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Novel nonpeptidic inhibitors of HIV-1 protease obtained via a new multicomponent chemistry strategy

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Abstract—Using a newly developed multicomponent chemistry strategy in combination with structure based drug design, a new class of HIV-1 protease inhibitors has been obtained.

Hardly has any single disease received so much attention, in such a short time since it was first reported, as the acquired immunodeficiency syndrome (AIDS). The discovery of the human immunodeficiency virus (HIV), as the causal agent of the illness, was quickly followed by the identification of proteins essential in the virus life cycle.

One of these proteins the HIV-1 protease, which belongs to the family of the aspartyl proteases, plays a crucial role in viral processing and maturation.

The high-resolution determination of the X-ray structure of the HIV-1 protease[1] opened the door for the development of inhibitors (HIV-PI), which were tailored after the target peptide sequences. Using peptidomimetic design, HIV-PI were developed in which the scissile-bond was replaced by a nonscissile transition state analog bond.[2] All HIV-PI currently approved for the treatment of HIV infection, namely atazanavir,[3] lopinavir,[4] amprenavir,[5] indinavir,[6] nelfinavir,[7] ritonavir,[8] and saquinavir,[2] belong to this type of compounds (statine motif). Inhibitors with peptidic character often suffer from low oral bioavailability and rapid biliary excretion. In order to improve the biopharmaceutic properties of future antiviral drugs, inhibitors of nonpeptidic or reduced peptidic character have been proposed. One of these compounds tipranavir[9] recently entered phase III clinical trials.

Initially, monotherapy was used to treat HIV infection and resistance quickly arose. When combinations of antiviral agents started to be used, HIV variants resistant to multiple HIV-PI then appeared.[10] This increase of cross-resistance dictates the need for the discovery of new classes of inhibitors. Current HIV-PI pose a major problem because of the lengthy and linear synthetic approach used to synthesize them.[11] Taking into account that the majority of infected people can barely afford treatment it would be of advantage to have drugs with low manufacturing costs.

Multicomponent chemistry offers a tool to produce analogs of existing drugs or completely novel chemical structures using very few steps.[12]

In this letter we report the synthesis and initial biological evaluation of a new class of HIV-PI, obtained by a previously undescribed three-component reaction.

The synthesis is composed of two steps; a combination of the classical three-component Passerini reaction[13] followed by a base catalyzed Dieckmann condensation

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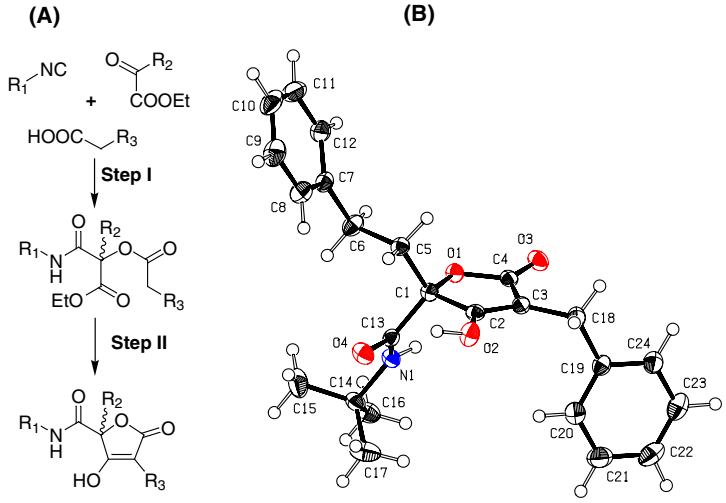

Figure 1. (A) Reagents and conditions: (step I) THF, rt; (step II) LDA, THF, −78 °C. (B) X-ray structure of compound **12**.

to achieve the ring closure[14] (Fig. 1A). Both steps can be carried out separately or in a one-pot manner.[15]

There is basically no preference for the substituents R_1, R_2, and R_3. The only prerequisite is the use of α-methylene carboxylic acids, since the methylene carbon gets incorporated into the five-member ring.

The central structural element of this new class of molecules is a 3-hydroxy-5-oxo-2,5-dihydrofuran-2-carboxamide moiety, which resembles the 4-hydroxy-2-pyrone element characteristic of previously described inhibitors.[16,17] However in contrast to the 4-hydroxy-2-pyrones the 3-hydroxy-5-oxo-2,5-dihydrofuran-2-carboxamide moiety is accessible to a very short MCR synthetic route. In order to assess the suitability of this compound class as potential HIV-PI, molecular modeling studies were carried out. For the studies the modeling tools implemented in the package Moloc[18] were used.

The X-ray structure of the HIV-1 protease reveals a dimer of two identical aspartic protease-like subunits, related by the intramolecular twofold axis.[1] The signature active-site triad (Asp25-Thr26-Gly27) of each of the domains is located in a loop and the two carboxylic acid groups of the symmetry-related aspartates (Asp25 and Asp25′) are practically co-planar and show close contacts. The determination of the X-ray structure of the HIV-1 protease with a substrate-based inhibitor[19] helped define the binding subsites for potential inhibitors in the enzyme. The S1–S3, and S1′–S3′ subsites are rather well defined. The S1, S2, S1′, S2′ pockets are hydrophobic in nature, but available structures of protease-inhibitor complexes show that while the S1 and S1′ subsites are occupied by hydrophobic groups, the S2, S2′ pockets can accommodate both hydrophilic and hydrophobic groups.

The structures of ritonavir[8] and tipranavir[9] in complex with the HIV-1 protease were used as basis for the modeling studies. Moreover to get structural insight the X-ray structure of a putative inhibitor was solved (Fig. 1B).[20]

In principle an inhibitor should address all four subsites S1, S2, S1′, and S2′ simultaneously and ideally further subsites as well. From a simple inspection of the core structure of the reaction products, it becomes readily apparent that the occupation of the four primary subsites can only be achieved if one of the reactants, R_3, addresses two of these subsites simultaneously (Fig. 1A). Based on the initial modeling studies a possible binding mode of a putative inhibitor was proposed (Fig. 2).

During the course of the reaction a stereocenter is formed at the 2 position of the central cycle. The carboxamide moiety, which stems from the isocyanide, would occupy in the case of the *R*-enantiomer the S2′ subsite while the other substituent, which stems from the ester would occupy the S1′ subsite. The *S*-enantiomer on the other hand would position the carboxamide moiety in the S1′ pocket where the carbonyl group could form a hydrogen bond with the backbone amide of Ile50 in the flap region and therefore is energetically favored. This is in contrast with the mode of binding of the 4-hydroxy-2-pyrone class of inhibitors,[17] where the carbonyl group of the central cycle makes hydrogen bonds to the backbone-amide nitrogens of Ile50/Ile50′.

The groups, which occupy the S1 and S2 subsites, can then only be provided by the α-methylene carboxylic acid. Both subsites are addressed simultaneously only if

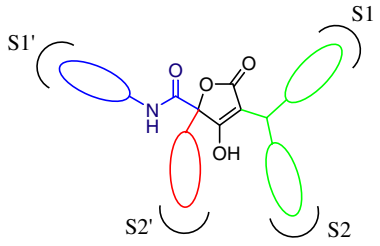

Figure 2. Schematic mode of binding for a putative HIV-1 protease inhibitor of the new 3-hydroxy-5-oxo-2,5-dihydrofuran-2-carboxamide class. The substituents are color-coded depending on the reactant they originate from. Red: ester, blue: isocyanide, and green: α-methylene acid. The four primary binding sites are denoted S1, S1′, S2, and S2′.

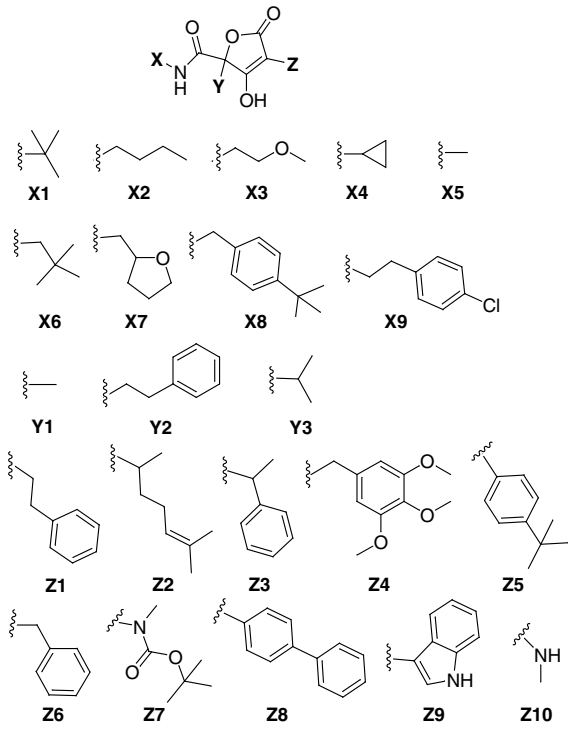

Figure 3. Substituents in the synthesized compounds.

there is a bisubstitution at the β position of the aforementioned reactant.

To test the hypothesis, a small number of commercially available ethylesters and α-methylene carboxylic acids, as well as isocyanides from our in house collection were chosen (Fig. 3).

The biological activity, IC_{50}, of the synthesized compounds was determined using a modified version of the method described by Matayoshi et al.[21]

All synthesized compounds were purified either by reverse phase HPLC or conventional silica gel chromatography and no attempt was made to separate the enantiomers. Table 1 summarizes the structural and biological data for the compounds.

Table 1. Biological activities of synthesized compounds

Compound	Components	IC_{50} (μM)
1	X1Y1Z1	70
2	X2Y1Z1	>100
3	X1Y1Z2	151
4	X1Y1Z3	105
5	X3Y1Z3	224
6	X4Y1Z4	>1000
7	X4Y1Z1	294
8	X5Y1Z3	244
9	X1Y2Z3	4
10	X1Y1Z4	>1000
11	X6Y2Z4	>1000
12	X1Y2Z6	11
13	X1Y2Z1	3
14	X1Y2Z5	17
15	X1Y2Z7	47
16	X1Y2Z8	27
17	X7Y2Z7	193
18	X8Y2Z3	5
19	X1Y2Z2	1
20	X9Y3Z3	31
21	X7Y2Z10	>1000
22	X1Y2Z9	107

The chemical formula of the individual compounds can be derived from the components and Figure 3.

A look at the values of inhibitory activity shows that a short and bulky substituent like Z4 leads to a complete loss of activity. A long hydrophobic substituent like Y2 leads to a better activity compared with the short Y1 substituent. Otherwise a wide variety of substitutions do not lead to a complete loss of activity.

In order to investigate the mode of action of the compound class, kinetic studies using compounds **1** and **9** as representatives were performed. A competitive mode of inhibition could be determined for both compounds based on the Cornish-Bowden and Dixon plots.[22]

Figure 4 shows a model of compound **9** docked in the active site of the HIV-1 protease. Residues that form the S1, S2, S1′, and S2′ are colored yellow, pink, orange, and magenta, respectively. Residues Ile84 and Ile84′ have been assigned the colors of the S2′ and S2 subsites but could be considered as part of the S1 and

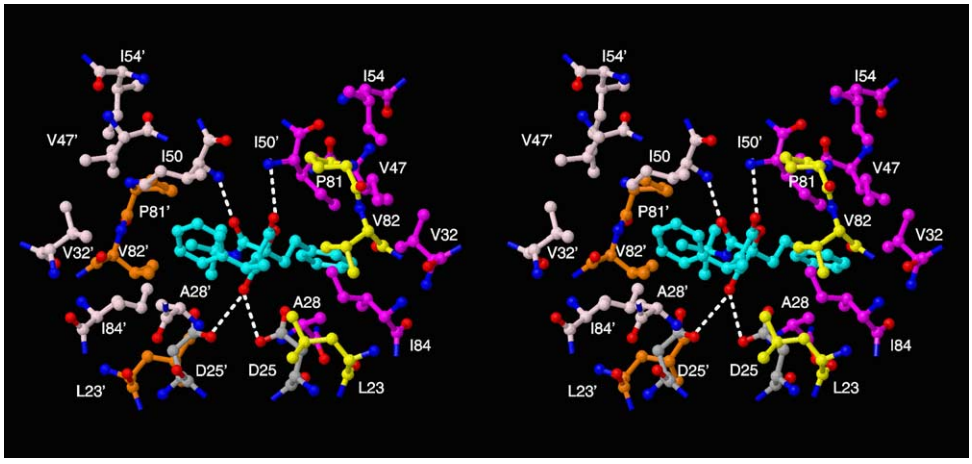

Figure 4. Compound **9** docked in the active site of the HIV-1 protease. See text for details. Figure was generated with Molmol.[24]

Table 2. Comparison of different activities dependent of the purification state

Compound	Components	IC_{50} I (μM)	IC_{50} II (μM)	IC_{50} III (μM)
23	X1Y2Z3	6	4.1	2.0
24	X1Y2Z2	15	1.3	1.3
25	X8Y2Z3(*R*)	84	1.2	4.6
26	X8Y2Z3(*S*)	84	1.2	5.4

The chemical formula of the individual compounds can be derived from the components and Figure 3 (*R* and *S* are the corresponding enantiomers of the methylene acid). IC_{50} I determined from the crude plate mixture; IC_{50} II after CLND/MS correction from the crude mixture; IC_{50} III of the compounds after resynthesis and purification.

S1′ subsites as well. The two active site aspartate residues are colored gray. The hydrogen bond interactions of the carboxamide moiety with the backbone NH of Ile50, of the carboxy group of the central ring with the NH of Ile50′ and of the 3-hydroxy group with the active site aspartates are represented by dashed lines. The occupancy of the subsites by the ring substituents is readily clear. The *t*-butyl group of the carboxamide binds in the S1′ pocket and the dimethyl-phenyl in the S2′ pocket. Whereas the phenyl and methyl groups provided by the α-methylene acid occupy the S2 and S1 subsites, respectively. It becomes apparent that larger substituents could be accommodated at the S1 and S2 subsites and should lead to an increase in the inhibitory activity.

In parallel to the modeling studies, array synthesis was performed. We optimized the two step sequence for performance in 96 well plates. The biological activity of the raw plates was determined after evaporation of the solvent.

A daughter plate was analyzed with a nitrogen specific CLND–MS coupling.[23] The herein determined yield was used to correct the crude IC_{50} value. In several cases the compound was resynthesized and purified. Encouragingly the corrected IC_{50} values were very similar to the ones of the purified compounds. A few examples are shown on Table 2. This developed procedure should allow for a faster lead optimization since the individual compounds can be directly tested without need for prior time-consuming purification.

In summary, we can show that a newly developed multicomponent reaction sequence could be used to easily synthesize a new class of competitive HIV-PI. Further studies with a wider variety of starting materials should lead to compounds with improved biological and pharmacokinetic activities.

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15. *Step I. Passerini product*: 1 equiv 2-substituted acetic acid derivative (5 mmol) were added to a stirred solution of 1 equiv α-ketoester (5 mmol) in 5 mL THF at room temperature. After 5 min, 1 equiv isocyanide was added and allowed to stir 24–48 h. The reaction mixture was then concentrated in vacuum. The residue was purified by silica gel chromatography, recrystallized from ethyl acetate/*n*-pentane, or by prep. HPLC to give an oil or a solid.
Step II. Cyclization: 2 equiv lithiumdiisopropylamide (1 mmol) was added to a stirred solution of 1 equiv Passerini product (0.5 mmol) in dry THF (5 mL) at

−78 °C and nitrogen atmosphere. After stirring at −78°C for 0.5–1 h, the mixture was allowed to stir at room temperature for 1 h.
The reaction was then quenched with water (10 mL) and acidified to pH 2 using 2 M HCl. The mixture was then extracted with ethyl acetate (4 × 10 mL), and the organic layer was the dried with Na_2SO_4 and concentrated in vacuum.
The residue was purified by silica gel chromatography, recrystallized from ethyl acetate/*n*-pentane, or by prep. HPLC to give an oil or a solid. Yield 12–95%.

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20. (a) X-ray structure determination and NMR characterization of compound **12** (a) $C_{24}H_{27}NO_4$, $M_r = 393.47$, colorless fragment ($0.53 \times 0.56 \times 0.64\,mm^3$), monoclinic, $P2_1/n$ (No 14), $a = 9.3647(1)$, $b = 11.6303(1)$, $c = 19.1562(2)$ Å, $\beta = 95.5617(4)$, $V = 2076.56(4)$ Å^3, $Z = 4$, $d_{calc} = 1.259\,g\,cm^{-3}$, $F_{000} = 840$, $\mu = 0.085\,mm^{-1}$. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no CCDC-235266 (12). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk); (b) Data Collection Software for Nonius kappa-CCD devices, Delft (The Netherlands), 2001; (c) Otwinowski, Z.; Minor, W. In *Methods in Enzymology*; Carter, W. C., Jr., Sweet, R. M., Eds.; Academic: New York, 1997; Vol. 276, pp 307–326; (d) SIR92: Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M. C.; Polidori, G.; Camalli, M. *J. Appl. Crystallogr.* **1994**, *27*, 435; (e) Spek, A. L. PLATON; Utrecht University: Utrecht (The Netherlands), 2001; (f) Sheldrick G. M. SHELXL-97. Universität Göttingen: Göttingen (Germany), 1998. 4-Benzyl-3-hydroxy-5-oxo-2-phenethyl-2,5-dihydro-furan-2-carboxylic acid *tert*-butylamide: 1 mmol gave colorless crystals 355 mg (90%) $C_{24}H_{27}NO_4$ MW: 393.47 g/mol; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.37$ (s, 9H, $C(CH_3)_3$), 2.06–2.16 (m, 1H), 2.43–2.67 (m, 3H), 3.52/3.57 (AB, $^2J = 14.9$ Hz, 2H, CH_2-Ph), 6.51 (s, 1H, NH), 7.112 (d, $J = 7.5$ Hz, 2H), 7.20–7.28 (m, 8H), 8.60 (br s, 1H, OH). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 27.37$ (CH_2), 28.39 ($C(CH_3)_3$), 29.43 (CH_2), 38.54 (CH_2), 52.63 ($C(CH_3)_3$), 82.12 (CCH_2CH_2), 126.36 (CH-ar), 128.24 ($C_{quart.}$), 128.38 (CH-ar), 128.43 (CH-ar), 128.51 (CH-ar), 138.33 ($C_{quart.}$), 139.62 ($C_{quart.}$), 168.64 (C═O), 172.11 (C═O). HPLC-MS (ESI): t_R ($\lambda = 220/254$ nm) = 3.99 min, $m/z = 394$ $[M+H]^+$, 416 $[M+Na]^+$.
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