Bioorganic & Medicinal Chemistry Letters 20 (2010) 1585–1588

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

N1-Heterocyclic pyrimidinediones as non-nucleoside inhibitors of HIV-1 reverse transcriptase

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ARTICLE INFO

Article history:
Received 4 December 2009
Revised 11 January 2010
Accepted 13 January 2010
Available online 21 January 2010

Keywords:
Human immunodeficiency virus
Non-nucleoside
Reverse transcriptase
Pyrimidinedione

ABSTRACT

A series of N1-heterocyclic pyrimidinediones were extensively evaluated as HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs). Inhibitor **1** is active against NNRTI-resistant viruses including RT mutant K103N. The co-crystal structure of inhibitor **1** with HIV-1 RT revealed that H-bonds are formed with K101 and K103. Efforts to improve the suboptimal pharmacokinetic profile of **1** resulted in the discovery of compound **13**, which represents the lead compound in this series with improved pharmacokinetics and similar potency as inhibitor **1**.

The use of non-nucleoside reverse transcriptase inhibitors (NNRTIs) for treatment of human immunodeficiency virus type 1 (HIV-1) infection has been well established.[1] Despite active research, there are only four marketed NNRTIs: efavirenz,[2] nevirapine,[3] delavirdine,[4] and most recently, etravirine.[5] Rapid emergence of drug-resistant mutant viruses that were cross-resistant to other NNRTIs limited the broad usage of this class of antiretroviral drugs. Efavirenz, the most prescribed NNRTI, is associated with side effects that include perturbation of central nervous system function and teratogenicity.[6] Despite its improved resistance profile, etravirine lacks the convenience of once-daily administration and is also associated with cutaneous and hypersensitivity reactions. Thus, our goal was to identify an NNRTI that possessed an improved safety profile with the convenience of once-daily dosing that remained active against mutant viruses, especially the reverse transcriptase (RT) K103N mutant that is selected by efavirenz.[7] We herein report the synthesis and evaluation of N1-pyridinyl pyrimidinediones as HIV-1 NNRTIs.

An N-pyridinyl pyrimidinedione (KRV-2110, **1**) was previously identified as a potent NNRTI (Fig. 1).[8] Antiviral profiling revealed that compound **1** also exhibits potent antiviral activity against RT Y181C and K103N mutant viruses. Detailed profiling of compound **1** indicated that it has moderate protein binding and high oral bioavailability (Table 1). However, pharmacokinetics in three species (rat, dog and monkey) suggested that once-daily dosing in humans was unlikely. Incubation of **1** with human hepatocytes produced a principal metabolite resulting from oxidation on the aminofluoropyridyl moiety. Hydroxylation of the C5 isopropyl was also observed.

Compound **1** was co-crystallized with HIV-1 reverse transcriptase as shown in Figure 2.[9] Hydrogen bonds are observed between the NH of the pyrimidinedione and the backbone carbonyl of K101

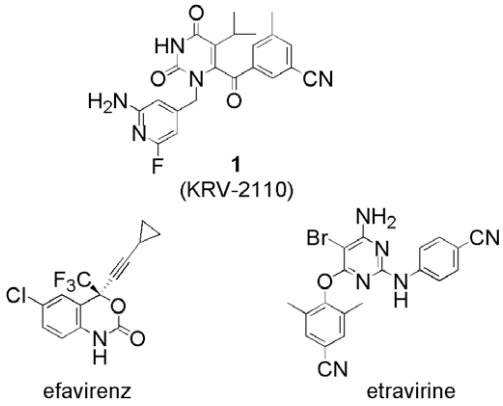

Figure 1. Structure of compound **1** (KRV-2110), efavirenz and etravirine.

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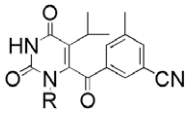
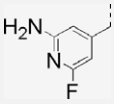
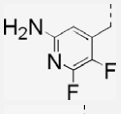
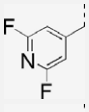
doi:10.1016/j.bmcl.2010.01.086

Table 1
Evaluation of pyrimidinedione NNRTI's



Compound	R	EC_{50} (nm)[a] WT fold change			% Protein Binding[b]	Human MS $t_{1/2}$ (min)[c]	$t_{1/2}$ (h)	Dog PK[d]	
		WT	Y181C	K103N				V_{ss} (L/kg)	% *F*
Efavirenz	—	1.1	2.6	35	99.9	98	ND	ND	ND
Efavirenz	—	1.6	2.6	0.9	99.8	140	8.87	1.6	13.3[e]
1		3.4	6.7	1.6	93.6	45	3.3	1.8	96[e]
9		4.5	8.2	7.3	93.3	51	ND	ND	ND
10		12.4	12	1.9	94.2	Stable	4.9	1.94	41.5[f]
13		6.2	16	2.4	87.7	281	4.7	1.61	80[g]

ND = not determined.
[a] Values are means of at least two experiments in MT-2 cells.
[b] Values determined from HSA column.
[c] Values determined by incubating with human microsomes; half-life greater than 395 min is reported as stable.
[d] Pharmacokinetic values determined from IV (0.5 mg/kg) or PO solution dosing in beagle dogs in triplicate.
[e] %*F* determined with 4 mg/kg oral dose.
[f] %*F* determined with 0.5 mg/kg oral dose.
[g] %*F* determined with 2 mg/kg oral dose.

as well as the NH_2 of the aminofluoropyridyl moiety and the backbone carbonyl of the K103 residue.

Analogs of compound **1** were designed to block the oxidation of the aminofluoropyridine as shown in Scheme 1. Compound **1** was synthesized starting from 3,5-dichloro-2,4,6-trifluoro-pyridine (**2a**) that was next converted to **3a** via dibenzyl malonate displacement and mono-decarboxylation. Hydrogenolysis of benzyl ester **3a** gave the corresponding acid and decarboxylation with concomitant bromination provided bromide **4a**. Bromide displacement with sodium benzoate, chloride removal via hydrogenolysis, and benzoate ester cleavage gave 2,6-difluoro-4-hydroxymethyl pyridine **5a**. Displacement of a fluoride with *p*-methoxybenzylamine, mesylation of the hydroxyl group, coupling with pyrimidinedione core **7**[8] and deprotection furnished compound **1**.

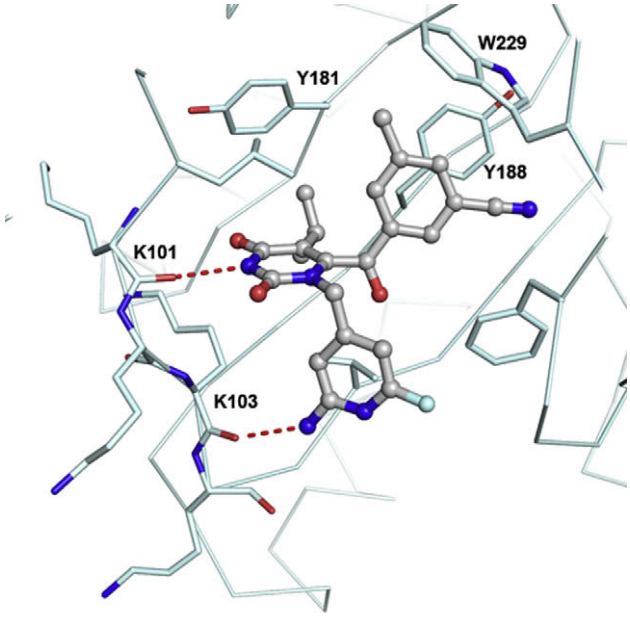
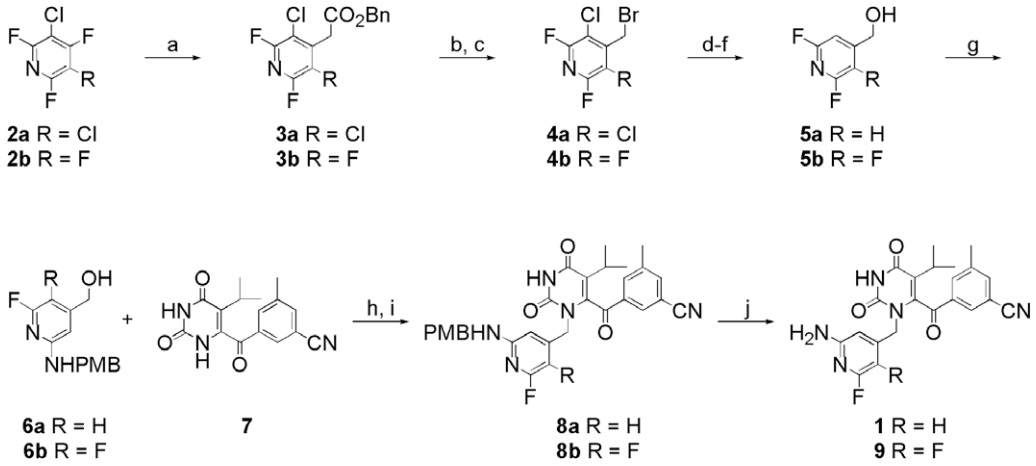


Figure 2. Compound **1** bound in the NNRTI binding pocket of HIV-1RT (2.3Å resolution).

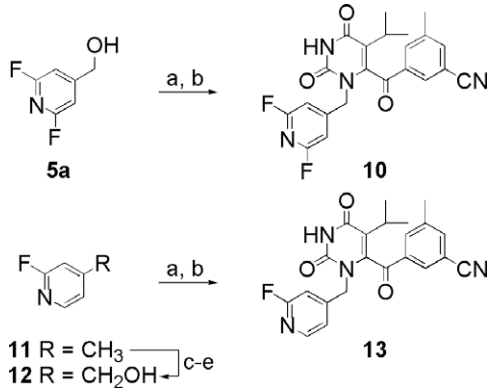
Compound **9** was prepared similarly to compound **1** starting from 3-chloro-2,4,5,6-tetrafluoro-pyridine (**2b**). Compound **9** would be expected to potentially block and/or slow down oxidation of the aminofluoropyridyl moiety, but no significant improvement in microsomal stability was observed. Potency of **9** against wild type and RT Y181C viruses was similar to **1** but compound **9** was less potent against RT K103N virus. We speculate that the ortho substitution may perturb the hydrogen bond formed between $-NH_2$ and the carbonyl of residue 103, either by electronic effects or torsional restriction.

Next, we investigated whether oxidation of the pyridyl nitrogen was contributing to the metabolic instability. To specifically address this issue, the corresponding phenyl analog lacking the pyridine nitrogen was prepared. The resulting compound showed only marginal improvement in microsomal stability.

The evidence assembled thus far indicated that the NH_2 in **1** was responsible for the microsomal instability. To test this hypothesis, difluoropyridine **10**, in which the amino group was replaced with fluoride (Scheme 2), was synthesized. Compound **10** proved to be more stable in both dog and human microsomes compared to **1**. However, in contrast to the in vitro observations, the half-life of compound **10** in dogs was only marginally improved relative to **1**. To reconcile this inconsistency, compound **10** was incubated in



Scheme 1. Reagents and conditions: (a) (i) dibenzyl malonate, NaH, DMF; (ii) DMSO/H_2O, 130 °C, 75%; (b) H_2, Pd/C, THF, 78–89%; (c) HgO, Br_2, chlorobenzene, 145 °C, 75–80%; (d) NaOBz, DMF, 66–81%; (e) H_2 (50 psi), Pd/C, EtOH, 61%; (f) NaOMe, MeOH, 95–98%; (g) $PMBNH_2$, 125 °C, 90–94%; (h) methanesulfonyl chloride, triethylamine, $CHCl_3$; (i) **7**, K_2CO_3, LiI, DMF, 32–55%; (j) CAN, ACN/H_2O, 77%.



Scheme 2. Reagents and conditions: (a) methanesulfonyl chloride, triethylamine, $CHCl_3$; (b) **7**, potassium carbonate, lithium iodide, DMF, 32%; (c) *N*-bromosuccinimide, benzoyl peroxide, CCl_4, reflux, 28%; (d) sodium benzoate, DMF, 79%; (e) sodium methoxide, MeOH, 94%.

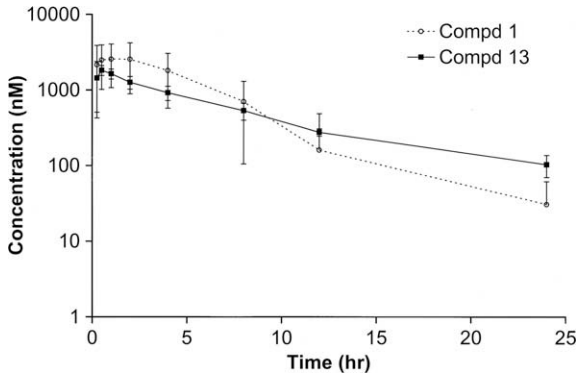


Figure 3. Comparison of PO PK for compounds **1** (4 mg/kg) and **13** (2 mg/kg) in beagle dogs.

fresh human hepatocytes which showed that the difluoropyridyl moiety of compound **10** underwent oxidative defluorination followed by glutathione adduct formation.

As a result of this observation, the stability of monofluoropyridine **13** was also evaluated in fresh human hepatocytes. Compound **13** did not form glutathione adducts in vitro and showed improved microsomal stability and a longer half-life compared to compound **1**. Compound **13** retained most of the potency against wild type and RT K103N viruses but displayed diminished potency against RT Y181C virus.

Since incubation of compound **1** with microsomes resulted in C5 hydroxylation, we substituted the isopropyl group for ethyl and *tert*-butyl in an attempt to blunt metabolism. Although we observed a modest improvement of in vitro and in vivo stability for the C5 ethyl analog, both ethyl and *tert*-butyl analogs were less potent against mutant viruses, in particular RT Y181C. The increased volume of the Y181C NNRTI binding pocket in the vicinity of the C5 substituent may explain why these compounds are less potent against the RT Y181C mutant virus.

Thus, the C5 isopropyl may provide the best balance of hydrophobic interactions with the NNRTI binding pocket and translational freedom to give the superior potencies against wild type and mutant viruses.[5]

Compound **13** from the N1-heterocyclic pyrimidinedione series displays improved properties compared to the original lead, compound **1**. It represents an improvement in microsomal stability, pharmacokinetic parameters while maintaining most of the antiviral potency of compound **1** against wild type virus. However, the half-life of **13**, as shown in Figure 3, together with its plasma adjusted antiviral potency, was insufficient to support once-daily dosing in humans.

Our parallel effort on N1-alkyl pyrimidinediones generated more promising results. Thus, research efforts were shifted to focus on the N1-alkyl pyrimidinedione series which is reported in the following Letter.[10]

Acknowledgements

The authors wish to thank Gerry Rhodes, Manoj Desai, Nilima Kutty, Candra Lancaster, and Stephanie Leavitt for their contributions.

References and notes

1. (a) Miyasaka, T.; Tanaka, H.; Bab, M.; Hayakawa, J.; Walker, R. T.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1989**, *32*, 2507; (b) Prajapati, D. G.; Ramajayam, R.; Yadav, M. R.; Giridhar, R. *Bioorg. Med. Chem.* **2009**, *17*, 5744.
2. Young, S. D.; Britcher, S. F.; Tran, L. O.; Payne, L. S.; Lumma, W. C.; Lyle, T. A.; Huff, J. R.; Anderson, P. S.; Olsen, D. B.; Carroll, S. S.; Pettibone, D. J.; O'Brien, J. A.; Ball, R. G.; Balani, S. K.; Lin, J. H.; Chen, I. W.; Schleif, W. A.; Sardana, V. V.; Long, W. J.; Byrnes, V. W.; Emini, E. A. *Antimicrob. Agents Chemother.* **1995**, *39*, 2602.

3. (a) Merluzzi, V. J.; Hargrave, K. D.; Labadia, M.; Grozinger, K.; Skoog, M.; Wu, J. C.; Shih, C. K.; Eckner, K.; Hattox, S.; Adams, J.; Rosenthal, A. S.; Faanes, R.; Eckner, R. J.; Koup, R. A.; Sullivan, J. L. *Science* **1990**, *250*, 1411; (b) Koup, R. A.; Merluzzi, V. J.; Hargrave, K. D.; Adams, J.; Grozinger, K.; Eckner, R. J.; Sullivan, J. L. *J. Infect. Dis.* **1991**, *163*, 966.
4. Dueweke, T. J.; Poppe, S. M.; Romero, D. L.; Swaney, S. M.; So, A. G.; Downey, K. M.; Althaus, I. W.; Reusser, F.; Busso, M.; Resnick, L.; Mayers, D. L.; Lane, J.; Aristoff, P. A.; Thomas, R. C.; Tarpley, W. G. *Antimicrob. Agents Chemother.* **1993**, *37*, 1127.
5. Das, K.; Clark, A. D., Jr.; Lewi, P. J.; Heeres, J.; De Jonge, M. R.; Koymans, L. M.; Vinkers, H. M.; Daeyaert, F.; Ludovici, D. W.; Kukla, M. J. *J. Med. Chem.* **2004**, *47*, 2550.
6. (a) Arendt, G.; de Nocker, D.; von Giessen, H. J.; Nolting, T. *Expert Opin. Drug Saf.* **2007**, *6*, 147; (b) Fumaz, C. R.; Muñoz-Moreno, J. A.; Moltó, J.; Negredo, E.; Ferrer, M. J.; Sirera, G.; Pérez-Alvarez, N.; Gómez, G.; Burger, D.; Clotet, B. *J. Aquir. Immune Defic. Syndr.* **2005**, *38*, 560; (c) SUSTIVA® (efavirenz) capsules and tablets: Package insert, Bristol-Myers Squibb Co, Princeton, NJ, USA (2008). package inserts. Bms/com/pi/pi_sustiva.pdf.
7. Bacheler, L.; Weislow, O.; Snyder, S.; Hanna, G.; D'Aquila, R. The SUSTIVA Resistance Study Team. *Abstract*, Presented at the 12th World AIDS Conference, Geneva, Switzerland, June 29–July 3, 1998.
8. Guo, H.; Kim, C. H.; Kim, H. S.; Lee, C.-K.; Lee, I. Y.; Mitchell, M. L.; Son, J. C.; Xu, L.; WO 2008016522 A2, 2008.
9. Coordinates have been deposited into the PDB accession code 3LAK.
10. Mitchell, M. L.; Son, J. C.; Guo, H.; Im, Y.-A.; Cho, E. J.; Wang, J.; Hayes, J.; Wang, M.; Paul, A.; Lansdon, E. B.; Chen, J. M.; Graupe, D.; Rhodes, G.; He, G.-X.; Geleziunas, R.; Xu, L.; Kim, C. U. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1589.