Inhibitors of Human Immunodeficiency Virus Type 1 Protease Containing 2-Aminobenzyl-Substituted 4-Amino-3-hydroxy-5-phenylpentanoic Acid: Synthesis, Activity, and Oral Bioavailability

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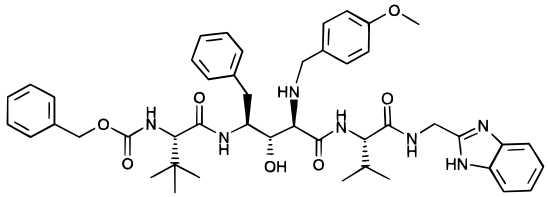
Systematic modifications of HIV protease inhibitor (2*R*,3*S*,4*S*)-4-[[(benzyloxycarbonyl)-L-valyl]-amino]-3-hydroxy-2-[(4-methoxybenzyl)amino]-5-(phenylpentanoyl)-L-valine 2-(aminomethyl)-benzimidazole amide led to a novel series of inhibitors with a shortened, modified carboxy terminus. Their synthesis, *in vitro* enzyme inhibitory data, and antiviral activities are reported. Of particular interest are derivatives featuring the (1*S*,2*R*)-1-amino-2-hydroxyindan moiety at the P2′-position since some of them exhibit substantial oral bioavailability in mice. The influence of aqueous solubility and structural parameters on the oral resorption of the inhibitors is discussed. Optimum enhancement of oral bioavailability was observed with L-*tert*-leucine in P2-position, resulting in the discovery of (2*R*,3*S*,4*S*)-4-[[(benzyloxycarbonyl)-L-*tert*-leucyl]-amino]-3-hydroxy-2-[(4-methoxybenzyl)amino]-5-phenylpentanoic acid (1*S*,2*R*)-1-amino-2-hydroxyindan amide which combines high antiviral activity (IC_{50} = 250 nM) with a good pharmacokinetic profile (AUC = 82.5 μM·h at a dose of 125 mg/kg po in mice).

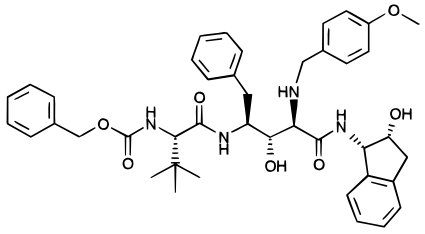
Introduction

The protease of the human immunodeficiency virus type 1 (HIV-1 PR) is essential for replication of the virus[1] and, hence, is regarded as one of the most promising targets for chemotherapy of HIV infection. First reports of clinical efficacy of HIV PR inhibitors[2−4] demonstrated the validity of this therapeutic principle. High *in vitro* potency is readily obtained with a variety of recently described inhibitors (see refs 5 and 6 for reviews), but clinical efficacy, in addition, strongly depends on pharmacological factors, such as oral bioavailability and duration of action. Unfortunately, in the field of peptidomimetic PR inhibitors, these factors are currently not amenable to rational prediction. For example, in the case of renin inhibitors no correlation between their partition coefficients and oral absorption or duration of action could be found.[7] It is thought, however, that lower molecular weight, reduced lipophilicity, diminished propensity to form hydrogen bonds, and a smaller number of amide bonds might contribute to improved pharmacokinetic properties of peptidic compounds. Since these are obviously only rough guidelines, the discovery of new chemotypes of HIV PR inhibitors exhibiting both potent anti-HIV activity and high oral bioavailability still remains a challenge.

In a previous report[8] we described structure−activity relationships in a series of HIV PR inhibitors containing 2-heterosubstituted 4-amino-3-hydroxy-5-phenylpentanoic acid (AHPPA). This study led to compounds with a benzimidazole group in P3′-position, *e.g.*, compound **1** (Figure 1) which selectively inhibits HIV-1 PR (K_i = 3.4 nM) and shows potent antiviral activity *in vitro* (IC_{50} = 27 nM), establishing 2-heterosubstituted AHPPA as a new HIV PR inhibitor core. However, these inhibitors did not show significant oral bioavailability in mice or rats.[9] In this report, we describe a novel series of derivatives with a reduced number of peptide bonds and lower molecular weight than **1**. The combination of 2-heterosubstituted AHPPA as dipeptide mimetic and the 2-aminoindanol moiety[10] in the P2′-position, as exemplified by compound **2** (Figure 1), led to orally bioavailable inhibitors of HIV-1 with potent antiviral activity.



1, lead compound from previous study [8]



2, orally bioavailable inhibitor of present study

Figure 1. Chemical structures of AHPPA-containing HIV PR inhibitors **1** and **2**.

Chemistry

The general route for the syntheses of 2-heterosubstituted derivatives of AHPPA is illustrated for compound **2** in Scheme 1. The key intermediate, a mixture of the diastereomeric oxiranes **3a,b** (ratio = 9:1), is readily available via a three-step procedure starting from *N*-(*tert*-butyloxycarbonyl)phenylalaninol.[8] As shown previously, this mixture can be used in the next step without further separation. When the mixture of epoxides **3a,b** was reacted with *p*-methoxybenzylamine in ethanol, only compound **4** was obtained in a diaste-

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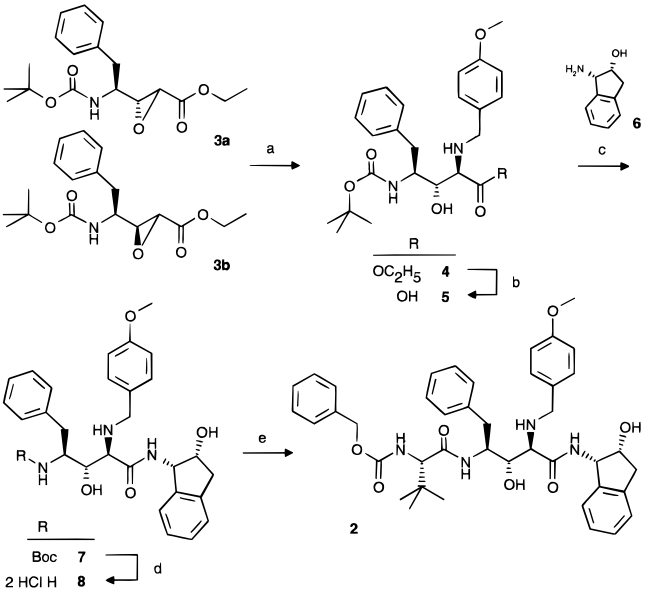
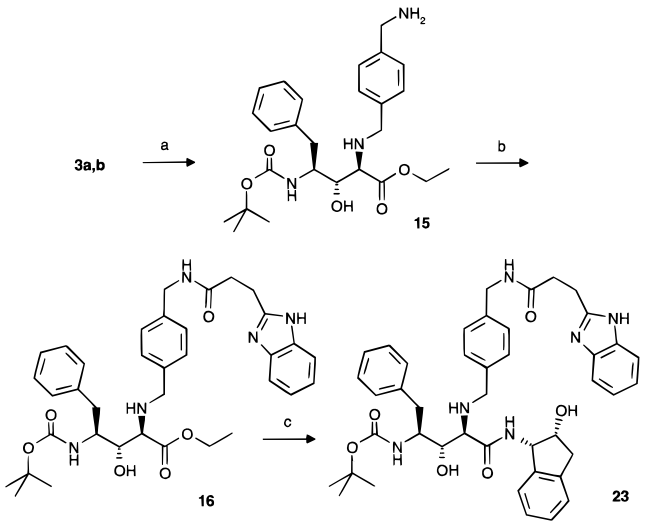
Scheme 1. Synthesis of HIV-1 PR Inhibitors Containing 2-Aminobenzyl-Substituted AHPPA[a]



[a] (a) 4-Methoxybenzylamine, EtOH, 70 °C; (b) 1 N LiOH, THF, room temperature; (c) (1*S*,2*R*)-1-amino-2-hydroxyindan (**6**), HOBt, EDC·HCl, DMF, room temperature; (d) 3 N HCl in diethyl ether, dichloromethane, room temperature; (e) Z-*tert*-leucine, PyBOP, diisopropylethylamine, DMF, room temperature.

Scheme 2. Synthesis of P1′-Modified HIV-1 PR Inhibitors Containing 2-Aminobenzyl-Substituted AHPPA[a]



[a] (a) α,α′-Diamino-*p*-xylene, EtOH, 80 °C; (b) (2-benzimidazolyl)propionic acid, HOBt, EDC·HCl, DMF, room temperature; (c) (i) 1 N LiOH, THF, room temperature, (ii) (1*S*,2*R*)-1-amino-2-hydroxyindan (**6**), HOBt, EDC·HCl, DMF, room temperature.

reomerically pure form after chromatographic workup. The ester group of **4** was saponified to give acid **5**, which was coupled with 1(*S*)-amino-2(*R*)-hydroxyindan (AHI, **6**)[10] to yield **7**. Deprotection of the amino group in **7** generated **8**, which was subsequently coupled with Cbz-*tert*-leucine to afford inhibitor **2**.

The P1′-modified inhibitors **20**−**23** (Table 2) were synthesized as exemplified for compound **23** in Scheme 2: Thus, epoxide **3** was treated with 1,4-diamino-*p*-xylene to produce amino derivative **15**, and subsequent acylation gave derivative **16**. Selective alkaline hydrolysis of the ethyl ester function in **16** followed by coupling with **6** led to inhibitor **23**.

Inspired by the procedure of Thompson *et al.*,[11] we developed an alternative stereospecific approach for the preparation of AHI (**6**). Instead of derivation of racemic *cis*-aminoindanol with a chiral auxiliary for separation of diastereomers at the end of the reaction sequence, we performed the resolution of racemic *trans*-aminoindanol at an early stage of the synthesis by diastereomeric salt formation.[12] Using (+)-dibenzoyl-D-tartrate, only the (+)-enantiomer of *trans*-aminoindanol crystallized from ethanolic solution. The pure enantiomer of the free base was transformed to AHI following the published protocol (see the Experimental Section for details).

Boc-*tert*-leucine derivative **28**, synthesized according to the general route (Scheme 1), was the starting point for the synthesis of compounds **29**−**39** (Table 4). After removal of the Boc group, the amino terminus was acylated with various carboxylic acids. Substituted thioacetic acids used in the preparation of derivatives **30**−**32** were synthesized by the method of Mikitenko *et al.*[13]

Results and Discussion

HIV PR inhibitor **1** (Figure 1) and its derivatives which we described previously[8,9] contain two amino acids filling the S2 and S2′[14] sites of HIV PR.[15] Replacement of these amino acids by N-terminal Boc and C-terminal benzyl groups yielded an inhibitor of small size (compound **9**, Table 1) showing weak activity against the enzyme but, interestingly, slightly higher bioavailability in mice (see Table 6) than the potent inhibitor **1**.

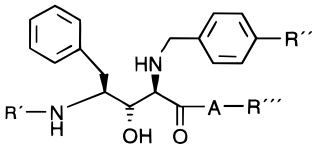
Since the corresponding ethyl ester of compound **9** (data not shown) was devoid of any activity at the enzymatic level, we investigated whether the C-terminal benzylamino group is responsible for the observed intrinsic activity. Compounds **12** with valine in position P2′ and **11** with phenylglycine replacing valine in **12** were synthesized. Equal potency in enzyme inhibition indicates that a phenyl substituent is well tolerated at this position.

In compound **10**, phenylglycinol was placed in P2′ replacing the benzylamino group in **9**. The hydroxy group of the phenylglycinol moiety was intended to interact with the NH of Asp29 of the enzyme. An analogous hydrogen bond is observed with the P2′-carbonyl oxygen in previously reported HIV PR inhibitors.[16] However, even at 3 μM, derivative **10** did not inhibit HIV PR. A similar result with phenylglycinol as terminus was reported by Tucker *et al.* in the case of HIV PR inhibitors containing the hydroxyethylamine isostere.[17]

Incorporation of AHI[10] in place of the P2′ benzylamino moiety led to inhibitor **7** with 40-fold increased potency relative to **9**. AHI had been described previously as an effective surrogate for the P2′ amino acid in HIV PR inhibitors containing a hydroxyethylene isostere[10] but was not beneficial in other inhibitors.[18] However, despite the improved K_i value, compound **7** was devoid of any antiviral activity.

In order to modify the pharmacokinetic properties, *e.g.*, oral uptake, we decided to elongate stepwise the P1′ side chain and the backbone of **9**. Molecular models of HIV PR complexed with **9** suggested that elongated moieties in the para-position of the P1′ benzylamino

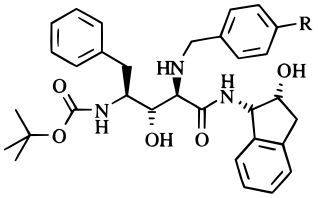
Table 1. Truncated AHPPA-Containing Inhibitors: Inhibitory Activity against HIV-1 PR and Antiviral Activity against HIV-1, IIIB, in MT4 Cells



no.	R′	R″	A	R‴	K_i (nM)[a]	IC_{50} (nM)[b]	mp (°C)	formula[c]
1	Z-Val	OCH_3	Val	$HNCH_2$-2-benzimidazolyl	3.4	25 ± 17 (17)	231−234	$C_{46}H_{57}N_7O_7$
7	Boc	OCH_3	bond	HN(2(*R*)-hydroxyindan-1(*S*)-yl)	65	*d*	183−185	$C_{33}H_{41}N_3O_6 \cdot 0.2\ H_2O$
9	Boc	H	bond	$HNCH_2Ph$	2500	*d*	50−53	$C_{30}H_{37}N_3O_4 \cdot 1.3\ H_2O$
10	Boc	OCH_3	bond	1(*S*)-HNCH(Ph)CH_2OH	*d*	*d*	65−68	$C_{33}H_{43}N_3O_6$
11	Boc	OCH_3	Phg	$HNCH_2$-2-benzimidazolyl	11	380 ± 52 (4)	104−109	$C_{40}H_{46}N_6O_6$
12	Boc	OCH_3	Val	$HNCH_2$-2-benzimidazolyl	7.7	510 ± 163 (5)	oil	$C_{37}H_{48}N_6O_6$
13	Boc	$O(CH_2)_2OH$	bond	$HNCH_2Ph$	1200	*d*	62−64	$C_{32}H_{41}N_3O_6 \cdot 0.5\ H_2O$
14	Boc	*e*	bond	$HNCH_2Ph$	680	*d*	111−115	$C_{41}H_{48}N_6O_5 \cdot 1.0\ H_2O$

[a] Usual standard deviation ± 20%. [b] Mean value ± standard deviation. Number of determinations given in parentheses. [c] Satisfactory elemental analyses within ± 0.4% of the calculated value for C,H,N were obtained. [d] No activity at 3 μM. [e] $CH_2NHCO(CH_2)_2$-2−benzimidazolyl.

Table 2. Truncated Inhibitors Modified in P1′: Inhibitory Activity against HIV-1 PR and Antiviral Activity against HIV-1, IIIB, in MT4 Cells



no.	R	K_i (nM)[a]	IC_{50} (nM)[b]	mp (°C)	formula[c]
7	O-CH_3	65	*d*	183−185	$C_{33}H_{41}N_3O_6 \cdot 0.2\ H_2O$
17	O-CH_2-CH_3	92	*d*	65−69	$C_{34}H_{43}N_3O_6$
18	O-CH_2-CH_2-OH	80	4000 (1)	77−82	$C_{34}H_{43}N_3O_7$
19	O-CH_2-CH_2(4-morpholinyl)	130	*d*	71−76	$C_{38}H_{50}N_4O_7$
20	CH_2-NH-CO-CH_3	70	2600 (1)	94−98	$C_{35}H_{44}N_4O_6$
21	CH_2-NH-CO-NH-Ph	33	*d*	100−104	$C_{40}H_{47}N_5O_6 \cdot 0.2$dioxane
22	CH_2-NH-CO-O-CH_2-Ph	19	*d*	73−76	$C_{41}H_{48}N_4O_7$
23	$CH_2NHCO(CH_2)_2$-2-benzimidazolyl	41	4400 (1)	128−132	$C_{43}H_{50}N_6O_6 \cdot 2.5H_2O$

[a] Usual standard deviation ± 20%. [b] Number of determinations given in parentheses. [c] Satisfactory elemental analyses within ± 0.4% of the calculated value for C,H,N were obtained. [d] No activity observed at 10 μM.

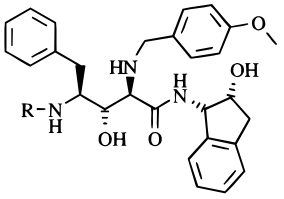
group might reach into the S3′ pocket of the enzyme.[15] Introduction of polar and charged residues in the para-position of the P1′ residues of HIV PR inhibitors was also used by Young *et al.* to enhance aqueous solubility and hence oral bioavailability.[19] Along these lines, compounds **13** and **14** were synthesized and found to be 2−4-fold better enzyme inhibitors than **9** (Table 1). These promising modifications were now applied to improve the anti-HIV activity of **7** bearing AHI in P2'. Introduction of the ethoxy (**17**), hydroxyethoxy (**18**), or morpholinoethoxy (**19**) group (Table 2) resulted in increase of the K_i value. In contrast, with acylated aminomethylene side chains, this activity was maintained (**20**) or, in the case of aromatic groups (**21**−**23**), even improved. Unexpectedly, no antiviral activity was detectable even for the best enzyme inhibitor in this series (**22**) exhibiting a K_i value of 19 nM.

In another set of derivatives, compound **7** was extended in the P2/P3-positions by Z-protected amino acids (**2**, **24**−**27**, Table 3); this modification generally yielded good enzyme inhibitors, with the best K_i values of about 8 nM. It is quite apparent that in this series antiviral activity does not strictly correlate with enzyme inhibition; *e.g.*, **24** and **25**, which are equipotent at the enzymatic level, differ in antiviral activity by a factor of 3. Compound **2** with *tert*-leucine in P2 was found to be the best inhibitor of HIV replication in this series (IC_{50} = 250 nM). The observation that the introduction of *tert*-leucine while slightly reducing the K_i value of the inhibitors leads to significantly better antiviral activity had been made by us before.[8] Also Schirlin *et al.* [20] found *tert*-leucine to be preferred in P2 of their HIV PR inhibitor but reported cellular toxicity of their *tert*-leucine-containing compounds. However, the analogues described here do not share this property.[21]

Studies of the substrate specificity of HIV-1 PR[22] suggested that aspartic or glutamic acid substituents might be beneficial for good enzyme inhibition; however, most likely poor cell penetration due to the negatively charged aspartic acid in **26** led to a lack of antiviral activity (Table 3).

Compound **2** was the starting point for further derivation, first in the P3-position (Table 4) where the Cbz group had been previously held constant. Therefore, compounds **28**−**39** were synthesized in order to evaluate the influence of heterocycles and heterosubstituted aromatic rings in P3 on antiviral activity. In addition, potential effects of this change in physicochemical parameters on solubility and oral bioavailability were investigated (Table 6, *vide supra*). Replacement of the Cbz group (**2**) by Boc (**28**) led to a marked drop in enzyme inhibition, while aromatic groups (**29**−**39**) introduced in P3 generally yielded good inhibitors with K_i values in the range from 6 to 18 nM. The

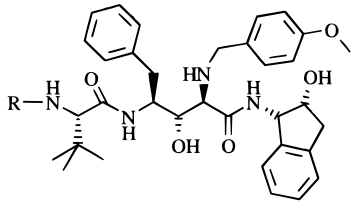
Table 3. Truncated Inhibitors Modified in P3−P2: Inhibitory Activity against HIV-1 PR and Antiviral Activity against HIV-1, IIIB, in MT4 Cells



no.	R	K_i (nM)[a]	IC_{50} (nM)[b]	mp (°C)	formula[c]
7	Boc	65	*d*	183−185	$C_{33}H_{41}N_3O_6 \cdot 0.2H_2O$
2	Z-Tle	9.5	254 ± 40 (5)	146−148	$C_{42}H_{50}N_4O_7 \cdot 0.3H_2O$
24	Z-Val	8.1	344 ± 128 (5)	81−91	$C_{41}H_{48}N_4O_7$
25	Z-Ile	8.2	1102 ± 474 (5)	112−115	$C_{42}H_{50}N_4O_7 \cdot 0.2H_2O$
26	Z-Asp	22	*d*	111−114	$C_{40}H_{44}N_4O_9 \cdot HCl \cdot 1.3H_2O$
27	Z-Asn	15	1400 (1)	158−162	$C_{40}H_{45}N_5O_8 \cdot 0.5\ H_2O$

[a] Usual standard deviation ±20%. [b] Mean value ± standard deviation. Number of determinations given in parentheses. [c] Satisfactory elemental analyses within ±0.4% of the calculated value for C,H,N were obtained. [d] No activity at 3 μM.

Table 4. Truncated Inhibitors Modified in P3: Inhibitory Activity against HIV-1 PR and Antiviral Activity against HIV-1, IIIB, in MT4 Cells



no.	R	K_i (nM)[a]	IC_{50} (nM)[b]	mp (°C)	formula[c]
2	Z	9.5	254 ± 40 (5)	146−148	$C_{42}H_{50}N_4O_7 \cdot 0.3H_2O$
28	Boc	210	*d*	180−195	$C_{39}H_{52}N_4O_7$
29	benzimidazol-2-yl-$(CH_2)_2$CO	9.4	98 ± 49 (6)	130−134	$C_{44}H_{52}N_6O_6 \cdot 1.1H_2O$
30	[2-(5-methyl-1,3,4-thiadiazolyl)]S-CH_2-CO	8.0	178 ± 72 (5)	88−95	$C_{39}H_{48}N_6O_6S_2$
31	[3-(4-methyl-1,2,4-triazolyl)]S-CH_2-CO	5.8	190 ± 60 (5)	95−108	$C_{39}H_{49}N_7O_6S$
32	[3-(2-methyl-1,2,4,5-tetrazolyl)]S-CH_2-CO	12	180 ± 120 (5)	98−105	$C_{38}H_{48}N_8O_6S$
33	(2,3-dimethoxyphenyl)NH-CO	13	532 ± 105 (5)	102−105	$C_{43}H_{53}N_5O_8$
34	1(*R*)-hydroxy-3-phenylpropionyl	14	278 ± 110 (5)	115−118	$C_{43}H_{52}N_4O_7 \cdot 0.7H_2O$
35	2-isoquinolinyl-CO	12	*d*	110−116	$C_{44}H_{49}N_5O_6$
36	(2-hydroxyphenyl)$(CH_2)_2$CO	13	160 ± 65 (5)	90−102	$C_{43}H_{52}N_4O_7$
37	2-hydroxy-4-methoxycinnamoyl	18	238 ± 115 (5)	117−120	$C_{44}H_{52}N_4O_8$
38	3-hydroxy-4-methoxycinnamoyl	5.8	125 ± 53 (5)	121−123	$C_{44}H_{52}N_4O_8$
39	(3-hydroxy-4-methoxyphenyl)CH_2-CH_2-CO	7.7	88 ± 30 (5)	95−97	$C_{44}H_{54}N_4O_8$

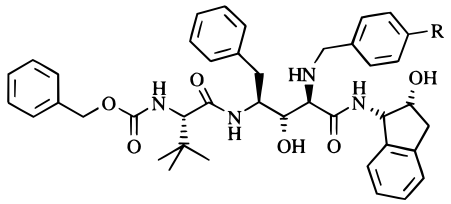
[a] Usual standard deviation ±20%. [b] Mean value ± standard deviation. Number of determinations given in parentheses. [c] Satisfactory elemental analyses within ±0.4% of the calculated value for C,H,N were obtained. [d] No activity at 3 μM.

importance of aromatic substituents in P3 for activity has been reported by others,[23] and our results with **28**−**39** support this finding. Again, for this series, antiviral activity cannot be predicted solely from the K_i values; the most drastic example is the unexpected lack of anti-HIV activity of compound **35** containing the isoquinolyl moiety (Table 4). The isoquinolyl group is part of potent inhibitors of HIV PR, eg., saquinavir,[24] which contains the hydroxyethylamine isostere. Remarkably, derivatives **29** and **39** show about 2.5-fold enhancement of antiviral activity as compared to **2**. In another series of derivatives an even more pronounced beneficial effect of the benzimidazole and hydroxy/methoxy-substituted phenyl moieties was observed.[8,25]

Finally, we tried to enhance the antiviral activity of **2** by elongation of the para-substitutent in the P1′-position (**40**−**46**, Table 5). Improved antiviral activity was obtained for compounds **41**−**46**, which agrees well with the results of Thompson *et al.*[11] In particular the ethoxymorpholinyl-substituted compound **42** showed significantly increased antiviral potency in cells (IC_{50} = 47 nM).

Selected compounds were tested for oral bioavailability in mice and for solubility in phosphate-buffered saline (Table 6). While the benzimidazole inhibitor **1** did not reach the systemic circulation after oral dosage to a significant extent (AUC = 0.6 μM·h), the truncated derivative **12** showed substantial oral bioavailability in mice (AUC = 126.6 μM·h, about 200-fold superior to **1**). In contrast to our initial assumption,[25] this finding indicates that the low oral adsorption of **1** cannot be attributed solely to the presence of the benzimidazole group since this structural feature is shared by **12**. The AHI-containing compounds **2** and **24**, too, were orally bioavailable. The absolute oral bioavailability of **2** in mice was determined to be 47%. Thus, the inhibitor levels after oral administration remained far above the concentrations needed to efficiently block HIV replication *in vitro* for a prolonged time.[21] Introduction of the benzimidazole group in P3 which markedly increased anti-HIV activity (compound **29**) led to a complete loss of oral bioavailability. Introduction of other P3 residues also resulted in significantly reduced oral uptake or even abolished it completely (**30**−**39**). The 3-hydroxy-4-methoxyphenyl group in **39**, which had led to enhanced antiviral activity and oral uptake in another series of inhibitors,[25] in the present case only led to an increase in the anti-HIV effect but concomitantly to loss of oral

Table 5. Truncated Inhibitors Modified in P1′: Inhibitory Activity against HIV-1 PR and Antiviral Activity against HIV-1, IIIB, in MT4 Cells



no.	R	K_i (nM)[a]	IC_{50} (nM)[b]	mp (°C)	formula[c]
2	O-CH_3	9.5	254 ± 40 (5)	146−148	$C_{42}H_{50}N_4O_7 \cdot 0.3H_2O$
40	O-CH_2-CH_3	9.9	256 ± 109 (5)	104−109	$C_{43}H_{52}N_4O_7 \cdot 1.0H_2O$
41	O-CH_2-CH_2-OH	16	155 ± 60 (5)	109−113	$C_{43}H_{52}N_4O_8 \cdot 1.9H_2O$
42	O-CH_2-CH_2-4-morpholinyl	9.5	47 ± 20 (5)	oil	$C_{47}H_{59}N_5O_8 \cdot 1.5H_2O$
43	CH_2-NHCO-CH_3	5.7	99 ± 68 (5)	96−99	$C_{44}H_{53}N_5O_7$
44	CH_2-NHCO-NH-Ph	3.3	175 ± 80 (5)	106−108	$C_{49}H_{56}N_6O_7$
45	CH_2-NHCO-O-CH_2-Ph	9.9	142 ± 63 (5)	94−97	$C_{50}H_{57}N_5O_8$
46	CH_2-NHCO$(CH_2)_2$-2-benzimidazolyl	4.1	161 ± 66 (5)	115−118	$C_{52}H_{59}N_7O_7 \cdot 0.9H_2O$

[a] Usual standard deviation ±20%. [b] Mean value ± standard deviation. Number of determinations given in parentheses. [c] Satisfactory elemental analyses within ±0.4% of the calculated value for C,H,N were obtained.

Table 6. Oral Bioavailability of AHPPA-Containing HIV PR Inhibitors in Mice

	oral bioavailability[a]		aqueous solubility[c]
no.	AUC (0 → ∞) (μM·h)	C_{max} (μM)	(μM)
1	0.6	0.2	<1
2	82.5	16.5	<1
9	0.8	0.4	<1
12	126.6	9.1	9
24	136.9	12.3	2.9
29	*b*	*b*	12.2
30	60.3	20.0	39
31	1.6	0.7	260
32	7.4	2.8	78
36	11.8	1.8	
38	*b*	*b*	
39	0.2	0.25	
40	23.9	4.3	<1
41	5.9	2.6	16.5
42	*b*	*b*	14.5
43	*b*	*b*	25.3
44	*b*	*b*	<1
45	*b*	*b*	<1
46	*b*	*b*	<1

[a] Compounds were administered in a liquid formulation to mice with an oral dose of 125 mg/kg. Concentration of compounds in whole blood were determined at various points of time. Maximal observed concentration (C_{max}) and area-under-the-curve (AUC) are given. [b] No compound detectable in blood above the detection limit of 0.1 μM. [c] Solubility in aqueous phosphate-buffered saline (pH 7.4).

uptake. Obviously, structural elements which seem to favor oral uptake cannot be exchanged between different groups of HIV PR inhibitors. Our findings indicate that rather the overall structural features of a compound govern the oral bioavailability.

Even more striking are the observed effects of subtle variations of the para substituents of the P1′ residue of compound **2**: Replacing the methoxy substituent by ethoxy (**40**) or an hydroxyethoxy group (**41**) reduced oral bioavailability by a factor of 4 and 14, respectively. All other variations in P1′ (**42**−**46**) abolished oral bioavailability totally. Whereas the aqueous solubility increased slightly by introduction of more polar substituents in the 4-position of the P1′ benzylamine residue ($CH_2NHCOCH_3$ (**43**) > $O(CH_2)_2$-(4-morpholinyl) (**42**) > $O(CH_2)_2$-OH (**41**) > OCH_3 (**2**)), the oral bioavailability was lowered. Enhancement of aqueous solubility due to the introduction of polar substituents in P3, *e.g.*, triazolylthioacetic acid (**31**), was also correlated with lower systemic uptake.

To summarize the results, systematic structural modifications of lead structure **1** led to compounds of different size and polarity and with a varying number of peptide bonds. From the effect of the structural modifications on oral bioavailability, the following conclusions may be drawn. 1. Reduced size of an inhibitor alone will not lead to enhanced oral bioavailability. 2. Structural features regarded as beneficial for oral uptake in one series of compounds may not enhance bioavailability in other types of inhibitors. 3. Only a very narrow band of tolerated modifications exists in the case of compound **2**. 4. In our series of compounds, there is no correlation between solubility and oral bioavailability. Due to the poor predictability of the pharmacokinetic behavior, only the testing of extended series of compounds will allow the identification of drug candidates with optimal combination of the desired properties.

In conclusion, we have synthesized a series of AHPPA-containing HIV PR inhibitors in a search to adequately balance antiviral activity and oral bioavailability, since only compounds which sufficiently fulfill both criteria are expected to be effective antiviral drugs *in vivo*. Compound **2** was chosen for further development due to its good pharmacokinetic profile, although it is not the most potent antiviral derivative of the present study. Additional antiviral and pharmacokinetic characterization of **2** has been already published elsewhere.[21]

Experimental Section

Chemistry. ^{1}H-NMR spectra were recorded with a Bruker WC-250 or AMX-500 spectrometer; chemical shifts are given in ppm (δ) relative to Me_4Si as internal standard. *J* values are given in hertz (Hz). Elemental analyses were performed by the Analytical Department, Sandoz Basle, Switzerland, and Mikroanalytisches Laboratorium, Institut für Physikalische Chemie, Universität Wien, and are within ±0.4% of the theoretical value. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Analytical thin-layer chromatography was performed on silica gel 60 F_{254} glass plates (HPTLC, E. Merck). Preparative column chromatography was performed on silica gel (40−63 μm) under pressure (~0.2 mPa). Solvents were AR grade and used without further purification. Evaporations were carried out *in vacuo* with a rotary evapora-

tor (Büchi). Melting points were determined with a thermovar apparatus (Reichert-Jung) and are not corrected. Representative methods for all compounds synthesized according to Schemes 1 and 2 are described.

(2*R*,3*S*,4*S*)-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-hydroxy-2-[(4-methoxybenzyl)amino]-5-phenylpentanoic Acid Ethyl Ester (4). To a solution of 20 g (59.6 mmol) of 4(*S*)-4-[[(1,1-dimethylethoxy)carbonyl]amino]-2,3-epoxy-5-phenylpentanoic acid ethyl ester (**3a,b**)[8] in 250 mL of ethanol was added 16.4 g (119.5 mmol) of 4-methoxybenzylamine. The mixture was stirred at 70 °C for 12 h. The solvent was distilled off *in vacuo*, and the residue was dissolved in 400 mL of ethyl acetate, washed with 1 N HCl, saturated $NaHCO_3$ solution, and brine, and dried over $MgSO_4$. The solvent was evaporated *in vacuo*, and the residue was chromatographed on silica gel (solvent: cyclohexane/ethyl acetate = 3/1): yield 24.9 g of **4** (88%) as an oil which crystallized upon prolonged storage; mp 73−77 °C; $[\alpha]^{20}_D = -15.4°$ ($c = 1$, CH_3OH); ^{1}H-NMR ($CDCl_3$) δ 1.26 (t, $J = 7$ Hz, 3 H), 1.37 (s, 9 H), 2.78−3.01 (m, 2 H), 3.30 (d, $J = 7$ Hz, 1 H), 3.53 and 3.73 (AB, $J = 12.5$ Hz, 2 H), 3.66−3.71 (m, 1 H), 3.80 (s, 3 H), 4.02 (q, $J = 8$ Hz, 1 H), 4.20 (q, $J = 7$ Hz, 2 H), 4.79 (d, $J = 9$ Hz, 1 H), 6.82 (s, 1 H), 6.85 (s, 1 H), 7.15−7.34 (m, 7 H). Anal. ($C_{26}H_{36}N_2O_6$) C,H,N.

(2*R*,3*S*,4*S*)-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-hydroxy-2-[(4-methoxybenzyl)amino]-5-phenylpentanoic Acid (5). To a solution of 42.6 g (90.1 mmol) of **4** in 500 mL of tetrahydrofuran was added 99 mL (99 mmol) of 1 N LiOH, and the reaction mixture was stirred for 12 h at room temperature. Neutralization with 1 N HCl led to a precipitate, which was filtered off and dried: yield 33.3 g of **5** (83%); mp 203−206 °C; ^{1}H-NMR ($CDCl_3$/DMSO-d_6 = 4/1) δ 1.38 (s, 9 H), 2.85−2.98 (m, 2 H), 3.34 (d, 1 H), 3.80 (s, 3 H), 3.85 (d, 2 H), 3.94 (d, 1 H), 4.02 (q, 1 H), 5.75 (bs, 1 H), 6.86 (d, 2 H), 7.15−7.40 (m, 7 H). Anal. ($C_{24}H_{32}N_2O_6$) C,H,N.

(2*R*,3*S*,4*S*,1′*S*,2′*R*)-4-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-hydroxy-2-[(4-methoxybenzyl)amino]-5-phenylpentan(2′-hydroxy-1′-indanyl)amide (7). **5** (11.9 g, 26.7 mmol) was dissolved in 1.3 L of dimethylformamide; 4.0 g (26.8 mmol) of **6**, 3.60 g (26.6 mmol) of 1-hydroxybenzotriazole, and 5.20 g (27.0 mmol) of *N*-ethyl-*N*′-[3-(dimethylamino)-propyl]carbodiimide hydrochloride were added at room temperature. The reaction mixture was stirred for 3 days at room temperature. The solvent was evaporated, and ethyl acetate was added. The solution was washed with 1 N HCl, saturated $NaHCO_3$ solution, and brine and dried over $MgSO_4$, and the solvent was evaporated; 70 mL of a mixture of cyclohexane/ethyl acetate (1/2) was added to the oily residue. The title compound crystallized overnight. The crystals were filtered off, washed with cold cyclohexane/ethyl acetate (1/2), and dried *in vacuo*: yield 8.45 g of **7** (55%); mp 183−185 °C; $[\alpha]^{20}_D = -6.7°$ ($c = 1$, methanol); ^{1}H-NMR ($CDCl_3$) δ 1.36 (s, 9 H), 1.91 (d, $J = 7.5$ Hz, 2 H), 2.99 and 3.09 (AB of ABX, $J_{AX} = 5.4$ Hz, $J_{BX} = 2.2$ Hz, $J_{AB} = 16$ Hz, 2 H), 3.38 (d, $J = 8$ Hz, 1 H), 3.56 and 3.61 (AB, $J_{AB} = 12$ Hz, 2 H), 3.79 (s, 3 H), 3.88 (d, $J = 7$ Hz, 1 H), 4.03−4.15 (m, 1 H), 4.60−4.68 (m, 1 H), 5.06 (d, $J = 10$ Hz, 1 H), 5.37 (dd, $J = 7$, 9.5 Hz, 1 H), 6.79 (d, $J = 8.5$ Hz, 2 H), 7.15 (d, $J = 8.5$ Hz, 2 H), 7.10−7.24 (m, 9 H), 8.22 (d, $J = 9$ Hz, 1 H). Anal. ($C_{33}H_{41}N_3O_6$) C,H,N.

(2*R*,3*S*,4*S*,1′*S*,2′*R*)-4-[[[*N*-[(Benzyloxy)carbonyl]-L-*tert*-leucyl]amino]-3-hydroxy-2-[(4-methoxybenzyl)amino]-5-phenylpentan(2′-hydroxy-1′-indanyl)amide (2). **7** (6 g, 10.4 mmol) was dissolved in a mixture of 20 mL of dichloromethane and 4 mL of methanol; 300 mL of 3 N hydrochloric acid in ether was added, and the mixture was stirred for 3 h at room temperature. The precipitate was filtered off, washed with ether, and dried *in vacuo* to yield 5.48 g of (2*R*,3*S*,4*S*,-1′*S*,2′*R*)-4-amino-3-hydroxy-2-[(4-methoxybenzyl)amino]-5-phenylpentan(2′-hydroxy-1′-indanyl)amide dihydrochloride (**8**) (96%), mp 147−149 °C, an aliquot of which was used in the next step without further purification: $[\alpha]^{20}_D = -8.1°$ ($c = 1$, methanol); ^{1}H-NMR (DMSO-d_6) δ 2.80−3.20 (m, 4 H), 3.80 (s, 3 H), 3.90−4.43 (m, 5 H), 4.50−4.60 (m, 1 H), 5.18−5.38 (m, 3 H), 6.70−7.05 (m, 3 H), 7.10−7.58 (m, 10 H), 8.08 (bs, 2 H), 8.45−8.60 (m, 1 H). Anal. ($C_{28}H_{35}Cl_2N_3O_4$) C,H,N.

Z-*tert*-Leucine (480 mg, 1.81 mmol), 1.0 g (1.82 mmol) of (2*R*,3*S*,4*S*,1′*S*,2′*R*)-4-amino-3-hydroxy-2-[(4-methoxybenzyl)-amino]-5-phenylpentan(2′-hydroxy-1′-indanyl)amide dihydrochloride (**8**), and 940 mg (1.81 mmol) of PyBOP was dissolved in 100 mL of dimethylformamide. Diisopropylethylamine (930 mg, 7.3 mmol) was added at room temperature, and the mixture was stirred for 12 h. The solvent was evaporated, and the residue was dissolved in ethyl acetate (140 mL). The solution was washed with 1 N HCl, saturated $NaHCO_3$ solution, and brine (60 mL each), concentrated *in vacuo* to 20 mL, and filtered over silica gel (40 g of silica gel 60, glass frit G3, eluent 300 mL of ethyl acetate). The filtrate was evaporated *in vacuo*, and the residue was crystallized from 60 mL of toluene/*tert*-butyl methyl ether (1/4): yield 932 mg of **2** (71%); mp 146−148 °C; $[\alpha]^{20}_D = -26.1°$ ($c = 1$, methanol); ^{1}H-NMR ($CDCl_3$) δ 0.89 (s, 9 H), 2.58 (bs, 1 H), 2.91−3.10 (m, 3 H), 3.17 (AX of ABX, $J_{AX} = 5$ Hz, $J_{AB} = 15$ Hz, 1 H), 3.35 (d, $J = 8$ Hz, 1 H), 3.61 (s, 2 H), 3.70 (d, $J = 10$ Hz, 1 H), 3.75 (s, 3 H), 3.89 (d, $J = 8$ Hz, 1 H), 4.38 (q, $J = 7$ Hz, 1 H), 4.62 (bs, 1 H), 4.69 (bs, 1 H), 4.90 and 5.02 (AB, $J_{AB} = 12$ Hz, 2 H), 5.14 (d, $J = 8$ Hz, 1 H), 5.40 (dd, $J = 5$, 8 Hz, 1 H), 6.36 (d, $J = 9$ Hz, 1 H), 6.78 (d, $J = 9$ Hz, 2 H), 7.12−7.36 (m, 16 H), 7.94 (d, $J = 9$ Hz, 1 H). Anal. ($C_{42}H_{50}N_4O_7$) C,H,N.

(2*R*,3*S*,4*S*)-2-[[(4-Aminomethyl)benzyl]amino]-4-[[(1,1-dimethylethoxy)carbonyl]amino]-3-hydroxy-5-phenylpentanoic Acid Ethyl Ester (15). A solution of **3**[8] (10 g, 30 mmol) and α,α-diamino-*p*-xylene (12.2 g, 90 mmol) in 600 mL of ethanol was kept at 80 °C for 20 h. The solvent was evaporated, and the residue was chromatographed on silica gel (ethyl acetate/methanol/aqueous ammonia = 10/10/1): yield 8.6 g of **15** (61%); ^{1}H-NMR ($CDCl_3$) δ 1.25 (t, $J = 7$ Hz, 3 H), 1.37 (s, 9 H), 2.45 (bs, 4 H), 2.78−2.90 (m, 2 H), 3.3 (d, $J = 8$ Hz, 1 H), 3.58 and 3.74 (AB, $J = 13$ Hz, 2 H), 3.66 (d, $J = 8$ Hz, 2 H), 3.8 (bs, 1 H), 4.00−4.23 (m, 3 H), 5.04 (d, $J = 9$ Hz, 1 H), 7.10−7.33 (m, 9 H). Anal. ($C_{26}H_{37}N_3O_5$) C,H,N.

(2*R*,3*S*,4*S*)-2-[[4-[[[2-(Benzimidazol-2-yl)propionyl]amino]methyl]benzyl]amino]-4-[[(1,1-dimethylethoxy)carbonyl]amino]-3-hydroxy-5-phenylpentanoic Acid Ethyl Ester (16). A solution of **15** (660 mg, 1.4 mmol) and 2-(benzimidazol-2-yl)propionic acid (266 mg, 1.4 mmol) in a mixture of dimethylformamide (5 mL) and 1,4-dioxane (5 mL) was treated with 1-hydroxybenzotriazole (229 mg, 1.4 mmol) and *N*-ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide hydrochloride (268 mg, 1.4 mmol) at 0 °C. The reaction mixture was stirred for 3 days at room temperature. After evaporation of the solvents the residue was chromatographed on silica gel (ethyl acetate/methanol = 20/1): yield 598 mg of **16** (76%); mp 85−90 °C; ^{1}H-NMR ($CDCl_3$) δ 1.22 (t, $J = 7$ Hz, 3 H), 1.35 (s, 9 H), 2.72 (t, $J = 7$ Hz, 2 H), 2.87 (d, $J = 7$ Hz, 2 H), 3.12 (t, $J = 7$ Hz, 2 H), 3.30 (d, $J = 8$ Hz, 1 H), 3.47 and 3.64 (AB, $J = 13$ Hz, 2 H), 3.65−3.78 (m, 4 H), 4.00−4.20 (m, 3 H), 4.28 (bs, 2 H), 5.14 (d, $J = 9$ Hz, 1 H), 6.95 and 7.04 (2 d, $J = 8$ Hz, 4 H), 7.10−7.30 (m, 8 H), 7.40−7.60 (bs, 2 H). Anal. ($C_{36}H_{45}N_5O_6$) C,H,N.

(2*R*,3*S*,4*S*,1′*S*,2′*R*)-2-[[4-[[[2-(Benzimidazol-2-yl)propionyl]amino]methyl]benzyl]amino]-4-[[(1,1-dimethylethoxy)carbonyl]amino]-3-hydroxy-5-phenylpentan(2′-hydroxy-1′-indanyl)amide (23). Saponification of **16** was carried out following the procedure given for **5**. Coupling of the free acid to **6** was done as described for **7**: yield 72%; mp 128−132 °C; ^{1}H-NMR ($CDCl_3$) δ 1.35 (s, 9 H), 2.18 (bs, 1 H), 2.44 and 2.62 (2 m, 2 H), 2.55 (bs, 3 H), 2.83 (m, 1 H), 2.95 and 3.02 (AB part of ABX, $J = 15$, 10, 2 Hz, 2 H), 3.05 and 3.17 (AB part of ABX, $J = 16$, 6 Hz, 2 H), 3.43 (d, $J = 5$ Hz, 1 H), 3.47 and 3.75 (AB, $J = 15$ Hz, 2 H), 3.94 and 4.43 (AB part of ABX, $J = 15$, 8, 6 Hz, 2 H), 4.18 (m, 2 H), 4.73 (bs, 1 H), 5.26 (d, $J = 10$ Hz, 1 H), 5.44 (dd, $J = 5$, 9 Hz, 1 H), 6.65 and 6.83 (2 d, $J = 8$ Hz, 4 H), 7.03 and 7.13 (2 t, $J = 7$ Hz, 2 H), 7.15−7.30 (m, 9 H), 7.44 (bs, 2 H), 8.28 (d, $J = 9$ Hz, 1 H), 8.73 (bs, 1 H). Anal. ($C_{40}H_{46}N_6O_6 \cdot 2.5H_2O$) C,H,N.

Stereospecific Synthesis of (1*S*,2*R*)-1-Amino-2-hydroxyindan (6). (1*S*,2*S*)-1-Amino-2-hydroxyindan Dibenzoyl-D-tartrate. A solution of 145 g (0.97 mol) of racemic *trans*-1-amino-2-hydroxyindan[17] in 3.5 L of 95% ethanol was added to a stirred solution of 348.4 g (0.97 mol) of (+)-dibenzoyl-D-tartaric acid monohydrate in 1.36 L of 95% ethanol at room temperature. The mixture was allowed to stir for an additional 5 min. After 3 h the precipitate was filtered off, washed with

900 mL of ethanol, transferred in a round-bottom flask, and refluxed for 3 h with 4.9 L of ethanol. After standing for 1 day at room temperature, the precipitate was filtered off, washed with 900 mL of ethanol, and dried in a vacuum oven at 50 °C. A recrystallized sample was kept as seed crystals: yield 167 g of (1*S*,2*S*)-1-amino-2-hydroxyindan dibenzoyl-D-tartrate (34%); mp 209−210 °C; $[\alpha]^{20}_D = +100.9°$ (methanol, $c = 1.11$). Anal. ($C_{27}H_{25}NO_9$) C,H,N.

(1*S*,2*S*)-1-Amino-2-hydroxyindan. A suspension of 166.9 g (329 mmol) of (1*S*,2*S*)-1-amino-2-hydroxyindan dibenzoyl-D-tartrate in 2 L of ethyl acetate and 600 mL (900 mmol) of 1.5 N NaOH was extracted in a perforator for 4 h. The organic layer was filtered, the solvent was evaporated, and the residue was dried *in vacuo* at 50 °C to give 47.3 g of (1*S*,2*S*)-1-amino-2-hydroxyindan (96%): mp 144−145 °C; $[\alpha]^{20}_D = +22.8°$ (methanol, $c = 1.12$); ^{1}H-NMR ($CDCl_3$) δ 2.30 (bs, 3 H), 2.95 and 3.10 (AB of ABX, $J_{AB} = 16$ Hz, $J_{AX} = 3$ Hz, $J_{BX} = 5$ Hz, 2 H), 4.3−4.5 (m, 2 H), 7.2−7.4 (m, 4 H). Anal. ($C_9H_{11}NO$) C,H,N.

(1*S*,2*S*)-1-(Benzoylamino)-2-hydroxyindan. A solution of 46.9 g (314 mmol) of (1*S*,2*S*)-1-amino-2-hydroxyindan and 65.3 mL (510 mmol) of triethylamine in 745 mL of DMF was cooled to 5 °C; 44.2 g (314 mmol) of benzoyl chloride was added via a dropping funnel within 30 min. The ice bath was removed, and the mixture was allowed to stir for 1 h at room temperature. The reaction mixture was poured on 2 L of ice water; the product was collected by filtration, washed with 2 × 200 mL of H_2O, and dried in a vacuum oven overnight: yield 72.5 g of (1*S*,2*S*)-1-(benzoylamino)-2-hydroxyindan (91%); mp 228−230 °C; $[\alpha]^{20}_D = +117.4°$ (methanol, $c = 0.57$); ^{1}H-NMR (DMSO-d_6) δ 2.75 and 3.17 (AB of ABX, $J_{AB} = 15$ Hz, $J_{AX} = 7$ Hz, $J_{BX} = 8$ Hz, 2 H), 4.35 −4.52 (m, 1 H), 5.24−5.38 (m, 1 H), 7.03−7.21, 7.40−7.60, 7.90−7.98 (m, 9 H), 8.76 (d, $J = 9$ Hz, 1 H). Anal. ($C_{16}H_{15}NO_2$) C,H,N.

(4*S*,5*R*)-2-Phenyl-3a,8a-dihydro-8*H*-indeno-[1,2-*d*]oxazole. (1*S*,2*S*)-1-(Benzoylamino)-2-hydroxyindan (71.8 g, 283 mmol) was suspended in 950 mL of dichloromethane and cooled in an ice bath; 60 mL of thionyl chloride was added via a dropping funnel. The ice bath was removed, and the mixture was allowed to stir at room temperature for 3 h and then concentrated to dryness. The residue was suspended in 200 mL of saturated $NaHCO_3$ and extracted into 4 × 250 mL of ethyl acetate. The combined organic layers were dried over $MgSO_4$ and filtered through Celite. The solvent was evaporated *in vacuo* to yield 66.0 g of (4*S*,5*R*)-2-phenyl-3a,8a-dihydro-8*H*-indeno[1,2-*d*]oxazole (92%): mp 113−115 °C; $[\alpha]^{20}_D = -177.1°$ (methanol, $c = 0.99$); ^{1}H-NMR (DMSO-d_6) δ 3.38 and 3.55 (AB of ABX, $J_{AB} = 16$ Hz, $J_{AX} = 8$ Hz, $J_{BX} = 8$ Hz, 2 H), 5.50 (t, $J = 8$ Hz, 1 H), 5.78 (d, $J = 8$ Hz, 1 H), 7.20−7.50 (m, 6 H), 7.60 (m, 1 H), 7.95 (m, 2 H). Anal. ($C_{16}H_{13}NO$) C,H,N.

(1*S*,2*R*)-1-Amino-2-hydroxyindan (6). A suspension of 66 g (280 mmol) of (4*S*,5*R*)-2-phenyl-3a,8a-dihydro-8*H*-indeno-[1,2-*d*]oxazole in 600 mL of 20% H_2SO_4 was heated to reflux for 10 h. The mixture was cooled in an ice bath; benzoic acid was filtered off. The filtrate was adjusted to pH >12 with 10 N NaOH and extracted with 2 × 500 mL of dichloromethane. The combined organic layers were washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to yield 37.7 g of **6** (92%): mp 115−117 °C; $[\alpha]^{20}_D = -38.0°$ (methanol, $c = 1.11$) (lit.[17] $[\alpha]^{20}_D = -62.0°$ (methanol, $c = 1.0$)); ^{1}H-NMR (DMSO-d_6) δ 2.75 and 2.83 (AB of ABX, $J_{AB} = 16$ Hz, $J_{AX} = 5$ Hz, $J_{BX} = 2$ Hz, 2 H), 4.03 (d, $J = 5$ Hz, 1 H), 4.21 (dt, $J = 2$, 5 Hz, 1 H), 7.20 (m, 3 H), 7.35 (m, 1 H). Anal. ($C_9H_{11}NO$) C,H,N.

Biology. All derivatives were dissolved in dimethyl sulfoxide to 10 mM. For cellular assays, these stock solutions were diluted into cell culture medium containing 10% fetal bovine serum and for enzyme assays into appropriate buffer (see below); the concentration of dimethyl sulfoxide did not exceed 0.3% in cellular assays and 5% in enzymatic assays.

HIV PR Inhibition Assay. HIV-1 PR was expressed in *Escherichia coli*, strain JM 105, using the expressor plasmid pTZprt$^+$ [26] and purified to homogeneity as described before.[27] Enzymatic activity was measured by following the cleavage of the substrate H-Lys-Ala-Arg-Val-Leu-pNph-Glu-Ala-Nle-NH_2, originally described by Richards *et al.*[28] Briefly, HIV-PR was incubated at 37 °C in 0.1 M morpholinoethanesulfonic acid, 0.37 M NaCl, and 4 mM EDTA, pH 6.25, with 280 μM substrate in the presence and absence of inhibitors. From the decrease of absorbance at 298 nm, initial reaction rates were calculated.

IC_{50} values for test compounds were obtained by fitting the initial velocity data (V) from the inhibition of substrate hydrolysis to the equation $V = V_0 \cdot IC_{50}/(I + IC_{50})$, where I denotes the inhibitor concentration and V_0 the velocity of the uninhibited reaction. Kinetic constants K_i were calculated from IC_{50} values by using the equation $IC_{50} = E_t/2 + K_i(1 + S/K_m)$, where E_t is the total enzyme concentration, S is the substrate concentration, and K_m is the Michaelis constant for the substrate.[29] K_i values reported here are the mean of two determinations, which usually yielded the same result within limits of ±20%.

Inhibition of HIV-1-Induced Cytopathic Effect in MT4 Cells. The assay procedure described by Pauwels *et al.*[30] was used with minor modifications. The HTLV I-transformed cell line MT4 was used as the target cell. Inhibition of HIV-1, strain IIIB,[31] induced cytopathic effect was determined by measuring the viability of both HIV- and mock-infected cells. Viability was assessed spectrophotometrically via *in situ* reaction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Virus-infected and uninfected cultures without compound were included as controls as were uninfected cells treated with compound. The cell concentration was chosen so that the number of cells/mL increased by a factor of 10 during the 5 days of incubation in mock-infected cultures. Virus inoculum was adjusted such to cause cell death in 90% of the target cells after 5 days of incubation. The virus was adsorbed to a cell suspension containing 1×10^6 cells/mL at 37 °C for 1 h. Then, the infected cells were added to microtiter plates containing the test compounds to give 1×10^5 cells/mL. Thus, compounds were added postadsorption.

Pharmacokinetic Studies. Female Balb/c mice were used. For oral administration (by gavage) the animals were fasted for 24 h prior to the start of and throughout the experiment; water was given *ad libitum*. For analysis, blood was collected in heparinized tubes. Samples (typically 0.4−1.0 mL) were frozen in liquid nitrogen and stored at −70 °C. After thawing, 200 μL of a suitable internal standard (0.01 mg/mL in methanol) and 4 vol of methanol were added. For calibration, blood samples were spiked with 10−10 000 ng of test compound, and then internal standard and methanol were added as above. Samples were mixed for 10 min and then centrifuged at 4000 rpm, 4 °C, for 10 min. The supernatants were removed and dried *in vacuo*.

For solid-phase extraction of the compounds, 1 mL disposable sulfobenzyl extraction columns (Baker) were used. The dried supernatant of the methanol extraction, resuspended in 10% methanol in 10 mM NH_4Ac, pH 4.0, was applied followed by successive washes (1 mL each) with 10 mM NH_4Ac, pH 4.0 (twice), methanol, hexane, and 10% trifluoroacetic acid. Compounds were eluted with 5% ammonia in methanol. Eluates were dried *in vacuo*.

For HPLC analysis, residues were taken up in 100 μL of 0.1% trifluoroacetic acid and 10% methanol in water. Aliquots (20−100 μL) were injected into the HPLC system (HP1500; column, Vydac RP-C18, 5 μm, 4 × 250 mm; isocratic elution with 50% or 60% acetonitrile in 10 mM NH_4Ac, pH 4.0; flow, 1 mL/min; 25 °C; UV detection at 200 nm). Drug concentration in the samples was calculated by least-squares linear regression analysis of the peak area ratio (inhibitor/internal standard) of the spiked blood standards versus concentration.

Determination of Aqueous Solubility. A weighed amount (about 1 mg) of inhibitor in amorphous form (lyophilized out of dioxane to constant weight) was combined with 1 mL of PBS. Samples were vortexed vigorously for 1 min and sonicated at 37 °C for 30 min. The suspensions were filtered through a G3 sinter funnel (or alternatively a Pasteur pipet filled with a cotton plug), and the filtrate was centrifuged at 100 000 rpm for 30 min. The supernatant was analyzed by HPLC (column,

RP-C18; isocratic elution with 10 mM aqueous NH_4OAc/CH_3CN = 50/50; flow rate, 1 mL/min; UV detection at 210 nm; room temperature). The concentration of the compound was calculated by external standardization of peak area versus concentration for each sample: minimal detectable concentration, 0.1 μg/mL; standard deviation, ±10%.

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