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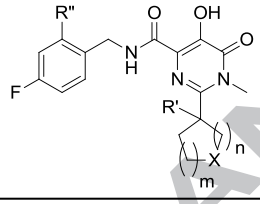

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Synthesis and evaluation of C2-carbon-linked heterocyclic-5-hydroxy-6-oxo-dihydropyrimidine-4-carboxamides as HIV-1 integrase inhibitors

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ABSTRACT

Integration of viral DNA into the host cell genome is an obligatory process for successful replication of HIV-1. Integrase catalyzes the insertion of viral DNA into the target DNA and is a validated target for drug discovery. Herein, we report the synthesis, antiviral activity and pharmacokinetic profiles of several C2-carbon-linked heterocyclic pyrimidinone-4-carboxamides that inhibit the strand transfer step of the integration process.

The HIV-1 integrase enzyme, one of three virus-encoded enzymes, plays a critical role in retroviral replication. After reverse transcription of viral RNA into a double stranded DNA, the integrase enzyme, as part of a pre-integration complex, migrates into the nucleus and catalyzes the insertion of the viral genome into the host chromosome via a multi-step process. The two enzymatic steps in this insertion process are: (1) cleavage of a dinucleotide from the 3'-ends of the viral DNA and (2) the subsequent insertion of these processed ends into the host DNA (strand transfer).[1] Although interrupting either of these steps could, in principle, lead to inhibition of viral replication, compounds that inhibit the strand transfer reaction were found to potently and specifically exhibit antiviral activity, leading to FDA approval of three strand transfer inhibitors, raltegravir,[2] elvitegravir[3] and dolutegravir[4] (Figure 1). We have examined pyrimidinone-4-carboxamides as inhibitors of HIV-1 integrase and a systematic survey of C2-substitutents led to the identification of tetrahydrofuran and tetrahydropyran substituents at C-2 as optimal moieties that combined high antiviral potency with good pharmacokinetic profiles in preclinical species. In this article, we disclose the synthesis, antiviral activity and pharmacokinetic properties of a series of C2-carbon linked heterocyclic-5-hydroxy-6-oxo-dihydropyrimidine-4-carboxamides as novel HIV-1 integrase strand transfer inhibitors.

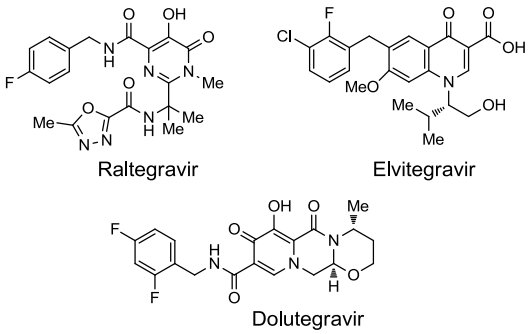
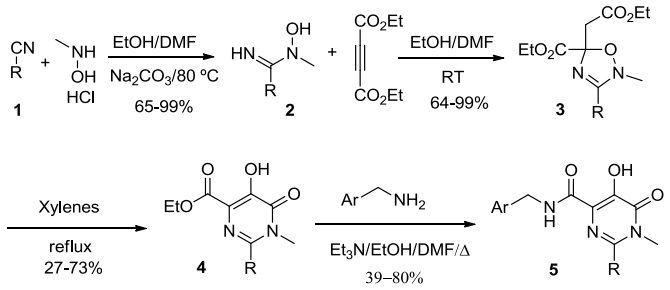


Figure 1. FDA approved strand transfer inhibitors.

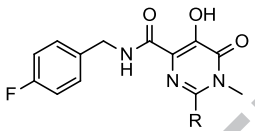
The desired pyrimidinone carboxamides were prepared in a short sequence as outlined in Scheme 1. Treatment of nitriles **1** with N-methylhydroxylamine provided the hydroxyamidine adducts **2** which upon exposure to diethyl acetylenedicarboxylate afforded the oxadiazolines **3**.[5] Pyrolysis of **3** in xylenes furnished the desired pyrimidinone esters **4**,[6] which upon heating with the appropriate benzylamine in the presence of triethylamine provided the final pyrimidinone-4-carboxamides **5**.

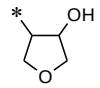
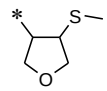
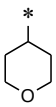
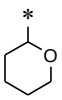


Scheme 1. Synthesis of pyrimidinone-4-carboxamides.

The initial structure-activity relationship (SAR) study focused on identifying suitable C2-substituents and a variety of five- and six-membered carbon-linked heterocycles were surveyed with the results summarized in Table 1. Both the furan and the 2,5-dihydrofuran analogs **6**-**7** exhibited good enzymatic inhibition in a biochemical strand transfer assay[7] but showed significantly reduced antiviral activity in a cell culture virus replication assay. In addition, compounds **6** and **7** displayed a large shift in antiviral activity in the presence of human serum albumin (HSA). For this inhibitor class, it was shown that HSA can substitute for human serum (HS), with 15 mg/mL mimicking results observed when 40% HS is added, while the addition of 45 mg/mL represents activity in 100% HS.[7] On the other hand, the C2-tetrahydrofuran-3-yl (THF) analog **8** exhibited excellent inhibitory activity in both the strand transfer and antiviral cell culture assays. Furthermore, compound **8** is significantly more potent than the unsaturated compounds **6** and **7** in the presence of HSA, suggesting lower protein binding.

Table 1. SAR of C2-heterocyclic substituents in a series of HIV-1 integrase inhibitors



Compound[a]	R	Inhibition of strand transfer IC_{50} (nM)	Antiviral EC_{50} in cell culture (nM)	
			FBS[b]	HSA[c]
6		21	424	14,490
7		19	206	1,783
8		4	9	60
9		6	48	126
10		3	7	33
11		5	10	120
12		5	15	153
13		5	6	28
14		10	49	301

[a]Compounds **8**-**14** were prepared and screened as racemic mixtures, [b]EC_{50} values were determined in the presence of 10% FBS, [c]HSA-EC_{50} values were determined in the presence of 15 mg/mL of human serum albumin and 10% FBS.

Interestingly, the nature of the substituent on the THF ring and the position of attachment of the THF moiety to the pyrimidinone core exert a significant influence on the antiviral activity of this series of pyrimidinone carboxamides. For example, the polar 4-hydroxy-THF derivative **9** maintained the potent enzymatic inhibitory activity associated with its progenitor **8** but exhibited diminished antiviral activity in the cell culture. On the contrary, the enzymatic and antiviral activities of the 4-thiomethyl-THF analog **10** are comparable with those of **8**. In addition, **10** is subject to a lower effect of HSA than that observed with **8** despite being more lipophilic. Interestingly, tetrahydrofuran **11** and tetrahydrothiophene **12** display comparable enzymatic and cell culture inhibitory activities to **8** but suffer from a higher HSA-shift. The tetrahydropyran-4-yl (THP) analog **13** exhibited excellent enzymatic and cell culture activities whereas the C2-tetrahydropyran-2-yl derivative **14** possesses diminished activities in both assays. Furthermore, **14** exhibited substantially reduced antiviral activity in the presence of HSA when compared with **13**. The data for compounds **8**, **10**-**14** suggest that the oxygen atom in the tetrahydrofuran-3-yl and tetrahydropyran-4-yl moieties increases the hydrophilic character of the molecule which, in turn, lowers the effect of HSA. In addition, it is possible that the oxygen atom in the tetrahydropyran-4-yl moiety may be engaging additional binding interaction with integrase, leading to improved antiviral activity for **13** when compared with **14**.

Select compounds were evaluated for pharmacokinetic (PK) evaluation; results are summarized in Table 2. In a rat IV study, **8**, **10** and **13** are characterized as low clearance compounds with values of 4.9, 3.6 and 2.6 mL/min/kg, respectively. All three compounds have short half-lives and a low volume of distribution. In the rat oral PK study, excellent oral exposure and good bioavailability was observed for **8** and **10**.

Table 2. Rat[a] pharmacokinetic data for **8**, **10** and **13**

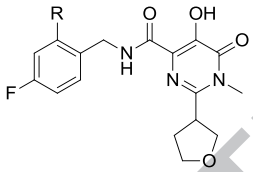
Compound	**8**	**10**	**13**
IV dose (mg/kg)[b]	1	1	1
CL (mL/min/kg)	4.9	3.6	2.6
$t_{1/2}$ (h)	1.63	1.08	0.58
V_{ss} (L/kg)	0.14	0.15	0.12
PO dose (mg/kg)[b]	5	5	nd[c]
C_{max} (μM)	10.45	8.04	
T_{max} (μM)	0.67	1.67	
AUC (μM*h)	33.89	39.45	
F (%)	68	67	

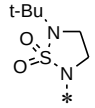
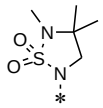
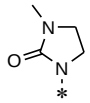
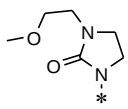
[a]Male Sprauge-Dawley rats, [b]Vehicle: PEG-400/Ethanol (90:10), [c]nd = not done

Although the tetrahydrofuran derivative **8** exhibited excellent antiviral activity and a good rat PK profile, it displayed greater than an 18-fold reduction in antiviral activity when tested in the presence of 45 mg/mL of HSA. Consequently, in order to reduce the HSA effect and improve overall drug-like properties, we synthesized and evaluated a variety of *ortho*-substituted

benzylamides based on this C2-THF substituted template and the results are summarized in Table 3. These data reveal that a variety of polar groups are well tolerated at the *ortho*-position and these moieties contributed to improved antiviral activity in cell culture with a lower HSA-shift. For example, the methylsulfonyl analog **15** and two sulfonamide derivatives **16** and **17** exhibited excellent antiviral activities[8] and displayed significantly reduced HSA-shifts compared to **8**. Similarly, the *ortho*-heterocyclic analogs **18-26** exhibited very potent antiviral activity and they are 5- to 15-fold more potent than **8** in the presence of 45 mg/mL HSA. The unsubstituted triazole compound **18** is equipotent with **8** both in the strand transfer and the FBS assays. Interestingly, the antiviral activity of the methyl triazole analogue **19** in HSA was similar to that of **18,** despite the fact that this compound exhibited reduced activity in both the strand transfer and the cell culture (FBS) assays. The lack of a shift in 45 mg/mL HSA for **19** may partly be due to the lower protein binding effected by the methyltriazole moiety. On the other hand, the 1,2,3-triazole analog **20** exhibited similar potencies both in the enzymatic and the FBS assays as **19,** but it displayed about a 2-fold shift in the HSA assay. Between the two carbon-linked methyl triazole derivatives **21** and **22**, the 1-methyl-1,2,4-triazole **21** is more potent than the 4-methyl-1,2,4-triazole **22** in all three assays. The thiadiazolidine analogs **23-24** and the imidazolidine compounds **25-26** displayed potent cellular activity in the FBS cell culture assay but, unfortunately, these compounds suffer from higher shifts in the presence of HSA (4 to 11-fold loss of activity).

Table 3. SAR associated with *N*-(arylmethyl)-5-hydroxy-1-methyl-6-oxo-2-(tetrahydrofuran-3-yl)-1,6-dihydropyrimidine-4-carboxamide derivatives

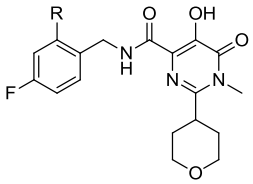


Compound[a]	R	Inhibition of strand transfer IC_{50} (nM)	Antiviral EC_{50} in cell culture (nM)	
			FBS[b]	HSA[c]
8	H	4	9	168
15	SO_2Me	9	14	33
16	SO_2NMe_2	10	4	25
17		9	5	19
18		4	7	18
19		12	14	18
20		10	13	28
21		6	5	11
22		18	28	27
23		16	2	22
24		10	2	22
25		6	7	30
26		3	5	27

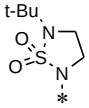
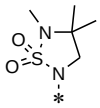
[a] Compounds were evaluated as racemic mixtures, [b]EC_{50} valued were determined in the presence of 10% FBS, [c]HSA-EC_{50} values were determined in the presence of 45 mg/mL human serum albumin and 10% FBS.

In parallel, the *ortho*-substituted benzylamide analogs **27**–**35** based on the C2-THP substituted core were prepared and evaluated, and the results are summarized in Table 4. Similar to the C2-THF series, several C2-THP derivatives showed improved antiviral activity in cell culture and displayed a lower HSA effect with less than a 4-fold loss of antiviral activity in the presence of 45 mg/mL HSA. Although, the C2-THP compounds **27-35** displayed comparable activities to those of the corresponding C2-THF derivatives, both in the strand transfer and cell culture (FBS) assays, the C2-THP analogs diverged from the C2-THF derivatives in the HSA assay. The majority of the C2-THP analogs exhibited 2-4 fold improved potency in presence of HSA than the corresponding C2-THF derivatives. Furthermore, the C2-THP compounds displayed reduced HSA shifts when compared with the corresponding C2-THF analogs.

Table 4. SAR associated with *N*-(arylmethyl)-5-hydroxy-1-methyl-6-oxo-2-(tetrahydro-2-pyran-4-yl)-1,6-dihydropyrimidine-4-carboxamide derivatives



Compound	R	Inhibition of strand transfer IC_{50} (nM)	Antiviral EC_{50} in cell culture (nM)	
			FBS[b]	HSA[c]
13	H	5	6	Nd[c]
27	SO_2Me	8	6	13
28	SO_2NMe_2	12	4	15
29		11	4	11
30		6	10	18
31		8	9	14
32		13	8	18
33		9	4	7

34		17	2	7
35		6	2	7

[a] Compounds **15-26** were evaluated as racemic mixtures, [b]EC_{50} valued were determined in the presence of 10% FBS, [c]HSA-EC_{50} values were determined in the presence of 45 mg/mL human serum albumin and 10% FBS, [c]nd = not determined.

Several *ortho*-substituted benzylamides were evaluated for their PK properties in rats and the results are compiled in Table 5. The cyclic sulfonamide **17** and methylsulfone **27** exhibited low clearance with short half-lives and low volumes of distribution. Compound **17** also demonstrated very good oral PK properties with high oral exposure and good bioavailability. On the other hand, in the rat IV screen, compounds **18**, **19**, **21**, **23**, **26** and **34** displayed moderate to high clearance, short half-lives and low volumes of distribution.

Table 5. Rat[a] pharmacokinetic data for C2-tetrahydrofuran-3-yl and C2-tetrahydropyran-4-yl pyrimidinone benzylamides

Compound	**17**	**18**	**19**	**21**	**23**	**26**	**27**	**34**
IV dose (mg/kg)[b]	1	1	1	1	1	1	1	1
CL (mL/min/kg)	6	47	37	12	31	15	3.9	41
$t_{1/2}$ (h)	0.54	0.42	0.23	0.5	0.24	0.46	0.58	0.22
V_{ss} (L/kg)	0.2	0.98	0.76	0.36	0.36	0.44	0.13	0.61
PO dose (mg/kg)[b]	5	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]
C_{max} (μM)	3.51							
T_{max} (μM)	0.63							
AUC (μM*h)	12.35							
F (%)	60							

[a]Male Sprauge-Dawley rats, [b]Vehicle: PEG-400/Ethanol (90:10), [c]nd = not done

In conclusion, we have identified tetrahydrofuran-3-yl and tetrahydropyran-4-yl moieties as optimal C2-substituents in a series of pyrimidinone HIV-1 integrase inhibitors by systematic investigation of various carbon-linked five and six membered heterocycles at the C2-position of the heterocycle core. In addition, in order to lower the effect of human serum albumin on the antiviral activity of the compound, we surveyed the effect of polar substituents at the benzylamide moiety using C2-THF and C2-THP derivatives as the vehicles. A variety of polar substituents at the *ortho*-position are well tolerated that exhibit improved antiviral activity and significantly lowered HSA shifts. Furthermore, several compounds were evaluated for their pharmacokinetic properties in rats, and compounds **8**, **10** and **17** displayed low clearance, high oral exposure and good bioavailability.

Acknowledgment

We are grateful to Mr. Henry Wong and Mr. Ashok Trehan of Department of Discover Synthesis for the contributions to the scale up of intermediates (4-fluoro-2-(1H-1,2,4-triazol-1-yl)phenyl)methanamine and (4-fluoro-2-(5-methyl-1H-1,2,4-triazol-1-yl)phenyl)methanamine. We also thank Mr. Fukang Yang for the scale up of (4-fluoro-2-(4-methyl-4H-1,2,4-triazol-3-yl)phenyl)methanamine.

References and notes

1. Craigie, R. *Future Virol.*, **2012**, *7*, 679.
2. Summa, V.; Petrocchi, A.; Bonelli, F.; Crescenzi, B.; Donghi, M.; Ferrara, M.; Fiore, F.; Gardelli, C.; Paz, O. G.; Hazuda, D. J.; Jones, P.; Kinzel, O.; Laufer, R.; Monteaguda, E.; Muraglia, E.; Nizi, E.; Orvieto, F.; Pace, P.; Pescatore, G.; Scarpelli, R.; Stillmock, K.; Witmer, M. V.; Rowley, M. *J. Med. Chem.* **2008**, *51*, 5843.
3. Sato, M.; Kawakami, H.; Motomura, T.; Aramaki, H.; Matsuda, T.; Yamashita, M.; Ito, Y.; Matsuzaki, Y.; Yamataka, K.; Ikeda, S.; Shinkai, H. *J. Med. Chem.* **2009**, *52*, 4869.
4. Johns, B. A.; Kawasuji, T.; Weatherhead, J. G.; Taishi, T.; Temelkoff, D. P.; Yoshida, H.; Akiyama, T.; Taoda, Y.; Murai, H.; Kiyama, R.; Fuji, M.; Tanimoto, N.; Jefferey, J.; Foster, S. A.; Yoshinaga, T.; Seki, T.; Kobayashi, M.; Sato, A.; Johnson, M. N.; Garvey, E. P.; Fujiwara, T. *J. Med. Chem*. **2013**, *56*, 5901.
5. Naidu, B. N.; Sorenson, M. E. *Org. Lett*. **2005**, *7*, 1391.
6. Naidu, B. N. *Synlett*, **2008**, 547. b) Pye, P. J.; Zhong, Y. L.; Jones, G. O.; Reamer, R. A.; Houk, K. N.; Askin, D. *Angew. Chem. Int. Ed*. **2008**, *47*, 4134.
7. Dicker, I.B., Walker, M.A., Lin, Z., Terry, B., Pajor, L., Zheng, M., Naidu, B.N., Banville, J., Meanwell, N.A., Krystal, M. A Simple and accurate *in vitro* method for predicting serum protein binding of HIV integrase strand transfer inhibitors. In *HIV-1 Integrase; Mechanism and Inhibitor Design;* Neamati, N., Ed.; John Wiley & Sons: Hoboken, New Jersey, 2011, pp275-286.
8. The individual epimers of **17** were evaluated for their antiviral activity and both epimers exhibited similar activities in strand transfer and cell culture assays.