Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 38 (2003) 495–499
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Orginal article

Structure-activity relationships of first bishydroxymethyl-substituted cage dimeric 4-aryl-1,4-dihydropyridines as HIV-1 protease inhibitors

Andreas Hilgeroth [a,*], Hauke Lilie [b]

[a] Department of Pharmacy, Martin-Luther-University Halle-Wittenberg, Wolfgang-Langenbeck-Str. 4, 06120 Halle, Germany
[b] Department of Biochemistry and Biotechnology, Martin-Luther-University Halle-Wittenberg, Kurt-Mothes-Str. 3, 06120 Halle, Germany

Received 9 December 2002; revised and accepted 27 February 2003

Abstract

A first series of novel bishydroxymethyl-substituted cage dimeric 4-aryl-1,4-dihydropyridines **5**–**8** has been synthesised and evaluated as HIV-1 protease and HIV-inhibitors in vitro assays. Moderate activity data of protease inhibition have been found for of the *N*-Boc substituted compound **8**. Reduced activity for compound **6** and almost no residual activity of **5** and **7** emphasise the importance of the *tert*. butyl substituent for protease inhibitory activity thus supporting a discussed probable binding of the *N*-acyloxy substituent to the S2/S2′ regions of protease.

Keywords: Cage dimeric 4-aryl-1,4-dihydropyridines; HIV-1 protease inhibitor; Binding region

1. Introduction

Since the discovery of HIV-1 protease (PR) as novel target enzyme for the development of HIV-1 protease inhibitors (PI) certain peptidic PIs have been established in HIV-therapy combined with nucleoside analogues or reverse transcriptase inhibitors [1,2]. Beside ongoing resistance development against peptidic PIs additional problems like poor oral bioavailability and severe side effects just like cardiovascular morbidity and mortality, strengthened the development of non-peptidic PIs as well as alternative therapeutic strategies of influencing virus-adsorption and especially virus-cell fusion processes [3]. Disappointing news of rapid emergence of resistance development against fusion inhibitor T-20 in clinical trials support a focussing on perspective series of non-peptidic PIs [4].

However, from the very few series of non-peptidic PIs, e.g. cyclic ureas **I**, hopeful candidates failed in clinical trials because of unsatisfying bioavailabilities Fig. 1 [5].

Molecular weights beyond 600 g mol^{-1} were found as decisively limiting factors of oral bioavailability within the cyclic ureas series [6]. We recently introduced dimeric 4-aryl-1,4-dihydropyridines as novel class of non-peptidic PIs **II** [7,8].

Their first bioanalytical evaluation of both poor protein binding as well as metabolism raised hopes for improved bioavailability compared to ureas [5]. However, molecular weights beyond 600 g mol^{-1} for first candidates within the dimeric series require a critical reduction of non-essential structural elements within the functionalised scaffold with respect to maintaining biological activity, thus leading to compounds of lowered molecular weights.

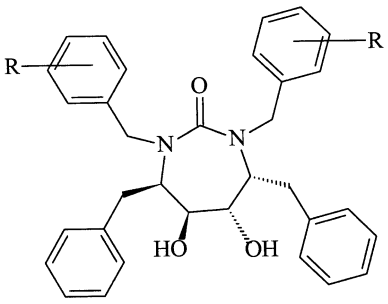
In the following we report first candidates of a novel series of bishydroxymethylene cage dimers **III** with a reduced number of hydroxymethylene functions compared to preceding candidates of the tetrakishydroxymethylene type and discuss their observed protease inhibitory activities with respect to structure-activity relationships as well as some antiviral data.

2. Chemistry

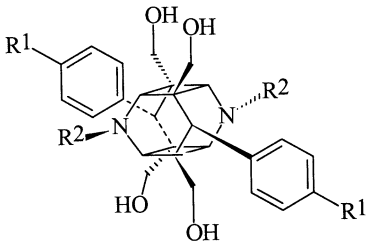
All target compounds **5**–**8** were accessible by a selective ester group reduction of those ester group functions bound to the substituted cyclobutane ring of

* Corresponding author.
E-mail address: hilgeroth@pharmazie.uni-halle.de (A. Hilgeroth).

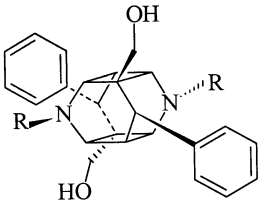
0223-5234/03/$ - see front matter
doi:10.1016/S0223-5234(03)00060-6



I



II



III

Fig. 1. Structural scaffolds of cyclic ureas **I**, cage dimeric 4-aryl-1,4-dihydropyridines of the tetrakishydroxymethylene type **II** and of the bishydroxymethylene type **III** discussed in the text.

recently reported cage dimers **1**–**4** which themselves were given by a solution photodimerization reaction of monomeric 4-aryl-1,4-dihydropyridine starting products [9] Fig. 2.

Selective ester group reduction was carried out with calcium borohydride at room temperature without any side product formation being observed as was followed by TLC. So the amide as well as the carbamide ester functions remained unaffected resulting in characteristical bonds in the infrared spectra between $v = 1628$ for the acetyl group of **5** and $v = 1709$ as highest carbamide ester vibration of compound **7**. As has been demonstrated for the precursor cage dimers **1**–**4** [9] the target compounds **5**–**8** exist in different rotameric forms due to a hindered rotability of the *N*-acyl groups resulting in a multiplicity of proton signals in the ^{1}H-NMR spectra of the compounds of three different rotamers **A**–**C**, two of a centrosymmetric structure (**A**, **B**) and one with a non-symmetric structure (**C**) with respect to the orientations of the two *N*-acyl groups (see Section 6).

3. Pharmacology

PR inhibitory activities of the synthesised cage dimers **5**–**8** have been determined in vitro using a spectrophotometric assay in comparison to *Saquinavir* as reference which showed a 99-percentual inhibition at 1 μM with an affinity constant K_i of 2.1 nM.

Dose–response curves have been calculated for target compounds **5**,**6** and **8** with resulting IC_{50} values as shown in Table 1. Dimer **7** showed poor solubility in the assay system so that triple inhibitory activity was measured at 8 μM and IC_{50} value was calculated according to Eq. (1) assuming parallel dose–response curves. The determination of K_i values (Table 1) followed Ref. [10].

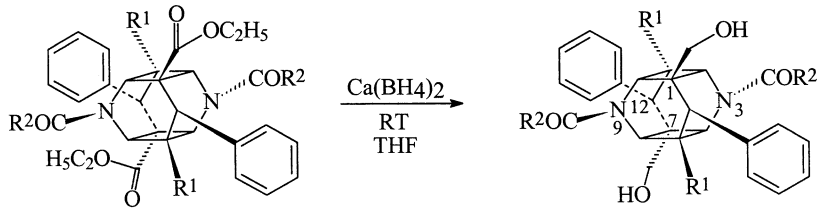
$$\frac{v}{v_{max}} = \frac{IC_{50}}{[I] + IC_{50}} \quad (1)$$

Antiviral activities of active compounds, **8** and **9**, against HIV-1, IIIB, have been determined in MT4 cells as described by measuring the inhibition of cytopathic effects on both HIV- and mock-infected cells in a spectrophotometric (MTT) assay compared to *Saquinavir* with EC_{50} of 6 nM [11,12].

4. Results and discussion

Comparing PR inhibitory activities of the *N*-acetyl compound **5** which itself was found inactive with $IC_{50} >$ 300 μM to those of the *N*-methoxycarbonyl derivative **6** some increased activity was measured with $IC_{50} < 200$ μM. Further structural variation of the alcoholic compound of the more favourable carbamidester substituent compared to the amide substitution proved the *tert*. butoxy group in **8** to be much better than the phenolic group in **7** which resulted in a loss of observed activity (Table 1).

Highest biological activities of PR and HIV inhibition of the *N*-Boc substituted compound **8** almost ly in the range of activity of the tetrakishydroxymethylene substituted equivalent derivative **9** [8] for which a binding mode of the *tert*. butoxy substituent to the hydrophobic binding region S2/S2′ of PR was suggested from its highest activity within a series of *N*-acyloxy substituted compounds of the tetrakishydroxymethylene type [8]. However, a possible alternative binding of the *N*-acyloxy substituents to the S1/S1′ regions of PR becomes almost unlikely with the results of this first



1-4 **5-8**

	R1	R2	
1	H	CH_3	**5**
2	H	OCH_3	**6**
3	H	OPh	**7**
4	H	O*tBu*	**8**
	CH_2OH	O*tBu*	**9**

Fig. 2. Synthesis of target compounds **5**–**8**.

Table 1
PR inhibition and antiviral activity of cage dimers **5**–**9**

Compound	IC_{50} (μM)	K_i (μM)	EC_{50} (μM)
5	312±7	130±3	n.d. [a]
6	180±2	75±1	n.d. [a]
7	300±21	125±6	n.d. [a]
8	72±2	30±1	>125
9	20±2	10±1	>83

[a] n.d., not determined.

series of derivatives of the bishydroxymethylene type presented now because the *N*-phenoxycarbonyl substituent proved to be unfavourable with respect to biological activity. With a possible binding of the *N*-phenoxycarbonyl substituent to the S1/S1′ regions of PR better biological results were expected because various reported results of structure-activity relationships of PIs promised an enhanced phenyl substituent to result in highest binding affinities to the S1/S1′regions of PR and thereby contributing to satisfying inhibitory activities [13].

So a possible binding of the *N*-phenoxycarbonyl substituent of compound **7** to the hydrophobic S1/S1′ regions of PR is unlikely and, in consequence, the assumption of a binding of the *N*-acyloxy substituent to the S2/S2′region of PR is strongly supported by the given results.

5. Conclusions

From a first series of bishydroxymethylene substituted cage dimeric 4-aryl-1,4-dihydropyridines with *N*-acyl substituents the *N*-Boc derivative shows highest PR inhibitory activities almost reaching biological results of PR inhibition as well as HIV inhibition like the corresponding tetrakishydroxymethylene compound. The lowered molecular weights of the representatives of the bishydroxymethylene series may result in increased bioavailability as has to be investigated. The given results of the *tert*. butoxy substituent with best activity as well as the observed inactivity the phenoxy substituent strongly support a binding of the *N*-acyl substituents to the S2/S2′ regions of PR. So the presented compounds with some moderate PR inhibitory activity achieve a valuable contribution to the structure-activity relationships within this class of novel non-peptidic PIs.

6. Experimental protocols

6.1. Chemistry

Commercial reagents were used without further purification. IR spectra were recorded as KBr disks on a Bruker IFS 28. ^{1}H-NMR (400 or 500 MHz) spectra were measured using tetramethylsilane as internal standard. TLC was performed on E. Merck 5554 silica gel plates. Mass spectra were measured with an AMD 402 mass spectrometer. Elemental analyses indicated by the symbols of the elements were within ± 0.4% of the theoretical values and were performed using a Leco CHNS-932 apparatus. The synthesis of compounds **1**–**4** and **9** was recently reported in Ref. [9] (**1**–**4**) and Ref. [8] (**9**).

*6.1.1. General procedure for compounds **5**–**8***

*6.1.1.1. 3,9-Diacetyl-1,7-dihydroxymethyl-6,12-diphenyl-3,9-diazahexacyclo[6.4.0.0$^{2.7}$.0$^{4.11}$. 0$^{5.10}$]dodecane (**5**).* **1** (0.04 g, 0.07 mmol) was dissolved in a freshly prepared suspension of calcium borohydride (0.04 g, 0.7 mmol) in dry THF (25 mL) which was given by stirring of calcium chloride (0.08, 0.7 mmol) and sodium borohydride (0.05 g, 1.4 mmol) for 1 h. Stirring was continued for 4 weeks. Then ice–water (2.5 mL) was added and the calcium borohydride excess was further hydrolysed by additional stirring for 1 h at 0 °C. Then the solution was extracted three times with chloroform (50 mL) and dried over sodium sulphate. After filtration and evaporation to dryness the remaining semi-solid residue was dissolved in chloroform. Addition of portions of diethylether followed by petrol ether finally resulted in a dimming of the solution from which crude **5** crystallised on cooling. Recrystallisation from methanol–water yielded 0.02 g (62%) of pure **5** as white powder (m.p.: 166–173). IR: *ν* 1628. ^{1}H-NMR ($(CD_3)_2SO$): *δ* 1.91, 1.94 (2 × s, 6H, *N*COCH_3, **C**), 2.03 (s, 6H, *N*COCH_3, **B**), 2.05 (s, 6H, *N*COCH_3, **A**), 2.95 (spl. d, 3H, CH_BOH, **B**, C7-CH_BOH, **C**, ^2J = 11.5 Hz), 2.96 (spl. d, 3H, CH_BOH, **A**, C7-CH_BOH, **C**, ^2J = 11.7 Hz), 3.01 (dt, 2H, 5-, 11-H, **A**, ^3J = 8.8/2.6 Hz), 3.08 (dt, 1H, 5-H, C, ^3J = 8.8/2.6 Hz), 3.15 (d, 6H, 6-, 12-H, **A**–**C**, ^3J = 2.6 Hz), 3.16 (dt, 3H, 5-, 11-H, **B**, 11-H, **C**, ^3J = 8.9/2.6 Hz), 3.17 (spl. d, 3H, CH_AOH, **B**, C7-CH_AOH, **C**, ^2J = 11.5 Hz), 3.20 (spl. d, 3H, CH_AOH, **A**, C7-CH_AOH, **C**, ^2J = 11.7 Hz), 3.96 (br s, 1H, C7-CH$_A$O*H*, **C**), 4.19 (d, 1H, 4-H, **C**, ^3J = 8.8 Hz), 4.32 (br s, 2H, OH, **B**), 4.35 (d, 1H, 10-H, **C**, ^3J = 8.9 Hz), 4.47 (d, 1H, 8-H, **C**, ^3J = 8.8 Hz), 4.50 (d, 2H, 4-, 10-H, **B**, ^3J = 8.9 Hz), 4.70 (br s, 2H, OH, **A**), 4.86 (d, 2H, 2-, 8-H, **B**, ^3J = 8.9 Hz), 5.02 (d, 2H, 4-, 10-H, **A**, ^3J = 8.8 Hz), 5.13 (br s, 1H, C1-CH$_A$O*H*, **C**), 5.23 (d, 1H, 2-H, **C**, ^3J = 8.9 Hz), 5.27 (d, 2H, 2-, 8-H, **A**, ^3J = 8.8 Hz), 7.06–7.47 (m, 30H, aromatic H, **A**–**C**). EIMS *m*/*z*: 458 (M^+). Anal. $C_{28}H_{30}N_2O_4 \cdot H_2O$ (C, H, N).

*6.1.1.2. 1,7-Dihydroxymethyl-3,9-dimethoxycarbonyl-6,12-diphenyl-3,9-diazahexacyclo[6.4 0.0$^{2.7}$.0$^{4.11}$.0$^{5.10}$]-dodecane (**6**).* Yield: 0.02 g (59%), white powder. (M.p.: 269–275 °C). IR: *ν* 1669. ^{1}H-NMR ($(CD_3)_2SO$): *δ* 2.99–3.07 (m, 12H, CH_BOH, 5-, 11-H, **A**–**C**), 3.34–3.38 (m, 12H, CH_AOH, 6-, 12-H, **A**–**C**), 3.60, 3.61 (2 × s, 18H, *N*COOCH_3, **A**–**C**), 4.51 (d, 1H, 4-H, **C**, ^3J = 9.0 Hz), 4.55 (d, 2H, 4-, 10-H, **A**, ^3J = 9.0 Hz), 4.57 (d, 2H, 4-, 10-H, **B**, ^3J = 10.2 Hz), 4.60 (d, 1H, 10-H, **C**, ^3J = 9.0 Hz), 4.72 (d, 1H, 8-H, **C**, ^3J = 10.9 Hz), 4.74 (d, 2H, 2-, 8-H, **B**, ^3J = 10.3 Hz), 4.82 (d, 3H, 2-, 8-H, **A**, 2-H, **C**, ^3J = 9.0 Hz), 5.03 (s, 1H, C1-CH$_2$O*H*, **C**), 5.04 (s, 5H, OH, **A**, **B**, C7-CH$_2$O*H*, **C**), 7.09–7.24 (m, 30H, aromatic H, **A**–**C**). EIMS *m*/*z*: 490 (M^+). Anal. $C_{28}H_{30}N_2O_4 \cdot 1.5\ H_2O$ (C, H, N).

6.1.1.3. 1,7-Dihydroxymethyl-6,12-diphenyl-3,9-diphenoxycarbonyl-3,9-diazahexacyclo-[6.4.0.0$^{2.7}$.0$^{4.11}$.0$^{5.10}$]dodecane (7). Yield: 0.02 g (57%), white powder. (M.p.: 269–279 °C). IR: *ν* 1709. ^{1}H-NMR ($(CD_3)_2SO$): *δ* 2.78–3.08 (m, 6H, CH_BOH, **A**–**C**), 3.10–3.15 (br m, 6H, 5-, 11-H, **A**–**C**), 3.27–3.32 (m, 12H, CH_AOH, 6-, 12-H, **A**–**C**), 4.33 (br d, 2H, 4-, 10-H, **A**), 4.48 (br d, 2H, 4-, 10-H, **B**), 4.64 (d, 1H, 4-H, **C**, ^3J = 9.0 Hz), 4.65 (d, 1H, 10-H, **C**, ^3J = 8.9 Hz), 4.68 (d, 1H, 8-H, **C**, ^3J = 9.0 Hz), 4.72 (br d, 2H, 2-, 8-H, **B**), 4.80 (d, 1H, 2-H, **C**, ^3J = 9.0 Hz), 4.84 (d, 2H, 2-, 8-H, **A**, ^3J = 8.5 Hz), 4.90 (br s, 1H, C7-CH$_2$O*H*, **C**), 4.92 (br s, 1H, C1-CH$_2$O*H*, **C**), 5.03 (br s, 2H, OH, **B**), 5.04 (br s, 2H, OH, **A**), 6.93–7.46 (m, 60H, aromatic H, **A**–**C**). EIMS *m*/*z*: 614 (M^+). Anal. $C_{38}H_{34}N_2O_6 \cdot 1\ H_2O$ (C, H, N).

*6.1.1.4. 3,9-Di(tert.)butoxycarbonyl-1,7-dihydroxymethyl-6,12-diphenyl-3,9-diazahexacyclo[6.4.0.0$^{2.7}$.0$^{4.11}$.0$^{5.10}$]dodecane (**8**).* Yield: 0.02 g (58%), white powder. (M.p.: 205–245 °C (decomposition)). IR: *ν* 1687. ^{1}H-NMR ($(CD_3)_2SO$): *δ* 1.36, 1.42 (2 × s, 54H, *N*COOC$(CH_3)_3$, **A**–**C**), 2.84–2.93 (m, 6H, CH_BOH, **A**–**C**), 2.97–3.07 (m, 6H, 5-, 11-H, **A**–**C**), 3.32–3.34 (m, 12H, CH_AOH, 6-, 12-H, **A**–**C**), 4.42 (d, 1H, 4-H, C, ^3J = 9.0 Hz), 4.47 (d, 1H, 10-H, **C**, ^3J = 9.4 Hz), 4.51 (d, 2H, 4-, 10-H, **A**, ^3J = 8.4 Hz), 4.54 (d, 2H, 4-, 10-H, **B**, ^3J = 8.1 Hz), 4.69 (d, 1H, 8-H, **C**, ^3J = 8.4 Hz), 4.71 (d, 2H, 2-, 8-H, **A** o. **B**, ^3J = 8.7 Hz), 4.81 (d, 3H, 2-, 8-H, **A** o. **B**, 2-H, **C**, ^3J = 7.5 Hz), 4.93 (s, 1H, OH, **C**), 4.95 (s, 3H, OH, **C**, **A** o. **B**), 4.96 (s, 2H, OH, **A** o. **B**), 7.12–7.78 (m, 30H, aromatic H, **A**–**C**). ESI-MS *m*/*z*: 613 ($M+K^+$). Anal. $C_{34}H_{42}N_2O_6 \cdot 1.5\ H_2O$ (C, H, N).

6.2. In vitro assay of PR inhibition

Enzymatic activity of PR was measured by following the cleavage of the substrate H–Lys–Ala–Arg–Val–Leu–p–nitrophenylalanine–Glu–Ala–Nle–NH_2 [14]. PR was incubated at 37 °C in 0.1 M MES, 0.37 M NaCl and 4 mM EDTA, pH 6.25, with 280 μM substrate in the presence or absence of inhibitor in a photometer cuvette. From the decrease of absorbance at 298 nm initial reaction rates were calculated [10]. Compounds were added from stock solutions in DMSO.

6.3. In vitro assay of anti-HIV activity

The HTLV I transformed cell line MT4 was used as the target cell. Inhibition of HIV-1, strain IIIB, 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 per millilitre 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-day incubation. The virus was adsorbed to a cell suspension containing 1×10^5 cells mL^{-1} 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^{-1}.

Acknowledgements

A. H. is grateful for the support of his work by the German Pharmaceutical Society (DPhG).

References

[1] A. Hilgeroth, Pharm. Uns. Zeit 27 (1998) 22–25.
[2] H.-J. Stellbrink, Dt. Ärzteblu. 94A (1997) 2497–2503.
[3] E. De Clercq, Nat. Rev. 11 (2002) 13–25.
[4] X. Wei, J.M. Decker, H. Liu, Z. Zhang, R.B. Arani, J.M. Kilby, M.S. Saag, X. Wu, G.M. Shaw, J.C. Kappes, Antimicrob. Agents Chemother. 46 (2002) 1896–1905.
[5] A. Hilgeroth, Min. Rev. Med. Chem. 2 (2002) 235–247.
[6] G.V. De Lucca, P.Y.S. Lam, Drugs Fut. 23 (1998) 987–994.
[7] A. Hilgeroth, A. Billich, Arch. Pharm. Pharm. Med. Chem. 332 (1999) 3–5.
[8] A. Hilgeroth, A. Billich, Arch. Pharm. Pharm. Med. Chem. 332 (1999) 380–384.
[9] A. Hilgeroth, F.W. Heinemann, U. Baumeister, Heterocycles 57 (2002) 1003–1016.
[10] D. Scholz, A. Billich, B. Charpiot, P. Ettmayer, P. Lehr, B. Rosenwirth, E. Schreiner, H.J. Gstach, J. Med. Chem. 37 (1994) 3079–3089.
[11] R. Pauwels, J. Balzarini, M. Baba, R. Snoek, D. Schols, P. Herdwijn, J. Desmyter, E. De Clercq, J. Virol. Methods 20 (1988) 309–321.
[12] S. Noble, D. Faulds, Drugs 52 (1996) 93–112.
[13] G.V. De Lucca, J. Liang, I. De Lucca, J. Med. Chem. 42 (1999) 135–152.
[14] A.D. Richards, L.H. Phylip, W.G. Farmeries, P.E. Scaborough, A. Alvarez, B.M. Dunn, P.-H. Hirel, J. Konvalinka, P. Strop, L. Pavlickova, V. Kosta, J. Biol. Chem. 265 (1990) 7733–7736.