Bioorganic & Medicinal Chemistry Letters 20 (2010) 5031–5034

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Development of 2-pyrrolidinyl-*N*-methyl pyrimidones as potent and orally bioavailable HIV integrase inhibitors

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ARTICLE INFO

Article history:
Received 20 June 2010
Accepted 11 July 2010
Available online 15 July 2010

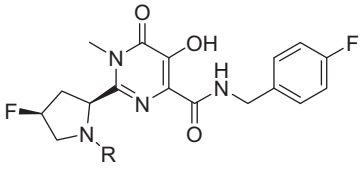
Keywords:
HIV integrase
N-Methyl pyrimidones

ABSTRACT

A series of 2-pyrrolidinyl-*N*-methyl pyrimidones HIV integrase inhibitors has been explored leading to the identification of derivative **13**, which showed high activity at inhibiting viral replication in cell culture, favorable pharmacokinetic profile in two preclinical species, and an attractive profile against a panel of HIV-integrase mutants.

HIV/AIDS continues to be a major threat with global estimates for 2008 of two million deaths, 33 million people infected worldwide with HIV and three million new infections, thus rendering the discovery of new drugs imperative. HIV integrase is one of the three enzymes encoded by the HIV genome and, being vital for the replication of the virus,[1] represents an attractive target for the management of HIV infection. We have reported the development of potent and orally bioavailable *N*-methyl pyrimidones,[2] which has also led to the discovery of Raltegravir[3] (brand name Isentress™), a first in class inhibitor of HIV integrase approved in both the US and Europe at the end of 2007. Within this effort, 2-pyrrolidinyl-*N*-methyl pyrimidones emerged as especially interesting and although the structure–activity relationship surrounding the pyrrolidine ring revealed a certain degree of tolerance, a (2*S*,4*S*)-4-fluoro-pyrrolidine **1** stood out as having strong enzymatic activity and efficacy at inhibiting viral spread in the cell-based assay (Table 1). The *N*-methyl pyrrolidine **1** was further evaluated in vivo (Table 2), where it showed low/moderate plasma clearance (21 and 4.4 mL/min/kg in rat and dog, respectively) and very high oral bioavailability (95% and 116% in rat and dog, respectively). Based on the attractive profile of **1** we sought to further optimize its antiviral activity, with our initial approach focusing on an evaluation of substituents other than a methyl group on the pyrrolidine N-atom (Table 1). Alternative *N*-alkyl substituents (e.g., **2** and **3**) consistently retained the intrinsic activity of the reference compound but cell-based antiviral activity was negatively impacted; **3** was fourfold less effective than **1** despite a twofold improvement in enzyme inhibition. In contrast evaluation of a variety of electron withdrawing groups on the pyrrolidine nitrogen

Table 1
Pyrrolidine N-substitution SAR



Compd	R	QUICKIN[a] IC_{50} (nM)	Spread CIC_{95}[b] (nM)	
			10% FBS	50% NHS
1	Me	23	63	125
2	*c*-Pr	20	63	250
3	CH_2(3-isoxazole)	10	156	625
4	COMe	15	14	17
5	CO_2Me	10	31	63
6	$CONMe_2$	10	125	125
7	SO_2Me	15	31	125
8	SO_2NMe_2	9	31	125
9	CO-pyrazine	10	16	16
10	$COCONMe_2$	21	16	16

[a] Results are the mean of at least three independent experiments. SD was always ±8% of the value. Assays were performed with recombinant HIV-1 integrase preassembled on immobilized oligonucleotides. Inhibitors were added after assembly and washings, and IC_{50} is the concentration of inhibitor that reduces HIV integrase activity by 50%. For details see Ref. 8.

[b] Spread assay: 95% cell culture inhibitory concentrations for inhibition of the spread of HIV-1 infection in cell culture, using HIV-1IIIb and MT-4 T-lymphoid cells, in a medium containing 10% fetal bovine serum (FBS) or 50% normal human serum (NHS). Results are the mean of at least three independent experiments, SD was always <±10% of the value. For details see Ref. 9.

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doi:10.1016/j.bmcl.2010.07.042

Table 2
Pharmacokinetic profile of **1** in rat[a] and dog[b]

Species	Cl[c] (mL/min/kg)	F[d] (%)	$t_{1/2}$[e] (h)	AUC[f] (μM h)
Rat	21	94	1.7	4.5
Dog	4.4	116	3.7	23.1

[a] Dose: iv = po, 3 mg/kg. Formulation: iv, DMSO/PEG400/H_2O (20–40–40%); po, 1% methylcellulose.
[b] Dose: iv, 1 mg/kg; po, 2 mg/kg. Formulation: iv, DMSO/PEG400/H_2O (20–40–40%); po, 1% methylcellulose.
[c] Terminal phase plasma half life following iv administration.
[d] Oral bioavailability.
[e] Terminal phase plasma half life following iv administration.
[f] Area under the curve following po administration.

Table 3
Pharmacokinetic profile of pyrrolidine N-substituted analogs in rat[a]

Compd	Cl[b] (mL/min/kg)	F[b] (%)	$t_{1/2}$[b] (h)	AUC[b] (μM h)
4	64	31	0.3	1.2
5	46	44	0.6	1.2
8	64	16	nd[c]	nd[c]
9	77	18	0.2	2.3
10	138	19	1.6	0.2

[a] Dose: iv = po, 3 mg/kg. Formulation: iv, DMSO/PEG400/H_2O (20–40–40%); po, 1% methylcellulose.
[b] For footnote details see Table 2.
[c] Not determined.

gave pleasing results. For compounds **4**–**10** not only was intrinsic activity improved, but antiviral activity was at least maintained and usually enhanced. Amide analog **4** and carbamate **5** led to superior antiviral activity compared to the urea **6,** the sulfonamide 7, or the sulfamide **8**. *N*-Acetyl analog **4** displayed a low shift both from the intrinsic enzymatic activity to the activity in the low serum conditions (suggesting it has good cell penetration) and also between the low and high serum cell-based assay conditions. This latter result is probably a consequence of the significant fraction of compound unbound in the presence of plasma proteins,[4] as indeed confirmed by human plasma protein binding measurements (79% for **4** vs 94% for **1**[5]). In light of the promising activity displayed by acetamide **4**, extensive screening of pyrrolidine *N*-amides was conducted. Alternative alkyl or arylamides typically had unacceptable shifts from intrinsic enzymatic activity to the cell-based potency (data not shown) but heteroaryl amides (e.g., **9**) and oxalamides (e.g., **10**) maintained the potency profile of **4**, confirming them as privileged substituents for pyrimidone-based HIV-integrase inhibitors.[3,4] The most interesting compounds from the potency screening were evaluated in rat pharmacokinetics, where all derivatives displayed moderate to very high plasma clearance and moderate bioavailability (Table 3). Carbamate **5** and acetamide **4** exhibited the best profile amongst these compounds, but in all cases inferior PK properties with respect to the lead compound **1** were observed. The metabolic stability in rat liver microsomes of **4** was studied in the presence of NADPH and UDPGA to evaluate the metabolism rate by oxidation and glucuronidation: modest stability was observed in both cases with 40% and 43% of initial remaining after 1 h, respectively.[6] These findings, together with the low value of rat plasma protein binding, 63%,[5] were consistent with the high plasma clearance observed in vivo.

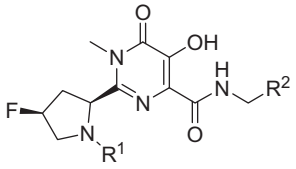
In order to align the attractive activity profile of our optimized pyrrolidine-*N*-amide and pyrrolidine-*N*-carbamoyl analogs we

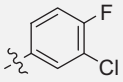
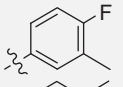
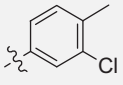
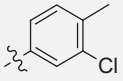
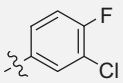
Table 5
Pharmacokinetic profiles of pyrrolidine amides and carbamates in rat[a] and dog[a]

Compd	Species	Cl[a] (mL/min/kg)	F[a] (%)	$t_{1/2}$[a] (h)	AUC[a] (μM h)
11	Rat	43	20	0.7	0.5
11	Dog	9.3	34	7.1	2.8
12	Rat	39	17	1.4	0.5
13	Rat	29	94	3.0	3.7
13	Dog	9.7	80	1.2	6.4
14	Rat	9.9	10	7.4	1.2
15	Rat	21	25	1.5	1.3

[a] For footnote details see Table 2.

Table 4
Benzylamide SAR on pyrrolidine amides and carbamates



Compd	R^1	R^2	QUICKIN[a] IC_{50} (nM)	Spread CIC_{95}[a] (nM)	
				10% FBS	50% NHS
11	COMe		24	3	17
12	COMe		27	16	31
13	COMe		32	5	46
14	CO_2Me		20	16	63
15	CO_2Me		19	16	31

[a] For footnote details see Table 1.

Table 6
Resistance profiles with HIV-1 containing site-directed integrase mutations[a]

Compd	T66I	V151I	F121Y	T125K	T66I[*b] M154I	T66I[*b] S153Y	N155S[*b]	T125K[*b] F121Y	T66I[*]/L74M[b] V151I
Raltegravir	1	1	3	1	1	1	10	8	6
4	nd[c]	nd[c]	nd[c]	nd[c]	2	5	15	15	10
11	2	1	4	1	3	3	31	17	31
13	1	1	1.5	1	0.8	0.8	8	3.5	2.5

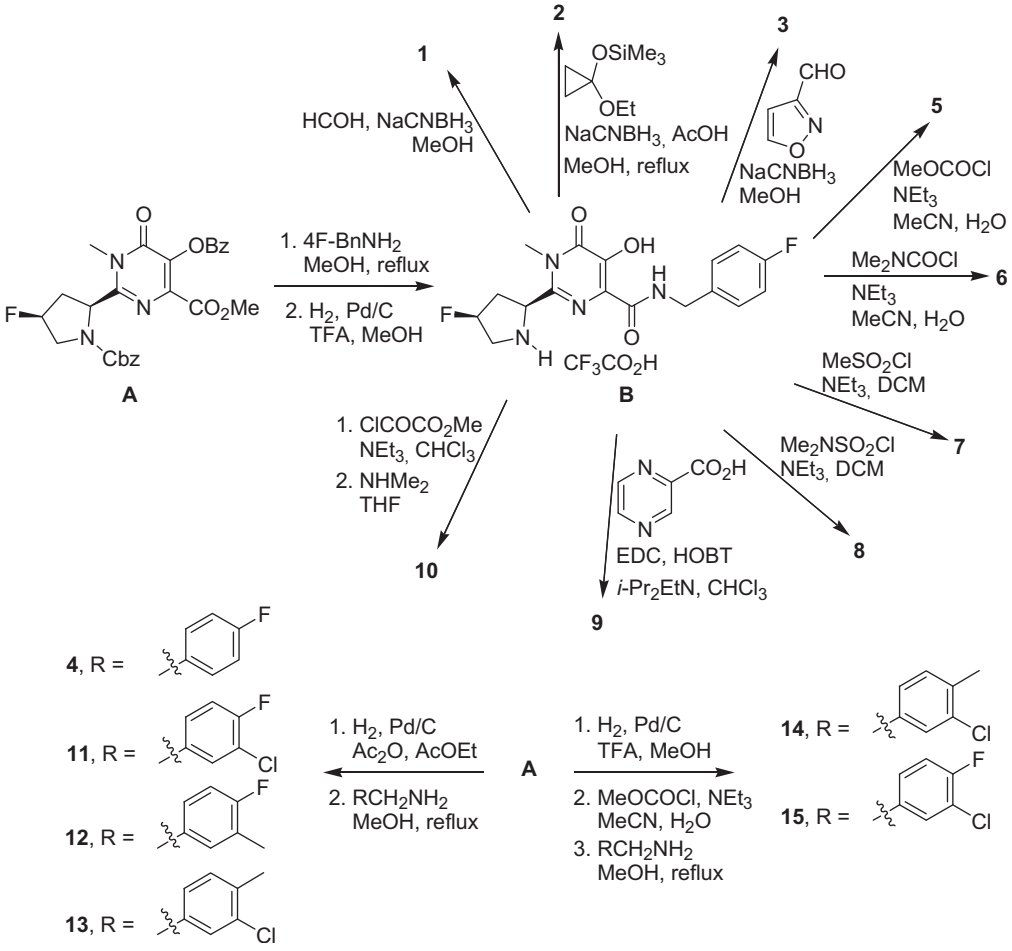
[a] Shift in IC_{50} relative to wild type HIV-1−single cycle infectivity assay.
[b*] Viruses that exhibit >50% reduction in specific infectivity.
[c] Not determined.

screened substituted benzylamides as alternatives to the 4-fluorobenzylamide fragment in the compounds **4** and **5**. The benzylamide moiety was known from previous work on the project to modulate the physicochemical properties of our inhibitors.[7]

After evaluating *N*-acetylpyrrolidine-based inhibitors containing an extensive variety of different benzylamides (results not shown), *meta-para*-di-substituted benzylamides **11**, **12,** and **13** emerged as the most interesting derivatives (Table 4). These compounds displayed an improvement in rat pharmacokinetics in terms of decreased plasma clearance when compared to the reference compound **4** and retained a similar level of cell-based potency (Tables 4 and 5).

Encouraged by these results, the benzylamide screening was extended to pyrrolidine carbamates leading to the identification of **14** and **15**. Similarly to what was observed for **5** when compared to **4**, these carbamates displayed a somewhat reduced antiviral potency when compared to the corresponding acetamides, but are characterized by a higher stability in rat pharmacokinetics being eliminated less rapidly (Tables 4 and 5). The improved stability in rat pharmacokinetic of **13** in comparison to the reference compound **4** may be attributed to its significantly larger fraction of compound bound to the plasma proteins (97.8% vs 63% bound, respectively),[5] and hence not available to be metabolized, which may exceed its reduced stability in liver microsomes (% of initial after 1 h: 8 vs 43 with UDPGA and 0.7 vs 40 with NADPH, respectively),[6] the net result being a reduced in vivo clearance.

Compounds **11** and **13** were selected for further evaluation, based on the best overall results in terms of potency and rat PK. Compounds **11** and **13** were then evaluated in dog pharmacokinetics study and both compounds were found to have moderate plasma clearance (9.3 and 9.7 mL/min/kg, respectively). In terms of oral bioavailability, compound **13** was however much superior to **11** (80% vs 34%, respectively) (Table 5). Compound **13** excelled also for its profile against a panel of HIV-integrase mutants selected in



Scheme 1.

our laboratories employing different classes of inhibitors (Table 6). In particular, **13** compared favorably on all the mutants to its analogs **11** and **4** and to the marketed drug Raltegravir.

The synthetic route used for **1**, comprising the advanced intermediate **A**, is fully described in a separate publication.[2] In Scheme 1 the synthetic routes for the assembly of the different derivatives are reported,[10] consisting of manipulation of **A** by benzylamide formation, pyrrolidine nitrogen deprotection, and functionalisation carried out in this or in an alternative order made using routine procedures that have been described in our previous work.[2]

In summary, optimization of **1** resulted in the identification of **13**, characterized by high potency in the cell-based assay, favorable PK profile when dosed orally to preclinical species and excellent mutant profile. Compound **13** thus represents a promising agent against HIV-1 and is the subject of further studies.

Acknowledgments

We thank Silvia Pesci for NMR analysis; Marina Taliani, Massimiliano Fonsi, and Francesca Naimo for plasma protein binding determination and microsome stability studies; Kara A. Stillmock, Peter J. Felock, and William A. Schlief for HIV biological testing; Steven Harper for proofreading the manuscript.

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4. Binding to plasma proteins reduces the effective concentration of drug at the target. See, for example: Muraglia, E.; Kinzel, O.; Gardelli, C.; Crescenzi, B.; Donghi, M.; Ferrara, M.; Nizi, E.; Orvieto, F.; Pescatore, G.; Laufer, R.; Gonzalez-Paz, O.; Di Marco, A.; Fiore, F.; Monteagudo, E.; Fonsi, M.; Felock, P. J.; Rowley, M.; Summa, V. *J. Med. Chem.* **2008**, *51*, 861. and references cited therein.
5. The test compound was dissolved in DMSO and incubated with human plasma at 37 °C for 1 h. The mixture was then spun in an ultrafiltration device and the filtrate was assayed for free drug by analytical LC/MS/MS detection.
6. The concentration of the test compound was 1 μM and microsomes 1 mg/mL. The degradation of the substrate was measured by LC/MS/MS after a 1 h incubation.
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10. Synthesis of **13**: **A** (102 mg, 0.20 mmol) dissolved in ethyl acetate (8 mL) was treated with Pd/C 10% (10 mg) and acetic anhydride (19 μL, 0.20 mmol) and submitted under H_2 atmosphere at room temperature. The reaction mixture was stirred at room temperature for 18 h and then was filtered through Celite. The filtrate was evaporated under reduced pressure, dissolved in MeOH (2.4 mL), treated with 3-Cl-4-Me-benzylamine (93 mg, 0.60 mmol) and refluxed for 48 h, then it was cooled down. The solvent was evaporated and the residue purified by RP-HPLC (stationary phase: column symmetry C18, 5 μm, 19 × 100 mm. Mobile phase: acetonitrile/H_2O buffered with 0.1% TFA). Fractions containing the pure compound were combined and freeze dried to afford **13** as a white powder (69.2 mg, 79% over two steps). ^{1}H NMR (DMSO-d_6, 300 MHz, 300 K) δ 12.06 (bs, 1H), 8.62 (t, J = 6.3 Hz, major rotamer) + 8.42 (t, J = 6.3 Hz, minor rotamer) (1H), 7.43–7.35 (m, 2H), 7.24–7.18 (m, 1H), 5.60–5.23 (m, 2H), 4.58–4.49 (m, 2H), 4.33–3.83 (m, 2H), 3.58 (s, 3H), 2.75–2.32 (m, 2H), 2.37 (s, 3H), 2.10 (s, major rotamer) + 1.91 (s, minor rotamer) (3H). MS: m/z 437 $(M+H)^+$.