Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 42 (2007) 567–579
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Synthesis of novel diarylpyrimidine analogues of TMC278 and their antiviral activity against HIV-1 wild-type and mutant strains

Céline Mordant [a], Benoit Schmitt [a], Elisabeth Pasquier [a], Christophe Demestre [a], Laurence Queguiner [a], Chantal Masungi [b], Anik Peeters [b], Liesbeth Smeulders [b], Eva Bettens [b], Kurt Hertogs [b], Jan Heeres [c], Paul Lewi [c], Jerome Guillemont [a,*]

[a] *Johnson & Johnson Pharmaceutical Research and Development, Medicinal Chemistry Department, Campus de Maigremont BP315, F-27106 Val de Reuil Cedex, France*
[b] *Tibotec BVBA, Generaal de Wittelaan L 11B 3, B-2800 Mechelen, Belgium*
[c] *Center for Molecular Design, Janssen Pharmaceutica, Antwerpsesteenweg 37, B-2350 Vosselaar, Belgium*

Received 20 September 2006; accepted 30 November 2006
Available online 15 December 2006

Abstract

Novel diarylpyrimidines (DAPY), which represent next generation of non-nucleoside reverse transcriptase inhibitors (NNRTIs), were synthesized and their activities against human immunodeficiency virus type I (HIV-1) assessed. Modulations at positions 2 and 6 of the left phenyl ring generated interesting derivatives of TMC278 displaying high potency against wild-type and mutant viruses compared to nevirapine and efavirenz. The pharmacokinetic profile of the best newly synthesized DAPY was evaluated and compared with TMC278 now in phase II clinical trials.

Keywords: NNRTI; HIV-1; Diarylpyrimidine (DAPY)

1. Introduction

Multi-drug therapy against the human immunodeficiency virus (HIV), known as highly active antiretroviral therapy (HAART) [1,2], has drastically reduced the morbidity and mortality of HIV-infected patients during the last decade and slowed down the progression of acquired immunodeficiency syndrome (AIDS). A number of inhibitors targeting reverse transcriptase (RT) and protease enzymes, and the fusion process are included in multi-drug therapy. Typically, reverse transcriptase inhibitors serve as a mainstay of most frontline HIV combination therapies. The reverse transcriptase enzyme can be inhibited by two classes of drugs belonging either to the nucleoside reverse transcriptase inhibitors (NRTIs) or to the non-nucleoside reverse transcriptase inhibitors (NNRTIs) [3]. NRTIs act at the active site of RT. They are incorporated in the growing DNA strand and prevent further elongation of the DNA-chain, which terminates DNA synthesis. This class of inhibitors is active in HIV-1 as well as HIV-2 strains. NNRTIs, however, bind to an allosteric site, in the proximity of the active site of the enzyme and are specifically active against HIV-1 [4,5].

The application of the approved NNRTIs is limited to some extent. This is mainly due to relatively low genetic barrier of this class of drugs, and cross-resistance between these structurally unrelated drugs. The intensive use of approved NNRTIs (efavirenz, nevirapine and delavirdine, Fig. 1) [6–8] has led to the emergence of HIV-resistant strains. Actually, for the first generation of NNRTIs, only a single mutation is sufficient to confer resistance, leaving some patients with no further NNRTI therapeutic solutions [9].

Issues related to the use of NNRTI drugs have stirred our effort and allowed the discovery of new series of NNRTIs that belong to the diarylpyrimidine (DAPY) family [10]. DAPY compounds, with TMC125 (Fig. 1) as prototype of the early generation of DAPY series, display a broad anti-HIV activity spectrum against wild-type and mutant strains. TMC125, which is currently in phase III clinical trials [11],

* Corresponding author. Tel.: +33 2 32 61 74 58; fax: +33 2 32 61 72 98.
E-mail address: jguillem@prdfr.jnj.com (J. Guillemont).

0223-5234/$ - see front matter © 2006 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2006.11.014

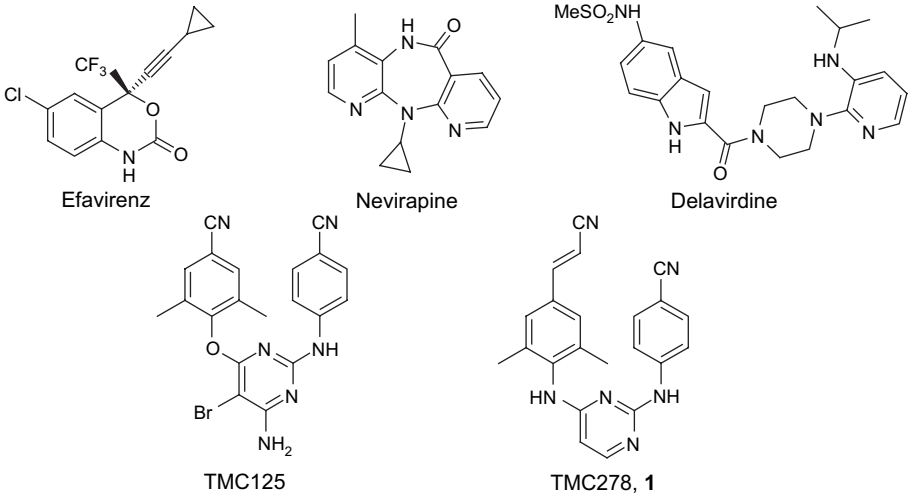


Fig. 1. Structure of reference compounds, TMC125 and TMC278.

is the first NNRTI to demonstrate beneficial effects on heavily experienced HIV-infected patients failing the traditional NNRTI therapies [12,13]. Structure–activity relationship studies combined with molecular modeling and X-ray analyses have provided keys on the capability of DAPY compounds to accommodate in the binding pocket, to amino acid residues that confer resistance to NNRTIs. This has resulted into the synthesis of a second-generation of DAPY compounds displaying high potency against a whole panel of HIV-1 relevant mutant strains. One of the second-generation of DAPY compounds, TMC278 (Fig. 1) [14,15], is now in phase II clinical trials [16].

To pursue our efforts in improving the anti-HIV activity of DAPY derivatives against a clinically relevant panel of mutant strains, the design and synthesis of TMC278 analogues were undertaken.

Modulations on the left wing A of the diarylpyrimidine were investigated (Fig. 2) on both sides:

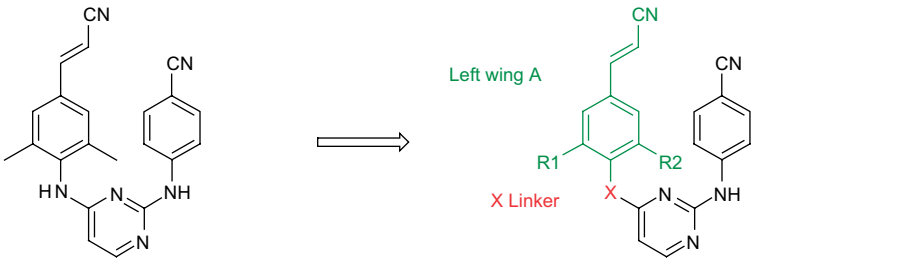
(i) The influence of an X spacer between the substituted phenyl and the position 4 of the pyrimidine was evaluated;
(ii) The effect of the nature of the substituents at positions 2 and 6 of the phenyl ring was assessed.

The results show that newly synthesized DAPY derivatives were endowed with nanomolar activity against a relevant panel of viruses, including single and double NNRTI mutant strains.

2. Chemistry

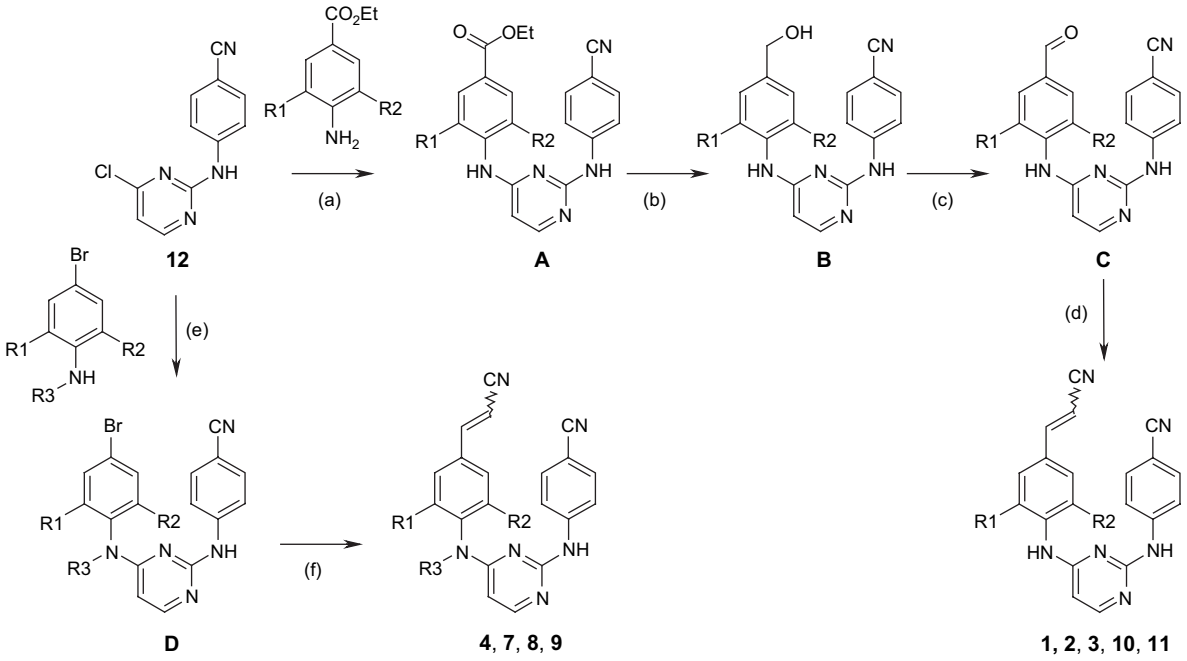
The new series of DAPY derivatives (compounds **2**–**11**) was synthesized following two main pathways based on classical reactions namely the Heck reaction and the Wittig–Wadsworth–Emmons reaction, which had been developed for the synthesis of **1** (TMC278) (Scheme 1) [14]. These two synthetic routes start from the same building block, chloropyrimidine derivative **12** [10].

On one hand, the Wittig precursor, the aldehyde derivative **C**, was synthesized in three steps from **12** with moderate yields. The ester intermediate **A** was first obtained by heating the chloropyrimidine **12** with the desired 3,5-disubstituted ethyl 4-aminobenzoate at 150 °C or 110 °C in the presence of 3 N hydrochloric acid. Thereafter, the ester function was classically reduced with lithium aluminum hydride and the resulting alcohol **B** underwent manganese-mediated oxidation to give the expected aldehyde **C**. The latter was thus involved in a key Wittig reaction and condensed with diethyl cyanomethyl phosphonate in the presence of *t*-BuOK to afford the following



2 R1=H, R2=Me, X=NH
3 R1=R2=H, X=NH
4 R1=H, R2=Me, X=NMe
5 R1=R2=Me, X=O
6 R1=R2=Me, X=S
7 R1=Me, R2=Et, X=NH
8 R1=Me, R2=iPr, X=NH
9 R1= Me, R2=OMe, X=NH
10 R1=Me, R2=Cl, X=NH
11 R1=OMe, R2=Cl, X=NH

Fig. 2. Modulations of an X spacer between the left wing A and the pyrimidine and introduction of various 2,6-substitutions on the phenyl ring.

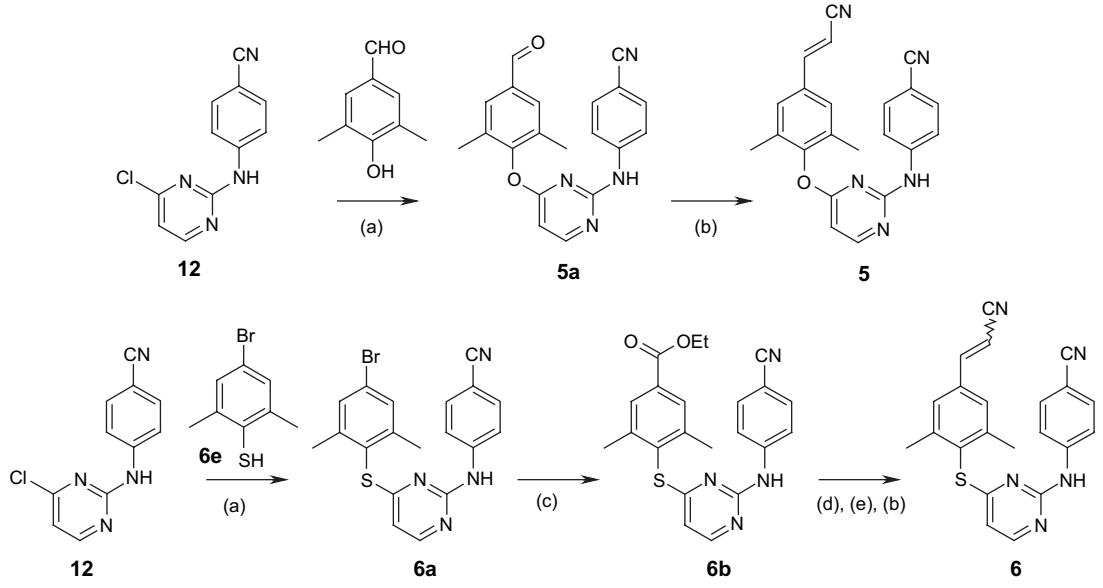


Scheme 1. Synthetic routes *via* Wittig or Heck reactions to 2,6-disubstituted rings. Wittig pathway: (a) **2**, **3**: 150 °C, 2–4 h; **10**, **11**: HCl 3 N, reflux, 5–16 h; (b) $LiAlH_4$, THF, 0 °C to RT, 20 h; (c) MnO_2, CH_2Cl_2/DMF, RT, 20 h; (d) $(EtO)_2P(O)CH_2CN$, *t*-BuOK, THF, 0 °C to RT, 4–20 h. Heck pathway: (e) **4**, **9**: NMP, 140 °C, 20 h; **7**: HCl 3 N, reflux, 20 h; **8**: heated neat; (f) acrylonitrile, $Pd(OAc)_2$, $P(o\text{-Tol})_3$, NEt_3, CH_3CN, 140 °C, 20 h.

acrylonitrile derivatives **2**, **3**, **10** and **11**. These products were isolated and further crystallized with a complete (*E*)-stereoselectivity, except for compound **3**, which exhibited a (*E*)/(*Z*) ratio of 80/20.

On the other hand, the Heck precursor was readily prepared from **12**. The bromo-derivative **D** was synthesized either by heating (140 °C) the chloropyrimidine **12** and the corresponding bromo-aniline in *N*-methyl-pyrrolidinone as solvent (compounds **4** and **9**) or according to the protocols described above for carboxylic esters of type **A** (**7** and **8**). The coupling reaction was then conducted on different bromo-derivatives of **D** according to classical Heck conditions using palladium(II) diacetate as a catalyst in the presence of triethylamine in acetonitrile. The following acrylonitrile derivatives **4**, **7**, **8** and **9** were thus prepared according to this procedure and obtained with moderate yields. The stereoselectivity ranged from 80/20 to 96/4 ratio in favor of the (*E*) diastereoisomer.

The synthesis of the corresponding phenoxy and phenylsulfanyl derivatives **5** and **6** was also based on a Wittig reaction in order to introduce the acrylonitrile function (Scheme 2).



Scheme 2. Synthesis of compounds **5** and **6**. (a) NaH, NMP, dioxane, 150 °C, 12–48 h; (b) $(Et_2O)_2P(O)CH_2CN$, *t*-BuOK, THF, 0 °C to RT, 20 h; (c) P(CO) 10 bar, $Pd(OAc)_2$, PPh_3, K_2CO_3, EtOH, DMF, 90 °C, 72 h; (d) $LiAlH_4$, THF, 0 °C to RT, 20 h; (e) MnO_2, CH_2Cl_2, RT, 72 h.

Compound **5** was synthesized in two steps from derivative **12**. Introduction of the phenoxy moiety on the pyrimidine scaffold was realized by heating (150 °C) the chloropyrimidine **12** and commercially available 3,5-dimethyl-4-hydroxybenzaldehyde with sodium hydride in a 1:1 mixture of NMP:dioxane. A Wittig reaction on **5a** allowed us to isolate **5** with a complete (*E*)-stereoselectivity and an excellent yield. The same coupling conditions were used, started from **12**, to synthesize bromo-derivative **6a**, which was then submitted to a palladium(II) catalyzed CO-insertion leading to the corresponding ethyl ester **6b** with a moderate yield. A standard sequence $LiAlH_4$-reduction/MnO_2-oxidation/Wittig reaction was finally performed to achieve the synthesis of **6** with a (*E*)/(*Z*) ratio of 85/15.

All these A-ring analogues of **1** were synthesized to allow further exploration of the influence of a heteroatom linker X and the effect of a 2,6-disubstitution on the activity.

3. Results and discussion

The anti-HIV activity of compounds **2**–**11** was measured using an HIV-1 replication assay and compared to TMC278 (**1**). The cells were infected with HIV-1 wild-type virus (LAI) or with single (L100I, K103N, Y181C, Y188L, F227C) or double mutant (L100I/K103N, K103N/Y181C, F227C/V106A) strains derived from wild-type LAI. The results are reported as the concentration required to achieve 50% inhibition of cellular activity (EC_{50}). In addition, the cytotoxicity (CC_{50}) of the compounds was determined. The selective index ($SI = CC_{50}/EC_{50}$), which indicates the specificity of the antiviral effect, is listed for the wild-type virus.

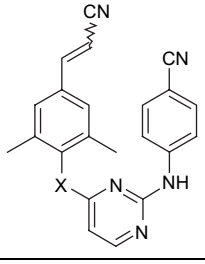
Tables 1 and 2 list the results for compounds **2**–**11** in comparison with those for compound **1** and three reference compounds efavirenz (**13**), nevirapine (**14**) and delavirdine (**15**). All DAPY derivatives were considerably more potent than **13**, **14** or **15** on the whole panel of viruses.

First the influence on activity of the X spacer connecting the left phenyl ring and the pyrimidine was evaluated (Table 1). Compounds **5** and **6**, in which the NH-linker of TMC278 (**1**) was respectively replaced by an O- and S-linker, were thus compared to TMC278 (**1**) and to the reference compounds (**13**, **14** and **15**).

In line with the results obtained for **1**, compounds **5** and **6** demonstrated high potency on the whole panel with an enhanced resistance profile on the double mutant strains as compared to marketed drugs (**13**, **14** and **15**). The K103N single mutant strain showed hypersusceptibility towards the DAPY compounds. However, the sulfanyl derivative **6** displayed a slight decrease of activity to some extent compared to TMC278 (**1**). These results prompted us to consider the NH-group as the most appropriate spacer to achieve high-level potency on single and double mutant strains. Therefore, we pursued the optimization process with this linker.

The effect of the left phenyl A-ring's 2,6-disubstitution on activity was then investigated. Modeling data with a 2,6-dimethyl DAPY derivative had shown that π–π main interactions were present between the phenyl ring A of the substrate and residues Tyr181 and Tyr188 of the binding pocket [12]. The conformation favoring these specific interactions was partially due to the presence of the two methyl groups at positions 2 and 6, which prevented great conformational shifts of this left wing A, especially by limiting the rotational freedom. Removal of the substituents might therefore increase the conformational degrees of freedom and weaken the π–π interaction. In addition, the two methyl groups were involved in hydrophobic interactions with a number of side chains exposed in the NNRTI pocket. One methyl group interacted with Pro95, Leu100 and Tyr181 and to a lesser extent with Glu138 and Trp229, while the other methyl group was located close to

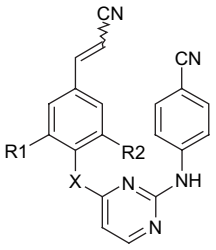
Table 1
Influence of the X spacer on the inhibition of wt LAI and mutant strains of HIV-1



Compound	X	*E*/*Z*	LAI	CC_{50}	SI[a]	EC_{50} (nM)							
						L100I	K103N	Y181C	Y188L	F227C	L100I + K103N	K103N + Y181C	F227C + V106A
1	NH	100/0	1.0	2000	2020	0.5	0.1	1.3	1.2	0.5	7.5	3.2	0.5
5	O	100/0	0.9	>25000	28302	0.6	0.2	3.2	1.6	0.7	3.7	4.3	0.6
6	S	85/15	3.7	>25000	>6821	2.6	0.7	5.8	2.8	1.4	79.2	18.6	1.1
13	Efavirenz		1.6	32314	19850	31.0	46.4	3.9	55.0	46.9	1460	55.0	4.1
14	Nevirapine		33.3	31631	>949	209	1980	7078	5910	827	3532	14505	2697
15	Delavirdine		122.1	67188	>550	9530	10220	9035	1022	4560	20583	38352	721

[a] $SI = CC_{50}/EC_{50}$.

Table 2
Influence of the 2,6-substitution on the inhibition of wt LAI and mutant strains of HIV-1



Compound	X	R1	R2	*E/Z*	LAI	CC_{50}	SI[a]	EC_{50} (nM)							
								L100I	K103N	Y181C	Y188L	F227C	L100I/K103N	K103N/Y181C	F227C/V106A
1	NH	Me	Me	100/0	1.0	2000	2020	0.5	0.1	1.3	1.2	0.5	7.5	3.2	0.5
2	NH	H	Me	100/0	0.9	>25000	>29372	9.2	0.6	13.4	43.6	1.2	478.7	89.6	1.2
3	NH	H	H	80/20	33.9	>25000	>737	585.1	57.7	1523	303.3	98.4	6440	3952	76.1
4	NMe	H	Me	80/20	1.0	11625	11351	2.5	0.7	12.0	16.8	1.0	103.8	160.3	1.5
7	NH	Me	Et	85/15	3.0	2553	841	0.9	0.5	4.0	2.6	1.1	8.2	5.3	0.9
8	NH	Me	*i*Pr	96/04	4.0	11791	2947	3.1	1.3	11.1	3.8	3.9	38.3	10.6	3.2
9	NH	Me	OMe	91/09	0.7	633	846	0.5	<0.1	1.9	0.9	0.3	21.1	4.3	0.3
10	NH	Me	Cl	100/0	0.8	2606	3316	0.4	0.1	2.3	2.3	0.5	6.6	4.1	0.5
11	NH	OMe	Cl	100/0	0.1	398	3981	0.2	0.3	1.0	2.5	0.8	15.8	2.0	0.3

[a] $SI = CC_{50}/EC_{50}$.

Val106 and Val179 and might also interact weakly with the side chains of K103 and Y188.

The antiviral results for the compounds with variations of the 2,6-substitution on the left phenyl ring are listed in Table 2.

The mono-substituted derivative **2** exhibited an excellent potency against wild-type virus but a rather moderate activity on mutant strains. The decrease in activity on the single mutant strains L100I, Y181C and Y188L was due to the loss of favorable hydrophobic interactions and the weakening of the π–π interactions. In the double mutant L100I/K103N, compound **2** was more than 100 times less active than in the wild-type enzyme. The non-2,6-substituted compound **3** had submicromolar activity against wild-type ($EC_{50} = 0.034$ μM) and was considerably less potent compared to compound **2**. This decrease in activity of **3** might be explained by additional degrees of freedom for the molecule due to the absence of substituents on the phenyl ring, causing further weakening of the π–π interactions.

Interesting results were also observed when considering the methylation of the NH-linker (compound **4**). This compound showed a nanomolar activity on wild-type and single mutant strains that was comparable to the other substituted DAPY derivatives. However, a drop in activity was observed when considering L100I/K103N and K103N/Y181C mutations. Superposition of **1** and **4** after docking in the NNRTI pocket showed that the methyl group of the N–CH_3 linker in compound **4** is located further away from residues Pro95, Leu100 and Tyr181.

It is also interesting to notice that introduction of an ethyl (**7**) or isopropryl (**8**) group at position 6 had only minor effects on the activity. In the series methyl–ethyl–isopropyl, the size of the substituent seems to be inversely proportional to the activity. This could be explained by the presence of some steric hindrance generated by the larger substituents. However, the double mutants L100I/K103N and K103N/Y181C, which were fully resistant to efavirenz (**13**) and nevirapine (**14**)/delavirdine (**15**), respectively, remained strongly inhibited by any of the substituents.

Introduction of small R1 and R2 groups, with electronic properties different from the previous alkyl substitutions, at positions 2 and 6 of the phenyl ring was also investigated. Electron-donating and electron-withdrawing groups were considered (**9**–**11**). The results showed antiviral profiles for these three compounds similar to the 2,6-dimethyl substituted compound TMC278 (**1**); the electronic nature of the substituents did not seem to highly impact the activity. The potency of compounds **9** and **11** on the double mutant strain L100I/K103N was slightly decreased compared to **1**.

Derivatives **10** and **11** were selected for further *in vitro* and *in vivo* studies and compared to TMC278 (**1**). The metabolic stability profile of these compounds was assessed using rat, dog and human liver microsomes (Table 3).

As outlined in Table 3, the metabolic stability in rat, dog and human liver microsomes of compounds **1**, **10** and **11** was similar. In rat and dog liver microsomes, high rates of recovery were observed (60–70%) whereas in human microsomes, **10** and **11** seemed to be less metabolically stable (up to 74% metabolized). However, since TMC278 demonstrated the same metabolic stability, further evaluation of the

Table 3
Metabolic stability of compounds **1**, **10** and **11**

Compound	Microsome stability (% recovered after 15 min)		
	Rat	Dog	Human
1 (TMC278)	73	65	26
10	72	60	39
11	53	58	26

pharmacokinetic profile of the two new compounds **10** and **11** in rat and dog species was sensible.

The results presented in Table 4 show mean values of pharmacokinetic parameters. Following a single dose of an oral formulation, TMC278 was well absorbed in rats and dogs with mean AUC values of 9650 and 23,790 ng.h/mL and high mean C_{max} levels of 1940 and 1046 ng/mL, respectively. Absorption of TMC278 was prolonged with a $T_{1/2}$ of 6 h in rats and 23 h in dogs. The derivatives **10** and **11** were highly absorbed in dogs as well. Compound **10** had lower mean AUC (17,600 ng.h/mL) and C_{max} (1040 ng/mL) values compared to **1** whereas the mean AUC (44,500 ng.h/mL) and C_{max} (2184 ng/mL) values of compound **11** were significantly higher. Nevertheless, the half-life of these derivatives was somewhat lower (14.5 h) compared to **1** in both animal species. The pharmacokinetic profile of TMC278 allows once-daily dosing in HIV-infected patients.

4. Conclusion

This exploratory study clearly highlights the necessity to maintain a 2,6-disubstitution pattern on the left wing A within the DAPY series to achieve a high level of inhibition on NNRTI-resistant viruses. Furthermore, it points out that the methyl groups in TMC278 could be efficiently replaced by other functionalities (**9**, **10**, and **11**) without any loss of activity. The newly synthesized derivatives had an enhanced potency to some extent on specific mutant strains. Compounds **10** and **11** not only demonstrate an excellent overall profile on a selection of clinically relevant mutant strains compared to the approved drugs, but also interesting pharmacokinetic characteristics in dogs. This promising broad-spectrum activity could thus support further developments of the DAPY series.

5. Experimental section

5.1. Chemistry

All analytically pure compounds were dried under vacuum in a drying pistol using a Buchi Glass Oven B-580 apparatus. Melting points were determined using a Leica VMHB apparatus and are uncorrected. TLC analyses were run on silica gel 60 F_{254} plates (Merck) using a variety of solvent systems and a fluorescent indicator for visualization. Spots were visualized under 254 nm UV illumination. Column chromatography was performed with silica gel 60 (Merck) (0.015–0.040 mm) or Kromasyl (Akzo Nobel) (0.010 mm).

Table 4
Pharmacokinetic parameters of compounds **1**, **10** and **11**

Compound	Rat (PO) normalized to 10 mg/kg			Dog normalized to 10 mg/kg		
	C_{max} (ng/mL)	AUC (ng.h/mL)	$T_{1/2}$ (h)	C_{max} (ng/mL)	AUC (ng.h/mL)	$T_{1/2}$ (h)
1 (TMC278)	1940	9650	6	1046	23,790	23
10	182	910	3.54	1040	17,600	14.5
11	199	410	3.83	2184	44,500	14.5

Proton NMR spectra were recorded on a Bruker Avance 300 (300 MHz) and a Bruker Avance 400 (400 MHz) spectrometer using internal deuterium lock. Chemicals shifts are reported to internal DMSO (δ 2.54) in parts per million and coupling constants (J) in hertz. Exact mass spectra (TOF) were recorded with a Micromass LCT instrument. Elemental analyses were performed with a Thermo Electron Corporation instrument EA 1110 or EA 1108 for C, H, and N, and the results were within ±0.4% of the theoretical values. Chemicals and solvents were purchased from either Acros Co. or Aldrich Chemical Co. Yields refer to purified products and are not optimized. The synthesis of TMC278 was described before [14].

5.2. General procedure 1 for the synthesis of 4-[2-(4-cyanophenylamino)-pyrimidin-4-ylamino]-3,5-disubstitutedbenzoic acid ethyl ester **A**

Method A: a mixture of 4-amino-3,5-disubstitutedbenzoic acid ethyl ester (1 eq.) and 4-(4-chloropyrimidin-2-ylamino)-benzonitrile **12** [10] (1 eq.) was heated at 150 °C in an oil bath for 2–4 h. The residue was extracted with CH_2Cl_2/MeOH and washed with 10% K_2CO_3. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from diisopropyl ether. *Method B*: a mixture of 4-amino-3,5-disubstituted-benzoic acid ethyl ester (1 eq.) and 4-(4-chloropyrimidin-2-ylamino)-benzonitrile **12** [10] (1 eq.) in the presence of 3 N hydrochloric acid was heated at reflux in an oil bath for 16 h. After cooling down, the precipitate was filtered and rinsed successively with water and diisopropyl ether.

5.2.1. 4-[2-(4-Cyanophenylamino)-pyrimidin-4-ylamino]-3-methylbenzoic acid ethyl ester **2A**

Compound **2A** was obtained according to general procedure 1, method A from **12** (0.0558 mol) and commercially available 4-amino-3-methylbenzoic acid ethyl ester (0.0558 mol). The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH/NH_4OH 96:4:0.1–90:10:0.1) and then recrystallized from diisopropyl ether (8 g, 39% yield). Mp 192 °C; ^{1}H NMR (DMSO-d_6) δ 1.35 (3H, t, $J = 7.7$ Hz), 2.30 (3H, s), 4.35 (2H, q, $J = 7.7$ Hz), 6.54 (1H, d, $J = 5.2$ Hz), 7.60 (2H, d, $J = 7.7$ Hz), 7.65–7.77 (3H, m), 7.88 (1H, d, $J = 8.0$ Hz), 7.91 (1H, s), 8.04 (1H, d, $J = 5.2$ Hz), 10.08 (1H, br s), 10.52 (1H, br s).

5.2.2. 4-[2-(4-Cyanophenylamino)-pyrimidin-4-ylamino]-benzoic acid ethyl ester **3A**

Compound **3A** was obtained according to general procedure 1, method A from **12** (0.003 mol) and commercially available ethyl 4-amino-benzoate (0.003 mol). The residue was crystallized from diisopropyl ether (0.91 g, 42% yield). Mp 233 °C; ^{1}H NMR (DMSO-d_6) δ 1.33 (3H, t, $J = 6.5$ Hz), 4.30 (2H, q, $J = 6.5$ Hz), 6.44 (1H, d, $J = 5.7$ Hz), 7.20 (2H, d, $J = 7.7$ Hz), 7.86–8.00 (6H, m), 8.18 (1H, d, $J = 5.7$ Hz), 9.85 (1H, s), 9.93 (1H, s).

*5.2.3. 4-[2-(4-Cyanophenylamino)-pyrimidin-4-ylamino]-3-chloro-5-methylbenzoic acid ethyl ester **10A***

Compound **10A** was obtained according to general procedure 1, method B from **12** (0.00332 mol), 4-amino-3-chloro-5-methylbenzoic acid ethyl ester (0.00332 mol) and 3 N HCl (4 mL). The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH/NH_4OH 98:2:0.1) and crystallized from CH_3CN/diisopropyl ether (0.44 g, 32% yield). Mp 195 °C; ^{1}H NMR (DMSO-d_6) δ 1.36 (3H, t, $J = 7.7$ Hz), 2.27 (3H, s), 4.37 (2H, q, $J = 7.7$ Hz), 6.28 (1H, br s), 7.45 (2H, d, $J = 7.2$ Hz), 7.60–7.75 (2H, m), 7.92 (1H, s), 7.95 (1H, s), 8.09 (1H, d, $J = 5.2$ Hz), 9.33 (1H, s), 9.68 (1H, s); MS ($C_{21}H_{18}N_5O_2Cl$): *m/z* 408 $(M + H)^+$.

4-Amino-3-chloro-5-methylbenzoic acid ethyl ester was previously synthesized according to the following protocol. A mixture of 4-amino-5-methylbenzoic acid ethyl ester (0.052 mol) and *N*-chlorosuccinimide (0.058 mol, 1.1 eq.) in acetonitrile (70 mL) was heated at reflux for 1 h. The resulting mixture was then basified with 10% K_2CO_3 and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (cyclohexane/ethyl acetate 85:15) to give 7.5 g of pure product (94% yield). Mp 130 °C; ^{1}H NMR (DMSO-d_6) δ 1.27 (3H, t, $J = 7.7$ Hz), 2.16 (3H, s), 4.22 (2H, q, $J = 7.7$ Hz), 5.92 (2H, s), 7.54 (1H, s), 7.65 (2H, s).

*5.2.4. 4-[2-(4-Cyanophenylamino)-pyrimidin-4-ylamino]-3-chloro-5-methoxy-benzoic acid ethyl ester **11A***

Compound **11A** was obtained according to general procedure 1, method B from **12** (0.0094 mol), 4-amino-3-chloro-5-methoxy-benzoic acid ethyl ester (0.0094 mol) and 3 N HCl (50 mL). The crude product was used without further purification in the next step (6.5 g, 77% yield). MS ($C_{21}H_{18}N_5O_3Cl$): *m/z* 424 $(M + H)^+$.

4-Amino-3-chloro-5-methoxy-benzoic acid ethyl ester was previously synthesized according to the following protocol. Thionyl chloride (5 mL) was added dropwise to an ice-cooled solution of 4-amino-3-methoxy-benzoic acid (0.060 mol) in ethanol (100 mL). The resulting mixture was heated at reflux for 3 h and then poured into ice. The mixture was then basified with 10% K_2CO_3 and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Next, the resulting 4-amino-3-methoxy-benzoic acid ethyl ester (10 g, 85% yield) was engaged in the next step. MS ($C_{10}H_{13}NO_3$): *m/z* 196 $(M + H)^+$. A mixture of 4-amino-3-methoxy-benzoic acid ethyl ester (0.0256 mol) and *N*-chlorosuccinimide (0.0282 mol, 1.1 eq.) in acetonitrile (20 mL) was heated at reflux for 1 h. The resulting mixture was then basified with 10% K_2CO_3 and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (cyclohexane/ethyl acetate 80:20) to give 4.3 g of pure product (73% yield). ^{1}H NMR (DMSO-d_6) δ 1.30 (3H, t, $J = 7.7$ Hz), 3.87 (3H, s), 4.25 (2H, q, $J = 7.7$ Hz), 5.78 (2H, br s), 7.27 (1H, d, $J = 2.5$ Hz), 7.48 (2H, d, $J = 2.5$ Hz); MS ($C_{10}H_{12}NO_3Cl$): *m/z* 230 $(M + H)^+$.

*5.3. General procedure 2 for the synthesis of 4-[4-(4-hydroxymethyl-2,6-disubstituted-phenylamino)-pyrimidin-2-ylamino]-benzonitrile **B***

Lithium aluminum hydride (2 eq.) was added portionwise to an ice-cooled solution of benzoic acid ethyl ester **A** (1 eq.) in THF (5 mL/mmol) under nitrogen. The reaction mixture was allowed to warm up to room temperature and stirred overnight. Ethyl acetate was then added followed by water and the resulting mixture was filtered over Celite. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure.

*5.3.1. 4-[4-(4-Hydroxymethyl-2-methyl-phenylamino)-pyrimidin-2-ylamino]-benzonitrile **2B***

Compound **2B** was obtained according to general procedure 2, from **2A** (0.0175 mol). The residue was crystallized from diisopropyl ether/isopropanol (4.7 g, 80% yield). Mp 210 °C; ^{1}H NMR (DMSO-d_6) δ 2.20 (3H, s), 4.50 (2H, d, $J = 5.7$ Hz), 5.19 (1H, t, $J = 5.7$ Hz), 6.15 (1H, d, $J = 5.7$ Hz), 7.20 (1H, d, $J = 6.8$ Hz), 7.25 (1H, s), 7.33 (1H, d, $J = 6.8$ Hz), 7.55 (2H, d, $J = 8.0$ Hz), 7.86 (2H, d, $J = 8.0$ Hz), 8.01 (1H, d, $J = 5.7$ Hz), 8.90 (1H, s), 9.63 (1H, s).

*5.3.2. 4-[4-(4-Hydroxymethyl-phenylamino)-pyrimidin-2-ylamino]-benzonitrile **3B***

Compound **3B** was obtained according to general procedure 2, from **3A** (0.00181 mol). The residue was engaged in the next step without further purification (0.59 g, 100% yield). Mp 215 °C; ^{1}H NMR (DMSO-d_6) δ 4.49 (2H, d, $J = 5.7$ Hz), 5.12 (1H, t, $J = 5.7$ Hz), 6.32 (1H, d, $J = 5.7$ Hz), 7.32 (2H, d, $J = 7.7$ Hz), 7.60 (2H, d, $J = 7.7$ Hz), 7.67 (2H, d, $J = 7.7$ Hz), 7.97 (2H, d, $J = 7.7$ Hz), 8.07 (1H, d, $J = 5.7$ Hz), 9.46 (1H, s), 9.74 (1H, s); MS ($C_{18}H_{15}N_5O$): *m/z* 318 $(M + H)^+$.

*5.3.3. 4-[4-(4-Hydroxymethyl-2-chloro-6-methyl-phenylamino)-pyrimidin-2-ylamino]-benzonitrile **10B***

Compound **10B** was obtained according to general procedure 2, from **10A** (0.0098 mol). The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH/NH_4OH 97:3:0.1) (1 g, 27% yield). Mp 246 °C; ^{1}H NMR (DMSO-d_6) δ 2.20 (3H, s), 2.27 (3H, s), 4.54 (2H, d, $J = 5.2$ Hz), 6.40 (1H, t, $J = 5.2$ Hz), 6.35 (1H, br s), 7.26 (1H, s), 7.38 (1H, s), 7.40–7.50 (2H, br m), 7.55–7.75 (2H, br m), 7.90–8.10 (1H, br m), 9.05 (1H, br s), 9.64 (1H, br s); MS ($C_{19}H_{16}N_5OCl$): *m/z* 366 $(M + H)^+$.

*5.3.4. 4-[4-(4-Hydroxymethyl-2-chloro-6-methoxy-phenylamino)-pyrimidin-2-ylamino]-benzonitrile **11B***

Compound **11B** was obtained according to general procedure 2, from **11A** (0.016 mol). The residue was engaged in the next step without further purification (4.1 g, 67% yield). MS ($C_{19}H_{16}N_5O_2Cl$): *m/z* 382 $(M + H)^+$.

5.4. General procedure 3 for the synthesis of 4-[4-(4-formyl-2,6-disubstituted-phenylamino)-pyrimidin-2-ylamino]-benzonitrile **C**

To a solution of benzoic methyl alcohol **B** in a CH_2Cl_2/DMF mixture was added portionwise MnO_2 (4 eq.). The resulting mixture was stirred overnight at room temperature then filtered over Celite and concentrated under reduced pressure.

5.4.1. 4-[4-(4-Formyl-2-methyl-phenylamino)-pyrimidin-2-ylamino]-benzonitrile **2C**

Compound **2C** was obtained according to general procedure 3, from **2B** (0.0136 mol) in 70 mL CH_2Cl_2. The residue was engaged in the next step without further purification (2.1 g, 46% yield). MS ($C_{19}H_{15}N_5O$): *m/z* 330 $(M+H)^+$.

5.4.2. 4-[4-(4-Formyl-phenylamino)-pyrimidin-2-ylamino]-benzonitrile **3C**

Compound **3C** was obtained according to general procedure 3, from **3B** (0.0016 mol) in a 1:1 CH_2Cl_2:DMF mixture (20 mL). The residue was crystallized from ethyl acetate/acetonitrile (0.25 g, 50% yield). Mp > 250 °C; ^{1}H NMR (DMSO-d_6) δ 6.45 (1H, d, $J = 5.7$ Hz), 7.73 (2H, d, $J = 7.7$ Hz), 7.88 (2H, d, $J = 7.7$ Hz), 7.96 (4H, d, $J = 7.7$ Hz), 8.21 (1H, d, $J = 5.7$ Hz), 9.85–9.95 (2H, m), 10.04 (1H, s); MS ($C_{18}H_{13}N_5O$): *m/z* 316 $(M+H)^+$.

5.4.3. 4-[4-(4-Formyl-2-chloro-6-methyl-phenylamino)-pyrimidin-2-ylamino]-benzonitrile **10C**

Compound **10C** was obtained according to general procedure 3, from **10B** (0.0027 mol) in a 10:1 CH_2Cl_2:DMF mixture (55 mL). The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH/NH_4OH 95:5:0.1) (0.24 g, 25% yield). MS ($C_{19}H_{14}N_5OCl$): *m/z* 364 $(M+H)^+$.

5.4.4. 4-[4-(4-Formyl-2-chloro-6-methoxy-phenylamino)-pyrimidin-2-ylamino]-benzonitrile **11C**

Compound **11C** was obtained according to general procedure 3, from **11B** (0.0107 mol) in a 7:1 CH_2Cl_2:DMF mixture (24 mL). The residue was crystallized from diisopropyl ether (1.9 g, 47% yield). Mp 196 °C; MS ($C_{19}H_{14}N_5O_2Cl$): *m/z* 380 $(M+H)^+$.

5.5. General procedure 4 for the synthesis of 4-{4-[4-(2-cyanovinyl)-2,6-disubstituted-phenylamino]-pyrimidin-2-ylamino}-benzonitrile (**2**, **3**, **5**, **10**, and **11**)

Potassium *tert*-butoxide (1.5 eq.) was added to an ice-cooled solution of diethyl cyanomethyl phosphonate (1.5 eq.) in THF (10 mL/mmol). The resulting mixture was stirred for 30 min at 0 °C, then at room temperature for another 30 min. A solution of aldehyde **C** (1 eq.) in THF (10 mL/mmol) was added dropwise to the reaction mixture. The stirring was maintained for 3–8 h. The reaction was then quenched with water and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Next, the residue was purified by silica gel column chromatography and crystallized.

5.5.1. 4-{4-[4-(2-Cyanovinyl)-2-methyl-phenylamino]-pyrimidin-2-ylamino}-benzonitrile **2**

Compound **2** was obtained according to general procedure 4, from **2C** (0.00096 mol). The residue was first crystallized from isopropanol/diisopropyl ether and then crystallized from hot ethanol followed by a last crystallization from isopropanol/diisopropyl ether to give pure product **2** ((*E*), 0.025 g, 7% yield). Mp > 260 °C; ^{1}H NMR (DMSO-d_6) δ 2.26 (3H, s), 6.38 (1H, d, $J = 5.0$ Hz), 6.43 (1H, d, $J = 16.0$ Hz), 7.51–7.61 (4H, m), 7.65 (1H, d, $J = 16.0$ Hz), 7.72 (1H, d, $J = 7.7$ Hz), 7.88 (2H, d, $J = 7.7$ Hz), 8.10 (1H, d, $J = 5.0$ Hz), 9.00 (1H, s), 9.73 (1H, s); MS ($C_{21}H_{16}N_6$) *m/z* 353 $(M+H)^+$.

5.5.2. 4-{4-[4-(2-Cyanovinyl)-phenylamino]-pyrimidin-2-ylamino}-benzonitrile **3**

Compound **3** was obtained according to general procedure 4, from **3C** (0.00047 mol). The residue was crystallized from diisopropyl ether/acetonitrile to give pure product **3** ((*E*)/(*Z*) 80/20, 0.061 g, 38% yield). Mp > 250 °C; ^{1}H NMR (DMSO-d_6) δ 5.73 (*Z*) (1H, d, $J = 12.1$ Hz), 6.35 (*E*) (1H, d, $J = 16.7$ Hz), 6.40 (1H, d, $J = 6.2$ Hz), 7.35 (*Z*) (1H, d, $J = 12.1$ Hz), 7.60 (*E*) (1H, d, $J = 16.7$ Hz), 7.63–7.77 (4H, m), 7.82 (2H, d, $J = 7.9$ Hz), 7.95 (2H, d, $J = 7.9$ Hz), 8.15 (1H, d, $J = 5.7$ Hz), 9.80–9.90 (2H, m).

5.5.3. 4-{4-[4-(2-Cyanovinyl)-2-chloro-6-methyl-phenylamino]-pyrimidin-2-ylamino}-benzonitrile **10**

Compound **10** was obtained according to general procedure 4, from **10C** (0.0025 mol). The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH/NH_4OH 98:2:0.1) and then crystallized from diisopropyl ether/acetonitrile to give pure product **10** ((*E*), 0.4 g, 20% yield). Mp 254 °C; ^{1}H NMR (DMSO-d_6) δ 2.22 (3H, s), 6.18–6.35 (1H, m), 6.60 (1H, d, $J = 16.7$ Hz), 7.40–7.50 (2H, m), 7.66–7.75 (4H, br m), 7.829 (1H, s), 8.06 (1H, d, $J = 5.7$ Hz), 9.23 (1H, br s), 9.68 (1H, br s).

5.5.4. 4-{4-[4-(2-Cyanovinyl)-2-chloro-6-methoxy-phenylamino]-pyrimidin-2-ylamino}-benzonitrile **11**

Compound **11** was obtained according to general procedure 4, from **11C** (0.0008 mol). The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH 98.5:1.5) and then crystallized first from diisopropyl ether followed by acetonitrile to give pure product **11** ((*E*), 0.045 g, 14% yield). Mp 264 °C; ^{1}H NMR (DMSO-d_6) δ 3.80 (3H, s), 6.22 (1H, br s), 6.70 (1H, d, $J = 16.7$ Hz), 7.45–7.50 (3H, m), 7.55 (1H, s), 7.65–7.78 (3H, m), 8.04 (1H, d, $J = 5.1$ Hz), 9.10 (1H, s), 9.67 (1H, s); MS ($C_{21}H_{15}N_6OCl$): *m/z* 380 $(M+H)^+$.

5.6. General procedure 5 for the synthesis of 4-[4-(4-bromo-2,6-disubstituted-phenylamino)-pyrimidin-2-ylamino]-benzonitrile **4D** and **9D**

A mixture of **12** (1 eq.) and 4-bromo-aniline (1 eq.) in *N*-methyl-pyrrolidinone (7 mL/mmol) was heated at 140 °C overnight. The reaction mixture was hydrolyzed and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was then purified by silica gel column chromatography.

5.6.1. 4-{4-[(4-Bromo-2-methyl-phenyl)-methylamino]-pyrimidin-2-ylamino}-benzonitrile **4D**

Compound **4D** was obtained according to general procedure 5 from **12** (0.008 mol) and (4-bromo-2-methyl-phenyl)-methylamine (0.008 mol). The residue was purified by silica gel column chromatography (CH_2Cl_2/*i*PrOH/NH_4OH 99:1:0.1) (0.25 g, 8% yield). Mp 200 °C; ^{1}H NMR (DMSO-d_6) δ 2.10 (3H, s), 3.35 (3H, s), 5.83 (1H, br s), 7.20 (1H, d, $J = 7.7$ Hz), 7.46–7.57 (3H, m), 7.53 (1H, d, $J = 2.2$ Hz), 7.72–7.87 (2H, br m), 8.00 (1H, d, $J = 5.1$ Hz), 9.43 (1H, s); MS ($C_{19}H_{16}N_5Br$): *m/z* 394 $(M + H)^+$.

(4-Bromo-2-methyl-phenyl)-methylamine was previously synthesized according to the following protocol. A mixture of *N*-methyl-*o*-toluidine (0.041 mol) and *N*-bromosuccinimide (0.045 mol, 1.1 eq.) in acetonitrile (20 mL) was stirred at room temperature for 3 h. The resulting mixture was then hydrolyzed with 10% K_2CO_3 and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (cyclohexane/CH_2Cl_2 50:50) to give 4 g of pure product (49% yield). ^{1}H NMR (DMSO-d_6) δ 2.04 (3H, s), 2.68 (3H, s), 5.20 (1H, br s), 6.39 (1H, d, $J = 7.7$ Hz), 7.10 (1H, s), 7.15 (1H, d, $J = 7.7$ Hz).

5.6.2. 4-{4-[4-Bromo-2-methyl-6-methoxy-phenylamino]-pyrimidin-2-ylamino}-benzonitrile **9D**

Compound **9D** was obtained according to general procedure 5 from **12** (0.014 mol) and 4-bromo-2-methyl-6-methoxy-phenylamine (0.014 mol). The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH 98.5:1.5) (2.6 g, 45% yield). Mp 185 °C; ^{1}H NMR (DMSO-d_6) δ 2.13 (3H, s), 3.74 (3H, s), 6.38 (1H, br s), 7.17–7.20 (2H, m), 7.40–7.80 (4H, br m), 7.97 (1H, d, $J = 5.1$ Hz), 8.68 (1H, br s), 9.60 (1H, s); MS ($C_{19}H_{16}N_5OBr$): *m/z* 410 $(M + H)^+$.

4-Bromo-2-methyl-6-methoxy-phenylamine was previously synthesized according to the following protocol. A mixture of 2-methoxy-6-methylaniline (0.036 mol) and *N*-bromosuccinimide (0.040 mol, 1.1 eq.) in acetonitrile (50 mL) was stirred at room temperature for 3 h. The resulting mixture was then hydrolyzed with 10% K_2CO_3 and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (cyclohexane/ethyl acetate 80:20) to give 3.15 g of pure product (40% yield). MS ($C_8H_{10}NOBr$): *m/z* 216 $(M + H)^+$.

5.6.3. 4-[4-(4-Bromo-2-ethyl-6-methyl-phenylamino)-pyrimidin-2-ylamino]-benzonitrile **7D**

Compound **7D** was obtained according to general procedure 1, method B from **12** (0.0084 mol), 4-bromo-2-ethyl-6-methyl-phenylamine (0.0084 mol) and 3 N HCl (20 mL). The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH/NH_4OH 98:2:0.1) (0.61 g, 18% yield). Mp 205 °C; ^{1}H NMR (DMSO-d_6) δ 1.10 (3H, d, $J = 7.7$ Hz), 2.15 (3H, s), 2.55 (2H, q, $J = 7.7$ Hz), 6.1 (1H, br s), 7.38 (1H, s), 7.40 (1H, s), 7.40–7.50 (2H, br m), 7.70–7.80 (2H, br m), 8.00 (1H, d, $J = 5.1$ Hz), 8.57 (1H, br s), 9.30 (1H, br s); MS ($C_{20}H_{18}N_5Br$): *m/z* 408 $(M + H)^+$.

4-Bromo-2-ethyl-6-methyl-phenylamine was previously synthesized according to the following protocol. A mixture of 2-ethyl-6-methylaniline (0.0344 mol) and *N*-bromosuccinimide (0.038 mol, 1.1 eq.) in acetonitrile (5 mL) was heated at reflux for 3 h. The resulting mixture was then hydrolyzed with 10% K_2CO_3 and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (cyclohexane/ethyl acetate 80:20) to give 2 g of pure product (27% yield). MS ($C_9H_{12}NBr$): *m/z* 214 $(M + H)^+$.

5.6.4. 4-[4-(4-Bromo-2-isopropyl-6-methyl-phenylamino)-pyrimidin-2-ylamino]-benzonitrile **8D**

A mixture of **12** (0.0044 mol) and 4-bromo-2-isopropyl-6-methyl-phenylamine (0.0044 mol) was heated neatly for 10 min. The residue was then purified by silica gel column chromatography (CH_2Cl_2/ethyl acetate 90:10) (0.26 g, 14% yield). Mp 210 °C; ^{1}H NMR (DMSO-d_6) δ 1.00 (3H, d, $J = 7.2$ Hz), 1.15 (3H, d, $J = 7.2$ Hz), 2.1 (3H, s), 3.07 (1H, sep, $J = 7.2$ Hz), 6.34 (1H, d, $J = 5.1$ Hz), 7.30–7.50 (3H, br m), 7.55–7.75 (2H, m), 7.90–8.1 (2H, br m), 8.70–8.90 (1H, br m), 9.55–9.75 (1H, br m); MS ($C_{21}H_{20}N_5Br$): *m/z* 422 $(M + H)^+$.

4-Bromo-2-isopropyl-6-methyl-phenylamine was previously synthesized according to the following protocol. A mixture of 2-isopropyl-6-methylaniline (0.0315 mol) and *N*-bromosuccinimide (0.035 mol, 1.1 eq.) in acetonitrile (5 mL) was heated at reflux for 3 h. The resulting mixture was then hydrolyzed with 10% K_2CO_3 and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (cyclohexane/ethyl acetate 80:20) to give 1.7 g of pure product (24% yield). ^{1}H NMR (DMSO-d_6) δ 1.13 (6H, d, $J = 7.7$ Hz), 2.08 (3H, s), 3.00 (1H, sep, $J = 7.7$ Hz), 4.73 (2H, s), 6.95 (2H, s).

5.7. General procedure 6 for the synthesis of 4-{4-[4-(2-cyanovinyl)-2,6-disubstituted-phenylamino]-pyrimidin-2-ylamino}-benzonitrile (**4**, **7**, **8**, and **9**)

A mixture of bromo-derivative **D** (1 eq.), acrylonitrile (10 eq.), $Pd(OAc)_2$ (0.2 eq.), tri-*o*-tolylphosphine (1 eq.) and triethylamine (4 eq.) in acetonitrile (20 mL/mmol) was stirred in a sealed tube at 140 °C overnight. The reaction mixture was hydrolyzed with water and extracted with CH_2Cl_2. The

organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography and then crystallized.

*5.7.1. 4-{4-[(4-(2-Cyanovinyl)-2-methyl-phenyl)-methylamino]-pyrimidin-2-ylamino}-benzonitrile **4***

Compound **4** was obtained according to general procedure 6, from **4D** (0.00076 mol). The residue was purified by silica gel column chromatography (toluene/isopropanol 94:6) and then crystallized from diisopropyl ether/isopropanol to give pure product **4** ((*E*)/(*Z*) 80/20, 0.073 g, 26% yield). Mp 239 °C; ^{1}H NMR (DMSO-d_6) δ 2.15 (3H, s), 3.37 (3H, s), 5.84 (1H, br s), 5.90 (*Z*) (1H, d, $J = 11.6$ Hz), 6.44 (*E*) (1H, d, $J = 16.7$ Hz), 7.32 (1H, d, $J = 7.7$ Hz), 7.38–7.57 (2H, m), 7.60–7.75 (3H, m), 7.76–7.85 (2H, m), 8.00 (1H, d, $J = 5.1$ Hz), 9.45 (1H, br s); MS ($C_{22}H_{18}N_6$) *m/z* 367 $(M + H)^+$.

*5.7.2. 4-{4-[4-(2-Cyanovinyl)-2-ethyl-6-methyl-phenylamino]-pyrimidin-2-ylamino}-benzonitrile **7***

Compound **7** was obtained according to general procedure 6, from **7D** (0.00072 mol). The residue was purified by silica gel column chromatography (toluene/isopropanol 93:7) and then crystallized from diisopropyl ether to give pure product **7** ((*E*)/(*Z*) 85/15, 0.069 g, 25% yield). Mp 159 °C; ^{1}H NMR (DMSO-d_6) δ 1.08 (3H, t, $J = 7.7$ Hz), 2.15 (3H, s), 2.50–2.60 (2H, m), 5.90 (*Z*) (1H, d, $J = 11.6$ Hz), 6.35 (1H, br s), 6.50 (*E*) (1H, d, $J = 16.7$ Hz), 7.35–7.55 (4H, br m), 7.60–7.75 (3H, br m), 8.02 (1H, br s), 8.95 (1H, br s), 9.60 (1H, br s); MS ($C_{23}H_{20}N_6$) *m/z* 381 $(M + H)^+$.

*5.7.3. 4-{4-[4-(2-Cyanovinyl)-2-isopropyl-6-methyl-phenylamino]-pyrimidin-2-ylamino}-benzonitrile **8***

Compound **8** was obtained according to general procedure 6, from **8D** (0.00071 mol). The residue was purified by silica gel column chromatography (first CH_2Cl_2/MeOH 99:1 then toluene/*i*PrOH 93:7) to give 0.058 g of product. The corresponding hydrochloride salt was formed (diisopropyl ether/ethanol + HCl in methanol) leading to pure product ((*E*)/(*Z*) 96/4, 0.033 g, 11% yield). Mp 224 °C; ^{1}H NMR (DMSO-d_6) δ 1.15 (6H, br s), 2.18 (3H, s), 3.05–3.15 (1H, br m), 6.47 (1H, d, $J = 16.7$ Hz), 7.40–7.60 (6H, m), 7.67 (1H, d, $J = 16.7$ Hz), 8.05 (1H, d, $J = 6.1$ Hz); MS ($C_{24}H_{22}N_6$) *m/z* 395 $(M + H)^+$. Anal. ($C_{24}H_{22}N_6$) Calcd C, 66.05; H, 5.97; N, 2.91. Found C, 65.05; H, 5.95; N, 17.73.

*5.7.4. 4-{4-[4-(2-Cyanovinyl)-2-methyl-6-methoxy-phenylamino]-pyrimidin-2-ylamino}-benzonitrile **9***

Compound **9** was obtained according to general procedure 6, from **9D** (0.0054 mol). The residue was purified by silica gel column chromatography (CH_2Cl_2/ethyl acetate 70:30) and then crystallized from diisopropyl ether/isopropanol to give pure product **9** ((*E*)/(*Z*) 91/9, 0.345 g, 17% yield). Mp 203 °C; ^{1}H NMR (DMSO-d_6) δ 2.17 (3H, s), 3.77 (3H, s), 5.92 (*Z*) (1H, d, $J = 12.1$ Hz), 6.55 (*E*) (1H, d, $J = 16.7$ Hz), 7.23 (1H, s), 7.30 (1H, s), 7.44–7.54 (2H, br m), 7.65 (*E*) (1H, d, $J = 16.7$ Hz), 7.70–7.80 (2H, m), 8.00 (1H, d, $J = 5.1$ Hz), 8.82 (1H, br s), 9.60 (1H, s); MS ($C_{22}H_{18}N_6O$) *m/z* 383 $(M + H)^+$.

*5.7.5. 4-[4-(4-Formyl-2,6-dimethyl-phenoxy)-pyrimidin-2-ylamino]-benzonitrile **5a***

Sodium hydride (0.0233, 1.1 eq.) was added to a solution of 3,5-dimethyl-4-hydroxybenzaldehyde (0.0233 mol, 1.1 eq.) in dioxane (35 mL) and stirring was maintained for 5 min. *N*-Methyl-pyrrolidinone (35 mL) was then added followed by **12** after 10 min. The reaction mixture was then heated to 150 °C for 12 h. Next, the mixture was hydrolyzed and extracted with CH_2Cl_2. The organic layer was washed twice with water and then dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2 100%) to give 3 g of product, which was crystallized from acetonitrile/diisopropyl ether (2.2 g, 27% yield). Mp 247 °C; ^{1}H NMR (DMSO-d_6) δ 2.17 (6H, s), 6.72 (1H, d, $J = 5.1$ Hz), 7.45 (2H, d, $J = 7.7$ Hz), 7.55 (2H, d, $J = 7.7$ Hz), 7.80 (2H, s), 8.50 (1H, d, $J = 5.1$ Hz), 10.03 (1H, s), 10.17 (1H, s).

*5.7.6. 4-{4-[4-(2-Cyanovinyl)-2,6-dimethyl-phenoxy]-pyrimidin-2-ylamino}-benzonitrile **5***

Compound **5** was obtained according to general procedure 4, from **5a** (0.0044 mol). The residue was first crystallized from diethyl ether to give 1.5 g of product ((*E*)/(*Z*) 90/10, 93% yield), which was then purified by silica gel column chromatography (acetonitrile/ammonium acetate 50:50). A last crystallization from diisopropyl ether led to pure product **5** ((*E*), 0.4 g, 25% yield). Mp 258 °C; ^{1}H NMR (DMSO-d_6) δ 2.09 (6H, s), 6.49 (1H, d, $J = 16.7$ Hz), 6.67 (1H, d, $J = 5.1$ Hz), 7.45 (2H, d, $J = 7.7$ Hz), 7.52–7.62 (4H, m), 7.68 (1H, d, $J = 16.7$ Hz), 8.47 (1H, d, $J = 5.1$ Hz), 10.15 (1H, s); MS ($C_{22}H_{17}N_5O$) *m/z* 368 $(M + H)^+$.

*5.7.7. 4-[4-(4-Bromo-2,6-dimethyl-phenylsulfanyl)-pyrimidin-2-ylamino]-benzonitrile **6a***

Sodium hydride (0.0291, 1.1 eq.) was added to a solution of 4-bromo-2,6-dimethyl-benzenethiol (0.0264 mol, 1 eq.) in dioxane (20 mL) and stirring was maintained for 15 min. *N*-Methyl-pyrrolidinone (20 mL) was then added followed by **12** (0.0264 mol, 1 eq.) after 10 min. The reaction mixture was heated at 150 °C overnight. The mixture was then poured into ice, filtered and washed successively with water and diethyl ether to give 7.18 g of product (61% yield), engaged without further purification in the next step.

*5.7.8. 4-[2-(4-Cyanophenylamino)-pyrimidin-4-ylsulfanyl]-3,5-dimethylbenzoic acid ethyl ester **6b***

A mixture of **6a** (0.00486 mol, 1 eq.), $Pd(OAc)_2$ (0.0005 mol, 0.1 eq.), triphenylphosphine (0.00972 mol, 2 eq.) and K_2CO_3 (0.00972 mol, 2 eq.) in a 1:1 DMF:EtOH mixture (100 mL) was heated at 90 °C under 10 bar of CO for 72 h. The reaction mixture was then filtered over Celite and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (cyclohexane/ethyl acetate

98:2) leading to 0.92 g of product (47% yield). Mp 200 °C; ^{1}H NMR (DMSO-d_6) δ 1.39 (3H, t, $J = 6.1$ Hz), 2.43 (6H, s), 4.40 (2H, q, $J = 6.1$ Hz), 6.76 (1H, d, $J = 5.0$ Hz), 7.35–7.45 (4H, m), 7.90 (2H, s), 8.28 (1H, d, $J = 5.0$ Hz), 10.20 (1H, s); MS ($C_{22}H_{20}N_4O_2S$): *m/z* 405 $(M + H)^+$.

5.7.9. 4-[4-(4-Hydroxymethyl-2,6-dimethyl-phenylsulfanyl)-pyrimidin-2-ylamino]-benzonitrile **6c**

Compound **6c** was obtained according to general procedure 2 from **6b** (0.000074 mol). The residue was purified by silica gel column chromatography (cyclohexane/ethyl acetate 60:40) to give 0.12 g of product (45% yield). Mp 173 °C; ^{1}H NMR (DMSO-d_6) δ 2.37 (6H, s), 4.56 (2H, d, $J = 5.1$ Hz), 5.42 (1H, t, $J = 5.1$ Hz), 6.53 (1H, d, $J = 5.0$ Hz), 7.30 (2H, s), 7.50–7.60 (4H, s), 8.25 (1H, d, $J = 5.0$ Hz), 10.19 (1H, s); MS ($C_{20}H_{18}N_4OS$): *m/z* 363 $(M + H)^+$.

5.7.10. 4-[4-(4-Formyl-2,6-dimethyl-phenylsulfanyl)-pyrimidin-2-ylamino]-benzonitrile **6d**

Compound **6d** was obtained according to general procedure 3 from **6c** (0.00124 mol). The residue was crystallized from diethyl ether to give pure product **6d** (0.43 g, 96% yield). Mp 184 °C; ^{1}H NMR (DMSO-d_6) δ 2.46 (6H, s), 6.68 (1H, d, $J = 5.0$ Hz), 7.42 (2H, d, $J = 7.7$ Hz), 7.50 (2H, d, $J = 7.7$ Hz), 7.89 (2H, s), 8.28 (1H, d, $J = 5.0$ Hz), 10.10 (1H, s), 10.21 (1H, s); MS ($C_{20}H_{16}N_4OS$): *m/z* 361 $(M + H)^+$.

5.7.11. 4-{4-[4-(2-Cyanovinyl)-2,6-dimethyl-phenylsulfanyl]-pyrimidin-2-ylamino}-benzonitrile **6**

Compound **6** was obtained according to general procedure 4 from **6d** (0.000361 mol). The residue was crystallized from diethyl ether to give pure product **6** (0.13 g, 94% yield). Mp 205 °C; ^{1}H NMR (DMSO-d_6) δ 2.38 (*E*) (6H, s), 2.40 (*Z*) (6H, s), 6.08 (*Z*) (1H, d, $J = 12.0$ Hz), 6.55 (*E*) (1H, d, $J = 16.7$ Hz), 6.55–6.60 (1H, m), 7.44 (2H, d, $J = 7.7$ Hz), 7.51 (2H, d, $J = 7.7$ Hz), 7.65 (2H, s), 7.75 (1H, d, $J = 16.7$ Hz), 8.37 (1H, d, $J = 5.1$ Hz), 10.2 (1H, s); MS ($C_{22}H_{17}N_5S$): *m/z* 384 $(M + H)^+$.

5.8. Test method

The antiviral activity of compounds was determined using an EGFP-based HIV-1 replication assay.

5.8.1. Assay principles

The HIV-1 replication assay measures virus replication as an induction of enhanced green fluorescent protein (EGFP) expression. The indicator MT4-LTR-EGFP cells contain an EGFP gene under the control of the HIV-1 LTR promoter sequence. Successful HIV-1 infection results in viral Tat protein expression and subsequent induction of EGFP expression. Compounds inhibiting HIV-1 infection are expected to reduce EGFP expression as compared to the untreated HIV-infected control. A parallel cytotoxicity assay is performed on MT4-CMV-EGFP indicator cells containing an EGFP gene under the CMV early promoter. These cells constitutively express EGFP and cytotoxicity is detected as a decreased reporter gene expression.

5.8.2. Methods

Serial 4-fold dilutions of test compounds were mixed with HIV-1 from a panel of different strains and MT4-LTR-EGFP cells, and incubated at 37 °C. After three days, the wells were examined for EGFP expression using an argon laser-scanning microscope. The effective compound concentration inhibiting 50% of the virus-induced EGFP signal (EC_{50}) was determined by linear interpolation of the EGFP signal versus logarithm of the compound concentration. Cytotoxicity was measured on MT4-CMV-EGFP cells using the same assay conditions as described above except that no virus was added. The 50% cytotoxic concentration (CC_{50}) was determined similarly to EC_{50}.

5.9. Metabolic stability assay

Sub-cellular tissue preparations were made according to Gorrod *et al*. [17] by centrifugation after mechanical homogenization of tissue. Tissue was rinsed in ice-cold 0.1 M Tris–HCl buffer (pH 7.4) to wash the excess of blood. When the whole animal liver was processed, a perfusion of the liver was performed with this buffer (~25 mL for rat, 100 mL for dog). Tissue was then blotted dry, weighed and chopped coarsely using surgical scissors. The tissue pieces were homogenized in three volumes of ice-cold 0.1 M phosphate buffer (pH 7.4) using either a Potter-S (Braun, Italy) equipped with a Teflon pestle or a Sorvall Omni-Mix homogenizer, for 7 × 10 s. In both cases, the vessel was kept in/on ice during the homogenization process. Tissue homogenates were centrifuged at 9000×*g* for 20 min at 4 °C using a Sorvall centrifuge or Beckman Ultracentrifuge. The resulting supernatant was stored at −80 °C and designated as "S9". The S9 fraction was centrifuged at 100,000×*g* for 60 min (4 °C) using a Beckman ultracentrifuge. The resulting supernatant was carefully aspirated, aliquoted and designated as "cytosol". The pellet was re-suspended in 0.1 M phosphate buffer (pH 7.4) in a final volume of 1 mL per 0.5 g original tissue weight and designated as microsomes. All sub-cellular fractions were aliquoted, immediately frozen in liquid nitrogen and stored at −80 °C until use. Incubations were performed in a phosphate buffer (pH 7.4) 0.1 M containing 5 μM substrate, 1 mg/mL of protein-active and 1 mg/mL of protein-inactive heat inactivated (control), and an NADPH-generating system comprised of 0.8 mM glucose-6-phosphate, 0.8 magnesium chloride and 0.8 U of glucose-6-phosphate dehydrogenase. The samples were incubated for 5 min at 37 °C prior to the addition of NADP in order to initiate the reaction. Incubations were terminated after 15 min by adding two volumes of DMSO (or acetonitrile). The samples were centrifuged (10 min, 900×*g*) and supernatants stored at room temperature (when stopped with DMSO; no longer than 24 h) or −20 °C (when stopped with acetonitrile; no longer than 24 h) before analysis. All incubations were performed in duplicates. Twenty microliters of assay supernatant was injected to a LC-MS LCQ Deca XP

equipped with a surveyor MS pump and an UV PDA detector (Thermofinnigan) in full loop using a Hypersil BDS C18 column (5 cm × 4.6 mm × 5 μm). The eluent(s) were solvent A: 0.01 M ammonium acetate (pH 7.5) and solvent B: 10% solvent A–90% acetonitrile with a gradient 0′ (95%A/5%B), 5′ (100%B), 9′ (100%B), 10′ (95%A/5%B), 12′ (95%A/%B) and a run time of 12 min. Data were analyzed using an Xcalibur (version 1.2) as follows: %Recovery = (protein-inactive ($T = 15$)/protein-inactive ($T = 0$)) × 100; %Metabolism = (1 − (protein-active ($T = 15$)/protein-active ($T = 0$)) × 100).

5.10. Pharmacokinetic studies

5.10.1. Animals

The pharmacokinetic studies of **1**, **10**, and **11** were conducted in Wistar rats and beagle dogs, using four animals (two males and two females or four males for **1**) per group. The body weights of rats and dogs ranged from 0.242 to 0.305 kg and 9.3 to 13.4 kg, respectively. The animals were housed under standard conditions and treated in accordance with the provisions of the Belgian law of 18 October 1991 on the approval of the European convention on the protection of vertebrates that are used for experimental and other scientific purposes. The compounds were administrated orally by gavage at a single dose of 5 or 20 mg/kg dissolved in PEG-400 to the fasting dogs and rats (deprived of food for 12–16 h before experimentation), respectively. For **1**, the dogs had free access to water and food throughout the entire experimental period.

5.10.2. Plasma sampling

Blood samples were withdrawn at 0, 0.3, 1, 3, 8, and 24 h for rats and extended to 32, 48, 72 and 96 h for dogs. Blood samples (1 or 3 mL) were taken into plastic tubes TAPVAL with 0.75% K_3EDTA (10 or 20 μL/mL blood) for plasma separation. Blood samples were centrifuged at 1900×*g* for approximately 10 min to allow plasma preparation. Plasma was separated immediately, transferred into Eppendorf tubes and stored at −180 °C ± 2 °C until analyzed by high performance liquid chromatography method.

5.10.3. Analytical methodology

The HPLC-UV method was developed for individual compounds and plasma samples were analyzed. Briefly for the compounds **10** and **11**, 0.15–0.3 mL aliquot sample of species plasma was spiked with 60 ng of internal standards in 30 μL methanol and was made alkaline with 0.54 mL NaOH (0.1 mol/L). The samples were vortex mixed for 10 s and eluted through the Extrelut 1 extraction column with 4 mL of the mixture *n*-heptane:*tert*-butylmethyl ether:-isoamyl alcohol (7:2:1). The column was dried and the extraction residue was dissolved in 0.1 mL methanol and 0.1 mL water. The solution was transferred in an autosampler vial for injection into the Agilent 1100 series HPLC system equipped with LC-3D Rev. A.0901, diode array detector, a high pressure quaternary pump, in-line four channel vacuum degasser, low pressure quaternary gradient mixer, variable volume temperature controlled autosampler and controlled column oven. The chromatography was performed on a Waters Symmetry C18 HPLC column (5 μm, 3.9 mm × 150 mm) preceded by a guard column Phenomenex C18 (4 mm × 2.0 mm ID). The mobile phase consisted of 0.2% aqueous ammonium acetate (A) and MeOH (B) for **11**. All gradients were linear. The UV detection was carried out at 305 nm, flow rate at 1.2 mL/min and injection volume of 100 μL. The calibration curves were linear over the concentration range of 0.03–1.5 μg/mL. The limit of quantification was 1, 20 and 30 ng/mL for **1**, **11**, and **10**, respectively. The accuracy of the method was >90%. The inter-day relative standard deviations were 9.54, 8.42 and 8.25% at 0.4, 1.5 and 6.0 μg/mL, respectively. The samples (0.1 mL aliquots of plasma) that contained **1** were extracted using solid phase columns (Bond Elut Certify, 130 mg, SPE, Varian). The SPE column was conditioned with 3 mL methanol, 3 mL water and 1 mL acetic acid 1 M. After addition of 3 mL acetic acid to 0.1 mL aliquots of plasma, the samples were extracted on the column followed by washing the column with 3 mL water, 1 mL acetic acid 1 M and 3 mL methanol. An internal standard was also used. The column was eluted with 3 mL methanol/NH_4OH 25% (98:2). The extract was evaporated to dryness and reconstituted in 150 μL ammonium formate 0.01 M (adjusted to pH 4 with formic acid/methanol 50/50). Twenty microliters of extracted samples was injected onto a reversed phase LC-column (100 × 4.6 mm ID, packed with 3μM-hypersil C18BDS) with a flow rate of 800 μL/min before splitting. The elution mixture was ammonium formate 0.01 M (adjusted to pH 4 with formic acid)/methanol (40:60). LC-MS/MS analysis was carried out on an API-3000 system (Applied Biosystems), which was coupled to an HPLC system (Agilent). The calibration curve was ranged from 1 to 2000 ng/mL. Depending on the sample dilution, the lower limit of quantification (LLOQ) was 1 or 10 ng/mL.

5.10.4. Pharmacokinetic data analysis

Individual plasma concentration–time profiles were analyzed using non-compartmental techniques. The maximum plasma and tissue concentration (C_{max}) and time of maximum concentration (t_{max}) were obtained by visual inspection of the data from the concentration–time profile. The area under the plasma concentration curve versus time (AUC_{0-t}) from time zero to the time of last measured concentration (C_{last}) was calculated by the linear trapezoidal rule. The AUC zero to infinite ($AUC_{0-\infty}$) was calculated as the sum of AUC $_{0-96h}$ and C_{96h}/β, with β, the elimination rate constant determined by log-linear regression of the terminal plasma concentration–time data. The half-($t_{1/2}$) was calculated as ln(2)/β on the 24–96 h time data.

Acknowledgements

We gratefully acknowledge Line Harmier for analysis and Sandrine Jolly for purification of compounds. We are also grateful to Luc Geeraert for proofreading of this manuscript.

References

[1] G.R. Kaufmann, D.A. Cooper, Curr. Opin. Microbiol. 3 (2000) 508–514.
[2] S. Vella, L. Palmisano, Antiviral Res. 45 (2000) 1–7.
[3] E. De Clercq, Chem. Biodivers. 1 (2004) 44–64.
[4] R. Esnouf, J. Ren, C. Ross, Y. Jones, D. Stammers, D. Stuart, Nat. Struct. Biol. 2 (1995) 303–308.
[5] C. Tantillo, J. Ding, A. Jacobo-Molina, R.G. Nanni, P.L. Boyer, S.H. Hughes, R. Pauwels, K. Andries, P.A.J. Janssen, E. Arnold, J. Mol. Biol. 243 (1994) 369–387.
[6] S.D. Young, S.F. Britcher, L.O. Tran, L.S. Payne, W.C. Lumma, L.A. Lyle, J.R. Huff, P.S. Anderson, D.B. Olsen, S.S. Carrol, D.J. Pettibone, J.A. O'Brien, V.V. Sardana, W.J. Long, V.W. Byrnes, E.A. Emini, Antimicrob. Agents Chemother. 39 (1995) 2602–2605.
[7] R.A. Koup, V.J. Merluzzi, J.L. Hargrave, J. Adams, K. Grozinger, R.J. Eckner, J.L. Sulliva, J. Infect. Dis. 163 (1991) 966–970.
[8] W.W. Freimuth, Adv. Exp. Med. Biol. 394 (1996) 279–289.
[9] E. De Clercq, Antiviral Res. 38 (1998) 153.
[10] D.W. Ludovici, B.L. De Corte, M.J. Kukla, H. Ye, C.Y. Ho, M.A. Lichenstein, R.W. Kavash, K. Andries, M.-P. De Bethune, H. Azijn, R. Pauwels, P.J. Lewi, J. Heeres, L.M.H. Koymans, M.R. De Jonge, K.J.A. Van Aken, F.F.D. Daeyaert, K. Das, E. Arnold, P.A.J. Janssen, Bioorg. Med. Chem. Lett. 11 (2001) 2235–2239.
[11] B.G. Gazzard, A.L. Pozniak, W. Rosenbaum, G.P. Yeni, S. Staszewski, K. Arasteh, K. De Dier, M. Peeters, B. Woodfall, J. Stebbing, G.A. Vant'Klooster, AIDS (London, England) 17 (18) (2003) F49–F54.
[12] K. Das, A.D. Clark Jr., P.J. Lewi, J. Heeres, M.R. De Jonge, L.M.H. Koymans, M. Vinkers, F.F.D. Daeyaert, D.W. Ludovici, M.J. Kukla, B. De Corte, R.W. Kavash, C.Y. Ho, H. Ye, M.A. Lichtenstein, K. Andries, R. Pauwels, M.-P. De Béthune, P.L. Boyer, P. Clark, S.H. Hughes, P.A.J. Janssen, E. Arnold, J. Med. Chem. 47 (2004) 2550–2560.
[13] K. Andries, H. Azijn, T. Thielemans, D. Ludovici, M. Kukla, J. Heeres, P.A.J. Janssen, B. De Corte, J. Vingerhoets, R. Pauwels, M.-P. De Béthune, Antimicrob. Agents Chemother. 48 (2004) 4680–4686.
[14] J. Guillemont, E. Pasquier, P. Palandjian, D. Vernier, S. Gaurrand, P.J. Lewi, J. Heeres, M.R. De Jonge, L.M.H. Koymans, F.F.D. Daeyaert, M.H. Vinkers, E. Arnold, K. Das, R. Pauwels, K. Andries, M.-P. De Béthune, E. Bettens, K. Hertogs, P. Wigerinck, P. Timmerman, P.A.J. Janssen, J. Med. Chem. 48 (2005) 2072–2079.
[15] P.A.J. Janssen, P.J. Lewi, E. Arnold, F. Daeyaert, M. De Jonge, J. Heeres, L. Koymans, M. Vinkers, J. Guillemont, E. Pasquier, M. Kukla, D. Ludovici, K. Andries, M.-P. De Béthune, R. Pauwels, K. Das, A.D. Clark Jr., Y.V. Frenkel, S.H. Hughes, B. Medaer, F. De Knaep, H. Bohets, F. de Clerck, A. Lampo, P. Williams, P. Stoffels, J. Med. Chem. 48 (2005) 1901–1909.
[16] F. Goebel, A. Yakovlev, A. Pozniak, E. Vinogradova, P. Lewi, G. Boogaerts, R. Hoetelmans, M.-P. De Béthune, M. Peeters, B. Woodfall, Conf. Retrovirus Opportun. Infect., Boston, 22–25 Febuary, 2005 (Abstract 160).
[17] J.W. Gorrod, D.J. Temple, A.H. Beckett, Xenobiotica 5 (1975) 453–463.