SUPPORTING INFORMATION

Pyridine-substituted desoxyritonavir is a more potent inhibitor of cytochrome P450 3A4 (CYP3A4) than ritonavir

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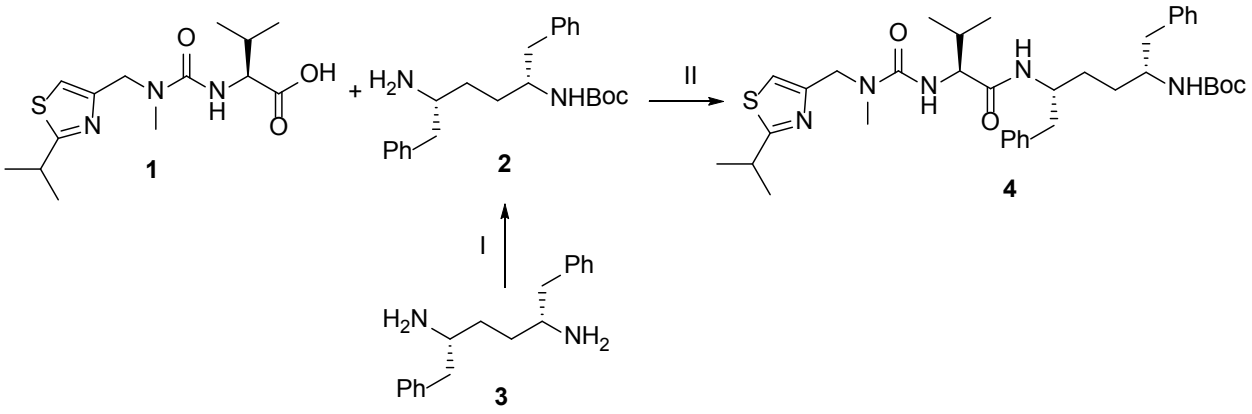
CONTENTS:

Synthesis of ritonavir analogs (carried out by Lianhong Xu and Hong Ye of Gilead Sciences)

Scheme 1. Synthesis of compound **4**.



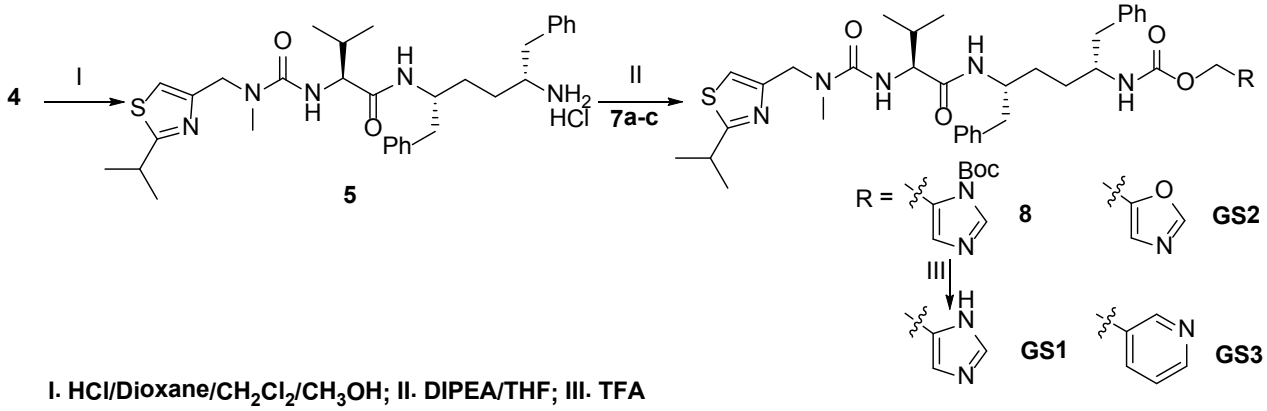
I. Boc_2O, TEA, MeOH; II. EDCI/HOBt/iPr_2NEt/THF

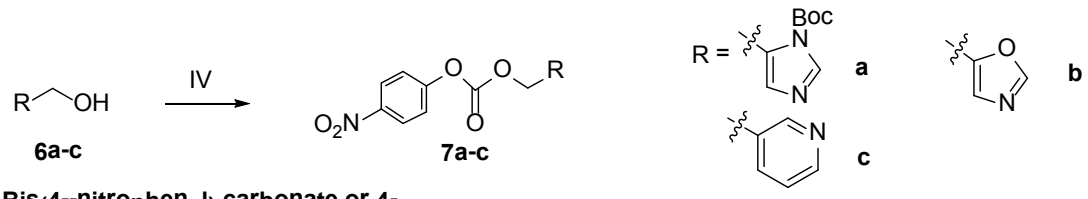
tert-Butyl (2R,5R)-5-amino-1,6-diphenylhexan-2-ylcarbamate (2). Boc_2O (1.28 g, 5.87 mmol) was added to a solution of HCl salt of (2R,5R)-1,6-diphenylhexane-2,5-diamine (**3**) (6 g, 17.6 mmol) and TEA (2.5 mL, 17.6 mmol) in CH_3OH (200 mL). The mixture was stirred for 16 hours. The solvent was evaporated and the residue was diluted with EtOAc. The organic layer was washed twice with saturated $NaHCO_3$, water and once with brine, dried over Na_2SO_4, filtered and concentrated. The crude material was purified by flash chromatography to give 2 g of the title compound **2**. Mass Spectrum (*m/z*): $(M+H)^+$ 369.0.

tert-Butyl (2R,5R)-5-((S)-2-(3-((2-isopropylthiazol-4-yl)methyl)-3-methylureido)-3-methylbutanamido)-1,6-diphenylhexan-2-ylcarbamate (4). HOBt (242 mg, 1.797 mmol), EDC (0.43 mL, 2.396 mmol), and DIPEA (0.84 mL, 4.792 mmol) were added to a solution of (S)-2-(3-((2-isopropylthiazol-4-yl)methyl)-3-methylureido)-3-methylbutanoic acid (**1**) (375 mg,

1.198mmol) and compound **2** (440 mg, 1.198 mmol) in THF (15 mL). The mixture was stirred for 16 hours and concentrated. Purification by column chromatography gave title compound **4** (600 mg). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.27-7.14 (11 H, m), 6.98 (1 H, S), 6.15-6.13 (1 H, d, J = 8.1 Hz), 4.63 (1 H, m), 4.44-4.37 (2 H, m), 415-3.72 (3 H, m), 3.26 (1 H, m), 3.00 (3 H, m), 2.71-2.66 (4 H, m), 2.23 (1 H, m), 1.67 (3 H, s), 1.52-1.36 (15 H, m), 0.94-0.86 (6 H, m). Mass Spectrum (*m/z*): $(M+H)^+$ 664.1.

Scheme 2. Synthesis of title compounds **1**, **2**, and **3** (labeled as **GS1**, **GS2** and **GS3**, respectively).





(S)-N-((2R,5R)-5-Amino-1,6-diphenylhexan-2-yl)-2-(3-((2-isopropylthiazol-4-yl)methyl)-3-methylureido)-3-methylbutanamide (5). tert-butyl (2R,5R)-5-((S)-2-(3-((2-isopropylthiazol-4-yl) methyl)-3-methylureido)-3-methylbutanamido)-1,6-diphenylhexan-2-ylcarbamate (**4**) (600 mg) was dissolved in dichloromethane/CH_3OH (10 mL/10 mL), and HCl in 4M dioxane (2.25 mL, 9 mmol) was added. The mixture was stirred at room temperature for 12 hours. The mixture was concentrated and dried to give title compound **5**. Mass Spectrum (*m/z*): $(M+H)^+$ 564.2.

tert-Butyl 5-(((4-nitrophenoxy)carbonyloxy)methyl)-1H-imidazole-1-carboxylate (7a). TEA (1.2 eq) was added drop wise to a solution of tert-butyl 5-(hydroxymethyl)-1H-imidazole-1-carboxylate (**6a**) (1eq.) and p-nitrophenyl carbonate or p-nitrophenylchloroformate (1eq.) in THF at room temperature. The mixture was stirred for 16 hours. The solvent was removed and the residue was diluted with EtOAc. The organic layer was washed twice with saturated aqueous $NaHCO_3$ and once with brine, and dried over Na_2SO_4. Concentration and purification by column chromatography gave title compound **7a**. **7b** and **7c** were prepared following the same procedure.

tert-Butyl 5-((5S,8R,11R)-8,11-dibenzyl-5-isopropyl-1-(2-isopropylthiazol-4-yl)-2-methyl-3,6,13-trioxo-14-oxa-2,4,7,12-tetraazapentadecan-15-yl)-1H-imidazole-1-carboxylate (8). DIPEA (25 mL, 0.134 mmol) was added to a solution of (S)-N-((2R,5R)-5-amino-1,6-diphenylhexan-2-yl)-2-(3-((2-isopropylthiazol-4-yl)methyl)-3-methylureido)-3-methylbutanamide (**5**) (40 mg, 0.067 mmol) and compound **7a** (23 mg, 0.08 mmol) in THF (1 mL). The mixture was stirred for 16 hours. The solvents were removed, and the residue was diluted with EtOAc. The organic layer was washed twice with saturated $NaHCO_3$, water and

once with brine, dried over Na_2SO_4, filtered and concentrated The crude material was purified with flash column chromatography gave title compound **8** (26 mg).

(1H-Imidazol-5-yl)methyl (2R,5R)-5-((S)-2-(3-((2-isopropylthiazol-4-yl)methyl)-3-methylureido)-3-methylbutanamido)-1,6-diphenylhexan-2-ylcarbamate (title compound 1). HCl in 4 M dioxane (0.42 mL, 1.65 mmol) was added drop wise to tert-butyl 5-((5S,8R,11R)-8,11-dibenzyl-5-isopropyl-1-(2-isopropylthiazol-4-yl)-2-methyl-3,6,13-trioxo-14-oxa-2,4,7,12-tetraazapentadecan-15-yl)-1H-imidazole-1-carboxylate (**8**) (130 mg, 0.165 mmol) in THF (2 mL). The mixture was stirred for 2 hours. The solvents were removed, and the residue was purified by HPLC to give title compound **1** (**GS1** in Scheme 2; 43 mg). ^{1}H-NMR (CD_3OD, 300 MHz) δ 7.64 (1 H, s), 7.19-7.15 (11 H, m), 7.01(1 H, s), 4.93 (2 H, s), 4.52-4.49 (2 H, m), 4.11-3.97 (3 H, m), 3.35-3.27 (1 H, m), 2.96 (3 H, s), 2.70-2.68 (4 H, m), 1.90(1 H, m), 1.51-1.36 (10 H, m), 0.86-0.84 (6 H, m). Mass Spectrum (*m/z*): $(M+H)^+$ 688.2.

Oxazol-5-ylmethyl (2R,5R)-5-((S)-2-(3-((2-isopropylthiazol-4-yl)methyl)-3-methylureido)- 3-methylbutanamido)-1,6-diphenylhexan-2-ylcarbamate (title compound 2). Title compound **2** (**GS2** in Scheme 2) was prepared following the procedure used to prepare compound **8**, except that **7b** was used instead of **7a**. ^{1}H-NMR (CD_3OD, 300 MHz) δ 8.19(1 H, s), 7.21-7.09 (12 H, m), 6.15-6.13 (1 H, d, J = 8.1 Hz), 5.02 (2H, s), 4.52-4.49 (2 H, m), 4.11-3.97 (3 H, m), 3.32-3.27 (1 H, m), 2.97 (3 H, s), 2.72-2.68 (4 H, m), 2.00-1.90 (1 H, m), 1.52-1.29 (10 H, m), 0.86-0.84 (6 H, m). Mass Spectrum (*m/z*): $(M+H)^+$ 689.3.

Pyridin-3-ylmethyl (2R,5R)-5-((S)-2-(3-((2-isopropylthiazol-4-yl)methyl)-3-methylureido)- 3-methylbutanamido)-1,6-diphenylhexan-2-ylcarbamate (title compound 3). Title compound **3**, (**GS3** in scheme 2) was prepared following the procedure used to prepare compound **8**, except that **7c** was used instead of **7a**. ^{1}H-NMR (CD_3OD, 300 MHz) δ 8.49(1 H, m), 7.73-7.70 (2 H, m), 7.43-7.39 (1 H, m), 7.21-7.15 (11 H, m), 6.15-6.13 (1 H, d, J = 8.0 Hz), 5.05-5.02 (2H, m), 4.52-4.49 (2 H, m), 4.11-3.97 (3 H, m), 3.32-3.27 (1 H, m), 2.96 (3 H, s), 2.71-2.69 (4 H, m), 2.00-1.90 (1 H, m), 1.53-1.28 (10 H, m), 0.86-0.84 (6 H, m). Mass Spectrum (*m/z*): $(M+H)^+$ 699.3.

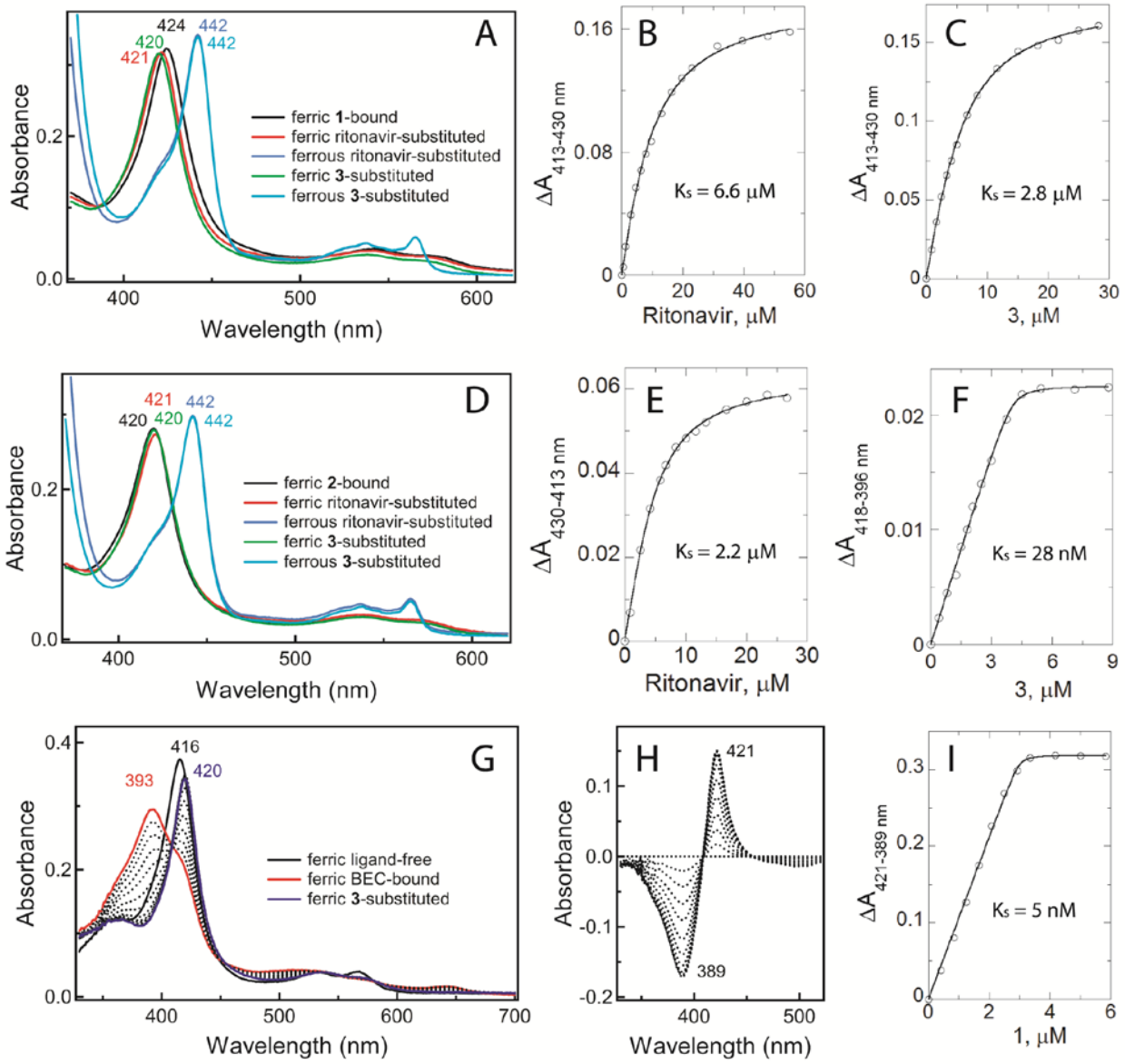


Figure 1S. *A-D*, Reversibility of the binding of compounds **1** and **2** to CYP3A4. Owing to spectral differences between the oxidized ligand-bound species and a characteristic 442 nm absorption of the ferrous ritonavir- and compound **3**-ligated CYP3A4, it can be demonstrated using displacement titrations that ritonavir and **3** can substitute **1** and **2**. Compound **3** has a several-fold higher affinity for CYP3A4 than ritonavir regardless of whether the protein is bound to **1**, **2** (plots B, C, E and F) or type I substrate bromoergocryptine (BEC, panels G-I). The affinity of ritonavir for BEC-bound CYP3A4 is 50 nM (*1)*.

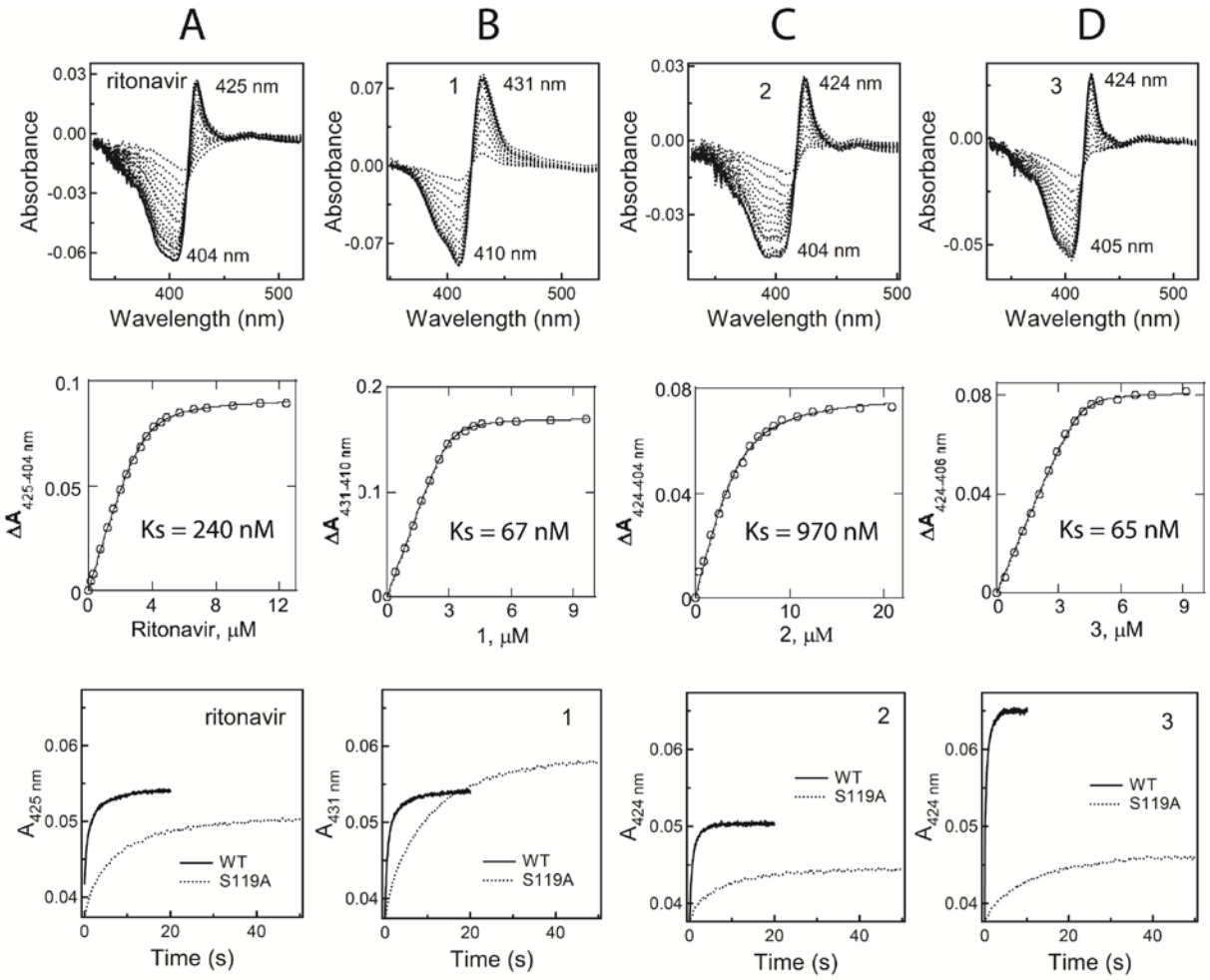


Figure 2S. Effect of the S119A mutation on the CYP3A4 affinity and kinetics of binding to ritonavir and compounds **1**, **2** and **3** (columns A-D, respectively). Lower panels are kinetic traces recorded at the highest ligand concentration used. The derived rate constants are listed in Table 2 of the main text.

References

1. Sevrioukova, I. F., and Poulos, T. L. (2010) Structure and mechanism of the complex between cytochrome P4503A4 and ritonavir, *Proc. Natl. Acad. Sci. U S A 107*, 18422-18427.