Experimental

1. Materials

Dry extract of *R. aculeatus* L. was obtained from overground and underground parts of plant material. Colloidal silice – Aerosil 200 (Degussa – Germany). Hydrochloric acid; sulphuric acid; *n*-butanol; methanol; ethylacetate; cyclohexan. All reagents were of analytical grade (Merck – Germany). chromatographic plate for HPTLC: Fertigplatten Kieselgel 60 10 × 20 cm (Merck – Germany); *p*-dimethylaminobenzaldehyde test.

2. Moisture determination

The measurements were made with a Moisture Analyzer Sartorius at 80 °C.

3. Assay of ruscogenin and neoruscogenin in dry extract

3.1. Acid hydrolysis

Approximately 100 mg of the extract was refluxed with 15 ml 1 N HCl for 3–3.5 h in a water bath at 95 ± 3 °C in the presence of 20 ml *n*-butanol. The *n*-butanol phase was washed several times with water and evaporated to dryness. The residue was dissolved in methanol in a 5 ml volumetric flask.

3.2. Densitometric high performance thin-layer chromatography

On a plate for HPTLC, with the help of a micropipette, 5 µl of the standard solution of ruscogenin (1 mg/ml) and of the solution of each sample were applied.
The plate was developed in a chamber saturated with the vapour of the system cyclohexane/ethylacetate (1 : 1) [3]. After the mobile phase reached the front line (8 cm), the plate was taken out, air-dried and sprayed with a solution of *p*-dimethylaminobenzaldehyde, after which it was dried at 110 °C for about 5 min and pink coloured spots appeared. The resulting coloured spots were submitted to densitometric chromatogram evaluation on CAMAG TLC SCANNER II at 520 nm.
The quantity of ruscogenins was calculated by determining the peak area of the sample solution with calibration curve obtained by chromatography of standards.

References

1 Capra, C.: Fitoterapia **43**, 99 (1972)
2 Mauss, H. J.: Phlebologie – Germany **21**, 200 (1992)
3 Nikolov, S.; Joneidi, M.; Panova, D.: Pharmazie **31**, 611 (1976)
4 Nikolov, S. et al.: Pharmacia **44**, 11 (1997)
5 Peneva, B. et al.: Pharmacia (S), **46**, 16 (1999)

Received December 23, 1999
Accepted February 5, 2000

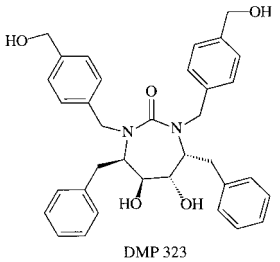
Dr. B. Peneva
Medical University
Department of Pharmaceutical Technology
2 Dunav St.
1000 Sofia
Bulgaria

Institut für Pharmazeutische Chemie, Fachbereich Pharmazie, Martin-Luther-Universität Halle-Wittenberg, Halle, Germany

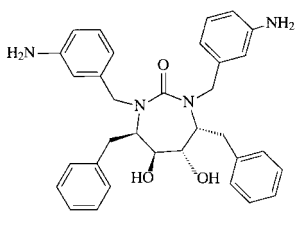
Plasma protein binding properties of dimeric 4-aryl-1,4-dihydropyridines as novel non peptidic HIV-1 protease inhibitors

A. Hilgeroth and A. Langner

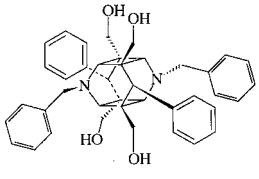
The plasma protein binding of drugs certainly influences their bioavailability. Protein binding of drugs with a small therapeutic range like the cardiac glycoside digitoxine is of great importance as such binding properties significantly lower the necessary blood levels [1, 2]. The bioavailability of drugs with poor intestinal absorption and, additionally, high plasma protein binding is often unsatisfying.
The peptidic HIV-1 protease inhibitors saquinavir and indinavir demand high doses due to their poor bioavailability that is partly caused by their high protein binding properties besides poor absorptions and extensive metabolism by the cytochrom P450 system [3].
Among the few non peptidic HIV-1 protease inhibitors, cyclic ureas like DMP 323 and DMP 450 with excellent anti-HIV activities in infected cell cultures failed in clinical trials because of unsatisfying bioavailabilities [4]. While DMP 323 showed poor absorption and extentsive oxidative metabolism, the therapeutic blood levels of DMP 450 were not achieved due to a high protein binding partly caused by interactions of the weakly basic anilino groups and α_1-acid glycoprotein.
Recently cage and *syn* dimeric 4-aryl-1,4-dihydropyridines have been introduced as novel non peptidic HIV-1 protease inhibitors with moderate activities [5–8]. With encouraging bioanalytical results of favourable poor metabolism and non-toxicity evaluated in Hep G2 cells those dimers hold promise as novel and perspective class of HIV-1 protease inhibitors [9, 10]. Nevertheless, their plasma protein binding properties had to be evaluated as determining factor of bioavailability.
For this study, the cage dimeric *N*-benzyl 4-phenyl-1,4-dihydropyridine (H 17) and the *syn*dimeric *N*-benzyl 4-phenyl-1,4-dihydropyridine (H 19) were selected.
Different concentrations of each compound were incubated with a solution of human serum albumin (4%) at 37 °C in a shaker. After separation of the protein bound share by centrifugation the drug concentrations were determined UV-spectroscopically. Comparing to the measured concentra-



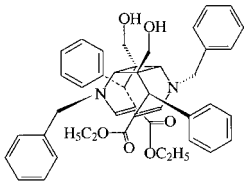
DMP 323



DMP 450



H17



H19

Table: Plasma protein binding of various HIV-1 protease inhibitors

inhibitors	Protein bound share (%)
saquinavir	99 [3]
indinavir	98 [3]
DMP 323	83 [4]
DMP 450	93 [4]
H17	n.d.[a]
H19	83.23 ± 4.39*

[a] n.d. = not detectable, * $\bar{x}$ ± SD, $n = 8$

tions without albumin, the protein bound share was calculated. The determined mean values are given in the Table.

H 17 was found in unchanged concentrations over the tested range, H 19 showed a mean protein binding of 83%. While the protein binding of H 19 is low compared to peptidic HIV-1 protease inhibitors or DMP 450, H 17 shows no detectable binding.

Obviously, the hydroxymethylene groups as possible functional groups for hydrophilic protein interactions are shielded by the neighbouring phenyl-substituents in the compact molecular structure as was recently derived from molecular modeling studies [11] and, additionally, proved by a low extent of phase-II metabolism of the compounds [9, 10].

Consequently, H 17 shows no protein binding while in H 19 the vinylogous carbamide ester moiety in certain peptidometic HIV-1 protease inhibitors may be the structural element decisive for binding.

In summary, protein binding of both cage or *syn* dimeric HIV-1 potease inhibitors investigated, should not be a limiting factor of their bioavailabilities which are currently determined.

Experimental

Protein binding was determined using a solution of human serum albumin (4%). For this purpose 10 ml of a solution of albumin (8%) was prepared using phosphate buffer pH = 7.4. The albumin solution was diluted with stock solutions of H 17 and H 19, respectively, in DMSO/buffer in terms of 1 : 1, so that finally 2.5 ml of each concentrations of 50, 100, 300 and 500 mg/l of the compounds were yielded. The mixtures were shaken for 2 h at 37 °C. Then the albumin was separated by a centrifugation procedure using a Zentrifugal-Ultrafilter Centrisart®. In the supernatant, concentrations of the compounds were determined UV-spectroscopically with two determinations for each concentration of H 17 (254 nm) and H 19 (245 nm).

Acknowledgement: Andreas Hilgeroth is grateful for the support of his work by the German Pharmaceutical Society (DPhG).

References

1 Verspohl, E. J.; Verspohl, J: Interaktionen, 3. Ed., p. 14, Wissenschaftliche Verlagsgesellschaft, Stuttgart 1995
2 Hilgeroth, A.: Pharm. Ztg. **145**, 100 (2000)
3 Hilgeroth, A.: Pharm. uns. Zeit **27**, 22 (1998)
4 De Lucca, G. V.; Lam, P. Y. S.: Drugs Fut. **23**, 978 (1998)
5 Hilgeroth, A.; Billich, A.: Arch. Pharm. Pharm. Med. Chem. **332**, 3 (1999)
6 Hilgeroth, A.; Billich, A.: Arch. Pharm. Pharm. Med. Chem. **332**, 380 (1999)
7 Hilgeroth, A.; Wiese, M.; Billich, A.: J. Med. Chem. **42**, 4729 (1999)
8 Hilgeroth, A.: Habilitationsschrift, p. 80, Martin-Luther-Universität, Halle 2000
9 Hilgeroth, A.; Langner, A.: Arch. Pharm. Pharm. Med. Chem. **333**, 32 (2000)
10 Hilgeroth, A.; Langner A: Arch. Pharm. Pharm. Med. Chem., in press
11 Hilgeroth, A.; Fleischer, R.; Wiese, M.; Heinemann, F. W.: J. Comput. Aid. Mol. Des. **13**, 233 (1999)

Received January 24, 2000
Accepted March 15, 2000

PD Dr. Andreas Hilgeroth
Institut für Pharmazeutische Chemie
Martin-Luther-Universität
Wolfgang-Langenbeck-Str. 4
D-06120 Halle
hilgeroth@pharmazie.uni-halle.de

Department of chemical-pharmaceutical R/D, Apogepha Arzneimittel GmbH, Dresden, Germany

Determination of atenolol/chlortalidone during dissolution of tablets with UV multicomponent analysis

W. WEHNER

Multicomponent pharmaceutical products are usually analyzed by HPLC. However this analysis is time-consuming, expensive and less accurate as a single UV method. Far the determination of a dissolution profile with many time intervals an automatic procedure is needed. We developed an UV method for the simultaneous determination of atenolol and chlortalidone during the dissolution of tablets with these active ingredients using the full spectrum quantitation (FSQ) from Beckman [1].

The UV spectra of the two substances are shown in Fig. 1. They are similare in the range of 220–260 nm. Maxima of atenolol are at 274 and 282 nm and maxima of chlortalidone are at 275 and 283 nm. The maxima of chlortalidone are very small in the typical concentration range of 10–25 mg/900 ml. In the range greater than 300 nm the spectra of both substances overlap and approach zero at 310 nm.

The full spectrum quantitation (FSQ), an advanced spectroscopic method for multicomponent analysis, uses principal component regression (PCR) with preprocessing in the Fourier domain of the absorbance spectrum.

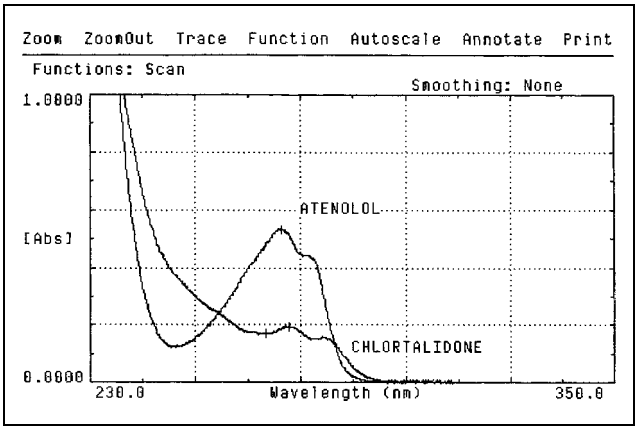


Fig. 1: UV spectra of atenolol and chlortalidone

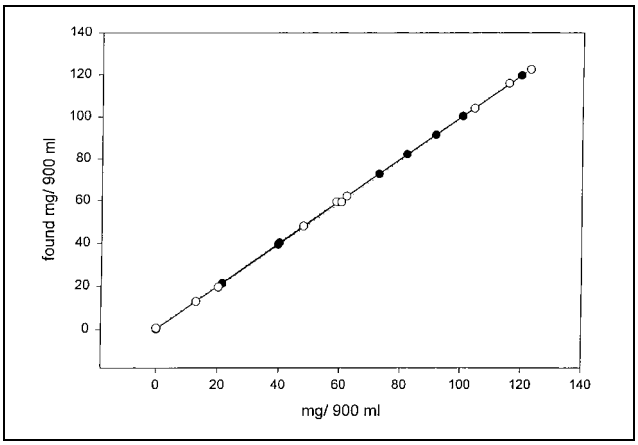


Fig. 2: Linearity of determination of chlortalidone/atenolol
—●— Atenolol, · · ○ · · Chlortalidone