Brief Articles

Microwave-Accelerated Synthesis of P1′-Extended HIV-1 Protease Inhibitors Encompassing a Tertiary Alcohol in the Transition-State Mimicking Scaffold

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Two series of P1′-extended HIV-1 protease inhibitors comprising a tertiary alcohol in the transition-state mimic exhibiting K_i values ranging from 2.1 to 93 nM have been synthesized. Microwave-accelerated palladium-catalyzed cross-couplings were utilized to rapidly optimize the P1′ side chain. High cellular antiviral potencies were encountered when the P1′ benzyl group was elongated with a 3- or 4-pyridyl substituent (EC_{50} = 0.18−0.22 μM). X-ray crystallographic data were obtained for three inhibitors cocrystallized with the enzyme.

Introduction

Increasing numbers of HIV/AIDS infected patients and related deaths, along with severe treatment-associated complications, make the HIV/AIDS pandemic more complex than ever.[1,2] The introduction of HIV protease inhibitors (PIs) in the mid 1990s dramatically changed the situation for HIV/AIDS patients.[3−6] Combination therapy initially including one protease inhibitor and two nucleoside reverse transcriptase inhibitors, the so-called highly active antiretroviral therapy (HAART), furnished a sharp decline in HIV/AIDS related mortality for patients receiving this therapy.[7,8] Seven PIs are available on the market today (December 2005), and yet another one has been granted accelerated approval by the FDA (tipranavir, June 2005).[9,10]

One of the major obstacles to overcome in the development of HIV protease inhibitors has been the low oral bioavailability due to insufficient membrane permeation properties, rapid metabolism, and/or protein binding.[11−13] The discovery of PIs that combine high oral bioavailability with potent anti-HIV activity remains challenging, although the identification of atazanavir represented a major step forward (Figure 1).[14−17]

We recently reported a new class of HIV-1 protease inhibitors which were structurally related to both atazanavir and indinavir[18,19] but comprising a tertiary alcohol as part of the transition-state mimicking unit, the best compound being **1** with a K_i value of 2.4 nM (Figure 1).[20] However, in assays with HIV-1 infected MT4 cells, the antiviral activities were low (EC_{50} values at best 1.1 μM). Thus, it was decided to further optimize inhibitor **1** in order to improve the potency on the cellular level. In this effort, structure **1** and the corresponding *m*-bromo derivative **8** were expected to serve as suitable arylpalladium precursors for microwave-assisted cross-coupling reactions (Scheme 1).[21−24] We herein report microwave synthesis and biological evaluations of two series of P1′-elongated analogues, demonstrating that compounds with improved activity in cell culture rapidly can be identified. X-ray crystallographic data for three of these new inhibitors bound to the HIV-1 protease are discussed.

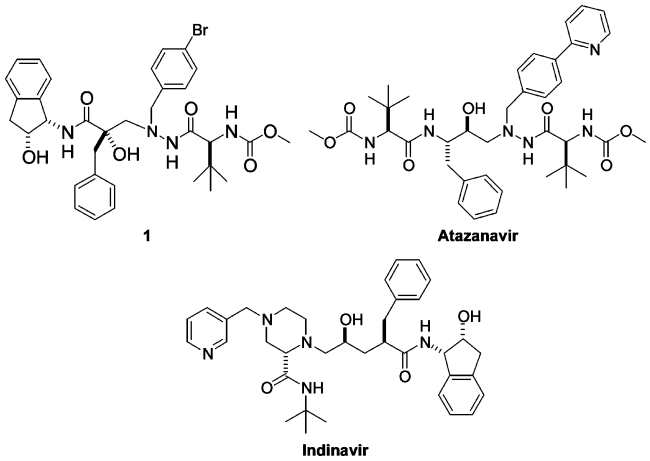

Figure 1. The new class of HIV protease inhibitors exemplified by the most potent compound from the first generation (**1**) and the approved inhibitors atazanavir and indinavir.

Results

Chemistry. The synthesis of bromo scaffolds **1** and **8** followed the protocol recently published for **1**[20] starting from 4-[25] or 3-bromobenzylhydrazine and *N*-(methoxycarbonyl)-L-*tert*-leucine, prepared according to published procedures (Scheme 1).[15,26] The hydrazide coupling was performed using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), *N*-methylmorpholine (NMM), and 1-hydroxybenzotriazole (HOBT), affording the bromo-substituted hydrazides **5** and **6** (Scheme 1). These hydrazides were then used to regioselectively ring-open the epoxide **7**[20] in Ti(O*i*Pr)$_4$-catalyzed reactions yielding key structures **1** and **8**, respectively.

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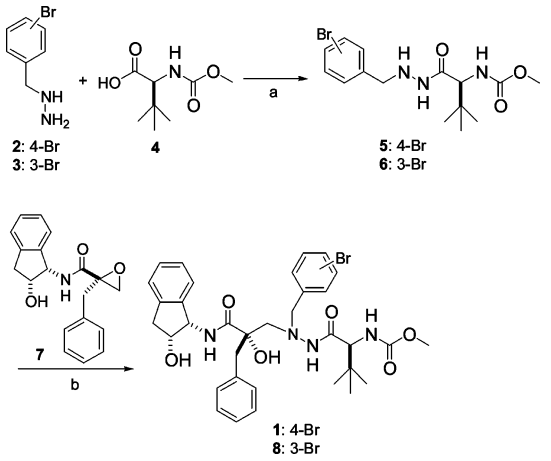
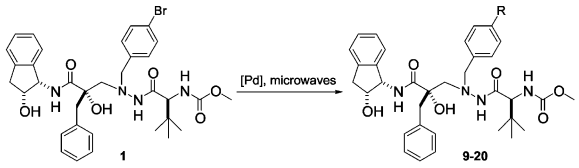
Scheme 1[a]

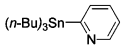
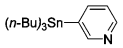
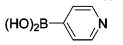
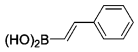
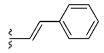
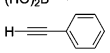
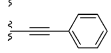
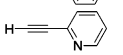
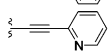
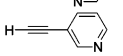
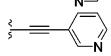
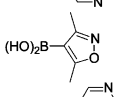
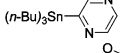
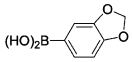
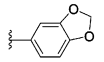
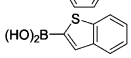
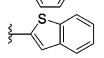


[a] Reagents: (a) EDC, HOBT, NMM, EtOAc, room temp, 54−76%; (b) Ti(O*i*Pr)$_4$, THF, 40 °C, 46−55%.

The P1′-elongated analogues **9**−**28** (Tables 1 and 2) were prepared by a series of sequential Suzuki,[27] Stille,[28] or Sonogashira[29] reactions performed under air with 20−60 min of controlled microwave irradiation in septum-sealed reaction vessels.[22,23,30] To enable comparison of the meta and para inhibitor sets, cross-coupling reactions yielding the phenyl, 2-, 3-, and 4-pyridyl, two-carbon elongated 2-phenylalkenyl, and phenyl- and 2- and 3-pyridylalkynyl-substituted analogues were performed with both **1** and **8**. For the para series, four additional heterocyclic and bicyclic compounds were prepared by palladium-catalyzed couplings (Table 1). Suzuki reactions were conducted with 5−10 mol % dichlorobis(triphenylphosphine)-palladium ($Pd(PPh_3)_2Cl_2$), 5 equiv of the appropriate organoboronic acid, and aqueous Na_2CO_3 as base in a DME/EtOH mixture.[31] Microwave heating at 120 °C for 30 min rendered compounds **9**, **12**, **13**, **17**, **19**-**21**, **24**, and **25** (Tables 1 and 2) in low to good isolated yields (26−62%), with the exception of sluggish pyridine-4-boronic acid which yielded para derivative **12** in only 17% yield. Stille coupling reactions were applied to afford compounds **10**, **11**, **18**, **22**, and **23** (Tables 1 and 2) using the corresponding tributyltin reagents and microwave irradiation for 20 min at 130 °C with 1 equiv of CuO[32] and 5 mol % $Pd(PPh_3)_2Cl_2$ in DMF. This generally furnished the target products in lower isolated yields (17−27%) compared to the Suzuki reactions, a trend which has been observed previously for symmetric HIV-1 PIs.[33] Alkynes **14**−**16** and **26**−**28** (Tables 1 and 2) were prepared using the Sonogashira reaction from phenylacetylene or ethynylpyridines in the presence of 5−8 mol % $Pd(PPh_3)_2Cl_2$ and 7−10 mol % CuI. Initially, a protocol including diethylamine as base and microwave processing for 30−40 min at 140 °C was applied for the Sonogashira reactions. However, this procedure promoted decomposition of the bromo precursors **1** and **8**. The conditions were therefore altered to include the less nucleophilic triethylamine as base at a lower temp (130 °C) for 60 min. This slightly milder method generally provided cleaner reactions and somewhat higher isolated yields of the acetylenic products, 22−45% (Table 1), compared to the 19−27% achieved with diethylamine as base (Table 2). All final compounds were purified by RPLC-MS with acetonitrile in 0.05% aqueous HCOOH as mobile phase to provide the pure products. Some of the analogues were found to partly form salts

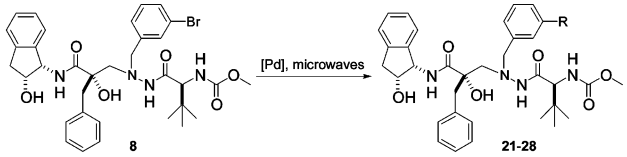
Table 1. Synthesis and Antiviral Activity of P1′ Para-Substituted Inhibitors **9**−**20**[a]



Cmpd	Reactant	Yield (%)	R-group	K_i[b] (nM)	EC_{50}[c] (μM)	CC_{50} (μM)
1	-	-		2.4	1.1	> 10
9		38		20	0.70	> 10
10		17		12	0.90	> 10
11[d,e]		27		5.0	0.18	> 10
12		17		5.5	0.22	> 10
13		59		11	> 10	> 10
14		22		15	> 10	> 10
15		34		9.0	1.0	4.9
16		45		7.8	0.75	> 10
17		31		3.8	1.1	> 10
18		19		2.1	1.0	7.8
19		49		11	1.0	> 10
20		62		3.5	> 10	> 10

[a] Microwave conditions: Suzuki reactions: $Pd(PPh_3)_2Cl_2$, Na_2CO_3 (aq), EtOH, DME, 120 °C, 30 min; Stille reactions: $Pd(PPh_3)_2Cl_2$, CuO, DMF, 130 °C, 20 min; Sonogashira reactions: $Pd(PPh_3)_2Cl_2$, Et_3N, CuI, DMF, 130 °C, 60 min. [b] Indinavir: $K_i = 0.52$ nM,[19] atazanavir: $K_i = 2.66$ nM.[16] [c] Indinavir: $EC_{50} = 0.041$ μM,[6,36] atazanavir: $EC_{50} = 0.0039$ μM.[37] [d] P_{app} (Caco-2) = 33×10^{-6} cm/s. [e] $Cl_{int} = 154$ μL/min/mg.

Table 2. Synthesis and Antiviral Activity of P1′ Meta-Substituted Inhibitors **21**−**28**[a]



Cmpd	Reactant	Yield (%)	R-group	K_i (nM)	EC_{50} (μM)	CC_{50} (μM)
8	-	-	Br	18	3.3	> 10
21	$(HO)_2B$–	26		23	> 10	> 10
22	$(n\text{-}Bu)_3Sn$–	18		15	> 10	4.4
23[b,c]	$(n\text{-}Bu)_3Sn$–	25		12	> 10	6.8
24	$(HO)_2B$–	31		93	2.0	> 10
25	$(HO)_2B$–	48		14	> 10	> 10
26	H–	27		23	> 10	> 10
27	H–	19		22	> 10	> 10
28	H–	23		18	> 10	> 10

[a] Microwave conditions: Suzuki reactions: $Pd(PPh_3)_2Cl_2$, Na_2CO_3 (aq), EtOH, DME, 120 °C, 30 min; Stille reactions: $Pd(PPh_3)_2Cl_2$, CuO, DMF, 130 °C, 20 min; Sonogashira reactions $Pd(PPh_3)_2Cl_2$, Et_2NH, CuI, DMF, 140 °C, 30−40 min. [b] P_{app} (Caco-2) = 11 × 10^{-6} cm/s. [c] Cl_{int} = 190 μL/min/mg.

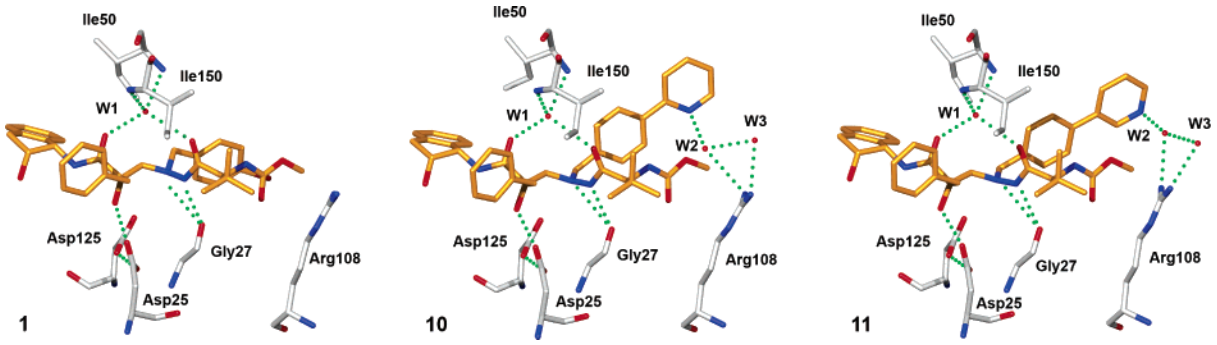


Figure 2. X-ray arrangement of inhibitor **1**, **10**, and **11** in the active site of the HIV-1 protease. The most relevant hydrogen bonding interactions between the inhibitors and the enzyme amino acid residues are represented by green dashed lines. The P1′ pyridyl groups of **10** and **11** are angled away from Arg108 via hydrogen bonding through a water molecule. The electronegative property of the bromine in **1** enables the P1′ group to close pack against Arg108.

with the formic acid. For these compounds, additional HRMS analyses were performed, and data presenting the actual mass within 5 ppm of the theoretical masses are included in the Supporting Information. The somewhat disappointing isolated yields can partly be explained by the rigorous purification procedure and partly by the complexity of the structures. Please note that all cross-couplings were performed without protection of the hydroxyl groups[34] and that no byproducts from water elimination were detected.

Biological Evaluations. Antiviral activities for compounds **8**−**28** are summarized as K_i and EC_{50} values in Tables 1 and 2. The previously investigated *p*-bromo compound **1** is included as a reference. Within the para collection (**1**, **9**−**20**, Table 1), all structures seemed to be well accommodated in the active site of the HIV-1 protease, and K_i values varying between 2.1 and 20 nM were encountered (Table 1). More interestingly, higher P1′ substituent dependence as deduced from the EC_{50} values was observed in the assay with HIV-1 infected MT4 cells. All compounds were active at concentrations up to 10 μM with EC_{50} values ranging from 0.18 to 1.1 μM except analogues **13**, **14**, and **20** with hydrophobic phenyl groups attached at a two-carbon distance from the parent P1′ benzyl group. The para 3- and 4-pyridyl derivatives **11** and **12** exhibited notably higher cellular activities (EC_{50} = 0.18 and 0.22 μM, respectively) than **1** (EC_{50} = 1.1 μM). In the meta library, (**8**, **21**−**28**, Table 2) slightly less potent enzyme inhibitors compared to the corresponding para analogues were obtained with K_i values ranging from 12 to 93 nM. Surprisingly, only two of the meta compounds exhibited antiviral activity on the cellular level, the best one being the 4-pyridine analogue **24** with an EC_{50} value of 2.0 μM (Table 2). Four compounds exhibited cell toxicity with CC_{50} values below 10 μM (**15**, **18**, **22**, and **23**, Tables 1 and 2). The 3-pyridyl analogues **11** and **23** (K_i = 5.0 and 12 nM, respectively) were further examined in a Caco-2 cell assay and for stability in liver microsome homogenate (Tables 1 and 2). Compound **11** exhibited excellent permeability (P_{app} = 33 × 10^{-6} cm/s) whereas the corresponding meta analogue **23** was more slowly transported across the Caco-2 cell membrane (P_{app} = 11 × 10^{-6} cm/s). The observed fast intrinsic clearance of these compounds in liver microsomes (Cl_{int} = 154 and 190 μL/min/mg, respectively) could be attributed to rapid oxidative metabolism of the P2 aminoindanol group.[35]

X-ray Crystallographic Data. The arrangements of compound **1**, **10**, and **11** in the active site of the HIV-1 protease including the most relevant hydrogen bonds, as deduced from X-ray crystallography, are presented in Figure 2 (PDB codes

2cej, 2cem, and 2cen, respesctively). The tertiary hydroxyl group in **1**, **10**, and **11** form a hydrogen bond to one of the catalytic aspartic acid residues (Asp25) in the enzyme and the hydrazide α- and β-nitrogens are hydrogen bonded to Gly27. Arg108 plays a crucial role for the positioning of the extended P1′ side chains, and in the case of **1**, the electronegative bromine can favorably close pack against the positively charged Arg side chain. Furthermore, in the complexes with compounds **10** and **11**, a water molecule is correctly positioned for hydrogen bonding between the pyridyl nitrogen atom and the Arg side chain. This arrangement forces the P1′ groups in **10** and **11** to be lifted up and away from Arg108. As a result, the position of the enzyme Pro181 residue differs (1.5 Å) between the three inhibitor/HIV-1 complexes. The P1′ groups are embedded in the enzyme S1′−S3′ cavity formed by amino acid residues 179−183.

Discussion

Generally, the para P1′ elongated structures exhibited higher enzyme affinity compared to the corresponding meta compounds, which could be due to an unfavorable angle of the P1′ side chains formed in the meta analogues for approach into the S1′−S3′ pocket. Not more than two inhibitors in the meta series were active on the cellular level, the best compound being **24** ($EC_{50} = 2.0\ \mu M$, Table 2). Thus, structure **24** was considerably less potent than the corresponding para derivative **12** ($EC_{50} = 0.22\ \mu M$, Table 1), although it should be taken into account that **24** also is a rather weak enzyme inhibitor ($K_i = 93$ nM, Table 2). The low cellular potencies of the meta analogues are not easy to explain, although a somewhat slower membrane transport, as deduced from the Caco-2 assay, is observed with the meta compound **23** ($P_{app} = 11 \times 10^{-6}$ cm/s, Table 2) as compared to the corresponding para derivative **11** ($P_{app} = 33 \times 10^{-6}$ cm/s, Table 1). By comparing the membrane permeation properties of compound **11** and **23** with data obtained from atazanavir and indinavir in the same assay the high P_{app} values of both **11** and **23** are exceptional.[20] This is probably due to intramolecular hydrogen bonding with the tertiary alcohol, which should prevent solvation of the hydroxyl group and result in a lower desolvation energy for entering the membrane phase.[38−40] In addition, conformational changes of these flexible molecules to attain a transient, more permeable structure could contribute to the fast membrane transport.[38] However, considering the favorable P_{app} values and in particular the K_i value of 12 nM, we can at present not explain why compound **23** was devoid of activity in the cell assay. Furthermore, it is notable that the 2-pyridyl derivative **10**, that is structurally most similar to atazanavir, is less potent than both the 3- and 4-pyridyl derivatives **11** and **12**.

Conclusion

Two series of HIV-1 protease inhibitors were synthesized from aryl bromide substituted templates by applying palladium-catalyzed cross-coupling reactions. Compared to conventional heating, high-density microwave irradiation reduced the reaction times of the cross-couplings from hours to minutes. The 21 investigated new inhibitors exhibited the following characteristics: (a) a tertiary alcohol based transition-state mimicking scaffold, (b) structural similarities to indinavir and atazanavir at the inhibitors P2 and P1′−P3′ side chains, and (c) a set of diverse elongations of the benzylic P1′ arm. The 3- or 4-pyridyl extensions in para position afforded structures with up to 7-fold increased potency on the cellular level compared to the previously reported tertiary alcohol based inhibitors. The overall best compound within this study was the para-substituted 3-pyridine analogue **11** which gave seven times higher cellular antiviral potency than parent compound **1**, showed no indications of cell toxicity and exhibited excellent membrane permeability properties.

Experimental Section

General Procedures for the Palladium-Catalyzed Reactions. Method A. Aryl bromide **1** or **8** (1 equiv), boronic acid (5 equiv), $Pd(PPh_3)_2Cl_2$ (0.05−0.1 equiv), 2 M Na_2CO_3 (aq, 3 equiv), EtOH (0.6 mL), and DME (2.4 mL) were stirred in a Smith process vial sealed under air at 120 °C for 30 min in the microwave cavity. Five drops of formic acid were added to the mixture, and then the solvent was evaporated followed by purification on RPLC-MS.

Method B. Aryl bromide **1** or **8** (1 equiv), tin reagent (4 equiv), $Pd(PPh_3)_2Cl_2$ (0.05 equiv), CuO (1−1.1 equiv), and DMF (2 mL) were stirred in a Smith process vial sealed under air at 130 °C for 20 min in the microwave cavity. CH_2Cl_2 was added to the mixture followed by washing with saturated $NaHCO_3$ (aq). The organic phase was dried (Na_2SO_4) and evaporated, and then the residue was redissolved in CH_3CN and washed with isohexane. The CH_3CN phase was evaporated, and the crude product was purified using RPLC-MS.

Method C. Aryl bromide **1** (1 equiv), alkyne (2 equiv), Et_3N (10 equiv), $Pd(PPh_3)_2Cl_2$ (0.05 equiv), CuI (0.1 equiv), and DMF (2.1 mL) were stirred in a Smith process vial sealed under air at 130 °C microwave heating for 60 min. Evaporation of most of the solvent was followed by purification using RPLC-MS.

Method D. Aryl bromide **8** (1 equiv), alkyne (1.2−1.3 equiv), Et_2NH (8.7 equiv), $Pd(PPh_3)_2Cl_2$ (0.08 equiv), CuI (0.07−0.09 equiv), and DMF (2 mL) were stirred in a Smith process vial sealed under air at 140 °C microwave heating for 30−40 min. Workup was performed by extracting the mixture with CH_2Cl_2 and H_2O. The organic phase was evaporated, and then the product was purified by RPLC-MS.

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Supporting Information Available: Experimental details and spectroscopic data for compounds **6** and **8**−**28**, elemental analysis data, X-ray structure determination details and statistics, procedures for enzyme, cytotoxicity and Caco-2 assays, and liver microsome stability evaluation. This material is available free of charge via the Internet at http://pubs.acs.org.

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