CHEMMEDCHEM

DOI: 10.1002/cmdc.200800183

Optimization of 5-Aryloxyimidazole Non-Nucleoside Reverse Transcriptase Inhibitors

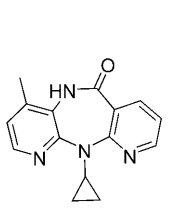
Lyn H. Jones,*[a] Gill Allan,[b] Romuald Corbau,[c] Duncan Hay,[a] Donald S. Middleton,[a] Charles E. Mowbray,[a] Sandra D. Newman,[a] Manos Perros,[c] Amy Randall,[a] Hannah Vuong,[a] Rob Webster,[b] Mike Westby,[c] and David Williams[a]

A major problem associated with non-nucleoside reverse transcriptase inhibitors (NNRTIs) for the treatment of HIV is their vulnerability to mutations in the allosteric binding site of reverse transcriptase that can result in the development of a resistant virus. Herein we present the optimization of a series of 5-aryloxy imidazoles, which possess a balanced pharmacological profile against both wild-type enzyme and the clinically relevant mutations K103N and Y181C. Subtle structural changes were used to probe structure–activity relationships relating to both potency and metabolic stability, which led to an imidazole derivative with an impressive overall profile.

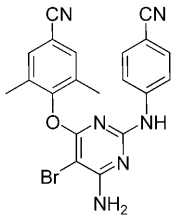
Introduction

Approximately 33 million people worldwide are living with HIV/AIDS and there were 2.5 million people newly infected with the HIV virus in 2007.[1] There is no cure for the infection caused by HIV, but a number of drugs slow or halt disease progression and treatment is aimed specifically at reducing viral load. As HIV can become rapidly resistant to any single antiretroviral drug, a combination of three or more drugs are usually required to effectively suppress the virus. This is known as highly active antiretroviral therapy (HAART) and as a treatment regimen it has dramatically reduced both morbidity and mortality among HIV patients. Reverse transcriptase (RT) is an essential enzyme in the infectious lifecycle of the virus, and inhibitors of this enzyme have become a regular component of a HAART regimen. However, established drugs called non-nucleoside reverse transcriptase inhibitors (NNRTIs: nevirapine, delavirdine, and efavirenz), which bind to an allosteric pocket outside the catalytic site, are particularly vulnerable to the development of viral resistance caused by mutations in RT that can retain viable enzymatic function.[2] Etravirine is a recently launched second-generation NNRTI drug that is active against the two most clinically relevant RT mutations, K103N and Y181C.[3]

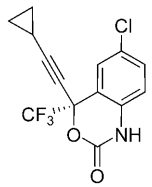
Capravirine is another second generation inhibitor that possesses an excellent mutant profile, which served as the inspiration for our work on 5-aryloxy imidazole NNRTIs and we have previously described our initial synthetic exploits in this area.[4] We developed a reliable synthetic route, which had the potential to furnish a number of related analogues to generate structure–activity relationships, and the targets **1**, **2**, and **3** illustrated the early potential of this series. A very attractive feature of the series is the high potency (against both wild-type and mutant enzymes) combined with low molecular weight (and hence high ligand efficiency), which is a desirable starting



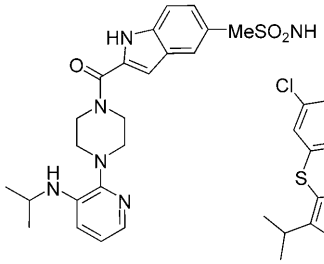
Nevirapine



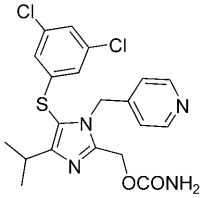
Etravirine



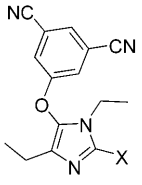
Efavirenz



Delavirdine



Capravirine



1 X=H
2 X=CH_2OH
3 X=CH_2OCONH_2

[a] *Dr. L. H. Jones, D. Hay, Dr. D. S. Middleton, Dr. C. E. Mowbray, S. D. Newman, A. Randall, H. Vuong, Dr. D. Williams*
Discovery Chemistry, Sandwich Laboratories
Pfizer Global Research and Development
Ramsgate Road, Kent, CT13 9NJ (UK)
Fax: (+44) 1304-651-821
E-mail: lyn.jones@pfizer.com

[b] *Dr. G. Allan, Dr. R. Webster*
Pharmacokinetics, Dynamics and Metabolism, Sandwich Laboratories
Pfizer Global Research and Development
Ramsgate Road, Kent, CT13 9NJ (UK)

[c] *Dr. R. Corbau, Dr. M. Perros, Dr. M. Westby*
Discovery Biology, Sandwich Laboratories
Pfizer Global Research and Development
Ramsgate Road, Kent, CT13 9NJ (UK)

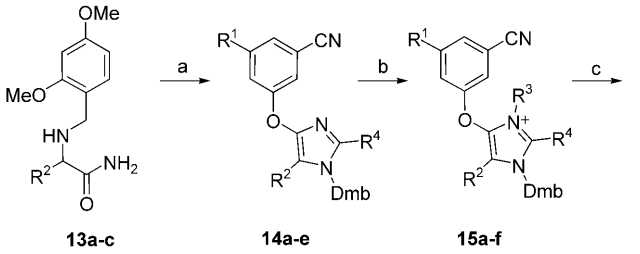
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.200800183.

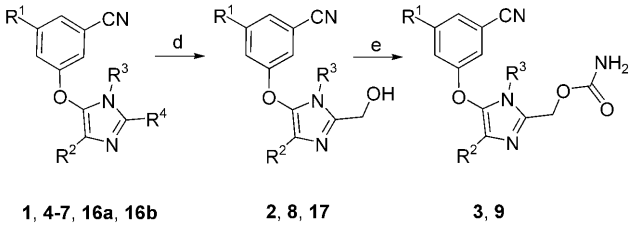
point for achieving orally absorbed drugs.[5] Herein we present our optimization of these novel imidazoles with particular emphasis on the interplay of structure, potency, and metabolic stability.

Synthetic Chemistry

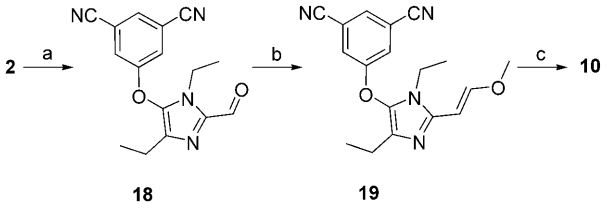
Preparation of **1**, **2**, and **3** has been reported previously.[4] **4** was prepared using analogous chemistry (Scheme 1): treatment of aminoamide **13a**[4] with triethylorthoacetate followed by arylation with commercially available 3,5-dicyanofluorobenzene gave **14a**, quaternization and deprotection furnished **4** (structure confirmed by NOE and C-H NMR correlation experiments—see Supporting Information). Similar chemistry was used to prepare derivatives **5**–**7**. Reaction of the C2-H imidazoles with paraformaldehyde proceeded smoothly to give the hydroxymethyl-substituted compounds **2** and **8**. Carbamoylation of the alcohol derivatives furnished **3** and **9**.

Derivative **10** was prepared using the procedure outlined in Scheme 2. Oxidation of **2** using Dess–Martin periodinane gave the aldehyde **18**. Wittig homologation provided enol ether **19**, which was hydrolyzed and reduced to provide **10**.





Scheme 1. Synthesis of 5-Aryloxy Imidazoles: a) 1. $(EtO)_3CCH_3$ or $(EtO)_3CH$, reflux; 2. Aryl fluoride, K_2CO_3, DMSO, 100 °C; b) MeI or EtI; c) BBr_3/DCM; d) Paraformaldehyde, DMSO; e) $Cl_3CCONCO$ then basic alumina; (Dmb = 2,4-dimethoxybenzyl).



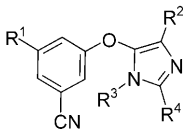
Scheme 2. Preparation of **10**: a) Dess–Martin periodinane, DCM; b) $Ph_3PCH_2OCH_3Cl$, KHMDS, THF; c) 1. aq HCl, THF; 2. $NaBH_4$, MeOH.

Results and Discussion

Derivatives **1**, **2**, and **3** possess good potency against the RT enzyme and impressive in vitro metabolic stability, assessed as half-life in human liver microsomes (HLM, Table 1). In fact, these compounds illustrate significant improvement in metabolic stability over capravirine, which has been achieved by replacing the hydrophobic chlorine atoms with nitrile moieties (ΔLog $P \sim -2$ from Rekker fragments[6]), removing a metabolically vulnerable pyridine ring and replacing a vulnerable sulfur atom with oxygen.[7]

Our aim to optimize the series initially focused on making further improvements in potency while retaining adequate metabolic stability. Our strategy was to achieve these improvements using relatively subtle changes to the aryloxy-imidazole

Table 1. Potency and metabolic stability of 5-aryloxy imidazole derivatives **1**–**10** and Capravirine.



Compd	R^1	R^2	R^3	R^4	wt RT IC_{50} [nM][a]	wt AV IC_{50} [nM][a]	K103N RT IC_{50} [nM][a]	Y181C RT IC_{50} [nM][a]	HLM (min)[b]	Log *D*
1	CN	Et	Et	H	2100	nd	7500	4200	>120	1.5
2	CN	Et	Et	CH_2OH	476	116	1350	850	>120	1.6
3	CN	Et	Et	CH_2OCONH_2	103	nd	110	155	>120	1.0
4	CN	Et	Et	Me	131	32	390	262	101	1.9
5	Cl	Et	Et	Me	18	3	7	14	16	3.2
6	CN	*i*Pr	Et	Me	59	nd	57	57	23	2.4
7	CN	*c*Pr	Et	Me	133	13	93	110	53	2.3
8	CN	*i*Pr	Me	CH_2OH	2090	nd	nd	nd	>120	1.6
9	CN	*i*Pr	Et	CH_2OCONH_2	71	23	69	71	>120	1.4
10	CN	Et	Et	CH_2CH_2OH	78	10	83	77	>120	1.6
Capravirine	–	–	–	–	47	3	110	61	7.5	4.5

[a] Geomean of $n = 3$ [b] half life in human liver microsomes. nd = not determined.

skeleton that would not compromise the impressive ligand efficiency or mutant profile. In particular, we were keen to retain the 3,5-disubstituted phenyl moiety, that is also present in capravirine, to optimize edge-to-face π interactions with the immutable tryptophan residue W229.[8] Indeed, the rational design of NNRTIs to interact specifically with W229 to improve mutant resilience has also been suggested by Balzarini and De Clercq[9] and was recently utilized in the development of pyrrolobenzoxazepine NNRTIs by Campiani and co-workers.[10]

Interestingly, we found that a simple methyl group located at the 2-position of the imidazole (derivative **4**) improved potency relative to the unsubstituted and hydroxymethyl derivatives **1** and **2** respectively, albeit with a slight reduction in metabolic stability (Table 1). Replacing one of the aryl nitrile groups with a chlorine atom to provide **5** increased the lipophilicity (ΔLog *D* 1.3), and improved the potency but sacrificed metabolic stability. Adding a methyl group to provide the 3-isopropyl substituted imidazole **6** (as in capravirine) similarly increased potency, but at the expense of metabolic turnover, and a cyclopropyl group at this position (derivative **7**) showed no improvement over **4**. A methylene "shuffle" performed on **2** to provide the isolipophilic derivative **8** retained good stability but the potency was reduced approximately fourfold. Addition of a methylene group to **3** to provide **9** and to derivative **2** to furnish **10** resulted in the desired, though modest, improvements in potency and retention of metabolic stability. Of particular note for all analogues (**1**–**10**) is that they retain potencies against the clinically relevant single-point mutations K103N and Y181C, which are within tenfold of the wild-type enzyme (Table 1).

Imidazoles **9** and **10** therefore represent our optimized derivatives within this series, which we were able to achieve in a relatively small number of design cycles (that is, small total number of compounds made). Our preferred compound for further analysis was imidazole **10**, based primarily on synthetic expedience, as the precursor **2** could be prepared on sufficient scale to readily furnish material for further studies.

The RT enzyme potency for imidazole **10** translated well in an antiviral assay, (Table 2) and although the compound is approximately 20-fold less active than capravirine (AV $IC_{50}=$ 0.5 nM) it is three orders of magnitude less lipophilic. The hydrophilicity of **10** and the other potent derivatives is significant as the NNRTI binding pocket is particularly hydrophobic, and therefore potent NNRTIs usually require a high Log *D*, which can lead to poor metabolic stability and low aqueous solubility.[11] Unsurprisingly, imidazole **10** displayed impressive solubility (Table 2).

A plot of HLM half life versus Log *D* (Figure 1) for the imidazole series illustrates a trend of increasing lipophilicity with decreasing metabolic stability, and compounds with Log $D \leq 1.6$ are stable for >120 min in this assay. Included on this plot is capravirine, which follows the same trend. **10** possesses excellent stability in human hepatocytes (HHep), which suggests there is no significant phase II metabolism.

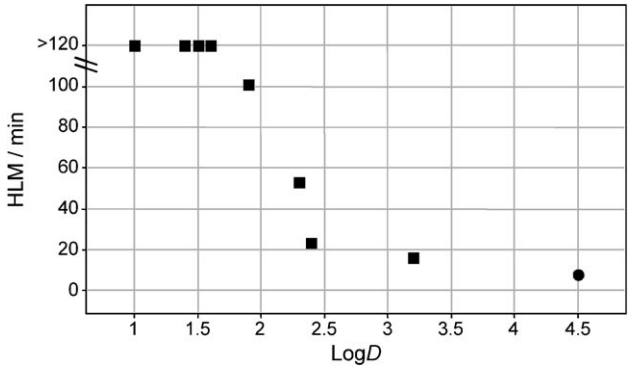


Figure 1. Trend of half-life in HLM (minutes) versus Log *D*: Compounds **1**–**10** (■), Capravirine (●).

Interestingly we also found that imidazole **10** formed a reactive metabolite conjugate with glutathione following its incubation with HLM, a feature that has been linked with hepato- and idiosyncratic toxicity.[12] The reasons underlying this observation warrant further investigation and will be reported in due course.

Additionally, **10** has high flux in the Caco-2 cell line, with no evidence of efflux, indicative of predicted high human absorption,[13] presumably a feature of its low molecular weight and topological polar surface area (tPSA).

Table 2. In vitro profile of NNRTI **10.**

wt RT IC_{50}[a]	78 nM
wt AV IC_{50}[b]	10 nM
HLM	>120 min
HHep	<5 μL min^{-1} per million
Log *D*	1.6
Solubility at pH 7.4	0.25 mg mL^{-1}
MW	310
tPSA	95 Å^2
Caco-2[c]	$>30\times10^{-6}$ cm s^{-1}
Reactive metabolite	Positive

[a] Geomean for $n=3$. [b] Compound **10** was not cytotoxic, $CC_{50}>$ 100 μM. [c] 10 μM dose.

Conclusions

We have optimized both the potency and metabolic stability of a series of novel 5-aryloxy imidazole NNRTIs yielding a number of interesting derivatives, which possess potent activity against the clinically relevant RT mutations K103N and Y181C. These compounds were readily prepared in a concise and selective manner, which was an important aspect of our design process to deliberately reduce synthetic complexity as a means of addressing the current cost of goods issued with existing HAART regimens. This also facilitated the generation of structure–activity relationships to probe the effects of subtle differences in structure on function. Further profiling of our optimized compound **10**, including a comparison of its profile with isomeric pyrazole NNRTIs, will be reported in due course.

Experimental Section

General: Melting points were determined on a Gallenkamp melting point apparatus using glass capillary tubes and are uncorrected. Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere, using commercially available anhydrous solvents. Thin-layer chromatography was performed on glass-backed precoated Merck silica gel (60 F254) plates and FCC (flash column chromatography) was carried out using 40–63 μm silica gel. NMR spectra were carried out on a Varian Mercury 400, Varian Inova 500, or Bruker Avance 600 spectrometer in the solvents specified. LC/MS were recorded on a Waters Micromass ZQ using atmospheric pressure chemical ionization (APCI) and evaporative light scattering detection (ELSD) techniques. Combustion analyses were conducted by Exeter Analytical UK, Ltd, Uxbridge, Middlesex. Other abbreviations are used in conjunction with standard chemical practice.

2-(2,4-Dimethoxy-benzylamino)-butyramide (R^2 = Et, 13a)

Compound **13a** was prepared according to the procedure described in reference [4].

2-(2,4-Dimethoxy-benzylamino)-3-methyl-butyramide (R^2 = *i*Pr, 13b).

2,4-Dimethoxybenzaldehyde (19.6 g, 118 mmol) and 2-amino-3-methyl butyramide hydrochloride (36 g, 236 mmol) were dissolved in methanol (800 mL), then $NaBH_3CN$ (7.4 g, 118 mmol) was added and the mixture was stirred at RT for 35 h. The reaction was quenched with 1 N aq NaOH, extracted with EtOAc, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was triturated with diethyl ether to yield **13b** as a white solid (14.3 g, 46%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39 (bs, 1H), 7.08 (d, 1H), 6.44 (m, 2H), 5.48 (bs, 1H), 3.80 (m, 8H), 3.60 (s, 1H), 3.00 (s, 1H), 2.12 (m, 1H), 0.90 ppm (m, 6H); APCI MS *m/z* 267 $[M+H]^+$; LCMS (ELSD) >95% (267, $[M+H]^+$).

2-Cyclopropyl-2-(2,4-dimethoxy-benzylamino)-acetamide (R^2 = *c*Pr, 13c)

2,4-Dimethoxybenzaldehyde (2.3 g, 13.9 mmol) was added portionwise to a stirred solution of 2-amino-2-cyclopropyl acetamide (1.8 g, 13.9 mmol) in THF (30 mL). After 2 h, $Na(OAc)_3BH$ (4.43 g, 20.9 mmol) was added portionwise. The resulting suspension was maintained at RT for 18 h, then diluted with DCM, washed with water, then saturated sodium bicarbonate solution, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (FCC) (5% MeOH/DCM) to provide **13c** as a pale yellow solid (2.3 g, 59%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.10 (d, 1H), 6.43 (m, 2H), 3.84 (s, 3H), 3.82 (s, 3H), 3.80–3.65 (m, 5H), 2.60 (d, 1H), 1.04 (m, 1H), 0.57 (m, 1H), 0.49 (m, 1H), 0.40 (m, 1H), 0.23 ppm (m, 1H); APCI MS *m/z* 280 $[M+H]^+$; LCMS (ELSD) >95% (280, $[M+H]^+$).

5-(3,5-Diethyl-2-methyl-3*H*-imidazol-4-yloxy)-isophthalonitrile (4)

Amino amide **13a** (300 mg, 1.2 mmol) was dissolved in triethylorthoacetate (2 mL), one drop of acetic acid was added, and the mixture was stirred at 150 °C for 24 h. The mixture was cooled to RT and the volatiles removed under reduced pressure. DMSO (2 mL) was added followed by potassium carbonate (164 mg, 1.2 mmol) and 3,5-dicyanofluorobenzene (174 mg, 1.2 mmol). The mixture was stirred at 100 °C for 18 h before the addition of water, extracted with EtOAc, washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by FCC (100% EtOAc) to provide **14a** as a pale yellow solid (125 mg, 33% over 2 steps): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.60 (s, 1H), 7.50 (s, 2H), 6.53 (s, 1H), 6.39 (s, 2H), 4.95 (s, 2H), 3.92 (s, 3H), 3.78 (s, 3H), 2.40 (q, 2H), 2.32 (s, 3H), 1.02 ppm (t, 3H); APCI MS *m/z* 403 $[M+H]^+$; LCMS (ELSD) >95% (403, $[M+H]^+$).

Compound **14a** (5-[1-(2,4-Dimethoxy-benzyl)-5-ethyl-2-methyl-1*H*-imidazol-4-yloxy]-isophthalonitrile, 100 mg, 0.25 mmol) was dissolved in EtI (2 mL) and heated to reflux for 18 h. The mixture was cooled to RT and the volatiles removed under reduced pressure. The residue was purified by FCC (2% MeOH/DCM) to yield **15a** as a white solid (100 mg, 71%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.16 (s, 2H), 7.73 (s, 1H), 7.07 (d, 1H), 6.48 (m, 2H), 5.28 (s, 2H), 4.08 (q, 2H), 3.80 (s, 6H), 2.83 (s, 3H), 2.55 (q, 2H), 1.41 (t, 3H), 0.98 ppm (t, 3H); APCI MS *m/z* 431 [parent+H]$^+$; LCMS (ELSD) >95% (431, [parent+H]$^+$).

Compound **15a** 5-(3,5-Dicyano-phenoxy)-3-(2,4-dimethoxy-benzyl)-1,4-diethyl-2-methyl-3*H*-imidazol-1-ium iodide, 900 mg, 1.6 mmol) was dissolved in DCM (20 mL) cooled to 0 °C, BBr_3 (1 M in DCM, 8.0 mL, 8.0 mmol) was added dropwise and allowed to warm slowly to RT. The mixture was then cooled to 0 °C, 2 N aq NaOH (100 mL) was added, and stirred for 30 min. The mixture was extracted with DCM, dried ($MgSO_4$), filtered. and concentrated under reduced pressure. The residue was purified by FCC (5% MeOH/EtOAc) to yield **4** as a white solid (260 mg, 58%); MP 145–146 °C (crystalline); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.68 (s, 1H), 7.25 (s, 2H), 3.70 (q, 2H), 2.38 (q, 2H), 1.25 (t, 3H), 1.15 ppm (t, 3H); APCI MS *m/z* 281 $[M+H]^+$; LCMS (ELSD) >95% (281, $[M+H]^+$); HMBC, HSQC, and NOE experiments included in Supporting Information.

3-Chloro-5-(3,5-diethyl-2-methyl-3*H*-imidazol-4-yloxy)-benzonitrile (5)

Amino amide **13a** (500 mg, 2.0 mmol) was dissolved in triethylorthoacetate (2.2 mL), one drop of acetic acid was added and the mixture was stirred at 120 °C for 4 h. The mixture was cooled to RT and the volatiles removed under reduced pressure. DMSO (1.5 mL) was added followed by potassium carbonate (330 mg, 2.4 mmol) and 3-cyano-5-chlorofluorobenzene (370 mg, 2.4 mmol). The mixture was stirred at 100 °C for 5 h before the addition of water, extracted with EtOAc, washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by FCC (100% EtOAc) to provide **14b** as a pale yellow gum (70 mg, 8.5% over 2 steps): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.25 (m, 2H), 7.18 (m, 1H), 6.50 (s, 1H), 6.42 (s, 2H), 4.95 (s, 2H), 3.83 (s, 3H), 3.80 (s, 3H), 2.43 (q, 2H), 2.28 (s, 3H), 1.02 ppm (t, 3H); APCI MS *m/z* 413 $[M+H]^+$; LCMS (ELSD) >95% (413, $[M+H]^+$).

Compound **14b** (3-Chloro-5-[1-(2,4-dimethoxy-benzyl)-5-ethyl-2-methyl-1H-imidazol-4-yloxy]-benzonitrile, 85 mg, 0.21 mmol) was dissolved in EtI (2 mL) and heated to reflux for 18 h. The mixture was cooled to RT and the volatiles removed under reduced pressure. The residue was purified by FCC (2% MeOH/DCM) to yield **15b** as a pale yellow solid (90 mg, 77%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.90 (m, 1H), 7.48 (m, 2H), 7.10 (d, 1H), 6.48 (m, 2H), 5.31 (s, 2H), 4.09 (q, 2H), 3.80 (s, 6H), 2.84 (s, 3H), 2.58 (q, 2H), 1.61 (t, 3H), 1.03 ppm (t, 3H); APCI MS *m/z* 438 [parent−H]$^-$. LCMS (ELSD) >95% (438, [parent−H]$^-$).

Compound **15b** (5-(3-Chloro-5-cyano-phenoxy)-3-(2,4-dimethoxy-benzyl)-1,4-diethyl-2-methyl-3H-imidazol-1-ium iodide, 90 mg, 0.16 mmol) was dissolved in DCM (2 mL) cooled to 0 °C, BBr_3 (1 M in DCM, 0.8 mL, 0.8 mmol) was added dropwise and allowed to warm slowly to RT. The mixture was then cooled to 0 °C, 2 N aq NaOH (10 mL) was added, and stirred for 30 min. The mixture was extracted with DCM, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by FCC (5% MeOH/EtOAc) to yield **5** as a white solid (30 mg, 65%); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.38$ (m, 1H), 7.18 (m, 1H), 7.12 (m, 1H), 3.65 (q, 2H), 2.42 (s, 3H), 2.40 (q, 2H), 1.23 (t, 3H), 1.18 ppm (t, 3H); APCI MS *m/z* 290 $[M+H]^+$; LCMS (ELSD) > 95% (290, *M*+H).

5-(3-Ethyl-5-isopropyl-2-methyl-3*H*-imidazol-4-yloxy)-isophthalonitrile (6)

Amino amide **13b** (4.5 g, 17 mmol) was dissolved in triethylorthoacetate (36 mL), one drop of acetic acid was added, and the mixture stirred at 140 °C for 5 h. The volatiles were removed under reduced pressure and the residue dissolved in DMSO (38 mL), potassium carbonate (2.3 g, 17 mmol), and 3,5-dicyanofluorobenzene (2.5 g, 17 mmol) were added and the mixture stirred at 100 °C for 18 h. Water was added and the mixture extracted with EtOAc, washed with brine, dried ($MgSO_4$), filtered, and the solvents removed under reduced pressure. The residue was purified by FCC (20% EtOAc/pentane) to provide **14c** as a cream solid (1.1 g, 15% over 2 steps): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.58$ (s, 1H), 7.52 (s, 2H), 6.47 (m, 3H), 4.97 (s, 2H), 3.86 (s, 3H), 3.79 (s, 3H), 2.76 (m, 1H), 2.30 (s, 3H), 1.11 ppm (d, 6H); APCI MS *m/z* 417 $[M+H]^+$; LCMS (ELSD) > 95% (417, $[M+H]^+$).

Compound **14c** (5-[1-(2,4-Dimethoxy-benzyl)-5-isopropyl-2-methyl-1*H*-imidazol-4-yloxy]-isophthalonitrile, 1.1 g, 2.5 mmol) was dissolved in EtI (11 mL) and heated to reflux for 18 h. The mixture was cooled to RT and the volatiles removed under reduced pressure. The residue was purified by FCC (2% MeOH/DCM) to yield **15c** as a pale yellow solid (1.3 g, 92%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.19$ (s, 2H), 7.75 (s, 1H), 7.05 (d, 1H), 6.50 (m, 2H), 5.32 (s, 2H), 4.05 (q, 2H), 3.81 (s, 6H), 3.12 (m, 1H), 2.80 (s, 3H), 1.21 (t, 3H), 1.10 ppm (d, 6H); APCI MS *m/z* 445 [parent+H]$^+$; LCMS (ELSD) > 95% (445 [parent+H]$^+$).

Compound **15c** (5-(3,5-Dicyano-phenoxy)-3-(2,4-dimethoxy-benzyl)-1-ethyl-4-isopropyl-2-methyl-3*H*-imidazol-1-ium iodide, 1.3 g, 2.3 mmol) was dissolved in DCM (100 mL) cooled to 0 °C, BBr_3 (1 M in DCM, 11.7 mL, 11.7 mmol) was added dropwise and allowed to warm slowly to RT. The mixture was then cooled to 0 °C, 2 N aq NaOH (10 mL) was added, and stirred for 30 min. The mixture was extracted with DCM, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by FCC (1% MeOH/EtOAc) to yield **6** as a white solid (373 mg, 54%); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.63$ (s, 1H), 7.42 (s, 2H), 3.62 (q, 2H), 2.66 (m, 1H), 1.21 (t, 3H), 1.17 ppm (d, 6H); APCI MS *m/z* 295 $[M+H]^+$; LCMS (ELSD) > 95% (295, $[M+H]^+$).

5-(5-Cyclopropyl-3-ethyl-2-methyl-3*H*-imidazol-4-yloxy)-isophthalonitrile (7)

Amino amide **13c** (605 mg, 2.29 mmol) was dissolved in triethylorthoacetate (6 mL), one drop of acetic acid was added, and the mixture stirred at 130 °C for 5 h. The volatiles were removed under reduced pressure and the residue dissolved in DMF (5 mL), potassium carbonate (313 mg, 2.27 mmol), and 3,5-dicyanofluorobenzene (332 mg, 2.27 mmol) were added and the mixture stirred at 90 °C for 18 h. Water was added and the mixture extracted with DCM, washed with brine, dried ($MgSO_4$), filtered, and the solvents removed under reduced pressure. The residue was purified by FCC (4% MeOH/DCM) to provide **14d** as a white solid (428 mg, 45% over 2 steps): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.55$ (s, 1H), 7.50 (s, 2H), 6.51 (m, 2H), 6.43 (dd, 1H), 5.10 (s, 2H), 3.87 (s, 3H), 3.80 (s, 3H), 2.30 (s, 3H), 1.34 (m, 1H), 0.74 (m, 2H), 0.58 ppm (m, 2H); APCI MS *m/z* 415 $[M+H]^+$; LCMS (ELSD) > 95% (415, $[M+H]^+$).

Compound **14d** (5-[5-Cyclopropyl-1-(2,4-dimethoxy-benzyl)-2-methyl-1H-imidazol-4-yloxy]-isophthalonitrile, 204 g, 0.49 mmol) was dissolved in EtI (1 mL) and heated to reflux for 18 h. The mixture was cooled to RT and the volatiles removed under reduced pressure to yield **15d** as a pale yellow solid (278 mg, 99%): ^{1}H NMR (400 MHz, CD_3OD): $\delta = 8.04$ (s, 1H), 7.87 (s, 2H), 7.15 (d, 1H), 6.63 (d, 1H), 6.53 (dd, 1H), 5.40 (s, 2H), 4.06 (m, 2H), 3.84 (s, 3H), 3.79 (s, 3H), 2.67 (s, 3H), 1.50 (m, 1H), 1.30 (m, 3H), 0.80 (m, 2H), 0.60 ppm (m, 2H); APCI MS *m/z* 443 [parent+H]$^+$; LCMS (ELSD) > 95% (443, [parent+H]$^+$).

Compound **15d** (4-Cyclopropyl-5-(3,5-dicyano-phenoxy)-3-(2,4-dimethoxy-benzyl)-1-ethyl-2-methyl-3H-imidazol-1-ium iodide, 200 mg, 0.35 mmol) was dissolved in DCM (6 mL) cooled to 0 °C, BBr_3 (1 M in DCM, 3.5 mL, 3.5 mmol) was added dropwise, and allowed to warm slowly to RT. The mixture was then cooled to 0 °C, 2 N aq NaOH (10 mL) was added, and stirred for 30 min. The mixture was extracted with DCM, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by FCC (10% MeOH/DCM) to yield **7** as a white solid (78 mg, 60%); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.65$ (s, 1H), 7.51 (s, 2H), 3.69 (q, 2H), 2.37 (s, 3H), 1.47 (m, 1H), 1.20 (t, 3H), 0.76 ppm (m, 4H); APCI MS *m/z* 293 $[M+H]^+$; LCMS (ELSD) > 95% (293, $[M+H]^+$).

5-(2-Hydroxymethyl-5-isopropyl-3-methyl-3*H*-imidazol-4-yloxy)-isophthalonitrile (8)

Amino amide **13b** (14.2 g, 53 mmol) was dissolved in triethylorthoformate (100 mL), one drop of acetic acid was added, and the mixture stirred at 150 °C for 4 h. The volatiles were removed under reduced pressure and the residue dissolved in DMSO (120 mL), potassium carbonate (7.4 g, 53 mmol), and 3,5-dicyanofluorobenzene (7.8 g, 53 mmol) were added, and the mixture stirred at 100 °C for 18 h. Water was added and the mixture extracted with EtOAc, washed with brine, dried ($MgSO_4$), filtered, and the solvents removed under reduced pressure. The residue was purified by FCC (20% EtOAc/pentane) to provide **14e** as a yellow solid (6.0 g, 29% over 2 steps): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.55$ (s, 1H), 7.50 (s, 2H), 7.21 (s, 1H), 6.87 (m, 1H), 6.52 (m, 2H), 5.01 (s, 2H), 4.85 (s, 3H), 4.78 (s, 3H), 2.90 (m, 1H), 1.21 ppm (d, 6H); APCI MS *m/z* 403 $[M+H]^+$; LCMS (ELSD) > 95% (403, $[M+H]^+$).

Compound **14e** (5-[1-(2,4-Dimethoxy-benzyl)-5-isopropyl-1H-imidazol-4-yloxy]-isophthalonitrile, 1.68 g, 4.18 mmol) was dissolved in MeI (15 mL) and heated to reflux for 17 h. The mixture was cooled to RT and the volatiles removed under reduced pressure. The residue was triturated with diethyl ether to yield **15e** as a pale yellow solid, which was used crude in the next step (1.48 g).

Compound **15e** (5-(3,5-Dicyano-phenoxy)-3-(2,4-dimethoxy-benzyl)-4-isopropyl-1-methyl-3*H*-imidazol-1-ium iodide, 1.47 g, 2.72 mmol) was dissolved in DCM (75 mL) cooled to 0 °C, BBr_3 (1 M in DCM, 25 mL, 25 mmol) was added dropwise, and allowed to warm slowly to RT. The mixture was then cooled to 0 °C, 2 N aq NaOH (20 mL) was added, and stirred for 30 min. The mixture was

extracted with DCM, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by FCC (5% MeOH/DCM) to yield **16a** as a white solid (295 mg, 27% over 2 steps); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.69–6.64$ (m, 2H), 7.43 (s, 2H), 3.45 (s, 3H), 2.77 (m, 1H), 1.18 ppm (d, 6H); APCI MS *m/z* 267 $[M+H]^+$; LCMS (ELSD) >95% (267, $[M+H]^+$).

Compound **16a** (5-(5-Isopropyl-3-methyl-3*H*-imidazol-4-yloxy)-isophthalonitrile, 295 mg, 1.11 mmol) was dissolved in DMSO (2 mL), paraformaldehyde (333 mg, 11.1 mmol) was added followed by powdered 4 Å molecular sieves (100 mg), and the mixture heated to 140 °C for 18 h. The mixture was cooled to RT, diluted with EtOAc, washed with water, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by FCC (10% MeOH/DCM) and triturated with diethyl ether to yield **8** as a white solid (188 mg, 58%); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.68$ (s, 1H), 7.46 (s, 2H), 4.71 (s, 2H), 3.47 (s, 3H), 2.71 (m, 1H), 1.13 ppm (d, 6H); APCI MS *m/z* 297 $[M+H]^+$; LCMS (ELSD) >95% (297, $[M+H]^+$).

Carbamic acid 5-(3,5-dicyano-phenoxy)-1-ethyl-4-isopropyl-1*H*-imidazol-2-ylmethyl ester (9)

Compound **14e** (3.3 g, 8.0 mmol) was dissolved in EtI (33 mL) and heated to reflux for 18 h. The mixture was cooled to RT and the volatiles removed under reduced pressure. The residue was purified by FCC (2% MeOH/DCM) to yield **15f** as a pale yellow solid (4.3 g, 94%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.08$ (s, 1H), 7.98 (s, 2H), 7.75 (s, 1H), 7.60 (d, 1H), 6.55 (d, 1H), 6.50 (s, 1H), 5.38 (s, 2H), 4.10 (q, 2H), 3.90 (s, 3H), 3.84 (s, 3H), 3.25 (m, 1H), 1.55 (t, 3H), 1.18 ppm (d, 6H); APCI MS *m/z* 431 [parent+H]$^+$; LCMS (ELSD) >95% (431, [parent+H]$^+$).

Compound **15f** (5-(3,5-Dicyano-phenoxy)-3-(2,4-dimethoxy-benzyl)-1-ethyl-4-isopropyl-3*H*-imidazol-1-ium iodide, 100 mg, 0.18 mmol) was dissolved in DCM (5 mL) cooled to 0 °C, BBr_3 (1 M in DCM, 1.8 mL, 1.8 mmol) was added dropwise, and allowed to warm slowly to RT. The mixture was then cooled to 0 °C, 2 N aq NaOH (5 mL) was added, and stirred for 30 min. The mixture was extracted with DCM, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by FCC (5% MeOH/DCM) to yield **16b** as a white solid (25 mg, 50%); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.68$ (s, 1H), 7.42 (d, 2H), 3.72 (q, 2H), 2.70 (m, 1H), 1.36 (t, 3H), 1.18 ppm (d, 6H); APCI MS *m/z* 281 $[M+H]^+$; LCMS (ELSD) >95% (281, $[M+H]^+$).

Compound **16b** (5-(3-Ethyl-5-isopropyl-3H-imidazol-4-yloxy)-isophthalonitrile, 200 mg, 0.71 mmol) was dissolved in DMSO (1 mL), paraformaldehyde (214 mg, 7.1 mmol) and powdered 4 Å molecular sieves (45 mg) were added, and the mixture heated to 140 °C for 18 h. Saturated sodium bicarbonate solution was added and the mixture extracted with EtOAc, washed with brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by FCC (5% MeOH/DCM) to yield **17** as a white solid (98 mg, 44%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.66$ (s, 1H), 7.42 (s, 2H), 4.71 (s, 2H), 3.84 (q, 2H), 2.66 (m, 1H), 1.28 (t, 3H), 1.10 ppm (d, 6H); APCI MS *m/z* 311 $[M+H]^+$; LCMS (ELSD) >95% (311, $[M+H]^+$).

Compound **17** (5-(3-Ethyl-2-hydroxymethyl-5-isopropyl-3H-imidazol-4-yloxy)-isophthalonitrile, 75 mg, 0.24 mmol) was dissolved in THF (3 mL), cooled to 0 °C, and trichloroacetyl isocyanate (34 μL, 0.29 mmol) was added dropwise. After 2 h saturated sodium bicarbonate solution was added and the mixture extracted with DCM, dried ($MgSO_4$), filtered, and the solution was filtered again through a pad of wet basic alumina to yield **9** as a white solid (60 mg, 71%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.70$ (s, 1H), 7.49 (s, 2H), 5.22 (s, 2H), 3.87 (q, 2H), 2.78 (m, 1H), 1.30 (t, 3H), 1.22 ppm (d, 6H); APCI MS *m/z* 354 $[M+H]^+$; LCMS (ELSD) >95% (354, $[M+H]^+$).

5-[3,5-Diethyl-2-(2-hydroxy-ethyl)-3*H*-imidazol-4-yloxy]-isophthalonitrile (10)

Compound **2** (990 mg, 3.34 mmol) was dissolved in DCM (25 mL), cooled to 0 °C, and Dess–Martin periodinane (1.4 g, 3.3 mmol) was added. After 3 h the reaction was quenched with saturated sodium bicarbonate solution, extracted with EtOAc, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by FCC (100% EtOAc) to yield **18** as a pale yellow oil (430 mg, 44%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.65$ (s, 1H), 7.73 (s, 1H), 7.42 (s, 2H), 4.22 (q, 2H), 2.38 (q, 2H), 1.29 (t, 3H), 1.18 ppm (t, 3H); APCI MS *m/z* 295 $[M+H]^+$; LCMS (ELSD) >95% (295, $[M+H]^+$).

Methoxymethyl triphenylphosphonium chloride (676 mg, 2.2 mmol) was dissolved in THF (5 mL), cooled to 0 °C, KHMDS (0.5 M in THF, 4.4 mL, 2.2 mmol) was added, and the mixture stirred for 30 min before the addition of **18** (5-(3,5-Diethyl-2-formyl-3H-imidazol-4-yloxy)-isophthalonitrile, 430 mg, 1.46 mmol) in THF (5 mL). The mixture was allowed to warm to RT and after 6 h the reaction was quenched with water, extracted with EtOAc, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by FCC (50% EtOAc/pentane) to yield **19** as a pale yellow oil (137 mg, 29%): ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.92$ (s, 1H), 7.63 (s, 2H), 7.40 (d, 1H), 5.71 (d, 1H), 3.77 (q, 2H), 3.75 (s, 3H), 2.34 (q, 2H), 1.19 (t, 3H), 1.10 ppm (t, 3H); APCI MS *m/z* 323 $[M+H]^+$; LCMS (ELSD) >95% (323, $[M+H]^+$).

Compound **19** (5-[3,5-Diethyl-2-((*E*)-2-methoxy-vinyl)-3H-imidazol-4-yloxy]-isophthalonitrile, 135 mg, 0.42 mmol) was dissolved in THF (2 mL), aq HCl (2 N, 1 mL) was added, and the mixture heated to 70 °C. After 2 days the solution was cooled to RT, saturated sodium bicarbonate solution was added, extracted with EtOAc, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was dissolved in methanol (2 mL), cooled to 0 °C, sodium borohydride (16 mg, 0.42 mmol) was added, and the mixture stirred for 1 h. The mixture was quenched with water, extracted with EtOAc, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified by FCC (5% MeOH/DCM) to yield **10** as a white solid (24 mg, 18% over 2 steps): MP 143–144.5 °C; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.92$ (s, 1H), 7.68 (s, 2H), 3.90 (t, 2H), 3.92 (q, 2H), 2.87 (t, 2H), 2.35 (q, 2H), 1.22 (t, 3H), 1.08 ppm (t, 3H); APCI MS *m/z* 311 $[M+H]^+$; LCMS (ELSD) >95% (311, $[M+H]^+$).

Reverse transcriptase assay

The inhibition of the RNA-dependent DNA polymerase activity of the HIV-1 reverse transcriptase enzyme was measured using a primer extension assay. Briefly, the reverse transcriptase enzyme was diluted in RT buffer (50 mM Tris, 5 mM $MgCl_2$, 0.025% Triton, pH 7.8, 20 nM) to a predefined activity where ~8,000 cpm are obtained after a 30 min incubation. The enzymes were prepared at four times the final concentration. In our experimental system, the K_m for poly r(A). oligo $d(T)_{16}$ and for TTP have been measured as 2.25 nM and 0.56 μM respectively for wt RT (data not shown). For the reaction, in a 96 well plate, 25 μL of the fourfold final concentration of test compound was incubated with 25 μL of a fourfold RT buffer solution (50 mM Tris, 5 mM $MgCl_2$, 0.025% Triton, pH 7.8, 20 nM poly r(A).oligo $d(T)_{16}$ and 1 μM TTP final concentration). This solution was then mixed with 50 μL of the substrate mix (for 6 mL

of substrate mix, add: 12.5 μL of 20 μM of poly r(A).oligo d(T)16, 60 μL of [^{3}H]TTP, 11.5 μL of TTP 1 M, 6 mL with dilution RT buffer). Each sample was prepared in duplicate. The reaction mixtures were incubated for 30 min at 37 °C. The reaction was stopped by addition of 25 μL of the stop solution (500 mM EDTA). The plate was then read using the Topcount NXT with a reading time of 1 min well^{-1} and IC_{50} values were deduced from the dose response curves obtained.

Antiviral assay

The ability of the compounds described in this paper to inhibit HIV replication was tested using the HeLaP4 reporter cell line infected with the CXCR4-tropic laboratory-adapted strain NL4-3 (obtained from the Centralized Facility for AIDS Reagents, National Institute for Biological Standards and Control [NIBSC], Potters Bar, Hertfordshire, United Kingdom). HeLaP4 cells, obtained from the NIH reagent program, were grown in Dulbecco's modified Eagle's medium (Gibco BRL, Belgium) containing 10% fetal calf serum (FCS; International Medical, Belgium), 100 μg mL^{-1} streptomycin, 100 U mL^{-1} penicillin, and 0.5 mg mL^{-1} Geneticin (Gibco BRL). They were maintained in approximately 25 mL culture medium, in 80 cm^2 flasks in a 37 °C CO_2 incubator and split (typically 1:7) twice weekly. Cells were split 1:2 the day before use in an assay to ensure cells are preconfluent. Cells were infected with HIV-1 NL4-3 using an MOI of 0.16 for 2 h. Infected cultures were then washed with RPMI and resuspended in 5 mL of RPMI medium at a final cell density of 2×10^5 cells mL^{-1} and added at 1×10^4 cells per well into 96-well plates containing half-logarithmic dilutions of the test compounds. Five days after infection, virus replication was measured using the FluorAceTM β-galactosidase reporter assay (Bio-Rad, 170-3150) following the manufacturer's recommendations. The EC_{50} value was calculated as the concentration of compound that effected a decrease in β-galactosidase activity of infected, compound-treated cells to 50% of that produced in the supernatants of infected, compound-free cells.

Acknowledgements

We thank Torren Peakman and Michael Kinns for running NOE, HMBC, and HSQC NMR experiments. We also thank Ian Burr, Alex Martin, and Amy Thomas for determining the potencies of these compounds.

Keywords: AIDS · antiviral agents · aryloxy imidazole · medicinal chemistry · NNRTI

[1] UNAIDS/WHO 2007 AIDS epidemic update.
[2] E. De Clercq, *Chem. Biodiversity* **2004**, *1*, 44.
[3] R. Haubrich, S. Gubernick, U. Yasothan, P. Kirkpatrick, *Nat. Rev. Drug Discovery* **2008**, *7*, 287.
[4] L. H. Jones, T. Dupont, C. E. Mowbray, S. N. Newman, *Org. Lett.* **2006**, *8*, 1725.
[5] A. L. Hopkins, C. R. Groom, A. Alex, *Drug Discovery Today* **2004**, *9*, 430.
[6] R. Rekker, *The Hydrophobic Fragmental Constant*, Elsevier, Amsterdam **1977**.
[7] H.-Z. Bu, W. F. Pool, E. Y. Wu, S. R. Raber, M. A. Amantea, B. V. Shetty, *Drug Disp. Met.* **2004**, *32*, 689.
[8] J. Ren, C. Nichols, L. E. Bird, T. Fujiwara, H. Sugimoto, D. I. Stuart, D. K. Stammers, *J. Biol. Chem.* **2000**, *275*, 14316.
[9] H. Pelemans, R. Esnouf, E. De Clercq, J. Balzarini, *Mol. Pharm.* **2000**, *57*, 954.
[10] C. Fattorusso, S. Gemma, S. Butini, P. Huleatt, B. Catalanotti, M. Persico, M. De Angelis, I. Fiorini, V. Nacci, A. Ramunno, M. Rodriquez, G. Greco, E. Novellino, A. Bergamini, S. Marini, M. Coletta, G. Maga, S. Spadari, G. Campiani, *J. Med. Chem.* **2005**, *48*, 7153.
[11] W. L. Jorgensen, J. Ruiz-Caro, J. Tirado-Rives, A. Basavapathruni, K. S. Anderson, A. D. Hamilton, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 663.
[12] D. C. Evans, A. P. Watt, D. A. Nicoll-Griffith, T. A. Baille, *Chem. Res. Toxicol.* **2004**, *17*, 3.
[13] S. Yee, *Pharm. Res.* **1997**, *14*, 763.

Received: June 16, 2008
Revised: August 13, 2008
Published online on October 15, 2008

