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Benzoxazole and benzothiazole amides as novel pharmacokinetic enhancers of HIV protease inhibitors

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ABSTRACT

A new class of benzoxazole and benzothiazole amide derivatives exhibiting potent CYP3A4 inhibiting properties was identified. Extensive lead optimization was aimed at improving the CYP3A4 inhibitory properties as well as overall ADME profile of these amide derivatives. This led to the identification of thiazol-5-ylmethyl (2S,3R)-4-(2-(ethyl(methyl)amino)-*N*-isobutylbenzo[*d*]oxazole-6-carboxamido)-3-hydroxy-1-phenylbutan-2-ylcarbamate (**C1**) as a lead candidate for this class. This compound together with structurally similar analogues demonstrated excellent 'boosting' properties when tested in dogs. These findings warrant further evaluation of their properties in an effort to identify valuable alternatives to Ritonavir as pharmacokinetic enhancers.

The current options for the treatment of Human Immunodeficiency Virus (HIV-1) infected patients consist of nucleoside analogue reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), CCR5 antagonists and a fusion inhibitor. A triple regimen comprising NRTIs as a backbone regimen and NNRTIs and/or PIs is considered the standard of care.[1,2] The use of PIs has been a major breakthrough in the therapy for HIV-1 infection, substantially reducing morbidity and mortality in infected individuals. The majority of PI regimens involve co-administration of a low dose of Ritonavir (Norvir®, RTV, **1**) with the PI in order to enhance patient exposure to the latter agent, thereby preventing or overcoming resistance and allowing less frequent dosing which potentially improves patient adherence.[3] RTV inhibits both the P-glycoprotein (P-gp) transport system and the cytochrome P450 enzyme CYP3A4.[4] In contrast, the majority of PIs are metabolised by CYP3A4 and most PIs are P-gp substrates. As a result, the pharmacokinetic profile of concomitantly administered PIs is beneficially influenced in RTV boosted regimens, resulting in an increased bioavailability of the boosted PI, slower elimination, and possibly, improved penetration into HIV reservoirs may be achieved.

While **1** is approved as an HIV PI itself (600 mg, BID (=twice a day)) it is hardly used as such, but more frequently as a pharmacokinetic enhancer. Being the only approved pharmacokinetic enhancer on the market, typical 'boosting' regimens contain a daily dose of 100–200 mg of **1**.

Unfortunately, the use of **1** is also associated with side effects such as gastrointestinal adverse events and changes in serum lipids, insulin resistance, lipoatrophy and CYP induction. Moreover, since **1** is a PI itself, resistance mutations might be induced that could contribute to resistance against other PIs. As a consequence, novel derivatives devoid of the drawbacks associated with the use of **1** are of major interest, and various groups have reported on their progress in this area (Fig. 1).[5–7] Cobicistat (**2**, GS-9350), a compound structurally related to RTV has emerged recently as a promising candidate. This compound has a comparable CYP3A4 inhibition to **1**, but more importantly, shows an improved tolerability and side effect profile. In addition, the superior physicochemical properties of **2** compared with **1** allowed co-formulation with tenofovir DF/elvitegravir/emtricitabine resulting in a single tablet known as Quad which is being evaluated in a phase-III clinical trial in HIV infected patients.[8] Here, we report on our efforts aimed at finding potential alternatives for **1**. This communication describes the discovery of a novel class of benzoxazole and benzothiazole amides that were designed to have no other primary activity than CYP3A4 inhibition together with an acceptable toxicity/side effect profile. This exercise has led to the selection of **C1**, also named TMC558445, as a lead candidate for the development of a new oral pharmaco-enhancer of HIV PIs.

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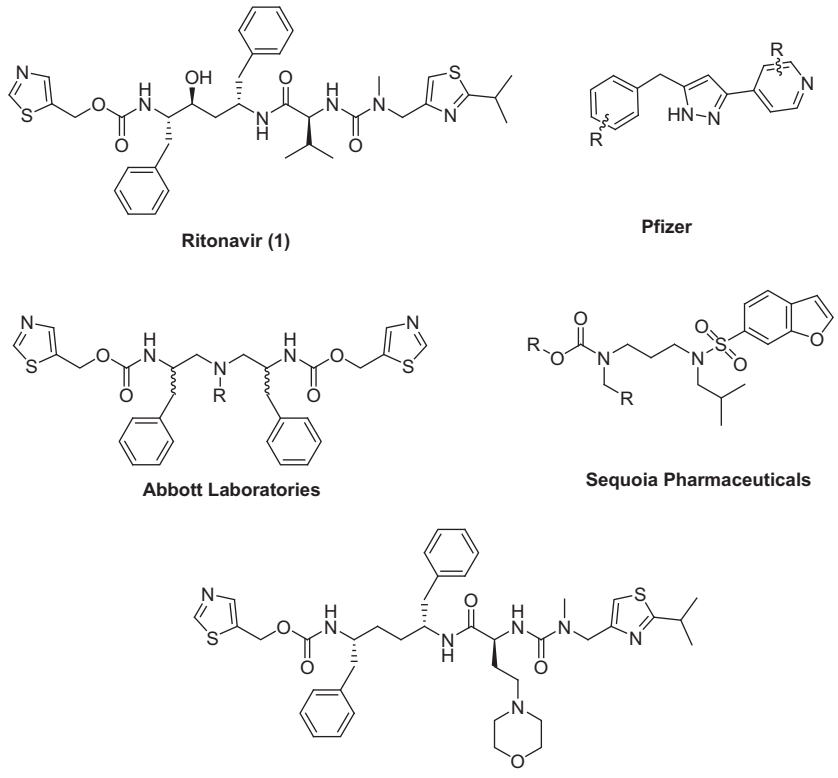


Figure 1. RTV (**1**), Cobicistat (**2**) and other CYP3A4 inhibiting scaffolds.

Our search for novel CYP3A4 inhibitors started from **3**, a previously identified PK-booster candidate which is also a potent HIV protease inhibitor (Fig. 2).[9] We based our design on three principles. First, we reasoned that preservation of the 5-thiazolyl moiety might be crucial to ensure ample CYP3A4 inhibition. This structural motif was identified as a major factor to explain the CYP3A4 inhibiting properties of **1** and related structures, as detailed spectroscopic studies showed that the N atom of the thiazole group is able to interact directly with the iron atom present in the heme group of CYP3A4. This interaction results in an impaired functionality of the enzyme.[10,11] Secondly, we envisaged that modification of the sulfonamide fragment of **3** would result in compounds devoid of HIV protease inhibition since the interaction of the sulfonamide group, either via the so called 'flap water molecule',[12] or directly,[13] with the backbone residues I50 and I50′ of the HIV protease is known to be essential for the class of sulfonamide based HIV protease inhibitors.

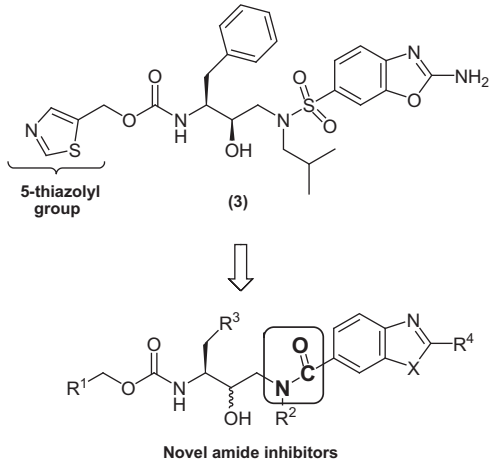
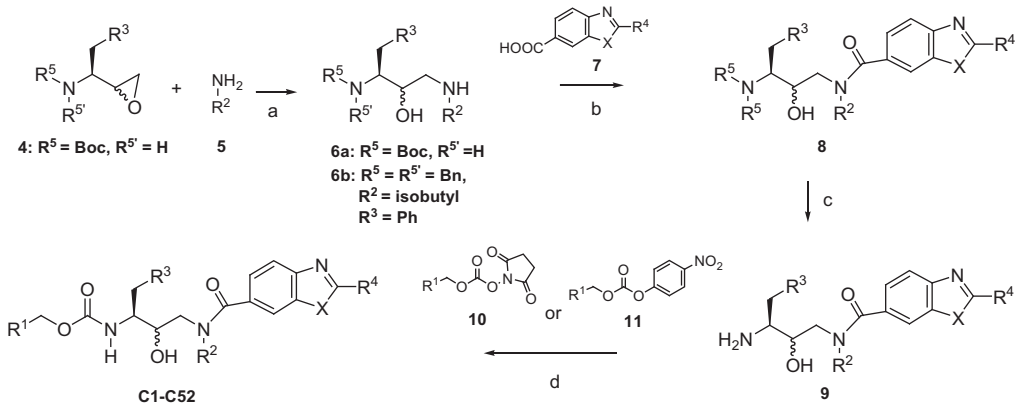


Figure 2. Design of new amide derivatives as CYP3A4 inhibitors.

Literature evidence shows that the modification or even complete removal of the secondary OH group has been used for this purpose given the crucial role this moiety plays in the interaction with the D25 and D25′ in the active site of HIV protease.[7,8a] However, switching from a sulfonamide group to an amide motif to deliberately abolish the antiviral activity is clearly different from any reported approach, and this concept is to the best of our knowledge, unprecedented. Finally, we foresaw that optimization of the R^2-, R^3- and R^4-substituent would allow us the identification of candidates with an optimal PK/PD profile.

A diverse set of derivatives was obtained using a convenient synthesis that allowed easy alteration of all substituents (Scheme 1, Table 1). Our initial method started from commercially available epoxides **4** that were reacted with an excess of primary amines **5** yielding derivatives **6a**. Alternatively, we started from dibenzyl protected intermediate **6b** (where R^2 = isopropyl, and R^3 = Ph). These were then coupled with acid derivatives **7** using HATU or BOP as activating agent yielding intermediates **8**.

Unexpectedly, the acid mediated deprotection of the Boc group (TFA or HCl/*i*PrOH) resulted in the formation of undesired side and decomposition products. Therefore, we reverted to a milder procedure using trimethylsilylchloride/sodium iodide which allowed the isolation of the amine after basic workup and purification by column chromatography.[14] Base mediated coupling of intermediates **9** with activated carbonates **10** or **11** yielded the target compounds **C1** to **C52**.[15] Contrary to the benzothiazole acid derivatives (**7**, where X = S), the corresponding benzoxazole derivatives were not commercially available. Therefore, starting from 4-amino-3-hydroxybenzoic acid **12**, the methylthio intermediate **13** was



Scheme 1. Reagents and conditions: (a) *i*-PrOH, reflux; (b) HATU or BOP, Et_3N, CH_2Cl_2; (c) TMSCl, NaI, CH_3CN or H_2, Pd/C, MeOH; (d) Et_3N, Me-THF, **10** or Et_3N, Me-THF, DMAP, **11**.

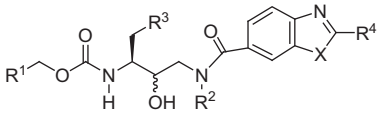
synthesized which served as an excellent precursor for various benzoxazole amine derivatives **14** (Scheme 2). The aryl and alkyl analogues were made via condensation of **12** with commercially available orthoesters under microwave irradiation.

The in vitro antiviral activity of all new compounds against wild type HIV-1 was evaluated in an acutely infected lymphoblastic cell line (MT4-LTR-EGFP) using a reporter gene assay.[16] As can be seen from Table 1, none of the derivatives showed any significant activity against HIV WT with EC_{50} values >10 μM in all cases. As a comparison (**3**), which is the sulfonamide analogue of **C8** has an EC_{50} value of 6.4 nM in this assay. These results thus confirmed that the switch from the sulfonamide moiety to the amide had the anticipated effect.

The CYP3A4 inhibition of all new compounds was determined in vitro using a human liver microsomes (HLM) based assay in which conversion of midazolam to 1′-OH- midazolam was measured (by LC/MS) in the presence and absence of the inhibitor. IC_{50} values shown in Table 1 were obtained by incubating the compounds across a concentration range with HLM's and the probe substrate.[17] Analysis of the results revealed several SAR trends. Overall, having a 5-thiazolyl fragment (R^1) present in the molecule resulted, in most cases, in potent CYP3A4 inhibition, with the 3-pyridyl and 5-benzo[1,3]dioxolyl fragment as good alternatives (compare **C1** with **C4** and **C10**). On the other hand, the 4-pyridyl group is clearly unfavorable as a 10-fold loss in inhibitory potency was observed (**C5**). Furthermore, it was observed that the presence of a basic nitrogen atom in the R^2 and R^4 substituents was not desirable (see **C6**, **C7** and **C9**) which directed our focus to less hydrophilic alternatives. For the R^4 substituent it was found that also carbon linked substituents (see **C11** and **C13**) as well as aromatic rings (**C12** and **C14**) were tolerated. Moreover, the unsubstituted derivatives (R^4 = H, **C3** and **C17**) maintained good CYP3A4 inhibition. Finally, comparison of **C1** with **C15** showed that the stereochemistry of the secondary OH group had no influence.

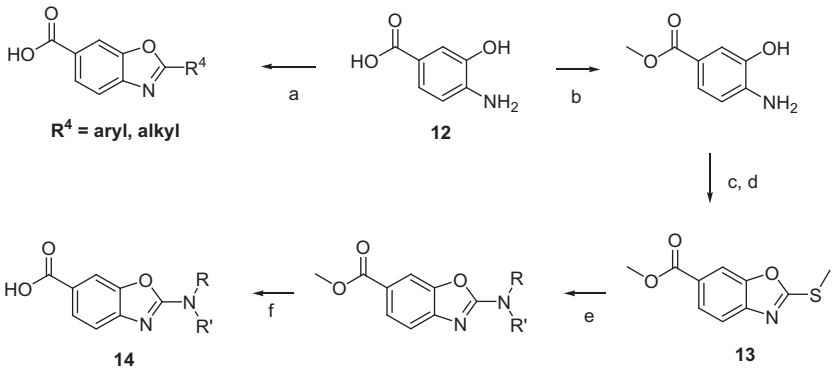
Based on their potent CYP3A4 inhibition, structural diversity as well as physicochemical differences, we decided to study compounds **C1**, **C2** and **C3** in more detail. First, the permeability and affinity for efflux transporters of the three compounds was evaluated in vitro, using a Caco-2 monolayer assay. As can be seen from Table 2, compound **C2** appeared to have the least attractive profile

Table 1
Structure, antiviral activity and CYP3A4 inhibition of selected benzoxazole and benzothiazole amide derivatives[a,b]



Compound	R^1	R^2	R^3	R^4	X	Antiviral activity HIV-1 WT (IIIB) (EC_{50}, μM)	CYP 3A4 inhibition (IC_{50}, μM)
C1	5-Thiazolyl	Isobutyl	Ph	Ethyl(methyl)amino	O	71	0.031
C2	5-Thiazolyl	Isobutyl	Ph	NH_2	S	76	0.019
C3	5-Thiazolyl	Isobutyl	Ph	H	S	95	0.023
C4	3-Pyridyl	Isobutyl	Ph	Ethyl(methyl)amino	O	48	0.022
C5	4-Pyridyl	Isobutyl	Ph	Ethyl(methyl)amino	O	49	0.2
C6	5-Thiazolyl	2-(dimethylamino)ethyl	Ph	Ethyl(methyl)amino	O	>100	0.41
C7	5-Thiazolyl	2-(4-morpholinyl)ethyl	Ph	Ethyl(methyl)amino	O	>100	0.14
C8	5-Thiazolyl	Isobutyl	Ph	NH_2	O	>100	0.042
C9	5-Thiazolyl	Isobutyl	Ph	1-Methylpiperidin-4-ylamino	O	>100	2.7
C10	5-Benzo[1,3]-dioxolyl	Isobutyl	Ph	Ethyl(methyl)amino	O	16	0.044
C11	5-Thiazolyl	Isobutyl	Ph	Methyl	O	>100	0.052
C12	5-Thiazolyl	Isobutyl	Ph	Phenyl	O	13	0.024
C13	5-Thiazolyl	Isobutyl	Ph	Isopropyl	O	45	0.037
C14	5-Thiazolyl	Isobutyl	Ph	4-Pyridyl	O	>100	0.024
C15	5-Thiazolyl	Isobutyl	Ph	Ethyl(methyl)amino	O	45	0.022
C16	5-Thiazolyl	Isobutyl	4-F-Ph	Ethyl(methyl)amino	O	47	0.023
C17	5-Thiazolyl	Isobutyl	3-Pyridyl	H	S	>100	0.090

[a] Only SAR relevant derivatives **C1**–**C17** are shown in this table. The data of other analogues (**C18–C52**) can be found in the Supplementary data file.
[b] In all cases the stereochemistry of the C–OH chiral center is 'R' with the exception of **C15** where it is 'S'.



Scheme 2. Reagents and conditions: (a) R^4-C(OMe)$_3$, μW; (b) CH_3COCl, MeOH; (c) $C_2H_5OCS_2K$, pyridine; (d) MeI, K_2CO_3, EtOAc; (e) HNRR′, THF; (f) LiOH, THF/H_2O.

Table 2
Apparant permeability (P_{app}) and efflux transporter affinity data for **C1**, **C2** and **C3** measured in Caco-2 cells[a]

Compound	Permeability (P_{app}, 10^{-6} cm/s)		Transport polarity ($P_{secretory}/P_{absorptive}$)	
	pH = 5.5	pH = 7.4	pH = 5.5	pH = 7.4
C1	10	11	2	2
C2	0.52	1.2	26	22
C3	11	9.8	2	3.6

[a] Compounds were tested at 10 μM, measurements were done after 60 min of incubation.

Table 3
PK parameters for **C1**, **C2** and **C3** following oral administration of a 10 mg/kg single dose to rats and dogs ($n = 3$)[a]

Compound	Species	C_{max} (μg/mL)	T_{max} (h)	$AUC_{0-\infty}$ (μg.h/mL)	F_{abs} (%)
C1	Rat	0.24	1.0	0.97	44
	Dog	1.01	0.5	2.24	81
C2	Rat	0.01	0.5	0.01	0.82
	Dog	0.14	1.0	0.20	1.37
C3	Rat	2.8	0.5	7.8	107[b]
	Dog	5.2	1.0	2.24	97

C_{max} = peak plasma concentration; T_{max} = time point at which C_{max} is reached; $AUC_{0-\infty}$ = area under the curve, total exposure; F_{abs} = absolute bioavailability.
[a]Formulation vehicles for all compounds were chosen based on solubility and tolerability; for **C1**: PEG400/2.5% VitE-TPGS (rat); PEG400/30% saline (dog). For **C2**: 20% hydroxypropyl-β-cyclodextrin (HP-β-CD) + 1% citric acid (rat) and 20% HP-β-CD (dog). For **C3:** 20% HP-β-CD (rat & dog).
[b] $n = 2$.

since it showed to be poorly permeable and had high efflux transporter affinity. In contrast, more favorable data were obtained for **C1** and **C3**.

Despite the clear differences between the compounds, we proceeded with a single dose PK experiment of **C1**, **C2** and **C3** in both rats and dogs. In all cases, the animals were given an oral dose of 10 mg/kg. Table 3 summarizes the results.

In general, the exposure was higher in dogs compared to rats. Compound **C2** showed a less favorable oral pharmacokinetic profile in both species, its overall exposure at this low dose being very limited. The low $AUC_{0-\infty}$-value for **C2** can be attributed to the low permeability of the compound (Table 2) since the metabolic stability for this compound in dog hepatocytes was found to high.[18] In rats, **C3** showed the best oral PK profile while in dogs, the overall exposure was found only to be similar to that of **C1** notwithstanding the fact that its C_{max} value was 5-fold higher. Subsequently, we performed a drug–drug interaction (DDI) study in dogs, to evaluate whether potent CYP3A inhibition and PK data would occur and result in 'boosted' plasma levels of a co-administrated compound. For the latter, Darunavir (DRV) was chosen as a probe molecule. DRV (Prezista®, formerly known as TMC114) is an HIV-1 protease inhibitor used for the treatment of naive as well as treatment experienced HIV-1 infected individuals, first approved in 2006.[16,19] The booster candidates **C1**, **C2**, or **C3** were dosed orally at 7.5 mg/kg, 5 min prior to oral dosing of DRV which was given at 30 mg/kg. The effect of **C1**, **C2**, or **C3** pre-administration on the exposure and absorption of DRV was assessed by evaluating the increase in C_{max} and AUC values compared to when DRV was dosed alone (Table 4).

Gratifyingly, we learned that all three compounds had a significant effect on the exposure of DRV as a 2-fold increase in C_{max} and a 5-fold increase in AUC was observed. Based on these data, none of the candidates appeared to differentiate itself from the others as the effects on the PK parameters of DRV were very similar for the three compounds. However, we considered **C1** as our preferred candidate because the C_{max} and AUC value were the lowest of the three candidates (C_{max} = 0.61 μg/mL; $AUC_{0-\infty}$ = 2.0 μg.h/mL).[20]

Overall, these findings suggest that **C1** has an intrinsically better profile as pharmaco-enhancer compared with the other two candidates.

Table 4
Effect of the pre-administration of **C1**, **C2** and **C3** on the PK parameters of DRV in dogs ($n = 3$)

DRV Parameter		Compound pre-administered[a]					
		C1		**C2**		**C3**	
C_{max} (μg/mL)	$AUC_{0-\infty}$ (μg.h/mL)	10.1	40.2	8.7	34.8	8.6	30.5
Fold increase		2.0	5.0	1.8	4.7	2.0	4.8

[a] Compounds were dosed as PEG400 solution.

In conclusion, we have identified a novel class of CYP3A4 inhibiting benzoxazole and benzothiazole amides that are devoid of HIV protease inhibiting activity following a key 'sulfonamide-to-amide' switch. Three representative examples were shown to be capable of substantially enhancing the plasma levels of DRV in dogs, making them attractive lead candidates in the search for novel pharmacokinetic enhancers. The results of further evaluation of these compounds on the pharmacokinetic profile of other HIV protease inhibitors as well as on their general preclinical safety and toxicity profile will be reported elsewhere.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.06.022.

References and notes

1. (a) Bartlett, J. A.; DeMasi, R.; Quinn, J.; Moxham, C.; Rousseau, F. *Aids* **2001**, *15*, 1369; (b) MacArthur, R. D.; Novak, R. M.; Peng, G.; Chen, L.; Xiang, Y.; Hullsiek, K. H.; Kozal, M. J.; van den Berg-Wolf, M.; Henely, C.; Schmetter, B.; Dehlinger, M. *Lancet* **2006**, *368*, 2125.
2. (a) Cane, P. A. *J. Antimicrob. Chemother.* **2009**, *64*, i37; (b) Deeks, E. D.; Perry, C. M. *Drugs* **2010**, *70*, 2315.
3. Xu, L.; Desai, M. C. *Curr. Opin. Investig. Drugs* **2009**, *10*, 775.
4. (a) Kumar, G. N.; Dykstra, J.; Roberts, E. M.; Jayanti, V. K.; Hickman, D.; Uchic, J.; Yao, Y.; Surber, B.; Thomas, S.; Granneman, G. R. *Drug Metab. Dispos.* **1999**, *27*, 902; (b) Youle, M. *J. Antimicrob. Chemother.* **2007**, *60*, 1195.
5. Sequoia Pharmaceuticals' SPI-452: Gulnik, S.; Eissenstat, M.; Afonina, E.; Ludtke, D. et al. Preclinical and early clinical evaluation of SPI-452, a new pharmacokinetic enhancer. 16th Conference on Retroviruses and Opportunistic Infections (CROI): Montreal, 2009, Abstract 41. A benzofuran series was also reported as PK enhancer; WO2008022345.
6. Pfizer reported on pyrazole derivatives as Cyp450 inhibitors. See: WO2008022345, WO2007034312, and WO2008004100.
7. Abbott Laboratories: Flentge, C. A.; Randolph, J. T.; Huang, P. P.; Klein, L. L.; Marsh, K. C.; Harlan, J. E.; Kempf, D. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5444.
8. (a) Xu, L.; Liu, H.; Murray, B. P.; Callebaut, C.; Lee, M. S.; Hong, A.; Strickley, R. G.; Tsai, L. K.; Stray, K. M.; Wang, Y.; Rhodes, G. R.; Desai, M. C. *ACS Med. Chem. Lett.* **2010**, *1*, 209; (b) http://www.gilead.com/pipeline.; (c) tenofovir DF means tenofovir disoproxil fumarate.
9. (a) Schueller, L.; Baert, L.; Lachau-Durand, S.; Borghys, H.; Clessens, E.; Van Den Mooter, G.; Vangyseghem, E.; Jonckers, T. H. M.; Van Remoortere, P.; Wigerinck, P.; Rosier, J. *Int. J. Pharm.* **2008**, *355*, 45.
10. Kempf, D. J.; Marsh, K. C.; Kumar, G. N.; Rodrigues, A. D.; Denissen, J. F.; McDonald, E.; Kukulka, M. J.; Hsu, A.; Granneman, G. R.; Baroldi, P. A.; Sun, E.; Pizzuti, D.; Plattner, J. J.; Norbeck, D. W.; Leonard, J. W. *Antimicrob. Agents Chemother.* **1997**, *41*, 654.
11. For a general overview of Cytochrome P450 inhibition see: Lin, J. H.; Lu, A. Y. H. *Clin. Pharmacokinet.* **1998**, *35*, 361.
12. King, N. M.; Prabu-Jeyabalan, M.; Nalivaika, E. A.; Wigerinck, P.; De Bethune, M. P.; Schiffer, C. A. *J. Virol.* **2004**, *78*, 12012.
13. For an example of the direct sulfonamide interaction with I50 & I50' see: Nalam, M. N. L.; Peeters, A.; Jonckers, T. H. M.; Dierynck, I.; Schiffer, C. A. *J. Virol.* **2007**, *81*, 9512.
14. Rigby, J. H.; Wilson, J. Z. *Tetrahedron Lett.* **1984**, *25*, 1429.
15. At the end of this work, a third synthetic route was devised starting from 6b. In this method, the thiazolyl carbamate was installed prior to the final HATU or BOP mediated coupling which allowed modification of the amide moiety at the end.
16. Surleraux, D. L. N. G.; Tahri, A.; Verschueren, W. G.; Pille, G. M. E.; de Kock, H. A.; Jonckers, T. H. M.; Peeters, A.; De Meyer, S.; Azijn, H.; Pauwels, R.; de Bethune, M.; King, N. M.; Prabu-Jeyabalan, M.; Schiffer, C. A.; Wigerinck, P. B. T. P. *J. Med. Chem.* **2005**, *48*, 1813.
17. (a) Compounds **C1**-**C52** were also tested for their inhibition of other CYP isoforms. **C1** was found to also inhibit CYP2C9 and CYP2C19 while not inhibiting CYP2D6 or CYP1A2. This data can be found in WO2009071650. (b) Ritonavir has an EC_{50} value of 0.0015 μM in this assay. More information on the practical setup of this assay can be found in the Supplementary data.
18. After 2 hours of incubation with 2μM of **C2**, only 12% of the dose was metabolized in dog hepatocyte cultures.
19. McCoy, C. *Clin. Ther.* **2007**, *29*, 1559.
20. In comparison, for **C2** and **C3** these values were C_{max} = 2.3 μg/mL & $AUC_{0-\infty}$ = 6.48 μg.h/mL and C_{max} = 2.1 μg/mL & $AUC_{0-\infty}$ = 7.07 μg.h/mL, respectively. The increased exposure of **C2** in the boosting studies could be related to the P-gp inhibiting effect of DRV. Studies with the P-gp substrate taxol demonstrated that DRV has P-gp inhibitory properties (internal report).