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Orally bioavailable prodrugs of a BCS class 2 molecule, an inhibitor of HIV-1 reverse transcriptase

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ABSTRACT

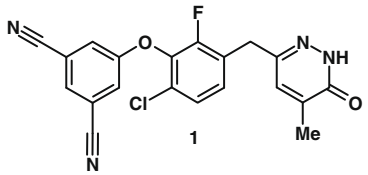
The *N*-2 position of pyridazinone **1**, a potent HIV-1 NNRTI that has limited aqueous solubility, was derivatized into a series of hydroxymethyl esters and carbonates as well as one phosphate. The derivatives served as prodrugs to effectively deliver **1** to rat plasma upon oral treatment at 50 mpk. Increases of 4.3- to 8.6-fold in 24-hour exposure of **1** (over that of parent) were observed while the prodrugs and the hydroxymethyl adduct **2** were undetectable.

We recently described the discovery and SAR of a new class of inhibitors of HIV-1 reverse transcriptase of which **1** is a representative.[1] These pyridazinones display high potency and bind at the allosteric site common to most nonnucleoside inhibitors (NNRTI). Compound **1** has very good Caco2 permeability and stability in human liver microsomes as well as low iv clearance rates in rat and dog, 16% and 14% of hepatic blood flow, respectively (see Fig. 1).

However, **1** displays poor aqueous solubility and a high melting point, both of which are typical characteristics for the chemotype. Compound **1** has some less than ideal pharmacokinetic properties that are likely influenced by these poor physical properties.

Panel 1 illustrates blood plasma levels achieved in rat following dosing of **1**. Plasma exposure was less than dose-proportional after oral administration with only a 3-fold increase in 24-hour AUC for the 10-fold increase in dose. These data for 5 and 50 mpk suggest that poor solubility and high crystallinity are playing significant roles to limit oral exposure. Furthermore, levels of **1** at the 50 mpk dose did not reach target plasma levels desired to provide a high antiviral inhibitory quotient of **1** that warranted its further development as an antiviral candidate.[2]

Prodrugs have been utilized to overcome delivery problems of drugs with limited solubility, for instance phenytoin.[3] Phenytoin is a molecule that displays poor solubility and acceptable permeability thus designated a BCS Class 2 molecule.[4] The prodrug fosphenytoin bears a solubilizing phosphate feature. The approach to generate a stable prodrug was achieved by linking that feature via a hydroxymethyl derivative. Phenytoin is a substituted hydantoin that is more acidic (calcd pK_a 8) than an amide and this property enables a rapid release of the parent upon cleavage of the



Caco2 perm: 19 x 10^{-6} cm/s
solubility at pH 6.5: 0.3 μg/mL

mp 230-232 °C
measured pK_a 10.5

Figure 1. Structure and selected properties of **1**.

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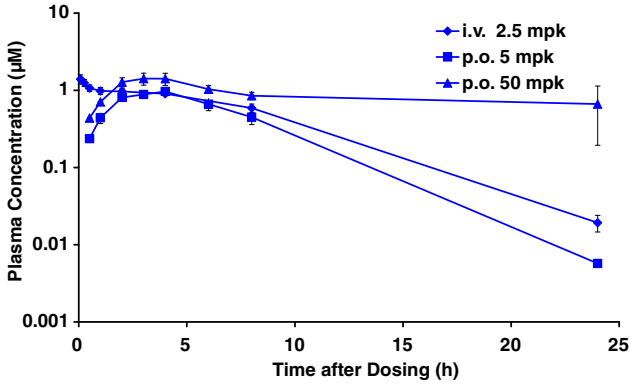
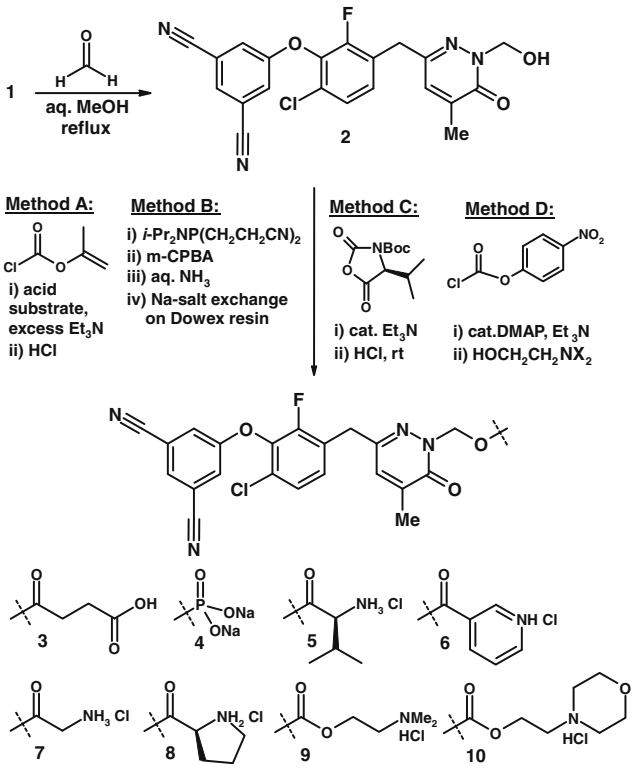


Panel 1. Pharmacokinetic curves after dosing **1** to rat.

phosphoryl bond of fosphenytoin. Inhibitor **1** displays acidity (pK_a 10.5) similar to that of phenytoin thus a rapid in vivo release of parent was anticipated upon application of this strategy.

We embarked on an investigation of prodrugs to address the solubility and crystalline issues of **1**. This letter describes a successful approach to improve the blood plasma levels of **1**. This was achieved by introducing solubilizing features and perturbing the crystallinity of **1** *via* the formaldehyde adduct **2** of which the prodrugs were produced. The opportunity to enhance exposure by active transport[5] was also examined.

Methods A (**3**, **6**–**8**), B (**4**), C (**5**), and D (**9**, **10**) enabled the generation of esters and carbonates and are highlighted in Scheme 1. These prodrugs were designed as anionic or cationic species so as to promote dissolution. Pyridazinone **1**[6] cleanly gave adduct **2** upon heating with aqueous formaldehyde and the complete removal of volatiles.[7] The hydroxyl group required rapid conversion in order to minimize reformation of **1** once a solution of **2** was generated. Isopropenyl chloroformate served as an excellent activating agent (for acid substrates) as shown for Method A. The succinate half ester **3** was generated and evaluated as the free acid, while the nicotinate **6** was evaluated as the hydrochloride salt. The requisite acid substrates for esters **7** and **8** were the *N*-Boc amino acids of Gly and Pro, respectively. The esters **7** and **8** were obtained as their HCl salts following the deprotection under acidic conditions. The phosphate ester **4** was generated as its di-sodium salt according to Method B. The *S*-Val derived *N*-carboxyanhydride readily consumed **2** at ambient temperature and ester **5** was obtained upon acid treatment as indicated in Method C. Method D illustrates the generation of carbonates by initial acylation of **2** with 4-nitrophenoxy chloroformate, followed by treatment with 2-(*N*,*N*-dimethylamino)ethanol or 4-(2-hydroxyethyl)morpholine to give **9** or **10**, respectively.[7]



Scheme 1. Preparation of hydroxymethyl derivatives of **1**.

Table 1 summarizes relevant physical and rat pharmacokinetic properties of **1** and prodrugs **3**–**10**. All compounds were dosed at 50 mpk except **1**, which was also dosed at 5 mpk (row 1). The prodrugs were dose adjusted to deliver 50 mpk of **1**.

The stability of **2** was also evaluated and the estimated half-life at either pH 2.0 or 6.5 was less than 60 s. Thus, it reverts to **1** rapidly in aqueous solutions.

One important characterization of the new derivatives was the determination of thermodynamic solubility, which is typically done by tumbling for 24 hours at pH 6.5. A prerequisite for this determination is adequate stability of the compounds, however almost all had half-lives less than 5 hours under these conditions. Therefore, exposing these prodrugs to the solubility assay would have produced variable amounts of **1** and thus generating data would have been uninterpretable.

All of the prodrugs displayed a mp less than the mp of the parent **1**. The crystallinity of these derivatives had been favorably altered thus hinting that dissolution may be favorably impacted to drive plasma exposure of **1**.

The succinate **3** and nicotinate **6** esters improved 24-hour plasma levels of **1**. In addition, **3** showed acceptable stability at physiological pH, whereas **6** hydrolyzed rapidly at pH 2. Phosphate **4** presented a clear improvement in stability as well as prodrug effectiveness. Prodrug **4** gave rise to the highest exposure levels with an 8.6-fold AUC increase of **1**. Interestingly, the T_{max} was delayed to 8 hours, likely a consequence of the higher stability of **4**. The action of nutrient processing enzymes such as phosphatases would be necessary to cleave the phosphoryl bond of **4** and thus the observation of continued absorption of **1** throughout the small intestine. It is possible that this metabolism had been saturated with this dose. However, this hypothesis was not fully evaluated in rat. Surprisingly, carbonate **9** showed unacceptable aqueous stability at pH 6.5 as well as **9** and **10** gave no improvement in PK properties.

Amino acid derivatives have previously been reported to increase absorption by targeting oligopeptide transporters.[5] Prodrugs **5**, **7**, and **8** were generated in this interest as well as the opportunity of their salts to enhance dissolution. Valinate ester **5** displayed acceptable aqueous stability with $t_{1/2}$ of 3.2 h at pH 6.5. However, **5** presented no benefit for the delivery of **1** within the broader set of prodrugs. The glycinate **7** and prolinate **8** esters showed 4- to 5-fold improvements in 24 h plasma levels but were excluded from further consideration due to insufficient stability.

The prodrugs and adduct **2** were not detected in rat plasma during the 24-hour course of the pharmacokinetic evaluations.

Pyridazinone **1** was found to be an excellent substrate to derivatize as hydroxymethyl linked prodrugs that in turn gave rapid in vivo liberation of parent. Substantially improved plasma levels of **1** were observed (AUC_{24h} > 50 μM h/mL) following the oral administration of prodrugs **3**–**10** to rat.

Table 1
Physical and pharmacokinetic properties of prodrugs of **1**.

Compound	Melting point (°C)	Method of prepn.	Stability $t_{1/2}$ (h)[a] pH 2	pH 6.5	Rat PK of 1[b]			AUC, fold increase[d]
					T_{max} (h)	C_{max} (μM)	AUC[c] (μM h/mL)	
1[e]	230–232	—	—	—	3.7	0.96	6.8	—
1	230–232	—	—	—	3.3	1.5	20.3	1
3	167–171	A	462	40	2.7	13.2	142	6.9
4	194–197	B	>24[f]	>24[f]	8.0	12.7	178	8.6
5	177–178	C	1415	3.2	4.0	9.1	134	6.5
6	173–174	A	10	4.7	4.7	8.8	101	4.9
7	162–163	A	38	1.0	4.0	6.3	93	4.6
8	163–165	A	17	<0.3	4.7	7.3	108	5.3
9	75–90	D	289	0.9	2.0	7.3	108	5.9
10	79–101	D	301	3.5	3.3	5.6	89	4.3

[a] Aqueous stability of prodrugs was assessed with a solution of 0.1 M HCl and 0.14 M KCl (pH 2) or a solution 25 mM of both NaH_2PO_4 and Na_2HPO_4 (pH 6.5).
[b] Three male Hanover–Wister rats were orally dosed with the test molecule at 50 mpk or the equivalent of prodrug to deliver 50 mpk of **1**. The values listed are the average of the three. The quantification limit for **1** was $\geqslant$5 ng/mL.
[c] Plasma was first sampled at 0.5 h while the animals were monitored for 24 h, at which time the last plasma sample was taken. At no time point was the prodrug or **2** detected and their quantification limits were 2–10 ng/mL.
[d] 24-hour exposure increase compared to same dose level of parent, **1**.
[e] PO dosed at 5 mpk.
[f] No degradation was detected between 0 and 24 h at 40 °C.

Phosphate prodrug **4** presented superior aqueous stability and the most efficient delivery of **1**. An 8.6-fold enhancement of the circulating NNRTI **1** was observed during the 24-hour exposure.

In contrast to previous experience with nucleoside-amino ester prodrugs, prodrugs of **1** bearing a lipophilic amino ester do not appear to be substrates for active transport, for example, **5**, as they did not enhance bioavailability relative to the other prodrugs.

Despite differences in aqueous stability, consistent and robust increases in 24-hour exposure, from 4.3- to 6.9-fold were obtained across the series of acyl prodrugs **3, 5–10** examined. Plasma concentrations of prodrugs were undetectable thus apparently consistent with prodrug conversion by ubiquitous esterase activity. Therefore, the proposal we forward is that the acyl cleavage of the prodrugs is initially subject to enzymatic action during absorption that likely was not saturated under the study conditions.

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We appreciate the early input provided by Professor Ronald T. Borchardt. This prodrug campaign would not have been possible if it were not for the efforts of the following excellent process research chemists, namely Michael Martin, Justin Vitale, Felicia Thai, Gary F. Cooper, Harlan Reese, Anton Constaninescu, and Jiang Zhu.

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7. A suspension of pyridazinone **1** (3.3 g, 8.4 mmol) in methanol (55 mL) and 37% aqueous formaldehyde (15 mL, 185 mmol) was heated to reflux for 2.5 h during which a solution formed. The reaction mixture was removed from heating and allowed to stand at rt for 1 h during which solids formed. The mixture was diluted with 20 mL of ice water and filtered. The solid was stored at 50 °C for 16 h in vacuo. The desired adduct **2** (3.3 g, 7.7 mmol, 92%) was obtained: mp 172–179 °C; ^{1}H NMR (d_6-DMSO, 300 MHz) δ 8.22 (t, J = 1.2 Hz, 1H), 7.93 (d, J = 1.2 Hz, 2H), 7.50 (dd, J = 1.5, 8.4 Hz, 1H), 7.35 (t, J = 7.5 Hz, 1H), 7.25 (apparent d, J = 1.2 Hz, 1H), 6.60 (t, J = 7.5 Hz, 1H), 5.27 (d, J = 7.5 Hz, 2H), 3.98 (s, 2H), 2.06 (d, J = 1.2 Hz, 3H); ^{13}C NMR (75 MHz) δ 160.3, 157.3, 154 (d), 144.4, 140.7, 137.3 (d), 131.4, 130.7, 129.5, 127.0 (d), 126.4, 123.7, 116.8, 114.8, 73.8, 33.5, 16.5; ESMS m/z 425 (M^{+1})+; Anal. calcd for $C_{21}H_{14}FClN_4O_3$: C, 59.37; H, 3.32; N, 13.19. Found: C, 59.08; H, 3.18; 13.14.

Method A. A CH_2Cl_2 (150 mL) solution of **2** (3.13 g, 5.2 mmol) was immediately added to a 0 °C solution of succinic acid (2.44 g, 20.6 mmol), triethylamine (3.6 mL, 26 mmol), 4-dimethylaminopyridine (127 mg, 1.05 mmol), and isopropenyl chloroformate (0.80 mL, 7.3 mmol) in CH_2Cl_2 (110 mL). The bubbling solution was stirred cold for 1.5 h and poured into 30 mL of 10% aq HOAc. The mixture was extracted with EtOAc (4× 50 mL) and stored over sodium sulfate. After removal of the volatiles, the residue was loaded onto a pad of silica and washed with 2:1 EtOAc/hexane. Acid **3** (1.36 g, 2.6 mmol, 50%) was eluted with 0.5% HOAc in 3:1 EtOAc/hexane and recrystallized from warm EtOAc and hexane giving the following characteristics: mp 167–171 °C; ^{1}H NMR (d_6-DMSO, 300 MHz) δ 12.2 (br s, 1H), 8.22 (t, J = 1.2 Hz, 1H), 7.95 (d, J = 1.2 Hz, 2H), 7.51 (dd, J = 1.5, 8.4 Hz, 1H), 7.35 (t, J = 7.5 Hz, 1H), 7.30 (apparent d, J = 1.2 Hz, 1H), 5.89 (s, 2H), 3.99 (s, 2H), 2.50 (t, J = 1.5 Hz, 4H), 2.07 (d, J = 1.2 Hz, 3H); ^{13}C NMR (75 MHz) δ 171.9, 169.9, 158.7, 155.6, 152 (d), 144.0, 139.2, 135.6, 135.4, 129.75, 129.6, 127.8, 127.7, 124.9, 124.8, 124.7, 122.1, 115.1, 113.1, 71.6, 31.8, 14.7; ESMS m/z 525 (M^{+1})$^+$; Anal. calcd for $C_{25}H_{18}FClN_4O_6$: C, 57.20; H, 3.46; N, 10.68. Found: C, 57.26; H, 3.61; N, 10.52.
Method B. 1-*H* tetrazole (225 mg, 3.2 mmol) and **2** (90% purity, 682 mg, 1.4 mmol) were suspended in CH_3CN (10 mL) immediately following by dropwise addition of bis(2-cyanoethyl)-*N*,*N*-di-*i*-Pr phosphoramidite (Spectrum Chemicals, 871 mg, 3.2 mmol) over 3 min. The mixture was stirred for 16 h at rt, filtered, and concentrated to yield a clear oil. The desired phosphite was purified (SiO_2, 60–90% ethyl acetate/hexanes) to yield (866 mg, quantitative) a clear oil and dissolved in CH_2Cl_2 (14 mL). Immediately, the solution was cooled to 0 °C and to this was added *m*-chloroperbenzoic acid in one portion (Aldrich Co. 77% purity, 326 mg, 1.45 mmol). The reaction was stirred for 4 h at 0 °C, treated with 1 M $Na_2S_2O_4$ and extracted twice with CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, filtered and concentrated. The desired material was purified (SiO_2, 0–2% methanol/EtOAc) to yield (620 mg, 69%) a white foam. The phosphate triester (612 mg, 1.0 mmol) was suspended in CH_3CN (2.5 mL) then treated with concentrated aq NH_3 (10 mL) and allowed to stir at rt. After 48 h, another portion of aq NH_3 (5 mL) was added and stirred for an additional 24 h. The suspension was then filtered, washed with CH_3CN to obtain the desired product as a white ammonium salt (456 mg, 86%). A portion of this was passed through a pad of Dowex 50 × 2 (Na^+) to obtain the **4** as an off-white sodium salt: mp 194–197 °C; Anal. calcd for $C_{21}H_{13}FClN_4O_6P\cdot(H_2O)_2$: C, 43.10; H, 2.93; N, 9.58. Found: C, 43.12; H, 2.67; N, 9.23.
Method C. A DMF (25 mL) solution of **2** (4.5 g, 10.6 mmol) was generated at rt and without delay treated with triethylamine (0.3 mL, 2.1 mmol) and a toluene (15 mL) solution of *N*-*tert*-butoxycarbonyl (*S*)-valine *N*-carboxyanhydride (3.1 g, 12.7 mmol). The resulting solution was stirred for 2 h and poured into water (120 mL) and extracted with 2:1 hexane/EtOAc (4 × 100 mL). The combined organic extracts was washed brine, stored over anhydrous sodium sulfate, and loaded onto a pad of silica gel (eluant gradient: 1:1 to 3:1 EtOAc/hexane). The desired *N*-Boc amino ester was obtained (5.6 g, 8.9 mmol, 84%) as a foam and was dissolved in EtOAc at rt and treated with hydrochloric acid (4 mL, 4 M solution in 1,4-dioxane). The solution deposited a white solid and stirring was continued for 14 h. Et_2O (25 mL) was added and the suspension was stored at 0 °C. The crude solid was collected and dissolved in warm *iso*-propanol and EtOAc and then stored at rt for 18 h. Amine HCl salt of **5** (1.3 g, 2.3 mmol, 22%): mp 176.7–178.3 °C; Anal. calcd for $C_{26}H_{23}FClN_5O_4HCl$: C, 55.72; H, 4.32; N, 12.50. Found: C, 55.71; H, 4.28; O, 12.35.
Method D. A CH_2Cl_2 (10 mL) solution of **2** (600 mg, 1.4 mmol) was cooled to 0 °C and immediately treated with triethylamine (0.6 mL, 4.2 mmol), 4-dimethylaminopyridine (85 mg, 0.71 mmol), and *para*-nitrophenoxy chloroformate (571 mg, 2.8 mmol). The yellow solution was stirred at 0 °C for 2 h and poured into 30 mL of aq $NaHCO_3$. The mixture was extracted with EtOAc (4 × 50 mL) and stored over sodium sulfate. The residue was loaded onto a pad of

silica and the intermediate carbonate (170 mg, 0.3 mmol) was eluted with 1:2 EtOAc/hexane and subsequently dissolved in CH_3CN (12 mL) and CH_2Cl_2 (10 mL) at rt and treated with 2-(*N*,*N*-dimethylamino)ethanol (0.1 mL, 1.1 mmol). After stirring for 16 h, the mixture was poured into aq $NaHCO_3$, extracted with EtOAc (3×25 mL) and stored over anhydrous sodium sulfate. The crude (85 mg, 11%) was dissolved in CH_2Cl_2 and treated with HCl (0.5 mL, 1 M Et_2O) and allowed to stand for 18 h at rt. Amine HCl salt of **9** was filtered and the volatiles were removed in vacuo: mp 75–90 °C; Anal. calcd for $C_{26}H_{23}FClN_5O_5HCl{\cdot}(CH_2Cl_2)_{0.65}$: C, 50.68; H, 4.04; N, 11.09. Found: C, 50.68; H, 3.95; O, 10.91.