Dimeric 4-Aryl-1,4-Dihydropyridines: Development of a Third Class of Nonpeptidic HIV-1 Protease Inhibitors

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Abstract: Cross-resistance development against most peptidic HIV-1 protease inhibitors (PI) forces the development of nonpeptidic alternatives. The classes of nonpeptidic protease inhibitors was limited so far to cyclic ureas and 4-hydroxy-2-pyrones with problems of limited bioavailability by extensive metabolism and protein binding. Cage dimeric 4-aryl-1,4-dihydropyridines have been developed as third class of nonpeptidic PIs. In the following synthesis, molecular modeling and biological activities of a first series of the novel PIs are reviewed. Bioavailability of the dimers will not be limited by protein binding and metabolism as far as evaluated.

1. INTRODUCTION

Since the discovery of the human immunodeficiency virus type-1 (HIV-1) as causative agent of acquired immunodeficiency syndrome (AIDS) [1], various peptidic PIs have been established in combined therapy regimes against the disease [2]. Those combined therapies were expected to lower an early observed resistance development against the peptidic PIs. However, meanwhile certain cross-resistances are described against the so far marketed PIs [3]. Severe side effects like cardiovascular morbidity and mortality are additional problems of the highly-dosed peptidic PIs with poor oral bioavailability [2]. Moreover, the lipodystrophy syndrome described during treatment with the peptidic PIs forces breaking off of the therapies. All these problems early strengthened the development of novel nonpeptidic PIs with promising better bioavailability.

First class of nonpeptidic PIs has been cyclic ureas which show different binding mode to the amino acids Ile50/50´ of the active site cavity of HIV-1 protease (PR) than all peptidic inhibitors [4]. While the peptidic PIs primary bind to a water molecule that binds to the *N*H-functions of Ile50/50´, cyclic ureas directly bind to the *N*H-functions of those amino acids *via* their urea carbonyl group (Fig. **1**).

Best candidate of the first generation **DMP 323** with C_2-symmetric properties like the active cage cavity of PR failed in clinical trials with insufficient oral bioavailability due to poor absorption and extensive oxidative metabolism by the cytochrom-P450 system [5]. C_2 symmetric **DMP 450** as candidate of the second generation showed improved absorption rates, but unsatisfying blood levels for effective therapy of HIV-1 mutants caused by high protein binding [5]. **DMP 851** as unsymmetric urea of the third generation with improved but not satisfying bioavailability (63% in dogs) presently undergoes clinical trials (Fig. **2**) [5].

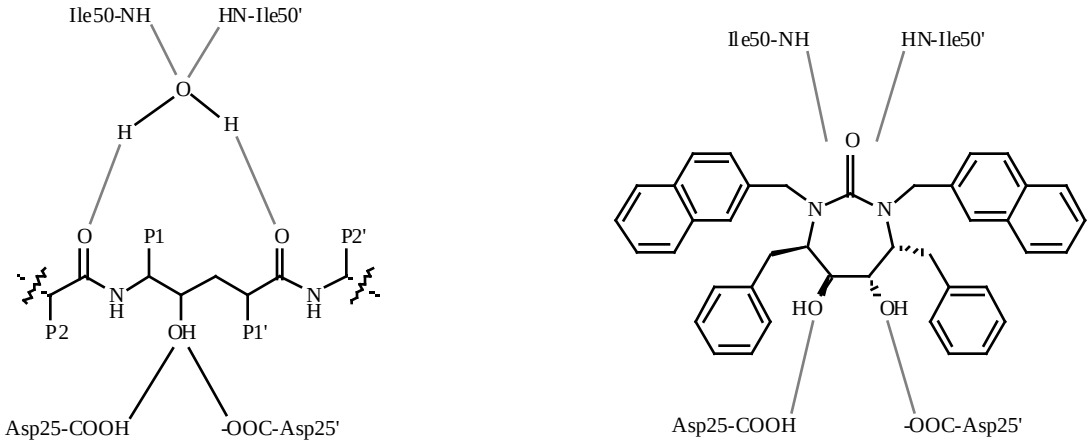

Fig. (1). Binding of one peptidic PI (above) and one cyclic urea (below) to the amino acids of PR.

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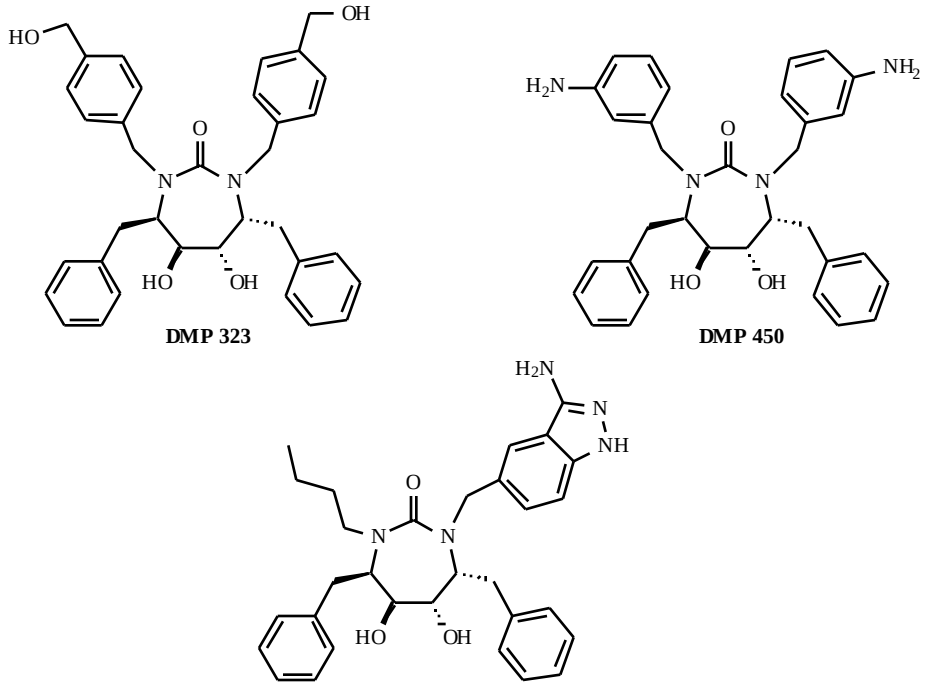


Fig. (2). Clinical candidates of cyclic ureas.

Broad screening efforts led to the discovery of 4-hydroxycoumarines with warfarin and phenprocoumon as first candidates of the second class of PIs. They also show direct binding to Ile50/50´ like cyclic ureas *via* oxygene atoms of the lactone structure [6]. Although PR inhibitory activities in the micromolar range was insufficient for therapy, excellent bioavailabilities and long half-lives encouraged for further developments (Fig. **3**) [6].

PNU-96988 with better PR inhibitory activity than warfarin and phenprocoumon disappointed in cell cultures. **PNU-103017** as candidate of the second generation with improved binding properties to hydrophobic regions (cyclooctyl residue) and amino acids Gly48 and Asp29 (sulfonamide residue) of PR failed in clinical trials because of high protein binding leading to insufficient blood levels [7]. Finally, **PNU-140690** named as **Tipranavir** was composed with structural elements of the preceded compounds as flexible phenyl alkyl side chain like in **PNU-96988**, aromatic sulfonamide residue from **PNU-103017** at a dihydropyrone scaffold. Protein binding was lower than that of **PNU-103017** [7]. **Tipranavir** presently undergoes clinical trials.

The poblems of the peptidic PIs, mainly cross-resistance development and the bioavailability problems of the so far

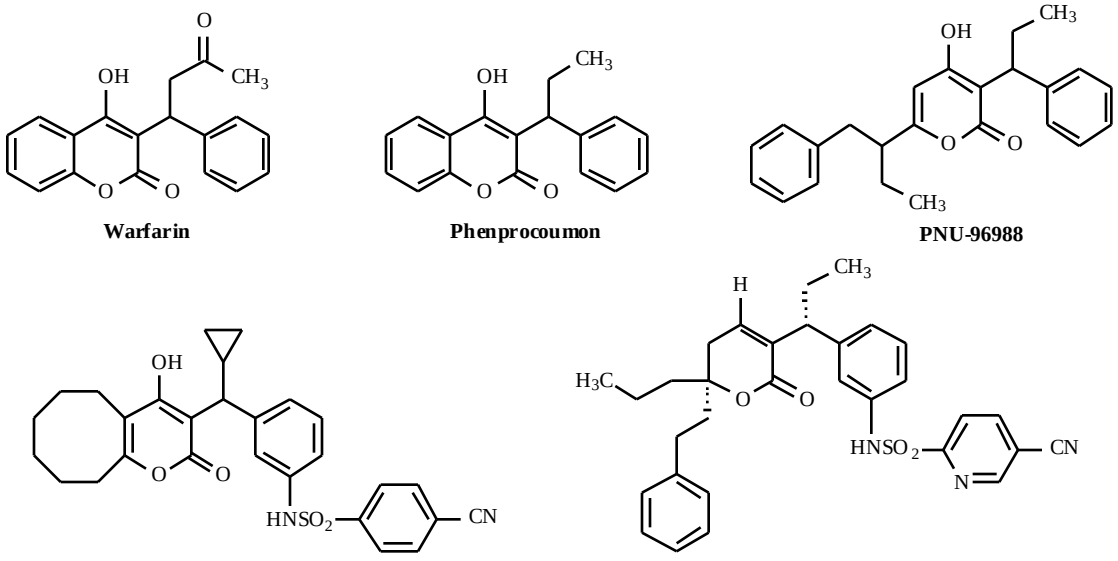
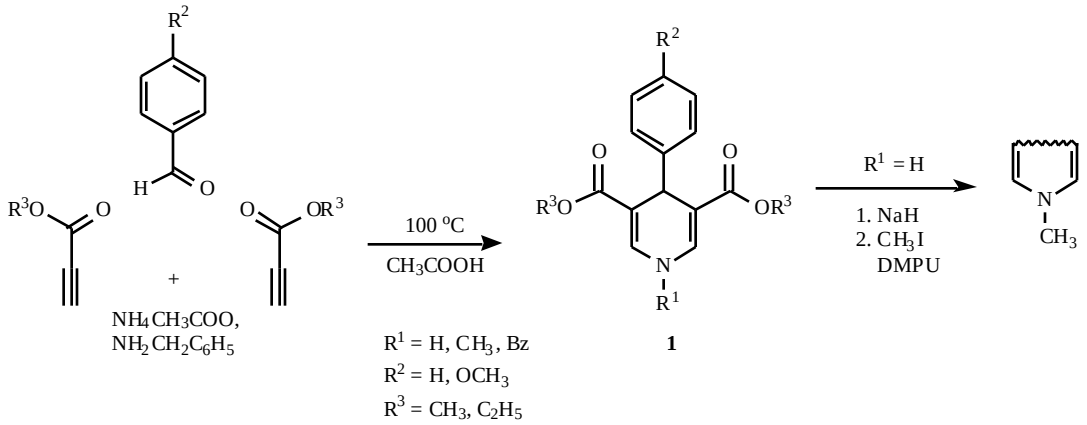


Fig. (3). 4-Hydroxycoumarine and 4-hydroxy-2-pyrone compounds in the development to clinical candidate **Tipranavir**.



Scheme 1.

unsatisfying nonpeptidic alternatives, strengthened the search for novel nonpeptidic lead structures with better bioavailabilities. Based on the discovery of topochemically dimerizing 4-aryl-1,4-dihydropyridines, discussed in the following, the conception and realization of a completely novel class of nonpeptidic PIs was developed.

2. CHEMISTRY

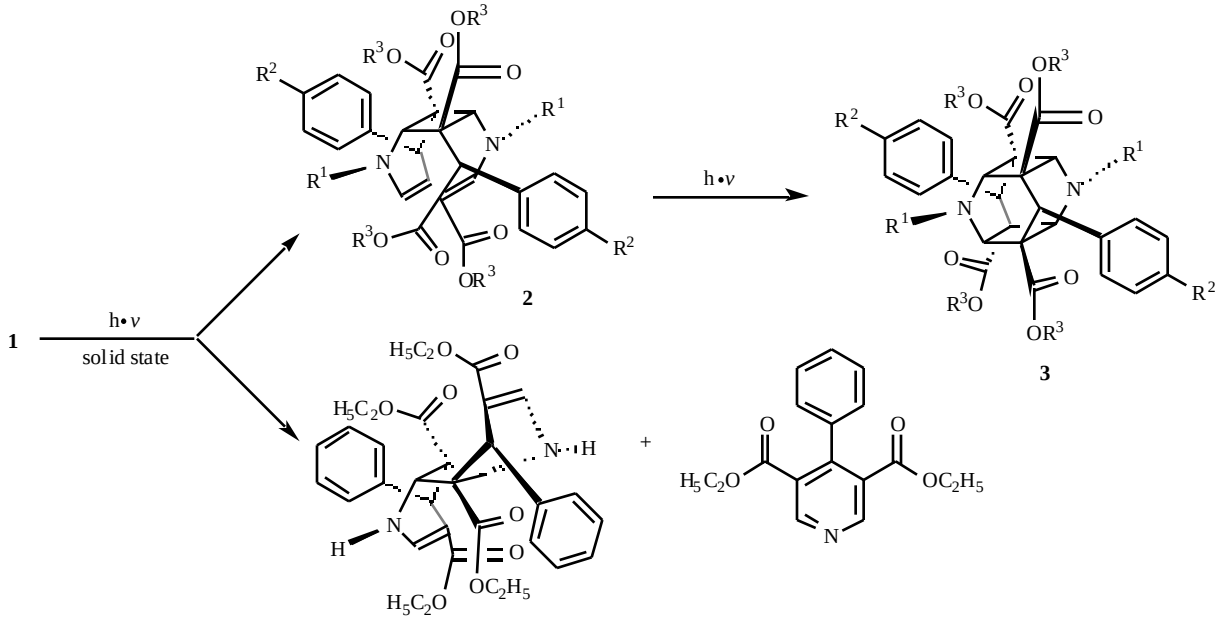
2.1. Conception

4-Aryl-1,4-dihydropyridine **1** with *N*H and *N*-alkyl substitutions were prepared either by cyclocondensation reaction of aromatic aldehyde, alkyl propiolate and ammonium acetate or benzylamine in acetic acid (Scheme 1) or by methylation of the corresponding 1,4-dihydropyridine anion [8].

Compounds **1** were found to be highly light-sensitive in the crystalline state. They almost exclusively yielded cage dimeric 4-aryl-1,4-dihydropyridines **3** except one investigated derivative with *N*H substitution that yielded an *anti* dimer besides small amounts of pyridine as photooxidation product (Scheme 2) [8]. The cage formation exclusively proceeded *via syn* dimeric intermediates **2** as was demonstrated by solid-state ^{13}C nmr spectroscopy [9].

The nearly quantitative cage formation was shown to result from crystal packing of the monomeric 4-aryl-1,4-dihydropyridines. X-Ray crystal structure analysis of several derivatives proved the nearest distance of potentially reacting double bonds as decisive criterion for the dimerization yielding centrosymmetric dimers from the reaction of centrosymmetrically arranged monomers (Fig. **4**) [10].

The cage dimers **3** possess favourable C_2-symmetric properties of their molecular structures like the active cage cavity of PR. After final ester group reduction to alcoholic functions the cage dimers show certain structural conformities with cyclic as well as azacyclic ureas that are developed as structural urea variants, e. g. **A-98881** (Fig. **5**) [11]. With respect to potential binding properties to the hydrophobic binding regions S1/S1´and S2/S2´ of PR the aryl substituents of the dimers were varied with 4-methoxyphenyl and phenyl, *N*-substituents with hydrogen,



Scheme 2.

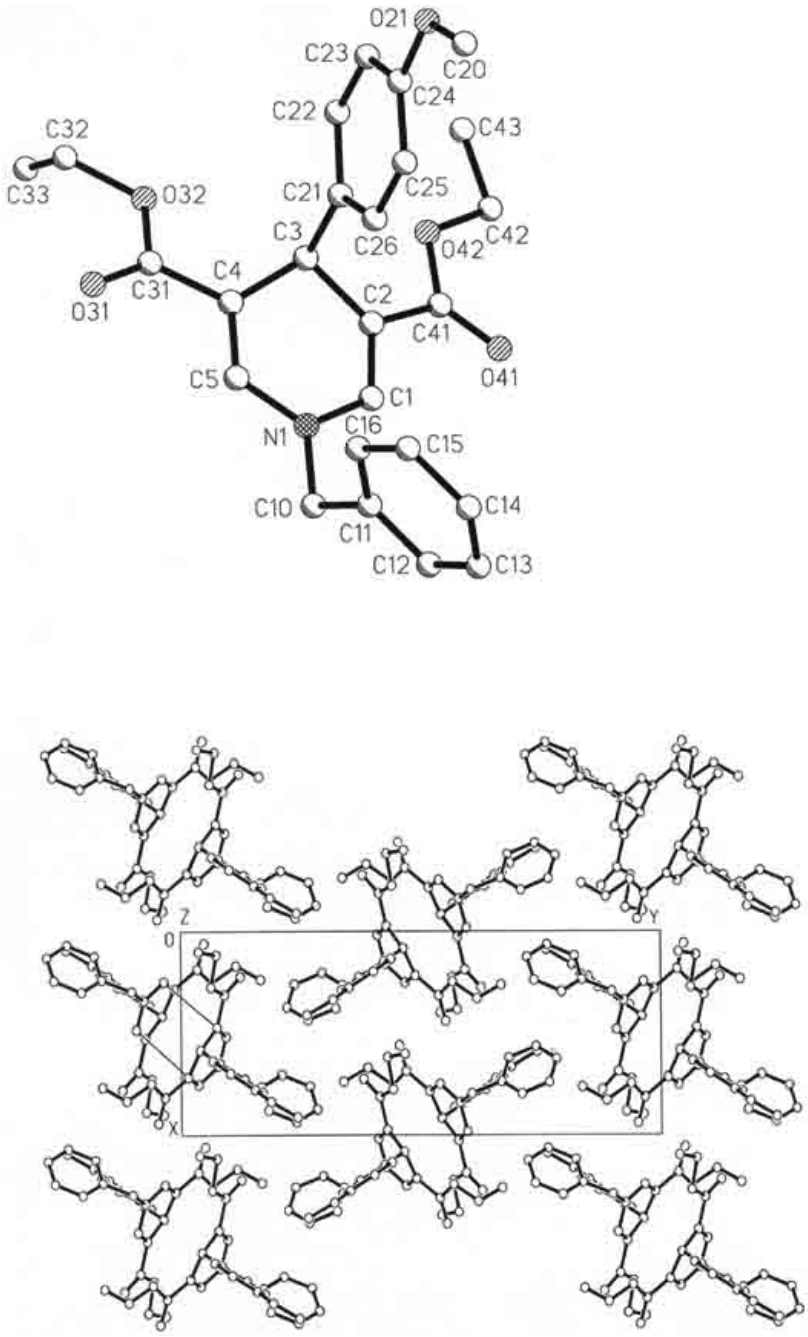


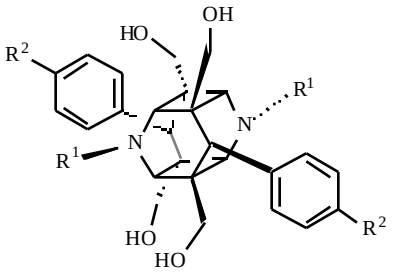
Fig. (4). Molecular structure of photoreactive *N*-benzyl-4-aryl-1,4-dihydropyridine **1** with $R^2 = OCH_3$ and $R^3 = OC_2H_5$ (above) and crystal packing of the compound with reacting double bonds of neighbouring molecules with distances of 3.490(6) Å (C1 - C4´) and 3.492(6) Å (C2 - C5´) (below).

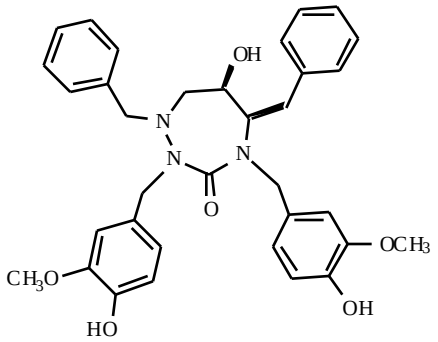
alkyl (methyl/benzyl), acyl (acetyl/phenylacetyl) and acyloxy (methoxy-/phenoxy-/tert. butoxycarbonyl).

2.2. Synthesis

2.2.1. N-Alkyl and NH-Cage Dimers

Cage dimers **3** have been given by solid-state photodimerization as discussed or, in the case of photostability resulting from unfavourable crystal packing, by solution dimerization reactions with lowered yields due to a partly observed *anti* dimer formation [12]. Ester group reduction of the *N*-alkyl derivatives to alcoholic target structures **4** succeeded with lithium aluminium hydride ($LiAlH_4$) at low temperatures (-8 °C) without any cage dimer fragmentation being observed (Scheme **3**) [13]. However, treatment of the *N*H-compounds with hydride agent leads to a primary proton abstraction followed by a dimer





A-98881

Fig. (5). Cage dimeric lead structure and azacyclic urea **A-98881**.

fragmentation to monomeric starting 1,4-dihydropyridine [13].

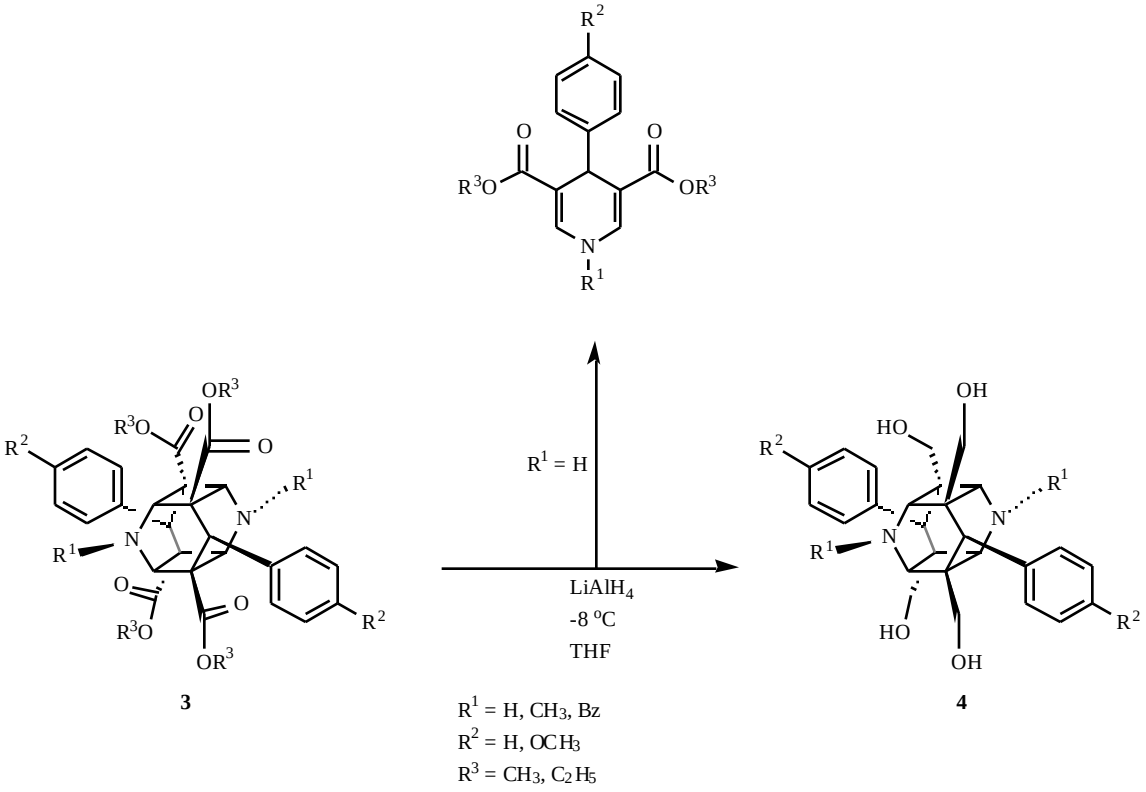
The desired *N*H-alcoholic structures of **4** were given by acylation of the *N*H-cage dimers **3** with benzylchloroformiate, followed by selective ester group reduction at the substituted cyclobutane using calcium borohydride and, finally, reductive carbamate ester cleavage with hydrogen on palladium/charcoal (Scheme **4**) [13].

2.2.2. N-Acyl and N-Acyloxy Cage Dimers

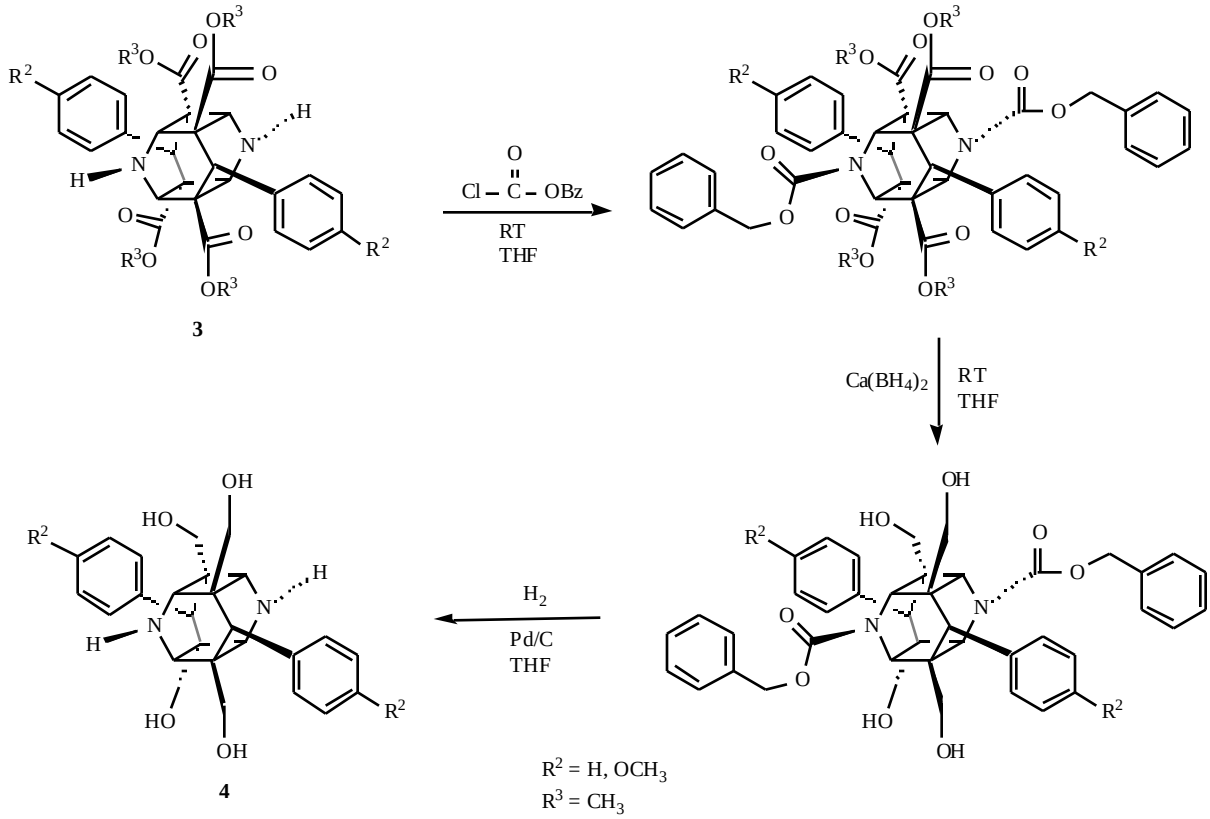
N-Acyl and *N*-acyloxy derivatives of alcoholic cage dimers (**4**) were given by primary *N*-acylation of the *N*H-substituted cage dimers with an excess of acyl- or acyloxychlorides to **3** and following selective ester group reduction using calcium borohydride (Scheme **5**) [14]. The *N*-Boc (tert. butoxycarbonyl) derivatives **3** (R^1 = H, OCH_3, R^2 = O*t*Bu) resulted from a solution photodimerization reaction of monomeric 4-aryl-1,4-dihydropyridine **1** (R^1 = H, OCH_3) which have been yielded by *N*-acylation of monomeric *N*H-dihydropyridines with di-tert. butyl-dicarbonate in triethylamine/DMF [14].

3. MOLECULAR MODELING

Molecular modeling studies have been made with alcoholic cage dimeric 4-(4-methoxyphenyl)-*N*-benzyl derivative (see Table 1) in comparison to cyclic as well as



Scheme 3.

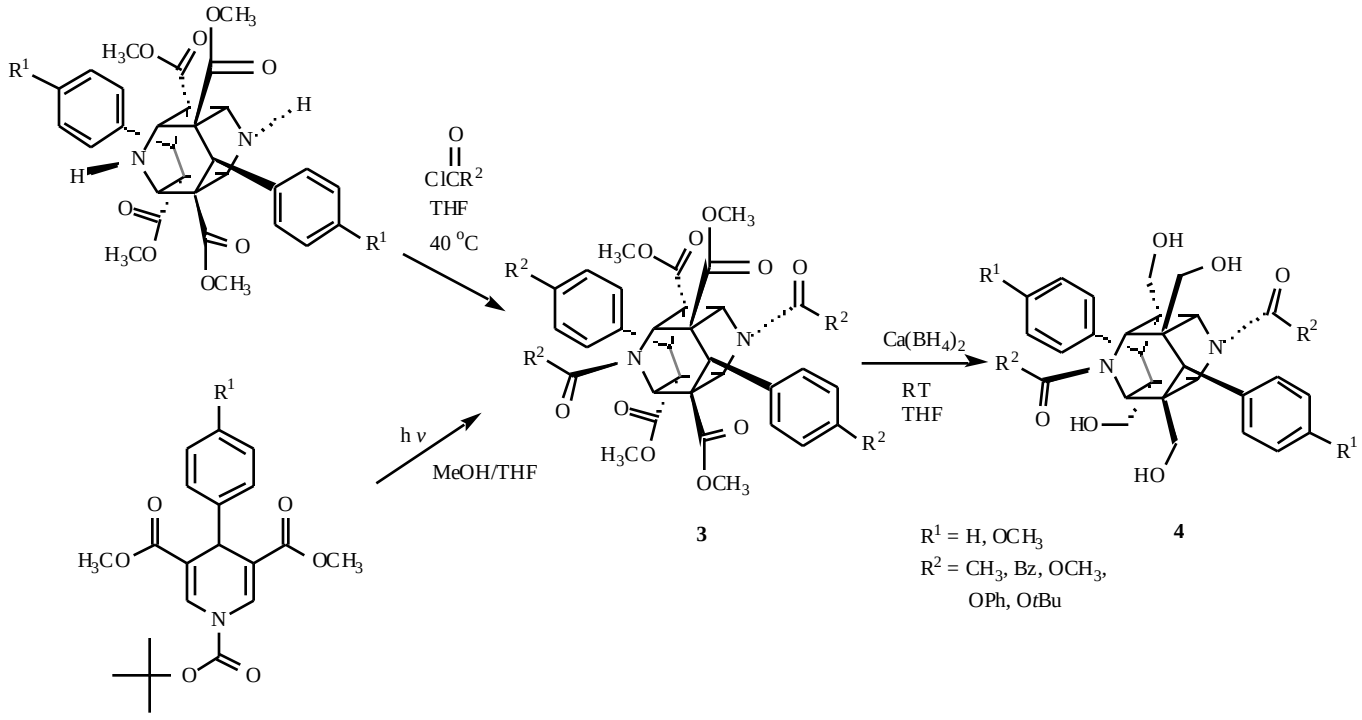


3 **4**

Scheme 4.

azacyclic ureas concerning molecular properties, i.e. electrostatic potentials (EP), and potential binding properties to PR. Ureas have been used in their enzyme-bound conformations [15].

Three symmetric conformations (**4A-C**) finally remained after a conformational search of the cage dimer and extensive minimization using Tripos force field. Two of them (**4A** and **4B**) possessed C_2 symmetric properties derived from either positive or negative values of each couple of important torsion angles RB1/RB1´, RB2/RB2´ and RB3/RB3´. The third one (**4C**) was centrosymmetric with both positive and negative values of a couple of torsion angles (Table 1) [15].



3 **4**

Scheme 5.

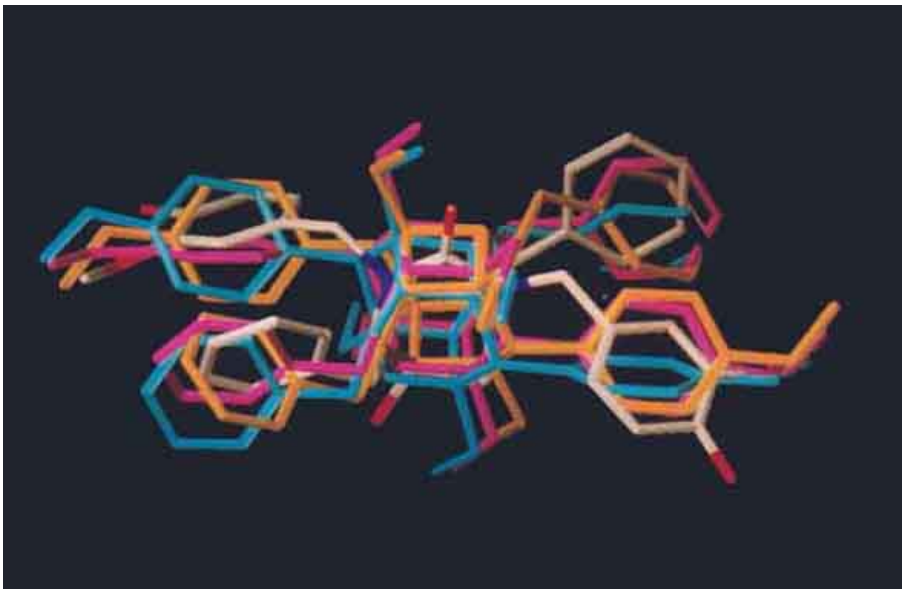


Fig. (6). Conformers **4A** (magenta), **4B** (cyan) and **4C** (yellow) fitted to **A-98881** after CoMFA field fit.

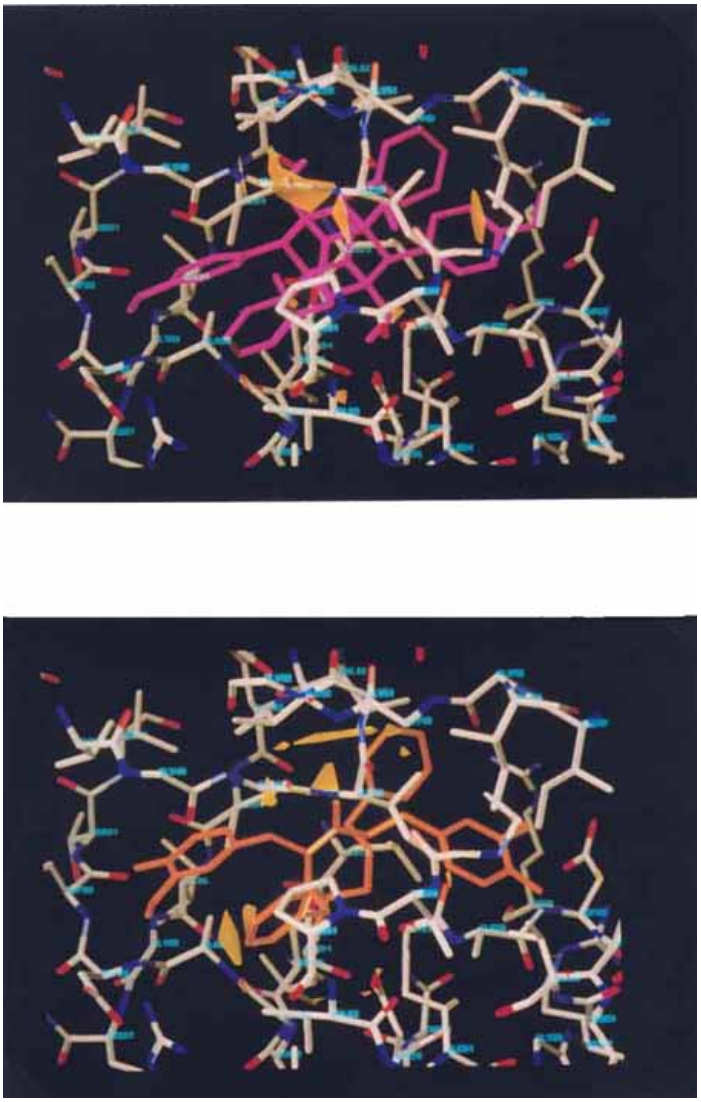
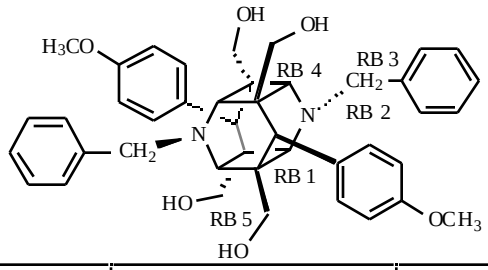


Fig. (7). Most favourable interaction regions for the *N*H-amide probe of conformer **4A** (above) and **A-98881** (below).

Table 1.



Conformation	Energy [kcal/mol]	RB1/RB1´ [°]	RB2/RB2´ [°]	RB3/RB3´ [°]
4A	74.98	-87.8/-88.1	103.1/104.5	72.5/75.1
4B	74.99	-136.3/-143.4	111.3/111.9	-75.7/-73.1
4C	79.97	-88.1/85.9	154.1/-148.4	165.8/-170.6

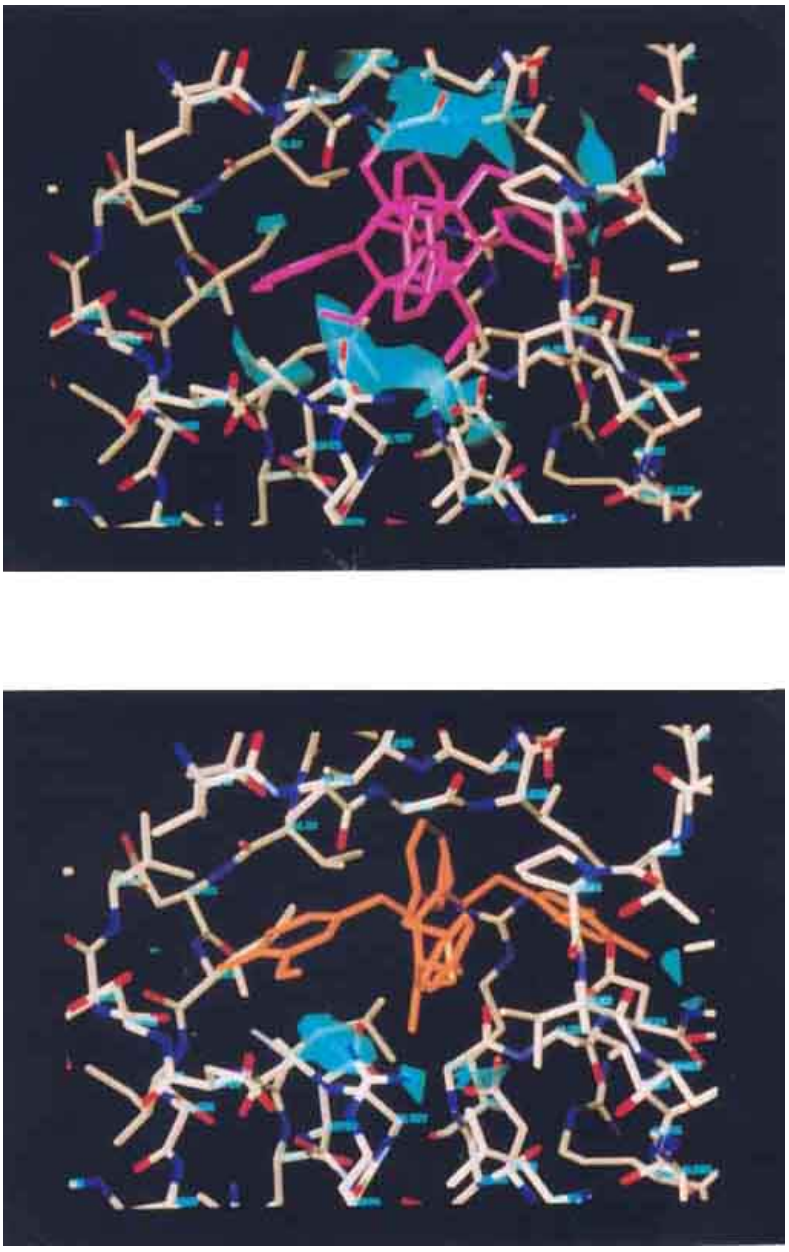


Fig. (8). Most favourable interaction regions for the carbonyl (COO^-) probe of conformer **4A** (above) and **A-98881** (below).

For comparison with azacyclic urea **A-98881** that itself showed perfect match with cyclic urea aromatic residues of the three conformers were fitted to corresponding residues of azacyclic urea. After Comparative Molecular Field Analysis (CoMFA)-field fit energetically most favourable conformer **4A** showed lowest deviation of the aromatic ring planes to those of azacyclic urea and thus the best fit (Fig. **6**).

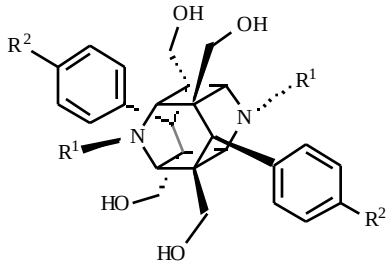
Molecular dynamic simulation (MDS) with conformer **4B** showed that the benzyl residues were freely rotable while the rotability of the 4-methoxyphenyl rings was limited as they did not flip. Another MDS carried out with the centrosymmetric conformer **4C** showed a change of symmetry from centrosymmetry to C_2 symmetry, so that the energetically most favourable conformer **4A** was selected for comparison of molecular and potential binding properties with azacyclic urea [15].

Calculated EPs displayed on the van-der-Waals surface of the molecules showed certain conformities in the negative potentials of corresponding aromatic residues as well as in the slight positive potentials of common hydroxy group regions. While the potential of the urea carbonyl group was directed and had a negative value the hydroxy function of the cage dimer shows a slight positive potential like the hydroxy function of the opposite molecule site.

Calculations of regions for optimized interactions with certain probes have been made using the program GRID [16] for comparison of binding properties of azacyclic urea with the cage dimer. In order to characterize decisive binding of hydroxy function and urea carbonyl group to Asp 25/25´and Ile50/50´ as shown in Fig. **1** interaction regions for an *N*H-amide and a carbonyl (of COO^-) probe have been considered [15].

Regions for optimized interactions for azacyclic urea with the *N*H-amide probe were found at a contour level of -5 kcal/mol near the *N*H function of Ile50 and another one with a slight distance to the *N*H function of Ile50´ (Fig. **7**). The cage dimer also shows corresponding regions near Ile50 and with distance to Ile50´. Comparing the location of regions for interactions with the carbonyl probe at a contour level of -4 kcal/mol conformities are found for both molecules with interaction fields near the carboxy functions of both amino acids Asp25 and 25´ [15].

Table 2.



R^1	R^2	Concentration [μM]	Inhibition [%]
H	H	50	18±3
CH_3	H	50	16±1
Bz	H	25	69±6
H	OCH_3	50	19±3
CH_3	OCH_3	50	20±4
Bz	OCH_3	10	23±2
$COCH_3$	H	50	6±1
COBz	H	50	52±6
$COOCH_3$	H	50	9±1
COOPh	H	25	17±4
Boc	H	25	49±5
$COCH_3$	OCH_3	25	7±3
COBz	OCH_3	50	18±3
$COOCH_3$	OCH_3	50	9±1
COOPh	OCH_3	50	18±2
Boc	OCH_3	50	32±6
Saquinavir		1	99

4. BIOLOGICAL ACTIVITY

First *in-vitro* evaluation of the cage dimers as PIs has been made at concentrations of 50 μM or 25 μM in the case of limited solubility. The resulting percentual inhibitions are given in Table 2.

Within the small series of *N*H and *N*-alkyl compounds the *N*H and *N*-methyl derivatives practically showed no PR inhibitory activity. Calculated IC_{50} values > 200 μM are comparable to inhibitory activities of first PIs **A-74702** or pepstatin A with about 200 μM [13]. The activity mainly increases with the introduction of *N*-benzyl substituents to IC_{50} = 16.2 μM for **4** with R^1 = Bz and R^2 = H, named as **H17** in the following. The significant difference may result from the possible hydrophobic interactions of all four aromatic residues with the hydrophobic regions S1/S1´and S2/S2´of PR as has been reported for ureas. In the case of *N*H and *N*-methyl substitution such interactions are unlikely as the substituents do not reach the hydrophobic amino acid regions for corresponding interactions. Within the group of *N*-acyl derivatives inhibitory activity increases from R^1 = COmethyl to -benzyl (*N*-acyl substitution) and COmethoxy to -phenoxy and, finally, -*t*-butoxy as most effective substituents in the series of *N*-acyloxy substitution [14]. Refering to a study of inhibitory activity of several PIs that found *N*-Boc substituents as the best ones for binding to the S2/S2´ region of PR the *N*-substituents of the cage dimers are suggested to bind to these hydrophobic regions of PR [17].

In order to characterize the mode of PR inhibition the velocity of substrate cleavage has been measured at varied substrate concentrations without and with presently best PI **H17** at a fixed concentration of 15 μM (Fig. **9**) [13].

Lineweaver-Burk plot of resulting data led to a calculated nearly unchanged maximum of the velocity of substrate

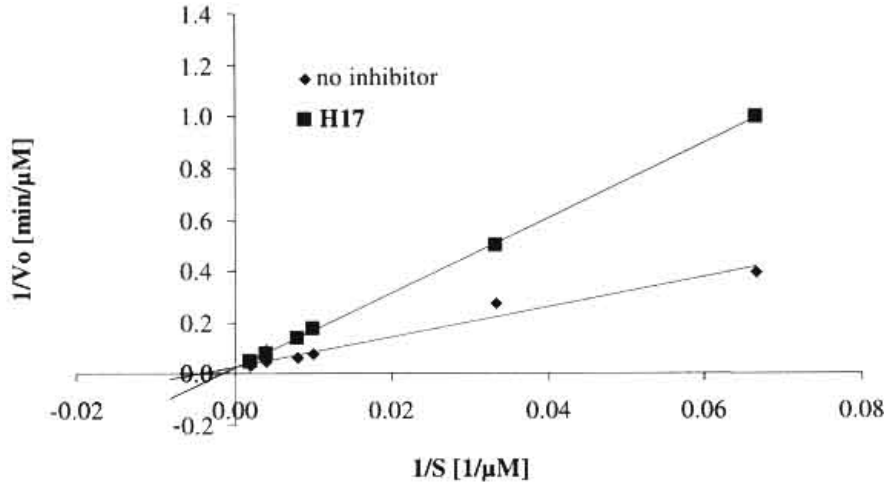


Fig. (9). Lineweaver-Burk plot for PI **H17**.

cleavage comparing uninhibited reaction (V_{max} = 37 µM min^{-1}) and the inhibited reaction with V_{max} = 46 µM min^{-1}. A significant difference was found for the resulting Michaelis-Menten constant making K_m = 214 µM for the uninhibited reaction and K_{app} = 670 µM for the inhibited one. Thus, the novel PIs turned out as competitive inhibitors of PR like the 4-hydroxycoumarines [6].

5. PROTEIN BINDING AND METABOLISM

Among the first two classes of nonpeptidic PIs high protein binding and extensive metabolism have been demonstrated as decisive features for clinical failure of most representatives. Thus, the development of novel PIs requires analysis of protein binding and metabolic investigations at the earliest state. Protein binding of various peptidic PIs with poor bioavailability, cyclic ureas and cage dimer **H17** are given in Table 3.

Table 3.

inhibitor	protein bound share [%]
saquinavir	99
indinavir	98
DMP 323	83
DMP 450	93
H17	- [a]

[a] undetectable

Comparing high protein binding of saquinavir, indinavir and **DMP 450** with dimer **H17** for which no binding was determinable it has to be concluded that bioavailability of the cage dimers will not be lowered by protein binding with respect to sufficient antiviral activity [18].

First metabolic investigations of cage dimer **H17** have been made using HepG2-cells that have been demonstrated to reproduce metabolic activities of hepatocyte cultures [19]. While no phase-I metabolites were found, the extent of phase-II metabolism, i. d. conjugation with activated glucuronic acid and sulfuric acid *via* hydroxymethylene functions, was poor. Obviously, the hydroxymethylene functions are shielded in the compact molecular structure by neighbouring aromatic residues, so that an attack of conjugating enzymes and complex substrates is sterically hindered [19].

SUMMARY

Cage dimeric 4-aryl-1,4-dihydropyridines have been developed as C_2 symmetric PIs by photodimerization reaction of monomeric 4-aryl-1,4-dihydropyridines. In comparison to cyclic ureas their symmetric properties qualify them for favourable binding to PR as has been demonstrated by molecular modeling. The presently best compounds from a first series of derivatives are characterized by four aromatic, respectively lipophilic, residues suggesting favourable interactions with lipophilic binding regions S1/S1´and S2/S2´ of PR. First bioanalytical evaluation of poor protein binding and metabolism encourages further development of this third class of PIs.

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