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*N*1-Alkyl pyrimidinediones as non-nucleoside inhibitors of HIV-1 reverse transcriptase

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ABSTRACT

A series of *N*1-alkyl pyrimidinediones were designed, synthesized and evaluated as HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs). Our efforts identified compound **10b**, which represents the lead compound in this series with pharmacokinetics and antiviral potency that may support once-daily dosing.

Nonnucleoside reverse transcriptase inhibitors (NNRTIs) of human immunodeficiency virus type 1 (HIV-1) are a key component of highly active antiretroviral therapy (HAART) for the long-term management of HIV infection. Efavirenz is the most widely prescribed drug in this class. Under sub-optimal conditions, treatment with efavirenz can lead to the development of resistant viruses that carry the reverse transcriptase (RT) mutation K103N. Efavirenz also causes CNS adverse events and is teratogenic.[1] These liabilities underscore the need for NNRTIs that are safer and better tolerated. Our goal was to identify an NNRTI that possessed an improved safety profile with the convenience of once-daily dosing.

In the preceding Letter,[2] we discussed SAR and properties of *N*1-heteoaryl/aryl pyrimidinediones. We herein report the synthesis and evaluation of *N*1-alkyl pyrimidinedione as HIV-1 NNRTIs.

To improve the intrinsic stability of this class of compounds, we first ascertained the stability of the pyrimidinedione core. We discovered that removal of the N1 substituent, which yielded **8b,** produced a molecule that was highly resistant to oxidation and glucuronidation in human microsomes. It also displayed favorable pharmacokinetics in dog but lacked antiviral activity (Table 1). To restore antiviral activity, we began by adding small alkyl groups at N1. We discovered that the addition of *N*1-ethyl (compound **10b**) produced a compound that had good HIV-1 antiviral activity ($EC_{50} = 6.1$ nM). This discovery prompted us to further explore SAR of *N*1-alkyl pyrimindindiones. The series of N-alkylated analogs that were prepared during these studies are illustrated in Scheme 1.

Three pyrimidinedione cores were prepared in a similar manner (Scheme 1). 5-Alkyl-barbituric acids[3] **1a–c** were converted to trichloropyrimidines **2a–c** by treatment with phosphorus oxychloride. Reaction of **2a–c** with sodium methoxide furnished 4-chloro-2,6-dimethoxypyrimidines **3a–d.**[4] Coupling partner **6** was prepared from benzonitrile **4** by bromination and followed by displacement of the resulting bromide using potassium cynanide. The anion of benzonitrile **6**, generated with sodium hydride, was reacted with chloropyrimidines **3a–d,** and subsequent oxidization of the resulting nitrile with oxygen produced dimethoxypyrimidines **7a–c.**[5] Deprotection using acetyl bromide gave the core pyrimidinediones **8a–c** that are C5-substituted with ethyl, isopropyl or *tert*-butyl groups. The core pyrimidinediones were then regioselectively alkylated using various alkyl halides and potassium carbonate in DMF.

We also examined different linkers between the pyrimidinedione and phenyl moieties to determine whether this linker plays any role in potency and/or stability. The carbonyl linker was replaced with moieties of varying polarity as well as with an ether linker to investigate linker flexibility. The synthesis of these analogs is illustrated in Scheme 2.

Dimethoxypyrimidine **7b** was treated with Deoxylfluor,[6] deprotected with acetyl bromide and alkylated to provide difluoromethylene analog **12**. The racemic monofluoro analog **16** was prepared

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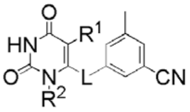
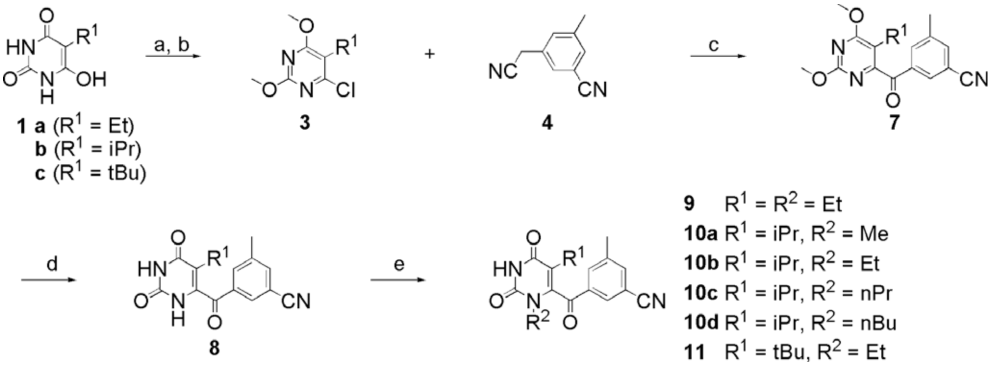
Table 1
Activity, protein binding and pharmacokinetic parameters of *N*-alkyl pyrmidinediones



Compd	L	R^1	R^2	EC_{50} (nM)[a] fold change			%Protein binding[b]	$t_{1/2}$ (h)	Dog PK[c]	
				WT	Y181C	K103N			Vss (L/kg)	%F
Efavirenz	—	—	—	1.1	2.6	35	99.9	ND	ND	ND
Etravirine	—	—	—	1.6	2.6	0.9	99.8	8.87	1.6	13[d]
8b	CO	*i*Pr	H	>500	>1	>1	ND	6.4	0.45	ND
10a	CO	*i*Pr	Me	187	>2.7	>2.7	85.9	ND	ND	ND
10b	CO	*i*Pr	Et	6.1	9.6	6.4	83.4	6.5	2.25	69[e]
10c	CO	*i*Pr	Pr	3.6	>58	>36	99.1	1.67	1.40	ND
10d	CO	*i*Pr	Bu	3.0	12	10	99.9	6.1	2.23	28[f]
9	CO	Et	Et	11.7	>43	>43	85.1	1.42	1.53	ND
11	CO	*t*Bu	Et	10	>50	2.7	99.3	ND	ND	ND
12	CF_2	*i*Pr	Et	4.2	4.3	9.0	98.7	ND	ND	ND
13	CHF	*i*Pr	Et	21	8.1	>24	98.0	ND	ND	ND
16	O	*i*Pr	Et	35	2.6	>14	96.0	ND	ND	ND

ND = not determined.
[a] Values are means of at least two experiments in MT-2 cells.
[b] Value determined from HSA column.
[c] Phamacokinetic values determined from IV (0.5 mg/kg) or PO solution dosing in beagle dogs in triplicate.
[d] %*F* determined with 4 mg/kg oral dose.
[e] %*F* determined with 0.5 mg/kg oral dose.
[f] %*F* determined with 2 mg/kg oral dose.



Scheme 1. Reagents and conditions: (a) diethylaniline or 2,6-lutidine, $POCl_3$, 90%; (b) NaOMe, MeOH, 65%; (c) NaH, DMF; NaH, O_2, 85%; (d) AcBr, 70 °C, 87%; (e) alkyl halide, K_2CO_3, DMF.

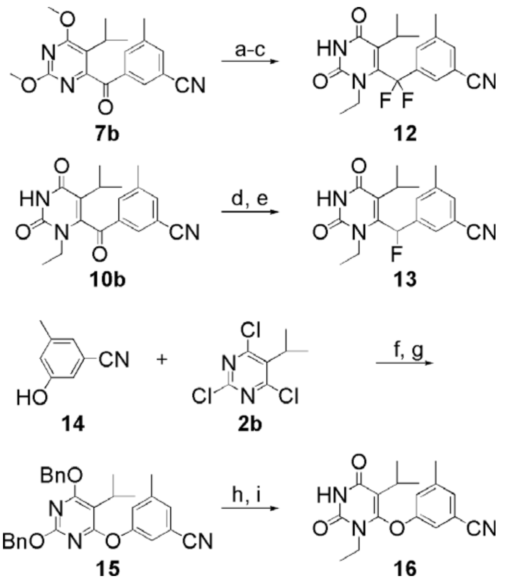
from **10b** by reduction with sodium borohydride and fluorination with DAST. Ether linked analog **16** was prepared by coupling of phenol **14** and chloropyrimidine **2b**, followed by benzyl protection/deprotection to aid purification and finally N1 alkylation.

The data for the prepared analogs are illustrated in Table 1. Addition of alkyl chains of increasing length to **8b**, from methyl to *n*-butyl improved potency against WT HIV-1. The *n*-butyl analog displayed the best antiviral activity in the series (compare **10a 10b**, **10c** and **10d**). Not surprisingly, protein binding also increased with alkyl length.

Curiously, the resistance profile of the *n*-propyl analog **10c** is inferior to that of the ethyl analog **10b** or *n*-butyl analog **10d**. X-ray crystallographic studies were performed to attempt to understand these differences.[7] Co-crystal structures with wild type RT showed that the pyrimidinedione cores of **10b**, **10c**, and **10d** bind in similar orientations but subtle differences exist in the direction of the alkyl group termini (Fig. 1).

Another difference observed between the co-crystal structures is that Y181 is flipped ‘down’ when **10b** is bound but ‘up’ with **10c** or **10d.** This conformational switching of Y181 has been observed by Stuart and co-workers in the co-crystal structures of HIV-1 RT with closely-related pyrimidinedione NNRTIs.[8] The authors observed that the larger 5-position isopropyl group in MKC-442 and TNK-651 force Y181 into a conformation enabling strong interactions with the 6-phenyl ring of the inhibitor whereas the smaller 5-position methyl group in HEPT cannot; this interaction leads to greater potency for MCK-442 and TNK-651 relative to HEPT.[8,9] Although **10b** contains the 5-position isopropyl group, Y181 adopts a similar conformation in the co-crystal structures of HEPT and **10b**. Thus, conformational switching of Y181 may also depend on the size of the N1 substituent as the co-crystal structures for **10c** and **10d** show Y181 adopts the ‘up’ conformation (as with MKC-442 and TNK-651) and the ‘down’ conformation with **10b** that contains the smaller *N*1-ethyl group.

The movement of the Y181 residue also has been observed in co-crystal structures with a different structural class of NNRTIs bound to RT.[10] Similar to an analysis of benzophenone NNRTI analogs, the ‘down’ position of Y181 may contribute to a favorable resistance profile. Compound **10b** relies less on the Y181 side chain for binding in the pocket than **10c** or **10d**. With the side chain ‘up’, the *n*-propyl has a poor resistance profile. The *n*-butyl regains the activity profile of **10b** in part due to the longer alkyl group



Scheme 2. Reagents and conditions: (a) deoxyfluor, cat. EtOH, 110 °C, 73%; (b) AcBr, 60 °C, 85%; (c) EtI, K_2CO_3, DMF; (d) $NaBH_4$, EtOH, 44%; (e) DAST, CH_2Cl_2, 68%; (f) NaH, DMF, 88%; (g) BnOH, NaH, DMF, 40%; (h) H_2, 10% Pd/C, EtOAc/EtOH, 29%; (i) EtI, K_2CO_3, DMF, 28%.

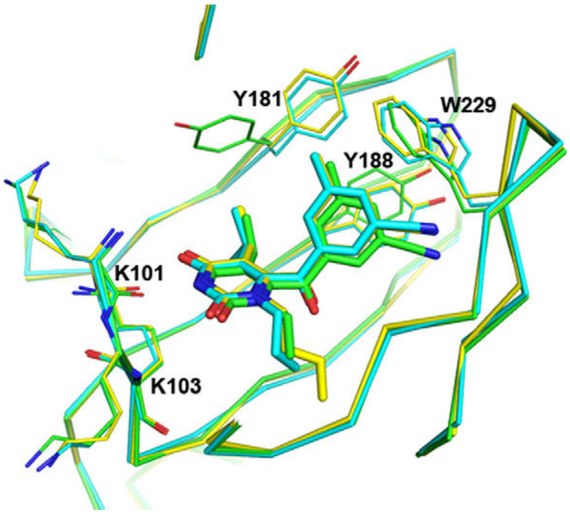


Figure 1. Overlay of X-ray co-crystal structures of wild type reverse transcriptase and compounds **10b** (green), **10c** (yellow), and **10d** (cyan).

anchoring **10c** within the NNRTI binding pocket. The combination of terminal methyl directionality differences with an apparent change in the NNRTI binding pocket could perhaps lead to restricted translational flexibility and diminished potency against mutant viruses.[11]

It was noteworthy that, the ethyl (**10b**), *n*-butyl (**10d**) and cyclopropylmethyl (**10f**) analogs displayed comparable potencies and PK properties but **10b** was less protein bound. We therefore fixed ethyl as the *N*1 alkyl substituent and examined C5 analogs.

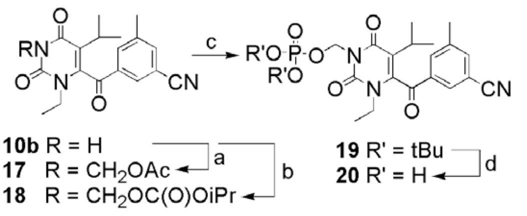
The 5-alkyl substituent was explored by changing the isopropyl group of **10b** to ethyl (**9**) or *tert*-butyl (**11**). Compound **10b** had a superior potency profile compared to **9** or **11**, suggesting the ethyl group may not occupy the NNRTI pocket optimally whereas the *tert*-butyl group may restrict ligand mobility, compromising potency against the Y181C mutant. Additionally, in vivo glucuronidation of compound **9** was observed for the first time in this series and resulted in suboptimal pharmacokinetics. Thus, we fixed the N1 and C5 positions and investigated other linkers.

Among the linkers investigated, the hydrophobic CF_2 group found in **12** yielded the most potent compound in terms of antiviral activity. Unfortunately, compound **12** was found to be unstable when tested in microsomes (data not shown). The racemic CHF linker (**13**) showed improved in vitro stability relative to **12** but lost antiviral potency. Analog **16** with an ether linker showed a small loss in potency for wild type and Y181C viruses but lost significant activity against K103N virus. Thus, of the linkers examined, the carbonyl linker offers the best balance of potency and stability.

Compound **10b** possesses the best balance of antiviral activity against wild type and mutant viruses, low protein binding and good pharmacokinetic properties. However, **10b** is highly crystalline (Table 2) and has low solubility. Thus, prodrugs of compound **10b** were prepared according to Scheme 3 to attempt to reduce crystallinity and/or improve solubility.

Alkylation of **10b** with bromomethyl acetate[12]/potassium carbonate or chloromethyl isopropylcarbonate[13]/sodium hydride gave prodrugs **17** and **18**, respectively. Reaction of **10b** with chloromethyl di-*tert*-butylphosphate[14] and potassium carbonate gave di-*tert*-butyl phosphate **19** that was deprotected with HCl in dioxane to give phosphate prodrug **20**.

The melting point, solubility and PK parameters of the prodrugs **17**, **18** and **20** are shown in Table 2. Methoxy acetate (**16**) and POC (**17**) prodrugs showed lower melting points than **10b** and higher oral bioavailability when administered as a suspension. Methoxy phosphate **20** displayed a ~1000-fold increase in solubility and



Scheme 3. Reagents and conditions: (a) bromomethyl acetate, K_2CO_3, DMF, 64%; (b) POC-Cl, NaH, DMF, 92%; (c) chloromethyl di-*tert*-butylphosphate, K_2CO_3, DMF, 50 °C, 61%; (d) TFA, CH_2Cl_2, 64%.

Table 2

Beagle dog pharmacokinetic parameters of parent **10b** and prodrugs **17**, **18** and **20** dosed from suspension versus tablet formulation (5 mg/kg)

Compds	MP[a] (°C)	Sol[b] (µg/mL)	Suspension		Tablet	
			AUC_{inf} (nM h)	*F* (%)	AUC_{inf} (nM h)	*F* (%)
10b	230.3	11	13,131	35.8	13,485	45.1
17	167.0	2.2	14,704	48.9	8781	35.3
18	154.7	<1	54,022	108.2	4543	24.0
20	ND	12,000	18,765	75.2	ND	ND

[a] Value determined using a differential scanning calorimeter (DSC).

[b] Aqueous solubility measured in 50 mM phosphate buffer.

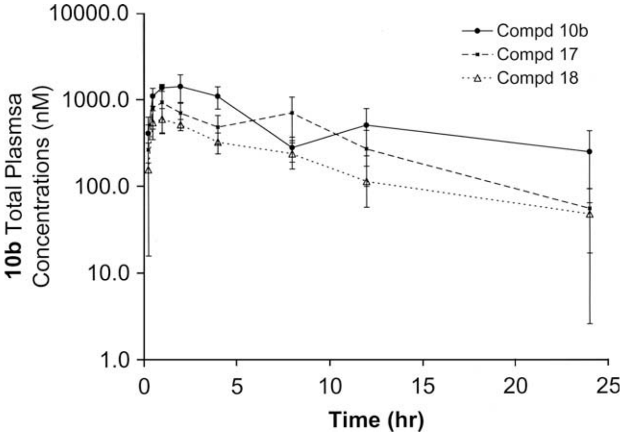


Figure 2. Comparison of exposure of compound **10b** from 5 mg/kg dose of parent (**10b**) or prodrugs **17** and **18** when dosing as tablet.

good oral bioavailability in dogs but resulted in emesis at doses higher than 5 mg/kg.

In contrast, when micronized material ($D_{50} = 3\mu$) was prepared and dosed in tablet form in dogs, the parent **10b** gave superior oral bioavailability compared to **17** and **18** (Fig. 2).

Compound **10b** (GS-9441) shows comparable protein adjusted antiviral potency ($EC_{50} = 16$ nM) against wild type HIV as etravirine ($EC_{50} = 11$ nM) but may have the advantage of once-daily administration.[15] While the resistance profile may be non-optimal, based on biological activity against wild type virus, favorable protein binding and good pharmacokinetic properties that suggest once-daily dosing, further profiling of compound **10b** for clinical evaluation is on-going.

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