (d) *N*-bromosuccinimide, acetonitrile, rt, o/n, $y \sim 40\%$; (e) $Pd(dppf)Cl_2$, CO, triethylamine, methanol, 100 psi, 100 °C, $y \sim 60\%$ (f) NaOH, dioxane, 55 °C, 3 h, $y \sim 80\%$; (g) HBTU, triethylamine, R_1R_2NH, DCM, DMF, $y = 40–90\%$; (h) *m*-CPBA, DCM, rt, 3 h; $y = 30–70\%$; (i) $POCl_3$, DCM; 50 °C, 3 h, $y \sim 70\%$ (j) R_3R_4NH, triethylamine, caesium fluoride, DMSO, 130 °C, $y \sim 70\%$; (k) $Pd(dppf)Cl_2$, CO, triethylamine, methanol, 100 psi, 100 °C, $y \sim 60\%$ (l) NaOH, dioxane, 55 °C, 3 h, $y \sim 80\%$; (m) HBTU, triethylamine, R_5R_6NH, DCM, DMF, rt, o/n, $y = 40–90\%$.

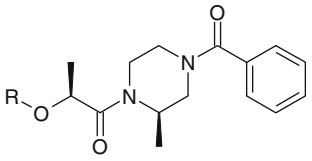
prepared from commercially available hydroxy heterocycles by the general methodology described in Scheme 1. Standard bromide displacement of intermediate **2** with the appropriate hydroxy heterocycles under basic conditions provided required targets **3–15** (Table 1).

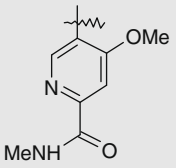
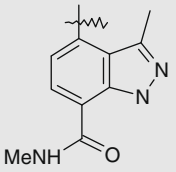
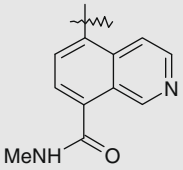
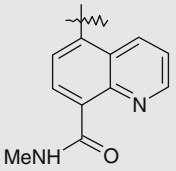
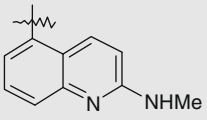
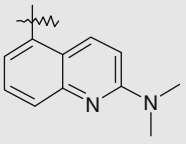
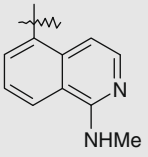
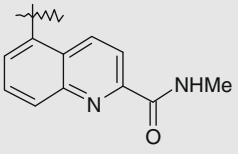
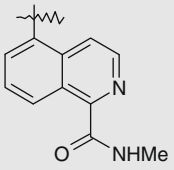
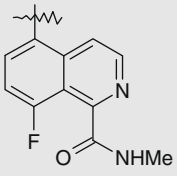
A number of derivatives showed promising activity in our gp160 fusion assay,[10] however, this did not always translate to equivalent antiviral activity.[11] Interestingly, indazole analogue **5** had promising potency in both assays as well as its N-methylated version **6** suggesting that the hydrogen on the indazole was not required for potency. Isoquinoline analogue **10** and quinoline analogue **11** had also promising potency in both assays, prompting us to explore the SAR around these novel heterocycles to enhance the potency of our targets. Structurally, the quinoline, isoquinoline and indazole templates looked very similar to the methoxy pyridine template; they just lack the amide feature which was essential for the potency of compound **1**.[2] Our priority was to introduce this key amide substituent onto the newly discovered rings to assess its influence on potency and lower the lipophilicity of the lead molecules. All single diastereoisomeric compounds were synthesised by the general method shown in Scheme 2.

Reaction of (*R*)-methyl lactate and the appropriate phenol in THF under standard Mitsunobu conditions afforded the aryl ether **16** generally in 70% yield. Ester hydrolysis under basic conditions followed by standard amide coupling with 2-(*R*)-4-benzoyl-2-methyl-piperazine provided the single diastereoisomer intermediate **17**. Intermediate **17** was then brominated with *N*-bromosuccinimide in acetonitrile. The corresponding bromo intermediate was carbonylated under carbon monoxide pressure to give the corresponding methyl ester intermediate. Ester hydrolysis under basic conditions followed by standard amide coupling provided target compounds **19–21**. Reaction of intermediate **17** with *m*-CPBA provided the N-oxide intermediate which was then chlorinated to give the chloro intermediate **18**. Carbonylation of **18** followed by ester hydrolysis and amide coupling provided target compounds **25–27**. The chloro intermediate **18** could also be displaced by amines under basic conditions to provide target compounds **22–24**.

Initial attempts to incorporate the potency enhancing amide substituent of compound **1** (Table 2) into the isoquinoline, quinoline and indazole templates resulted in a loss in potency (compounds **19–21**). Due to the difficulty in functionalising the indazole template, we subsequently focused our efforts on the isoquinoline and quinoline templates. Despites our initial disappointments, we continued to explore a range of substituents to build up SAR around the heterocyclic rings. Primary amines (compound **22**) improved potency by 20-fold in both fusion and anti-viral assays, while introduction of secondary amines resulted in a loss of potency (compound **23**) suggesting that the H-bond donor was essential for potency. Amides such as **25** were also tolerated albeit significantly weaker in the antiviral assay. Within the isoquinoline template, both amines and amides were tolerated (compounds **24** and **26**), with potency comparable to the quinoline **22**. The most

Table 2
gp160 fusion inhibitory activity and antiviral activity of functionalised piperidine analogues



Compound	R	Fusion IC_{50} (nM)	Anti-viral IC_{50} (nM)	clog *P*	MW
1		14	2	2.2	440
19		>10,000	ND	2.65	463
20		21,200	ND	2.71	460
21		1260	ND	2.92	460
22		5	13	4.12	432
23		3200	ND	4.34	446
24		61	ND	3.91	432
25		78	>4000	3.42	460
26		21	1.5	3.21	460
27		31	20	3.37	478

potent compound from each novel template, **22** and **26**, was progressed into rat pharmacokinetic studies (Table 3).

Compound **22** had a lower absorption, lower bioavailability and a higher unbound clearance compared to compound **1**, attributed to its high lipophilicity. This, coupled with a lower potency in the antiviral assay, resulted in an inferior profile to compound **1**. Compound **26** was equipotent to compound **1** in the antiviral assay and based on rat pharmacokinetic profile, we estimated that to cover 70% of clade B virus at IC_{90}, a 300 mg bid dose would be required, resulting in an overall inferior profile to compound **1**. Subsequently, compound **26** showed a positive signal in the glutathione trapping assay. The source of the reactive metabolite in compound **26** is believed to be P450-mediated oxidation *para* to the oxygen on the isoquinoline leading to a quinone-like species. We then prepared several analogues to block metabolism at this position. Unfortunately, the direct fluoro analogue **27** showed a significant drop in potency in the antiviral assay, putting an end to our efforts in exploring an alternative expression of the pyridine template.

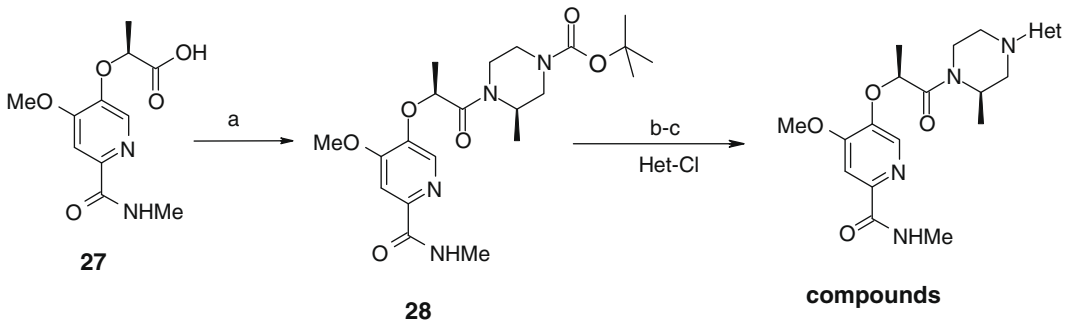
We then turned our attention to the benzamide group in compound **1**. Our strategy was to identify a conformationally restricted expression of the benzamide, the hypothesis being that if we could access a favoured locked conformation then significant potency gain could be realised. All targets were synthesised by the general method shown in Scheme 3 (Table 4).

Replacement of benzamide with directly attached imidazole **30** or purine **33** gave disappointing potency in the fusion assay. Replacement with pyridine **29,** indazole **31** or azaindole **32** gave some encouraging potency in the fusion assay; however this did not translate into antiviral activity. The most encouraging replacement was with polar heterocyclic systems such as azaindole **34** and azabenzimidazole **35**, which had similar polarity and antiviral potency to compound **1**. Unfortunately, both compounds **34** and **35**

Table 3
Rat pharmacokinetics (IV 1 mg/kg, po 1 mg/kg)

	Compound **1**	Compound **22**	Compound **26**
Cl (mL/min/kg)	18	27	41
Clu (mL/min/kg)	26	129	90
$T_{1/2}$ (h)	1	0.9	0.5
Vdu (L/kg)	2.2	10.5	3.5
F (%)	65	42	45
Fabs (%)	100	60	55

Cl = Clearance; Clu = unbound clearance; Vdu = unbound volume of distribution, *F* = bioavailability; Fabs = fraction of compound absorbed.



Scheme 3. Reagents and conditions: (a) WSCDI, HOBt, DIPEA, 2-(*R*)-4-*N*-BOC-2-methyl-piperazine, THF, rt, o/n, *y* = 80%; (b) HCl 4 M dioxane, rt, 3 h, *y* = Q; (c) triethylamine, butanol, 120 °C, 3 h, *y* ~ 50%.

Table 4

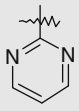
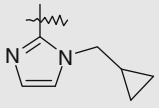
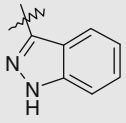
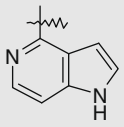
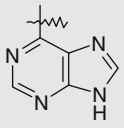
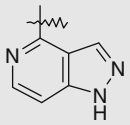
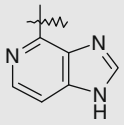
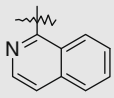
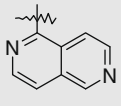
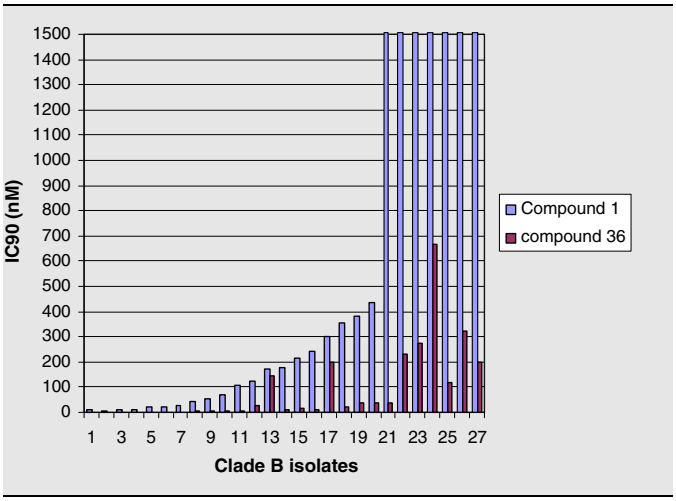
Compound	Het	Fusion IC_{50} (nM)	Anti-viral IC_{50} (nM)	clog *P*	MW
29		71	1620	1.32	414
30		1900	ND	1.7	458
31		42	979	2.87	452
32		29	>1000	2.61	452
33		296	ND	1.46	454
34		24	6	2.3	453
35		24	7	2.24	453
36		0.29	0.12	3.26	463
37		29	126	2.1	464

Table 5
Rat pharmacokinetic (IV 1 mg/kg, po 1 mg/kg)

	Compound **36**
Cl (mL/min/kg)	48
Clu (mL/min/kg)	198
$T_{1/2}$ (h)	0.5
Vdu (L/kg)	9.4
F (%)	21
Fabs (%)	100

Table 6
Antiviral activity of compound **1** and compound **36** against a panel of 25 clade B isolates



had liver blood flow clearance and zero oral exposure in the rat making both inferior to compound **1**. Both compounds were believed to be substrates for aldehyde oxidase.[12] To our surprise, the isoquinoline modification (compound **36**) produced a dramatic increase in potency in both fusion and antiviral assays. However the compound is not very stable metabolically with a half-life of only 4 min in human liver microsomes due to its high lipophilicity. Further heterocyclic analogues were prepared in the hope of maintaining the excellent antiviral potency whilst reducing metabolic vulnerability but all had disappointing activities as exemplified by compound **37**. With such an exquisite antiviral potency, compound **36** emerged as our most promising agent and was subjected to wider pharmacokinetic and virologic profiling. Compound **36** had a higher clearance and a lower bioavailability than compound **1** resulting in a half life of 0.5 h in the rat (Table 5). Scaling from the rat, compound **36** was predicted to exhibit a half life in humans of 3.5 h.

Compound **36** was then tested against a panel of 25 clade B isolates (Table 6). The compound was active against R5-, X4- and dual-tropic viruses.[13] Most importantly, compound **36** was active against all 25 isolates tested and had an improved spectrum profile compared to compound **1**. Based on human predictions and antiviral spectrum data, a 100 mg bid dose was predicted to give a C_{min} of 50 nM, covering 70% of clade B at IC_{90}. Assuming a linear pharmacokinetic profile across the dose range, a greater coverage could be achieved by higher but still reasonable doses, for example, 600 mg bid had a predicted C_{min} of 300 nM would cover 96% of clade B at IC_{90}. Although compound **36** had an inferior pharmacokinetic profile compared to compound **1**, its exquisite potency provided increased antiviral spectrum coverage. Further studies done on compound **1** showed that cross clade inhibition[14] would be very difficult with this chemical class, so more resistance studies would be required to progress compound **36** further.

In summary, a number of gp120-CD4 inhibitors for the treatment of HIV-1 were synthesized. Evaluation of the potency and pharmacokinetic properties of these compounds led to the identification of a broad antiviral spectrum agent with subnanomolar levels of potency against several clade B isolates.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.102.

References and notes

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11. (a) Antiviral assays. Activity against NL4-3 virus in HeLa-P4 cells was determined as follow. 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; (b) Antiviral assay against all other strains was carried out by Monogram Biosciences using their phenosense assay.
12. Oxidized metabolite formed in rat cytosol but not in rat liver microsome. Inhibition of metabolism observed in the presence of an aldehyde oxidase inhibitor, Raloxifene (data not shown).
13. Antiviral activity of compounds **1** and **36** against 25 Clade B isolates reported in Supplementary data.
14. Antiviral activity of compound **1** against Clade A, B, C and D isolates presented at the twenty first International Conference on Antiviral Research, Montreal, Quebec, Canada, April 13–17, 2008.