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Structure–activity relationships of diamine inhibitors of cytochrome P450 (CYP) 3A as novel pharmacoenhancers. Part II: P2/P3 region and discovery of cobicistat (GS-9350)

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ABSTRACT

The HIV protease inhibitor (PI) ritonavir (RTV) has been widely used as a pharmacoenhancer for other PIs, which are substrates of cytochrome P450 3A (CYP3A). However the potent anti-HIV activity of ritonavir may limit its use as a pharmacoenhancer with other classes of anti-HIV agents. Ritonavir is also associated with limitations such as poor physicochemical properties. To address these issues a series of compounds with replacements at the P2 and/or P3 region was designed and evaluated as novel CYP3A inhibitors. Through these efforts, a potent and selective inhibitor of CYP3A, GS-9350 (cobicistat) with improved physiochemical properties was discovered.

Cytochrome P450 (CYP) 3A4 is the most abundant human CYP enzyme in the liver and plays a key role in both the detoxification of xenobiotics and the metabolism of signaling molecules.[1–4] Most importantly, it metabolizes >50% of all approved drugs and, in many cases, limits their ability to achieve sufficient steady-state minimum drug concentration required for durable efficacy. In particular, all approved HIV protease inhibitors (PIs) are metabolized primarily by CYP3A4 and the related enzyme, CYP3A5, in the liver and intestine and thus, have unfavorable pharmacokinetic profiles, including poor and/or variable oral bioavailability and relatively short plasma elimination half-lives.[5] As a result, early PI-containing regimens were complex—they required frequent dosing, had high pill burdens, and were associated with significant side effects and undesirable toxicities. Utilization of ritonavir (RTV, **14**), an approved PI (antiviral therapeutic dose 600 mg twice daily) and also a potent mechanism-based inhibitor of CYP3A, as a pharmacoenhancer at subtherapeutic doses (100 or 200 mg daily) results in improved pharmacokinetics (PK) of concomitantly dosed PIs, thereby improving effectiveness while simplifying the regimens. This unique clinical practice that reduces the pill burden and enhances the adherence of the coadministered PIs is a cornerstone of PI-containing highly active antiretroviral therapy (HAART).[5] Ritonavir therefore has served a critical function in the development of PI-based HAART regimens and in the chronic management of HIV infection. However, as discussed in the preceding Letter,[6] RTV has many limitations when used as a pharmacoenhancer, so we set out to identify a novel pharmacoenhancer that can maintain effectiveness as a CYP3A inhibitor while minimizing RTV's limitations.

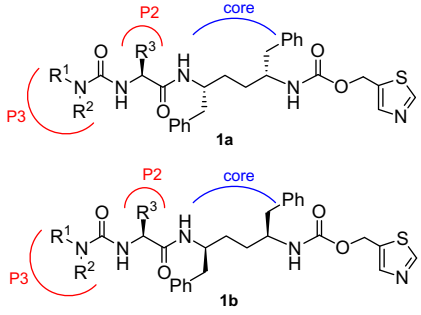

Figure 1. Structure of compound **1a** and compound **1b**.

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† These authors are no longer with Gilead Sciences.

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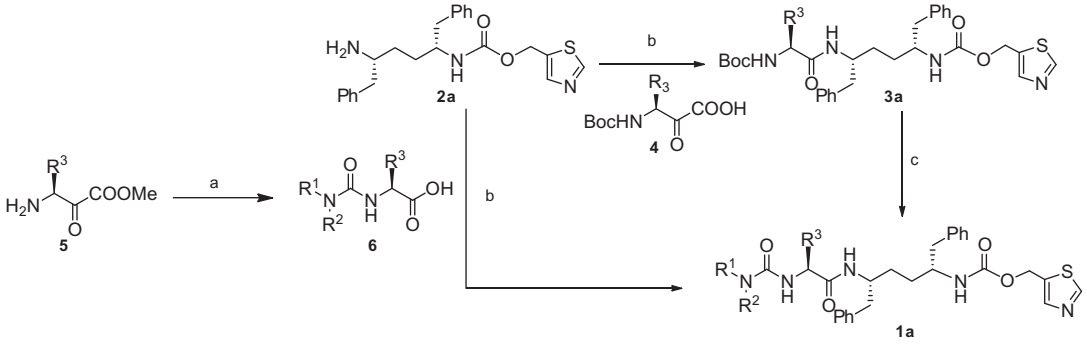


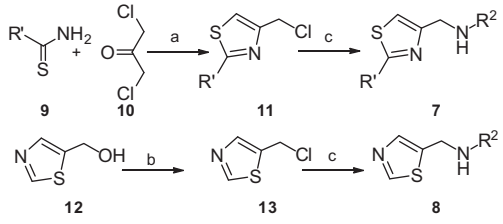
Scheme 1. Synthesis of compound **1a**. Reagents and conditions: (a) (1) CDI/iPr$_2$NEt; (2) R^{1}R^{2}NH; 3. NaOH/CH$_3$OH; (b) EDCI, HOBt, iPr$_2$NEt; (c) (1) TFA or HCl/dioxane; (2) CDI, iPr$_2$NEt; (3) R$_1$R$_2$NH.

In the preceding letter, we identified *R*,*R*- and *S*,*S*-1,4-diamine cores (exemplified by **1a** and **1b** Fig. 1) as our optimal central pieces. We herein report the synthesis and exploration of P2 and P3 regions of the diamine CYP3A inhibitors; these investigations led to the discovery of a novel pharmacoenhancer cobicistat (compound **35**, GS-9350).

A general sequence for the synthesis of compound **1a** analogs is outlined in Scheme 1. Compounds with formula **1b** were prepared using the same procedure except replacing the *R*,*R*-core of **2a** with the corresponding *S*,*S*-core. Compound **1a** can be constructed using two different routes. Amine **2a**[6] was coupled with Boc-protected amino acid **4** to give amide **3a**. Removal of the Boc-group yielded the corresponding amine, which was then acetylated with one equivalent CDI and subsequently reacted with amine R$_1$R$_2$NH to afford **1a**. Alternatively, compound **1a** can be prepared through coupling of acid **6**, which already contains a urea moiety, with amine **2a**. Acid **6** was prepared by treatment of protected amino acid ester **5** with CDI followed by R$_1$R$_2$NH. Subsequent ester hydrolysis generates acid **6** which is then coupled to **2a** using standard conditions. The amino acid **4** or **5** with various R$_3$ side chains were either commercially available in protected forms suitable for use in the described sequences, or prepared following conventional methods for synthesis of protected amino acids.

As shown in Scheme 2, 4′- or 5′-thiazolyl methyl amines (R$_1$R$_2$NH) **7** or **8** were synthesized from 4- or 5-chloromethyl thiazoles **11** or **13**, respectively, by treatment with primary amines. 2-Substituted 4-chloromethyl thiazole **11** was prepared from condensation of thioamide **9** with 1,3-dichloroacetone **10**.[7] 5-Chloromethyl thiazole **13** was obtained from commercial available 5-hydroxymethyl thiazole **12** by reacting with methanesulfonyl chloride.

As discussed in the previous communication,[6] we identified desoxy-RTV (**15**) as a lead compound for structure-activity relationship (SAR) studies. Although the antiviral potency of desoxy-RTV is greatly reduced compared to RTV, it still possesses modest anti-HIV activity with an EC$_{50}$ of 290 nM (Table 1). While building our understanding of the SAR for the diamine core, we simultaneously studied the SAR at the P3 and P2 portions of the lead compound **15**.



Scheme 2. Synthesis of compounds **7** and **8**. Reagents and conditions: (a) MgSO$_4$/acetone/reflux. (b) MsCl/Et$_3$N/MeCN; (c) R^{2}NH$_2$.

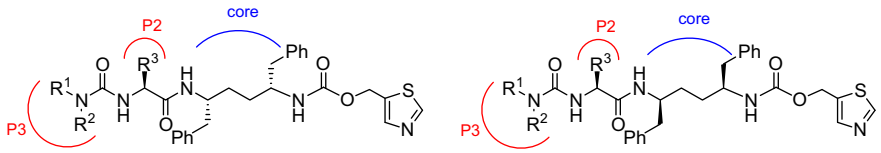
To establish SAR on P2/P3, our initial goal was to use modifications at P3 and/or P2 portion to dissociate the anti-HIV activity from the inhibitory potency against CYP3A possessed by the lead compound. Combination of the optimized P2 and P3 moieties with the best core would then allow us to identify an analog that is active against CYP3A but devoid of anti-HIV activity. The results are summarized in Table 1.

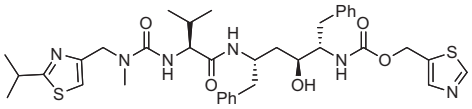
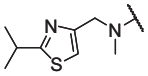
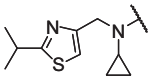
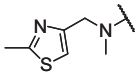
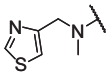
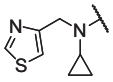
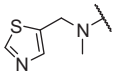
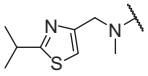
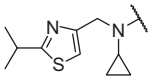
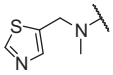
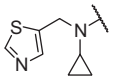
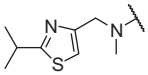
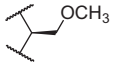
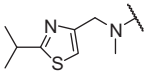
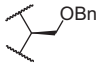
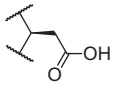
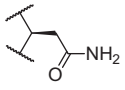
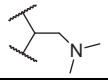
Reducing the size or removing the 2′-isopropyl group of the P3 portion 4-thiazolyl moiety (**17**, **18** vs **15**), in attempts to minimize interaction of the analogs with the HIV protease, only marginally decreased the binding with HIV protease and antiviral activity. Similarly, replacing the *N*-methyl with *N*-cPr (**16** vs **15**), aiming to interrupt the interactions of the compound with HIV protease, resulted in only minor reduction of anti-HIV activity. Compounds **16**–**18** with modified P3 possess comparable activity towards CYP3A. Combining these two modifications provided compound **19** which had a ~14-fold reduction of inhibitory activity against HIV yet maintained potency against CYP3A. This represents an increase in selectivity of approximately 80-fold compared to RTV. Compound **19** now has clinically marginal anti-HIV EC$_{50}$ of 4 μM, but we desired a compound inactive against HIV. Using 5-thiazole instead 4-thiazole at P3 (**20** vs **18**) did not offer any advantage in terms of selectivity, but simplified synthesis, as it can be obtained from the same intermediate as the right-side (P2′) thiazole.

We then turned our attention to the P2 portion of the molecule. Replacing valine with β-alanine at P2 (**21**) provided ~8-fold reduction of anti-HIV activity to give a compound with micromolar activity against HIV. Replacement of the valine at the 2-position with a serine, containing a more polar moiety, offered 5-fold reduction in activity against HIV. With serine at P2, the SAR trend at the P3 portion was consistent with that observed in the P2 valine series (compounds **22**–**25** vs **15**–**19**). Compound **25** has an EC$_{50}$ of 20 μM and shows over 400-fold improvement of selectivity compared to RTV. Further exploration at the P2 site showed that polar and bulky moieties can be tolerated by CYP3A but are less well tolerated by HIV protease (compounds **27**–**29**). The fact that that increased steric bulk at P2 can significantly reduce inhibitory activity against HIV protease and virus replication is exemplified by comparing compound **27** to **26**. The selectivity and activity of compound **27** met the minimum criterion for a selective CYP3A inhibitor we set at the outset of the program. However the physicochemical properties of compound **27**, especially its aqueous solubility, were not desirable.

After understanding the SAR for disassociating the HIV activity from CYP inhibitory potency, our next goal was to improve other properties including physicochemical properties. We thus incorporated moieties with potential to provide analogs with desirable

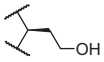
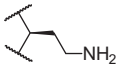
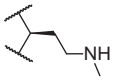
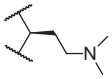
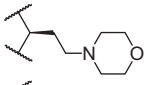
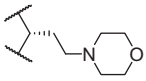
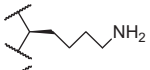
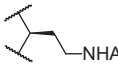
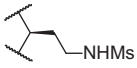
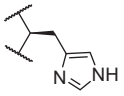
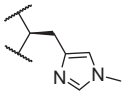
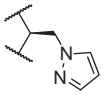
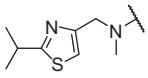
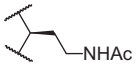
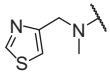
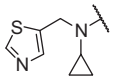
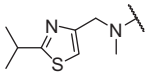
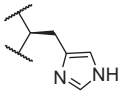
Table 1
Evaluation of pharmacoenhancers



Compound	R^1, R^2	R^3	Core	HIV protease IC_{50} (μM)	HIV replication EC_{50} (μM)	CYP3A[a] IC_{50} (μM)
14 Ritonavir				0.0006	0.05	0.11
15			*R,R*	0.005	0.29	0.13
16			*R,R*	0.55	0.75	0.21
17			*R,R*	0.035	0.4	0.1
18			*R,R*	0.242	0.8	0.14
19			*R,R*	3.59	4	<0.1
20			*R,R*	0.386	0.7	0.08
21			*R,R*	ND	2.5	0.13
22			*R,R*	0.05	1.5	0.15
23			*R,R*	1.41	2.5	0.11
24			*R,R*	4.3	12	0.17
25			*R,R*	19.3	20	0.12
26			*R,R*	ND	0.65	0.15
27			*R,R*	11.8	>30	0.17
28			*R,R*	0.01	10	0.50
29			*R,R*	0.143	1	0.13
30[b]			*R,R*	ND	3	0.14

(*continued on next page*)

Table 1 (*continued*)

Compound	R^1, R^2	R^3	Core	HIV protease IC_{50} (μM)	HIV replication EC_{50} (μM)	CYP3A[a] IC_{50} (μM)
31			*R,R*	0.039	2.7	0.14
32			*R,R*	>30	>30	0.21
33			*R,R*	>30	>30	0.13
34			*R,R*	>30	20	0.14
35			*R,R*	>30	>30	0.15
36			*R,R*	>30	>30	0.15
37			*R,R*	>30	>30	0.12
38			*R,R*	2	18	0.15
39			*R,R*	8	>30	0.12
40			*R,R*	3	>10	0.12
41			*R,R*	>30	>30	0.11
42			*R,R*	0.03	5	0.10
43			*S,S*	>30	>30	0.14
44			*S,S*	>30	>30	0.12
45			*S,S*	>30	>30	0.12
46			*S,S*	>30	>30	0.08
47			*S,S*	>30	>30	0.09

ND = not determined.

[a] Compounds are mechanism-based CYP3A inhibitors so IC_{50} values are representative and were all determined under the same conditions; Inhibitory potency against midazolam 1′-hydroxylase activity catalyzed by CYP3A.[9]

[b] Single compound but the absolute stereochemistry at P2 was not assigned.

physicochemical properties (compounds **30**–**34**). We were gratified to discover that introduction of basic moieties at the P2 site (compound **32** vs **31**) can significantly reduce the binding with HIV protease while maintaining activity against CYP and conferring greatly improved aqueous solubility. However, further detailed profiling of compound **32** indicated that it has poor permeability resulting in minimal oral bioavailability. Modification of the P2 of **32** to a secondary or tertiary amine moiety, such as methyl amine (**33**), dimethyl amine (**34**) or morpholine (**35**) can simultaneously offer much improved permeability while maintaining the desired selectivity profile. Compound **35** possesses both appropriate basicity and bulkiness at P2, therefore has the best overall selectivity against HIV. In addition, it maintained the favorable physicochemical properties of compound **32**. A broader group of analogs with

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Table 2
Evaluation of selectivity of pharmacoenhancers

Compound	CYP enzyme IC_{50} (μM)					PXR activation %[9]
	3A	1A2	2C19	2C9	2D6	
RTV	0.11	>25	12.7	4.9	2.3	51
32	0.11	>25	>25	>25	0.8	5
35	0.15	>25	>25	>25	9.2	10
36	0.15	>25	1.3	8	3.1	32
41	0.11	>25	2.2	4.7	0.6	15
43	0.16	>5	>25	>25	1.3	23
44	0.12	>25	>25	15.5	0.8	15
47	0.09	15	2.2	4.7	0.4	15

neutral and weakly basic to basic P2 moieties were also evaluated. Consistent with our earlier findings, basic and/or bulky P2 groups have significantly reduced activity against HIV (compounds **36**–**41**), while P2 groups that are weakly basic and/or less sterically demanding retain some residual anti-HIV activity (compound **42**). Incorporation of moieties possessing both basicity and bulkiness can generate analogs devoid of anti-HIV activity (**35**–**37**, **41**). As discussed in our previous Letter,[6] incorporation of a *S,S*-diamine core can afford favorable selectivity between anti-HIV potency and CYP potency. Such *S,S*-analogs containing P2 modifications with desirable CYP activity identified in *R,R*-series were prepared (compounds **43**–**47**). Consistent with previous observations, these analogs maintain CYP3A inhibitory potency and have no anti-HIV inhibitory activity.

Analogs that are devoid of anti-HIV activity and possess desired inhibitory activity against CYP3A were further profiled for their selectivity against the most important human drug metabolizing CYP enzymes and for their potential to activate PXR (Table 2).[8] As discussed in the preceding Letter, minimal PXR activation is desirable to avoid additional potential for drug-drug interactions and reducing the effectiveness of the pharmacoenhancer in vivo.

Compounds **32**, **43** and **44** demonstrated generally favorable CYP selectivity and less induction potential than RTV but had IC_{50} values for CYP2D6 of ~1 μM. Overall, compound **35** exhibits the best selectivity compared with RTV (Table 2). It has no significant activity against the tested CYP enzymes at concentrations likely to be achieved clinically, and also has minimal induction potential, indicating that it may have a lower potential for causing undesired drug-drug interactions compared to RTV.

Compound **35** (GS-9350, cobicistat) was further profiled in vivo. Results indicated that the absorption of **35** is above 50% in all non-clinical species, comparable to that of RTV.[9] Compound **35** was therefore expected to have high absorption potential in humans.

Extensive in vitro studies[9] comparing compound **35** and RTV have shown that **35** is a potent and selective CYP3A inhibitor that lacks significant anti-HIV activity. Compound **35** shares RTV's property of potent inhibition of the metabolism of a wide range of CYP3A substrates and retains the characteristic of mechanism-based inhibition of CYP3A. In addition, compound **35** also has favorable physicochemical properties, especially high aqueous solubility, allowing it to be formulated as a tablet and co-formulated with other drugs.[9]

Compound **35** was advanced to clinical studies. It was investigated in humans compared to RTV as a pharmacoenhancer for drugs metabolized by CYP3A to improve their systemic exposure.

In a randomized, placebo-controlled, double-blind, multicenter, 48-week Phase 3 study, cobicistat (**35**) demonstrated comparable efficacy to RTV as a pharmacoenhancer for atazanavir. The study found that an HIV regimen containing a cobicistat-boosted PI was non-inferior to a regimen containing a ritonavir-boosted PI at 48 weeks of therapy.[10] Currently, cobicistat is a component of an approved fixed-dose combination Stribild® in which it acts as a pharmacoenhancer for the HIV integrase strand transfer inhibitor, elvitegravir. It is also under regulatory review in USA as an individual agent. If approved, cobicistat may be an effective option for boosting the pharmacokinetics in HIV regimens that are based on PIs.

In summary, using desoxy-RTV as the lead, extensive studies on core and P2/P3 regions lead to the discovery of cobicistat (**35**, GS-9350). Cobicistat is a novel, selective and potent CYP3A inhibitor without any detectable antiviral activity and with desirable physicochemical properties. It is expected cobicistat will have broader applications in HIV patients compared to RTV once it is approved by regulatory agencies. In addition, cobicistat is expected to have broader utility compared to RTV as a pharmacoenhancer in treatment of other life-threatening diseases, such as anti-HCV, given its more desirable profile.

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