

Madurahydroxylactone Derivatives as Dual Inhibitors of Human Immunodeficiency Virus Type 1 Integrase and RNase H[∇]

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A series of 29 madurahydroxylactone derivatives was evaluated for dual inhibition of human immunodeficiency virus type 1 (HIV-1) integrase and RNase H. While most of the compounds exhibited similar potencies for both enzymes, two of the derivatives showed 10- to 100-fold-higher selectivity for each enzyme, suggesting that distinct pharmacophore models could be generated. This study exemplifies the common and divergent structural requirements for the inhibition of two structurally related HIV-1 enzymes and demonstrates the importance of systematically screening for both integrase and RNase H when developing novel inhibitors.

Madurahydroxylactone (MHL) (Fig. 1), a secondary metabolite from the soil bacterium *Nonomuraea rubra*, belongs to the family of benzo[*a*]naphthacenequinone antibiotics. The benzo[*a*]naphthacenequinones have a broad spectrum of biological activities and include the antifungal and antiviral agents benanomycin and pradimicin (10, 11, 18). Some MHL derivatives have been reported to offer improved antibacterial activities compared to those of the natural parent MHL (7). Some other derivatives have also been found to inhibit estrone sulfatase, an enzyme involved in regulating the supply of estrogens to estrogen-dependent breast tumors (8).

Human immunodeficiency virus type 1 (HIV-1) integrase and the HIV-1 RNase H domain of reverse transcriptase are two novel antiviral targets (9, 13) that share structural similarities (1). DNA aptamer inhibitors of RNase H can inhibit HIV-1 integrase (4), and conversely, HIV-1 RNase H can be inhibited by some diketo acid inhibitors of integrase (17, 19). Recently, tropolone derivatives have been reported to inhibit both enzymes (2, 5, 16). These results represent a proof of concept for the dual inhibition of integrase and RNase H by structurally related compounds and provide a rationale for discovering and elucidating the mechanisms of action of inhibitors of these two enzymes. Here we report a comparison of a series of MHL derivatives for the inhibition of HIV-1 integrase and HIV-1 RNase H. The structural requirements for the inhibition of integrase versus those of RNase H are discussed.

A 29-compound series of novel MHL derivatives (7, 8) (Fig. 1) was tested against HIV-1 integrase using an electrochemi-

luminescent, high-throughput strand transfer assay (6). In this 96-well-plate-based assay, a biotinylated 3'-end-preprocessed donor DNA substrate is incubated for 30 min at 37°C with 250 nM of recombinant integrase. After the addition of the drug, the reaction is initiated by the addition of a ruthenium-labeled duplex target DNA. The reaction is carried out for 60 min at 37°C, and the plates are subsequently read on a BioVeris M series analyzer (BioVeris Inc., Gaithersburg, MD). The same series of compounds was tested against HIV-1 RNase H, using a fluorescence resonance energy transfer high-throughput assay (12). In this 384-well-plate-based assay, the drug is added to 0.16 nM of a 3'-fluorescein 5'-DABCYL RNA/DNA hybrid, and the reaction is initiated by the addition of 7.5 nM of HIV-1

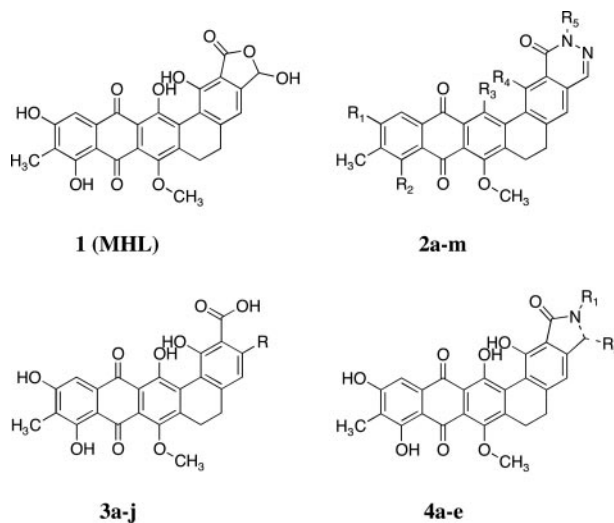
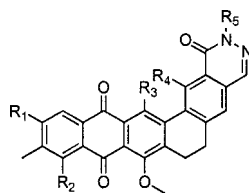


FIG. 1. Chemical structures of the MHL derivatives tested.

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TABLE 1. IC₅₀ values for HIV-1 integrase and RNase H inhibition by compounds 2a to 2m

Compound	Chemical structure ^d at position:					IC ₅₀ (μM) for:	
	R1	R2	R3	R4	R5	Integrase ^a	RNase H ^b
2a	OH	OH	OH	OH	Ph	0.41 ± 0.14	2.58 ± 0.48
2b	OH	OH	OH	OH	CH ₂ -COOEt	2.67 ± 1.26	2.11 ± 0.43
2c	OH	OH	OH	OH	C-(NH)NH ₂	3.02 ± 0.39	5.28 ± 0.18
2d	OH	OH	OH	OH	Ph-COOH	3.45 ± 0.25	9.11 ± 1.94
2e	OH	OH	OH	OH	H	4.06 ± 0.10	4.06 ± 0.17
2f	OH	OH	OH	OH	CH ₂ -COOH	4.62 ± 0.92	10.20 ± 1.68
2g	OH	OH	OH	OH	(CH ₂) ₂ -O-COMe	6.28 ± 0.38	8.63 ± 0.41
2h	OH	OH	OH	OH	(CH ₂) ₂ -CH ₃	6.34 ± 1.41	8.96 ± 1.69
2i	OBn	OBn	OBn	OBn	CH ₂ -COOH	106.00 ^c	22.4 ± 7.5
2j	OCOOMe	OH	OH	OH	Ph	200.00 ^c	1.43 ± 0.21
2k	OCOOMe	OAc	OH	OH	(CH ₂) ₂ -CH ₃	>333.00 ^c	8.98 ± 1.04
2l	OAc	OAc	OAc	OAc	Ph	>333.00 ^c	3.14 ± 0.36
2m	OAc	OAc	OAc	OAc	(CH ₂) ₂ -CH ₃	>333.00 ^c	10.50 ± 0.48

^a Values are means ± standard deviations from at least three independent experiments, unless otherwise noted.

^b Values are means ± standard errors from a single experiment.

^c Values are from a single experiment.

^d Ph, phenyl; Et, ethyl; Bn, benzyl; Me, methyl; Ac, acetyl.

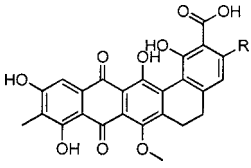
RNase H. The reaction is carried out for 30 min at room temperature and the fluorescence intensity assessed after EDTA quenching.

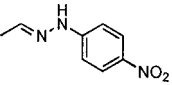
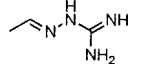
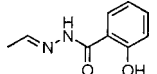
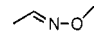

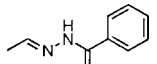
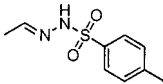
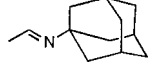
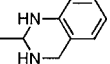
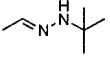
Fifty percent inhibitory concentration (IC₅₀) values for both assays and the chemical structures are presented in Tables 1 to 3. All compounds inhibit HIV-1 RNase H, with IC₅₀ values ranging from 0.3 to 22 μM and three compounds showing submicromolar IC₅₀ values. The IC₅₀ values for compounds 3j (Table 2), 4d, and 4e (Table 3) against RNase H are 0.7, 0.3, and 0.8 μM, respectively. In contrast, not all of the compounds inhibit HIV-1 integrase. Compounds 2k, 2l, and 2m do not show any integrase inhibition at concentrations up to 333 μM (Table 1). Compound 2a is the most potent integrase inhibitor, with an IC₅₀ value of 0.41 μM (Table 1). It also exerts a 20-fold strand transfer selectivity compared to 3'-end-processing inhibition (data not shown). The replacement of the hydroxyl group at the R1 position of compound 2a with a methoxycarbonyl group is sufficient to abolish HIV-1 integrase inhibition without affecting the potency for RNase H (compare compounds 2a and 2j in Table 1). Another requirement for integrase selectivity is the presence of an aromatic ring on the R5 position of compound 2a. The removal of this phenyl ring results in a 10-fold decrease in integrase selectivity (compare compounds 2a and 2e in Table 1), indicating a possible hydrophobic interaction between this portion of the molecule and integrase residues. Another structural requirement for selectivity can be derived from the compound series 3a to 3j (Table 2). The replacement of the nitrophenyl group on integrase-selective compound 3a by a phenylketone group from compound 3f abolishes selectivity for integrase (Table 2). Subsequent replacement of the phenylketone group with a *t*-butyl group leads to compound 3j, which now exhibits a >100-fold

increase in selectivity for RNase H (Table 2). This result is also in agreement with a potential hydrophobic interaction between this region of the molecule and integrase residues. By the same token, the replacement of the 1,3-piperazine ring of compound 4c by the phenylthiazole group of compound 4d or by the phenyldiazine group of compound 4e increases the selectivity for RNase H of these compounds by approximately 40- or 20-fold, respectively (Table 3). These results indicate that subtle structural modifications of the MHL derivatives can influence their potency against HIV-1 integrase and HIV-1 RNase H. They also suggest that the structural requirements for integrase selectivity seem more stringent than those for RNase H. All together, these results demonstrate that within the same chemical family, a compound can be "tuned" to favor integrase and/or RNase H inhibition.

Figure 2 shows a reciprocal plot of IC₅₀ values for integrase and RNase H. Most of the compounds are clustered in the center of the graph, highlighting the dual-inhibitor feature of the MHL derivatives. Almost half of the compounds (12 out of 29) fall within a threefold range of dual activities against both integrase and RNase H. Two compounds, 2a and 4d, show submicromolar IC₅₀s for integrase and RNase H, respectively, while being approximately 10- to 100-fold more selective for the other enzyme (Fig. 2). Four compounds (2l, 2j, 4e, and 3j) offer an approximately 100-fold increase in selectivity for RNase H over integrase (Fig. 2). These results indicate differential structural requirements for the inhibition of the two structurally related HIV-1 integrase and RNase H enzymes. They also suggest that a distinct pharmacophore model could be generated for each enzyme.

MHL derivatives, due to their broad spectrum of activities, may represent a useful platform for the development of anti-

TABLE 2. IC₅₀ values for HIV-1 integrase and RNase H inhibition by compounds 3a to 3j


Compound	Chemical structure	IC ₅₀ (μM) for:	
		Integrase ^a	RNase H ^b
3a		2.20 ± 0.74	14.0 ± 5.2
3b		3.15 ± 1.54	5.65 ± 0.40
3c		6.09 ± 0.21	3.54 ± 0.45
3d		12.50 ^c	10.10 ± 0.77
3e		13.20 ^c	3.48 ± 0.28
3f		14.60 ^c	3.10 ± 0.76
3g		22.00 ^c	5.88 ± 0.28
3h		37.00 ^c	9.41 ± 0.70
3i		40.00 ^c	5.86 ± 0.72
3j		122.00 ^c	0.69 ± 0.13

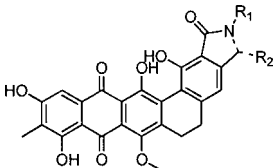
^a Values are means ± standard deviations from at least three independent experiments, unless otherwise noted.





^b Values are means ± standard errors from a single experiment.

^c Values are from a single experiment.

integrase or anti-RNase H inhibitors. One of the two integrase inhibitors currently in phase III clinical trials has been developed based on a quinolone antibiotic core (3, 14, 15). These MHL derivatives will have to be further characterized to determine their mechanisms of action and to better understand the mechanisms driving selectivity.

Finally, the fact that a large fraction of the MHL derivatives inhibited both integrase and RNase H demonstrates the importance of systematically screening for both HIV enzymes when developing novel inhibitors for either one of them. Although it is suitable to develop a highly specific inhibitor for a particular pharmacological target, it is also plausible that dual inhibitors of HIV-1 integrase and RNase H could be used in the treatment of HIV/AIDS (1). Recently, "portmanteau" inhibitors merging a diketo acid moiety with a nonnucleoside reverse transcriptase inhibitor have rationally been designed to achieve dual inhibition of HIV-1 integrase and reverse transcriptase (20). A similar approach could also be applied to

TABLE 3. IC₅₀ values for HIV-1 integrase and RNase H inhibition by compounds 4a to 4e


Compound	Chemical structure at position:		IC ₅₀ (μM) for:	
	R1	R2	Integrase ^a	RNase H ^b
4a	N(CH ₃) ₂	OH	7.70 ± 2.36	6.97 ± 0.72
4b			20.00 ^c	15.7 ± 1.2
4c			56.00 ^c	7.16 ± 0.66
4d			96.00 ^c	0.31 ± 0.11
4e			108.00 ^c	0.78 ± 0.12

^a Values are means ± standard deviations from at least three independent experiments, unless otherwise noted.

^b Values are means ± standard errors from a single experiment.

^c Values are from a single experiment.

HIV-1 integrase and RNase H. In such cases, antiviral activity might justify further mechanistic studies to validate the dual targeting of HIV-1 integrase and HIV-1 RNase H in the development of novel anti-HIV drugs.

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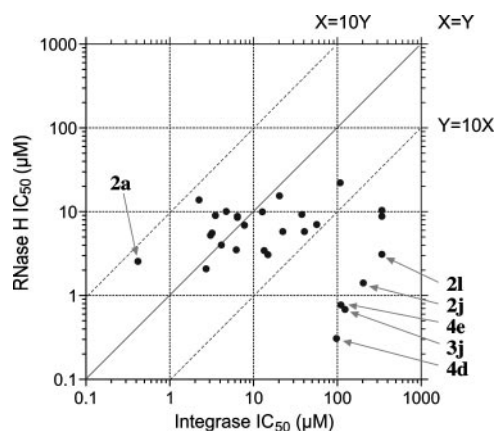


FIG. 2. Correlation between HIV-1 integrase and RNase H inhibition.

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