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6-Benzylamino 4-oxo-1,4-dihydro-1,8-naphthyridines and 4-oxo-1,4-dihydroquinolines as HIV integrase inhibitors

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ABSTRACT

SAR studies on the quinolone carboxylic acid class of HIV-1 integrase inhibitors focused on improving the metabolic stability and led to the discovery of **27** and **38**.

Human immunodeficiency virus type 1 (HIV-1) integrase is an enzyme that facilitates the insertion of the viral DNA into the host cell genome. The enzyme achieves this via strand transfer, which involves removal of the terminal dinucleotide from each 3′-end of the viral DNA followed by subsequent joining of the 3′-end of the viral DNA to the host DNA.[1] This virally encoded enzyme is necessary for viral replication and thus represents a very attractive target for antiretroviral drugs.[2,3] There have been numerous reports of HIV-1 integrase strand transfer inhibitors (INSTIs) in the literature (Fig. 1), and one compound, raltegravir (**1**, Isentress) has been approved for the treatment of HIV.[4,5] Elvitegravir (**2**), another INSTI is currently in phase III trials.[6,7] This Letter outlines efforts to understand the SAR of **2** in order to develop novel integrase inhibitors with improved microsomal stability, while maintaining equal or better potency in human serum.

The general method for preparation of the compounds described herein is shown in the scheme below (Scheme 1).[8] Starting from an appropriately substituted nicotinic acid (**3**), bromination to give **4** followed by a one-pot sequence involving acid chloride formation and addition of ethyl-3-(dimethylamino)acrylate yielded intermediate **5**. Displacement with an amino alcohol to give **6**, followed by cyclization and TBDMS protection provided the core compound **7**. This core was then converted into benzyl-linked compounds **9** by Negishi coupling followed by hydrolysis or benzylamine-linked compounds **11** by Buchwald coupling followed by hydrolysis.

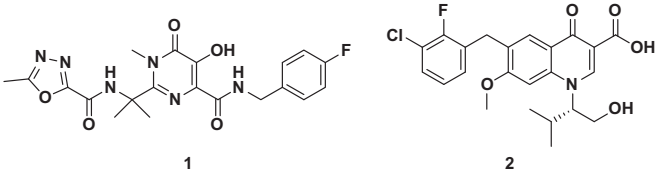

Figure 1. HIV-1 integrase inhibitors.

Table 1 shows the activity[9] of various compounds where a N atom has been incorporated into different positions around the quinoline core of (**2**), and demonstrates that the addition of the N atom was tolerated in only select positions. For example, addition of nitrogen in the 8 position while retaining the 7-methoxy group (**12**) gave a compound that displayed somewhat lower potency in human serum (136 nM vs 36 nM), but much improved metabolic stability. Other modifications were not as well tolerated. For example incorporation of nitrogen in the 8 (compound **14**) or 5 (**15**) positions in combination with a hydrogen replacing the methoxy in the 7 position resulted in a much greater loss of activity.

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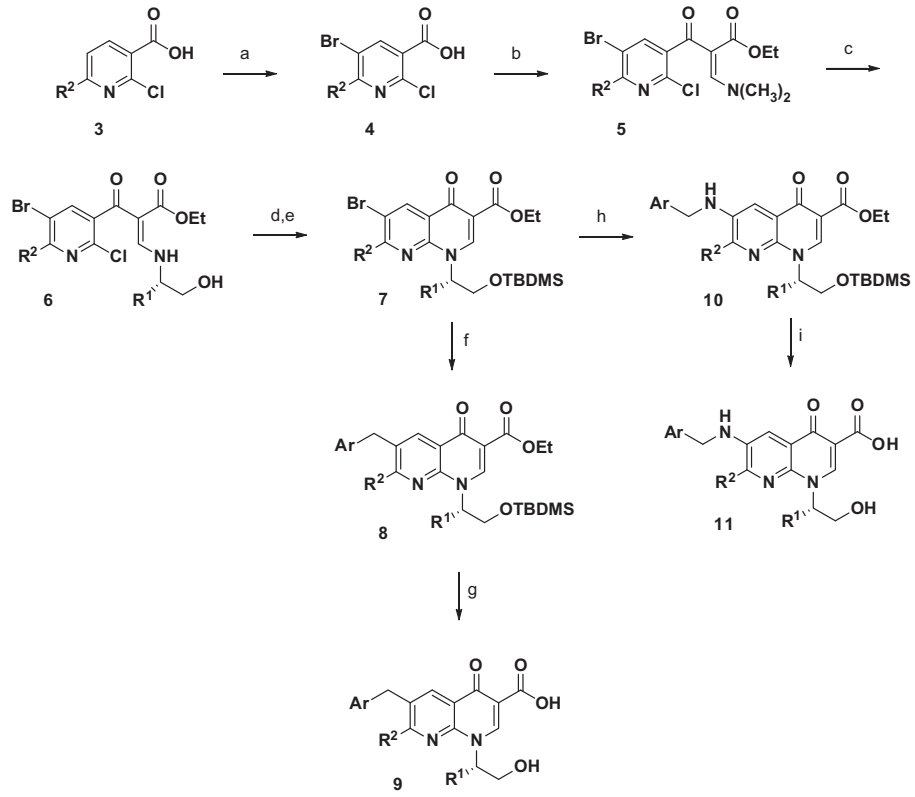
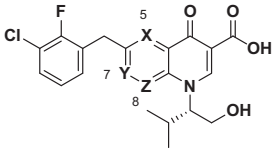


Scheme 1. (a) Br_2, AcOH; (b) (i) thionyl chloride, DMF, $PhCH_3$; (ii) ethyl-3-(dimethylamino)acrylate, Et_3N, THF; (c) amino alcohol, THF; (d) K_2CO_3, DMF; (e) TBMDS-Cl, imidazole, DMF; (f) benzylzinc bromide, $Pd(PPh_3)_2Cl_2$, THF; (g) NaOMe, H_2O, MeOH; (h) amine, $Pd(OAc)_2$, BINAP, Cs_2CO_3, dioxane; (i) NaOMe, H_2O, MeOH.

Table 1
SAR of the quinolone core



Compds	X	Y	Z	EC_{50}[a] (nM) (0/40% HS)	Microsomal stability[b] (% remaining)
2	CH	$C(OCH_3)$	CH	0.7/36	38
12	CH	$C(OCH_3)$	N	11/136	100
13	CH	N	CH	54/425	100
14	CH	CH	N	65/1937	99
15	N	CH	CH	>2500	ND
16	CH	N	$C(OCH_3)$	291/1772	ND

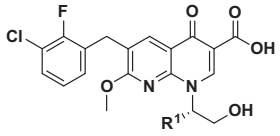
[a] Values are means of two or more experiments, coefficient of variation (CV) is <25%. HS = human serum.
[b] Human liver microsomes, 30 min incubation; ND = not determined.

Nitrogen incorporation at the 7 position gave a moderately active compound (**13**, 425 nM) which also displayed improved metabolic stability, but attempting to improve potency by adding a methoxy group (**16**) was not successful. Addition of multiple nitrogen atoms in the ring in all combinations led to inactive compounds (data not shown). Novel naphthyridine compound (**12**), with its high metabolic stability, thus became a new lead and the focus of further investigation.

The pendant amino alcohol group of naphthyridine (**12**) was examined, as shown in Table 2. Smaller groups such as methyl (**17**) were much less potent (1049 nM vs 136 nM for **12**). Ethyl (**18**) and butyl (**20**) groups lost approximately twofold activity. Interestingly, an *n*-propyl group (**19**) showed a much larger drop in activity compared to the parent *i*-propyl compound (1015 nM vs 136 nM). Other branched aliphatic groups were also more potent, including *t*-butyl (**21**) and isobutyl (**22**). In particular, *t*-butyl analog **21** showed an approximately twofold increase in potency in human serum (61 nM vs 136 nM) and thus became interesting for further investigation. Both **21** and **22** also displayed good microsomal stability.

Replacement of the methoxy group at the 7 position of naphthyridine (**21**) was examined and met with varying degrees of success, as shown in Table 3. Many groups in this position showed good to excellent activity, including ethoxy (**24**, 19 nM), hydroxyethyl (**26**, 2.4 nM) and morpholino (**27**, 19 nM), while hydroxyl (**23**, 199 nM) and isopropoxy (**25**, 195 nM) were less well tolerated. The microsomal stability of these compounds was good, with

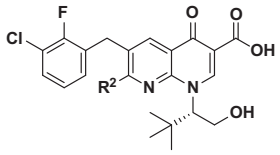
Table 2
SAR around the amino alcohol



Compds	R^1	EC_{50}[a] (nM) (0/40% HS)	Microsomal stability[b] (% remaining)
12	$-CH(CH_3)_2$	11/136	100
17	$-CH_3$	177/1049	ND
18	$-CH_2CH_3$	40/307	ND
19	$-CH_2CH_2CH_3$	180/1015	ND
20	$-CH_2(CH_2)_2CH_3$	20/245	ND
21	$-C(CH_3)_3$	8.6/61	82
22	Isobutyl	5.9/129	92

[a] Values are means of two or more experiments, coefficient of variation (CV) is <25%. HS = human serum.
[b] Human liver microsomes, 30 min incubation; ND = not determined.

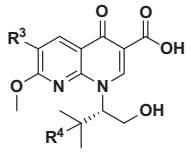
Table 3
Modification at the 7 position

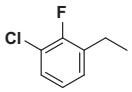
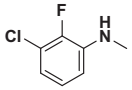
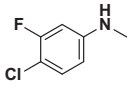
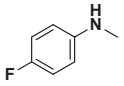
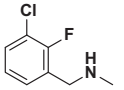
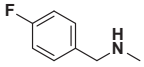
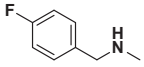


Compds	R^2	EC_{50}[a] (nM) (0/40% HS)	Microsomal stability[b] (% remaining)
21	$-OCH_3$	8.6/61	82
23	–OH	2.7/199	87
24	$-OCH_2CH_3$	1.4/19	84
25	$-OCH(CH_3)_2$	16/195	ND
26	$-OCH_2CH_2OH$	0.9/2.4	45
27		9.9/39	97

[a] Values are means of two or more experiments, coefficient of variation (CV) is <25%. HS = human serum.
[b] Human liver microsomes, 30 min incubation; ND = not determined.

Table 4
Linker modification



Compds	R^3	R^4	EC_{50}, nM (0/40% HS)[a]	Microsomal stability (% remaining)[b]
12		–H	11/136	100
28		–H	>2500/ND	ND
29		–H	>2500/ND	ND
30		–H	>2500/ND	ND
31		–H	490/2684	ND
32		–H	98/421	87
33		$-CH_3$	150/385	ND

[a] Values are means of two or more experiments, coefficient of variation (CV) is <25%. HS = human serum.
[b] Human liver microsomes, 30 min incubation; ND = not determined.

the exception of **26** (45% remaining after 30 min incubation). In particular, morpholino substituted naphthyridine compound **27** exhibited high microsomal stability (97% remaining after 30 min incubation) and relatively low (fourfold) serum shift. The further profiling of this compound is described below.

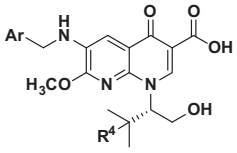
The benzyl linker between the naphthyridine core ring system of compound (**12**) and its pendant aromatic ring was examined, as shown in Table 4. Single heteroatom linkers such as anilines (**28**, regioisomer **29**, and 4-fluoro analog **30**) were not at all tolerated. Surprisingly, however, incorporation of an additional spacer to give a benzyl amine led to active compounds. Initially we synthesized benzyl amine **31** as a direct analog to **12** with 2-fluoro-3-chloro substitution on the aromatic ring. This gave a weakly active compound (2684 nM). However, incorporation of a 4-fluoro substituted benzyl amine as in **32** and **33** gave compounds that were only approximately three and sixfold less potent than **12** and **21** (421 and 385 nM vs 136 and 61 nM, respectively). In addition, compound **32** showed good microsomal stability (87% remaining after 30 min incubation). Thus, we decided to further investigate the benzyl amine portion of **32**.

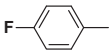
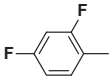
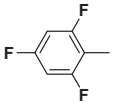
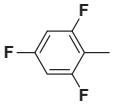
A wide range of substituents on the benzyl amine group of **32** were prepared, including methyl, methoxy and halo (data not shown), but in general fluoro substitution proved to be optimal (Table 5). For monosubstituted compounds, 4- (**32**) and 2- (**35**) substituted compounds were equipotent (421 and 283 nM) but 3-substitution (**34**) led to a fivefold loss of activity. 2,4-Disubstitution further increased potency (**36**, 139 nM vs 421 nM), and 2,4,6-trisubstitution proved optimal (**37**, 52 nM). Incorporating a *t*-butyl group on the amino alcohol portion (as optimized from Table 2) gave a compound (**38**) that was also potent (42 nM) and showed good microsomal stability (75% remaining after 30 min incubation). Compound **38** therefore also became one of our leads for further studies along with compounds **21** and **27**.

We initially examined the rodent pk for **21**, **27**, and **38**, with the results summarized in Table 6. The rat pk for **21**, **27**, and **38** was similar compared to **2**, with clearance, volume of distribution as well as half-life and bioavailability all falling within a roughly comparable range. As the rodent pk for all the compounds was similar, we then investigated higher species pk to attempt to differentiate between our lead compounds.

The monkey and dog pk after iv dosing for **27** and **38** is summarized in Table 7. In dog, the clearance and half-life of **27** (0.60 L/h/kg, 2.6 h) and **38** (0.93 L/h/kg, 2.0 h) were similar compared to **2** (0.60 L/h/kg, 3.6 h), although the clearance of both compounds in monkey was higher (1.18 L/h/kg and 2.65 L/h/kg for **27** and **38**, respectively, compared with 0.43 L/h/kg for **2**). This tracks well with the stability of **27** and **38** in dog liver microsomes (97% and 57%, respectively, remaining after 1 h incubation vs 84% for 2)

Table 5
Benzylamine modification



Compds	Ar	R^4	EC_{50}, nM (0/40% HS)[a]	Microsomal stability (% remaining)[b]
32		–H	98/421	87
34		–H	1458/1593	ND
35		–H	81/283	97
36		–H	4.6/139	87
37		–H	1.4/52	82
38		$-CH_3$	7.6/42	75

[a] Values are means of two or more experiments, coefficient of variation (CV) is <25%. HS = human serum.
[b] Human liver microsomes, 30 min incubation; ND = not determined.

Table 6
Rat PK for selected compounds

Compds	Cl_p[a] (L/h/kg)	V_d[a] (L/kg)	C_{max}[b] (ng/mL)	AUC[b] (ng h/mL)	$t_{1/2}$[b] (h)	%F[b]
2	0.23	2.74	1140	5630	2.9	26
21	0.16	2.31	1230	8500	2.9	31
27	0.42	4.02	414	2700	2.6	26
38	0.35	4.18	666	2890	3.7	23

[a] Dosed 2 mpk iv.
[b] Dosed 5 mpk po.

Table 7
Dog and monkey PK for selected compounds

Compds	Cl_p[a] (L/h/kg)	V_d[a] (L/kg)	$t_{1/2}$[a] (h)	Cl_p[b] (L/h/kg)	V_d[b] (L/kg)	$t_{1/2}$[b] (h)
2	0.60	2.38	3.6	0.43	1.99	3.2
27	0.60	1.03	2.6	1.18	3.06	8.1
38	0.93	1.93	2.0	2.65	3.93	2.2

[a] Dosed 1 mpk iv in beagle dogs.
[b] Dosed 1 mpk iv in rhesus monkeys.

and somewhat well in monkey liver microsomes (83% and 8%, respectively, remaining after 1 h incubation vs 4% for **2**).

In conclusion, we have developed a new series of naphthyridinone-containing HIV-1 integrase inhibitors that show much improved human microsome stability (75–97% vs 38% for compound **2**), excellent potency in the low nanomolar range in the absence or presence of 40% human serum, as well as acceptable pk parameters in multiple species. From these efforts two compounds, **27** and **38**, were selected for further development.

References and notes

1. Craigie, R. *J. Biol. Chem.* **2001**, *276*, 23213; Engelman, A.; Mizuuchi, K.; Craigie, R. *Cell* **1991**, *67*, 1211.
2. Pommier, Y.; Johnson, A. A.; Marchand, C. *Nat. Rev. Drug Disc.* **2005**, *4*, 236.
3. Serrao, E.; Odde, S.; Ramkumar, K.; Neamati, N. *Retrovirology* **2009**, *6*, 25.
4. Deeks, S. G.; Kar, S.; Gubernick, S. I.; Kirkpatrick, P. *Nat. Rev. Drug Disc.* **2008**, *7*, 117.
5. 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.; Monteagudo, 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.
6. 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.
7. Sato, M.; Motomura, T.; Aramaki, H.; Matsuda, T.; Yamashita, M.; Ito, Y.; Kawakami, H.; Matsuzaki, Y.; Watanabe, W.; Yamataka, K.; Ikeda, S.; Kodama, E.; Matsuoka, M.; Shinkai, H. *J. Med. Chem.* **2006**, *49*, 1506.
8. For complete schemes and experimental procedures, including for compounds **13**–**15**, see: Chen, H.; Song, J.; Vernier, J.-M.; Pinkerton, A. B.; Nagasawa, J. Y. PCT WO 2009/089263.
9. The antiviral activity of the compounds was characterized essentially as previously described[10] using VSV-G protein pseudotyped HIV-1 luciferase reporter virus isolates. Antiviral assays were carried out in 96-well tissue culture plates with compounds serially diluted from DMSO stocks into culture medium supplemented with 2% fetal bovine serum and either 0% or 40% human serum. The compounds were added to a mixture of HeLa-JC53 cells and virus and maintained at 37 °C and 5% CO_2 for 48 h. Following incubation, luciferase detection reagent was added to each well and luminescence was measured on a luminometer plate reader. The EC_{50} values were determined by non-linear regression analysis using GraphPad Prism4 software. The SAR was guided by the potency of the compounds in the presence of 40% human serum as an estimation of the antiviral activity of the compound while circulating in the presence of plasma proteins in vivo.
10. Zhang, Z.; Xu, W.; Koh, Y.-H.; Shim, J. H.; Girardet, J.-L.; Yeh, L.-T.; Hamatake, R. K.; Hong, Z. *Antimicrob. Agents Chemother.* **2007**, *51*, 429.