Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2569–2572

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Novel 3-Dodecanoylindole-2-carboxylic Acid Inhibitors of Cytosolic Phospholipase A_2

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Received 8 May 2001; revised 3 July 2001; accepted 5 July 2001

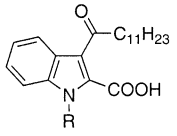
Abstract—Derivatives of 1-[2-(4-carboxyphenoxy)ethyl]-3-dodecanoylindole-2-carboxylic acid (**4**) with modified substituents at the indole-1-position were synthesized and evaluated for their ability to inhibit the arachidonic acid release in human platelets mediated by the cytosolic phospholipase A_2. One of the most active compounds obtained was **26** with an IC_{50} of 0.44 μM.

Introduction

Phospholipase A_2 (PLA_2) constitutes a large and diverse family of enzymes that catalyze the hydrolysis of membrane phospholipids at the *sn*-2 position to release fatty acids and lysophospholipids. When the liberated fatty acid is arachidonic acid, subsequent metabolism through the cyclooxygenase or the lipoxygenase pathways leads to the formation of eicosanoids, including prostaglandins and leukotrienes. Distinct lysophospholipids (1-*O*-alkyl-substituted choline glycerolysophospholipids) can be converted to the platelet-activating factor (PAF). Prostaglandins, leukotrienes, lysophospholipids and the PAF are potent mediators of inflammation.[1–3] Thus, inhibition of PLA_2 is considered as an attractive target for the design of new anti-inflammatory drugs.[4–7]

One problem associated with the in vitro search for PLA_2 inhibitors is the selection of the appropriate enzyme, since many different PLA_2s are present in the mammalian organism.[8] They can be divided in PLA_2s utilizing a catalytic histidine and in PLA_2s having a serine in the active site. The small molecular weight secretory PLA_2s ($sPLA_2$) are members of the first group. The second group consists of the cytosolic PLA_2s ($cPLA_2$), the calcium-independent PLA_2 ($iPLA_2$) and the lipoprotein-associated PLA_2s, which have higher molecular weights than the $sPLA_2$s. From all these PLA_2s the α-subtype of $cPLA_2$ seems to play the central role in the arachidonic acid cascade and during the inflammatory response as supported by experiments with animals which overexpressed $cPLA_2$[9] and with $cPLA_2$ knockout animals.[10–12]

Recently, we have found that several 3-acylindole-2-carboxylic acid derivatives are inhibitors of the $cPLA_2$-mediated arachidonic acid release induced by calcium ionophore A23187 in bovine platelets.[13] One of these compounds was the indole derivative **1**, which showed an IC_{50} of 8 μM in this assay (Fig. 1). An increase of inhibitory potency could be achieved by replacement of the *N*-methyl group of **1** by a longer carboxylic acid moiety. For the compounds **2** and **3** IC_{50} values of 1.6 μM were evaluated. The indoles **2** and **3** have also



Comp.	R
1	-CH_3
2	-$(CH_2)_7COOH$
3	-CH_2CH_2OPhenyl(4-CH_2COOH)
4	-CH_2CH_2OPhenyl(4-COOH)

Figure 1. Structures of indole-2-carboxylic acid inhibitors of $cPLA_2$.

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PII: S0960-894X(01)00472-3

shown activity in vivo in the carrageenan-induced rat paw edema model and in the phorbol ester-induced and the croton oil-induced ear edema models of the mouse.[14,15]

The replacement of the carboxylic acid side chain of **2** or **3** in position 1 of the indole by a 4-ethoxybenzoic acid residue led to a further increase of in vitro activity.[13] With an IC_{50} of 0.5 μM compound **4** was about 3-fold more active than **2** and **3** in the assay using bovine cells. Applying human platelets the same discrepancy in activity was assessed.[16] For **2** and **3** IC_{50} values of 2.5 and 2.6 μM, respectively, were evaluated, while the IC_{50} of **4** was 0.86 μM.

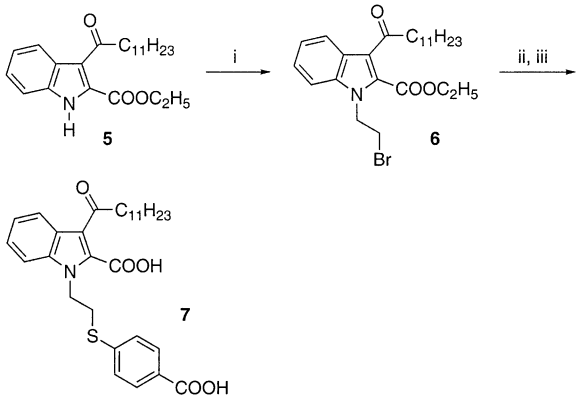
Here, we report the effects of the systematic variation of the (4-carboxyphenoxy)ethyl moiety of **4** on its $cPLA_2$-inhibitory activity in human platelets.

Chemistry

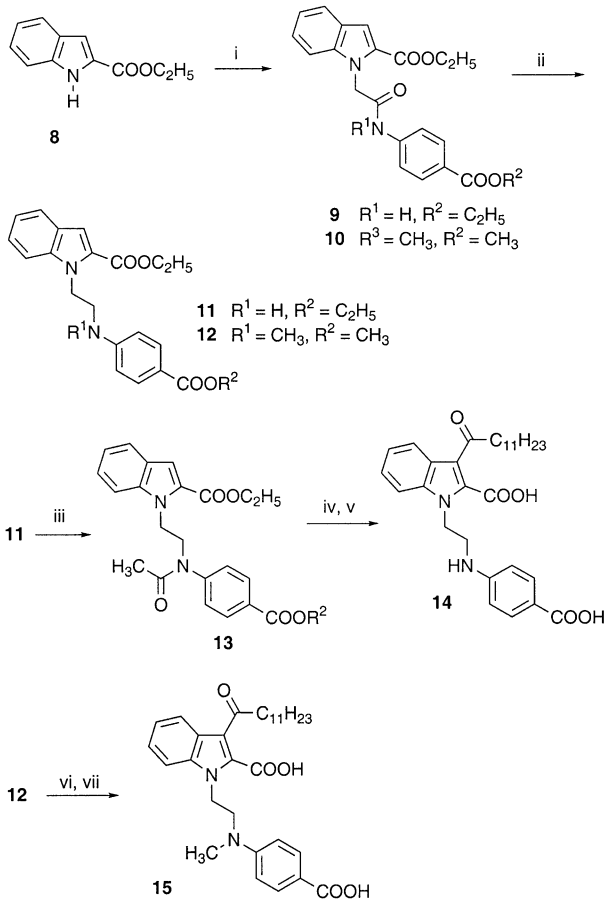
The synthesis of **7**, in which the oxygen of the phenoxy moiety of **4** was replaced by sulfur, started from ethyl 3-dodecanoylindole-2-carboxylate (**5**).[13] This was converted to **6** by treatment with *t*-BuOK and 1,2-dibromoethane in DMSO at 110–120 °C (Scheme 1). Reaction of **6** with ethyl 3-sulfanylbenzoate[17] and *t*-BuOK in ethanol/THF led to the diethylester of **7**, which was saponified by KOH to yield the desired test compound.

The indole derivatives **14** and **15**, in which the benzoic acid moiety was attached to the indole via a 2-aminoethyl or 2-(methylamino)ethyl spacer, were prepared by the routes shown in Scheme 2. Ethyl indole-2-carboxylate (**8**) was reacted in DMSO at 110–120 °C with the appropriate ethyl 4-[(chloroacetyl)amino]benzoates[18] in the presence of *t*-BuOK. The carbamoyl groups of the resulting compounds **9** and **10** were reduced to the amines **11** and **12** by $NaBH_4$ and BF_3–etherate in refluxing THF. For the synthesis of **14** the intermediate **11** was acetylated at the aniline nitrogen with acetylchloride in pyridine, then acylated in position 3 of the indole with dodecanoic acid, polyphosphoric acid and trifluoroacetic anhydride in dichloromethane[13] and at least heated in 10% aqueous NaOH to hydrolyze the ethyl ester groups and to cleave the acetyl group protecting the amine moiety. The target compound **15** was obtained by Friedel–Crafts acylation of **12** with dodecanoyl chloride and $AlCl_3$ followed by saponification with KOH.

The remaining indoles (**17**–**31**) were prepared by reacting ethyl 3-dodecanoylindole-2-carboxylate (**5**) with ethyl 4-(3-bromopropyl)benzoate[19] (**16a**) and 4-(ω-bromoalkoxy)benzoates (**16b**–**16n**), respectively, either in DMSO with *t*-BuOK as base or with powdered KOH, 18-crown-6 ether in refluxing benzene[20] followed by saponification in each case (Scheme 3). The procedure using crown ether usually gave better yields. The derivative with a hydroxy group in position 2 of the phenyl ring (**24**) was obtained by cleaving the ethoxygroup of **23** with BBr_3 in hexane/CH_2Cl_2 allowing the mixture to warm up from 0 °C to room temperature. The 4-(ω-bromoalkoxy)benzoic acid esters necessary for the synthesis of **18**, **19**, **22**–**26**, **28**, **29** and **31** could be synthesized by refluxing the substituted 4-hydroxybenzoic acid esters with an excess of 1,3-dibromopropane,



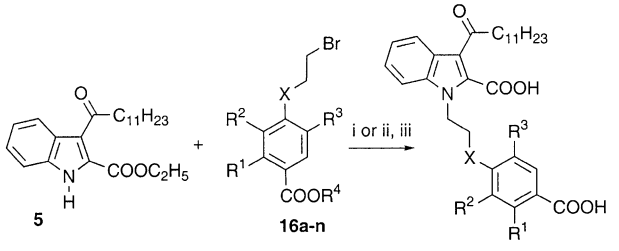
Scheme 1. (i) *t*-BuOK, DMSO, 1,2-dibromoethane, 110–120 °C, 10 min; (ii) *t*-BuOK, EtOH, THF, ethyl 4-sulfanylbenzoate, reflux, 1 h; (iii) EtOH, 10% aq KOH, reflux, 1 h.



Scheme 2. (i) *t*-BuOK, DMSO, ethyl 4-[(chloroacetyl)amino]benzoate or ethyl 4-[(chloroacetyl)(methyl)amino]benzoate, 110–120 °C, 5 min; (ii) $NaBH_4$, BF_3–etherate, THF, reflux, 1 h; (iii) pyridine, acetyl chloride, rt, 15 min; (iv) dodecanoic acid, polyphosphoric acid, trifluoroacetic anhydride, CH_2Cl_2, rt, 5 days; (v) 10% aq NaOH, reflux, 2 h; (vi) dodecanoyl chloride, $AlCl_3$, CH_2Cl_2, rt, 20 h; (vii) EtOH, 10% aq KOH, reflux, 1 h.

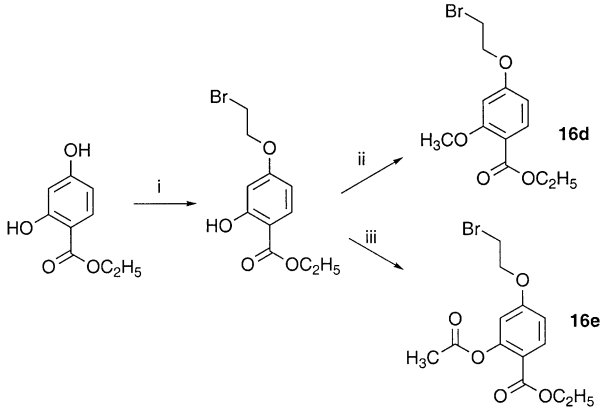
1,4-dibromobutane and 1,2-dibromoethane, respectively, in an ethanolic sodium ethoxide solution for 5 h to 3 days.[13] Since the yield of the nitro compound **16k** was very low (3%), the reaction was repeated with the recovered starting material in this case.

Deviating, the 4-(ω-bromoalkoxy)benzoates used for the preparation of **20** and **21** were synthesized as shown in Scheme 4 starting from ethyl 2,4-dihydroxybenzoate. This was converted to ethyl 4-(2-bromoethoxy)-2-hydroxybenzoate by reaction with 1,2-dibromoethane. The remaining free hydroxy group in *ortho*-position of the ester moiety was then methylated with methyl *p*-toluenesulfonate or acetylated with acetyl chloride to give **16d** and **16e** (Scheme 4). The synthesis of the 4-(2-bromoethoxy)benzoate intermediates **16j** and **16m** started from 4-hydroxyacetophenone derivatives (Scheme 5). These were reacted with 1,2-dibromoethane and sodium ethoxide in ethanol. Subsequently the acetyl group was



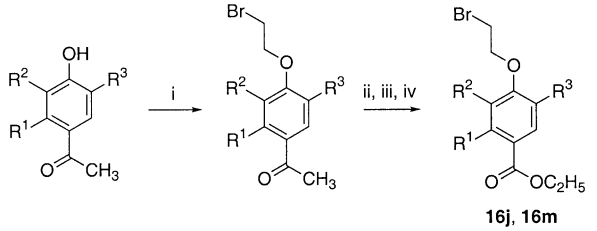
16a R^1,R^2,R^3=H; R^4=C_2H_5; X = CH_2;
16b R^1,R^2,R^3=H; R^4=C_2H_5; X = OCH_2;
16c R^1,R^2,R^3=H; R^4=C_2H_5; X = OCH_2CH_2;
16d R^1=OCH_3; R^2,R^3=H; R^4=C_2H_5; X = O;
16e R^1=$OOCCH_3$; R^2,R^3=H; R^4=C_2H_5; X = O;
16f R^1=Cl; R^2,R^3=H; R^4=C_2H_5; X = O;
16g R^1,R^3=H; R^2=OCH_3; R^4=C_2H_5; X = O;
16h R^1,R^3=H; R^2=Cl; R^4=CH_3; X = O;
16i R^1,R^3=H; R^2=F; R^4=CH_3; X = O;
16j R^1,R^3=H; R^2=CH_3; R^4=C_2H_5; X = O;
16k R^1,R^3=H; R^2=NO_2; R^4=C_2H_5; X = O;
16l R^1= H; R^2,R^3=Cl; R^4=C_2H_5; X = O;
16m R^1= H; R^2,R^3=F; R^4=C_2H_5; X = O;
16n R^1= H; R^2,R^3=CH_3; R^4=C_2H_5; X = O;

Scheme 3. (i) *t*-BuOK, DMSO, 110–120 °C, 5 min; (ii) powdered KOH, 18-crown-6 ether, benzene, reflux, 5–12 h; (iii) EtOH, 10% aq KOH, reflux, 1 h.



Scheme 4. (i) $NaOC_2H_5$, EtOH, 1,2-dibromoethane, reflux, 2 h; (ii) *t*-BuOK, DMSO, methyl *p*-toluenesulfonate, rt, 2 h; (iii) pyridine, acetyl chloride, rt, 30 min.

oxidized to a carboxylic acid moiety in a haloform reaction with Br_2 and NaOH. The obtained acid was esterified with thionyl chloride/ethanol to yield the benzoate components necessary for the synthesis of **27** and **30**.



Scheme 5. (i) $NaOC_2H_5$, EtOH, 1,2-dibromoethane, reflux, 7–14 h; (ii) Br_2, 12% aq NaOH, dioxane, 0 °C, 1.5 h; (iii) $SOCl_2$, benzene, reflux, 3h; (iv) EtOH, reflux, 15 min.

Biological Evaluation

The $cPLA_2$-inhibitory potency of the test compounds was evaluated by measuring the calcium ionophore A23187-induced arachidonic acid release from human platelets with HPLC and UV-detection at 200 nm.[16,21] To avoid metabolism of arachidonic acid via the cyclooxygenase-1 and the 12-lipoxygenase pathways, the dual cyclooxygenase-1/12-lipoxygenase inhibitor 5,8,11,14-eicosatetraynoic acid (ETYA) was added to the platelets in these experiments. Since lysis of the platelets by a test compound may misleadingly indicate enzyme inhibition, we also measured the cell lytic potency of each compound by turbidimetry.[22] In these experiments, it was found that none of the compounds showed cell lytic properties at concentrations near its IC_{50} against $cPLA_2$.

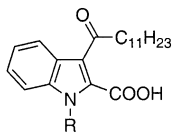
Results and Discussion

To explore the importance of the ether oxygen of the (4-carboxyphenoxy)ethyl substituent for the $cPLA_2$-inhibitory activity of the indole **4**, we replaced this atom by sulfur (**7**), nitrogen (**14**), methylated nitrogen (**15**) and methylene (**17**). As shown in Table 1, all these variations led to a decrease of activity. The IC_{50}-values of the compounds **7**, **15** and **17** lay between 2 and 3 μM. The greatest loss of inhibitory potency occurred when the oxygen was exchanged by nitrogen. With an IC_{50} of 5.4 μM **14** was about 6-fold less active than **4**.

An elongation of the ethoxy moiety of the (4-carboxyphenoxy)ethyl substituent by one (**18**) or two (**19**) carbons also reduced in vitro potency.

Modifications on the 4-carboxyphenoxy residue had different effects. While a methoxy (**20**), hydroxy (**21**) and chloro (**22**) substituent in *ortho*-position or a methoxy (**23**), hydroxy (**24**) and nitro (**28**) group in *meta*-position to the carboxylic acid functionality lowered activity, a chloro (**25**), fluoro (**26**) or methyl moiety (**27**) in *meta*-position slighthly increased it. Introduction of an additional chloro (**29**), fluoro (**30**) or methyl substituent (**31**)

Table 1. Inhibition of the $cPLA_2$-mediated arachidonic acid release from human platelets stimulated with calcium ionophore A23187



Compd	R	IC_{50} (μM)[a]
2	$-(CH_2)_7COOH$	2.5
3	$-CH_2CH_2OPhenyl(4-CH_2COOH)$	2.6
4	$-CH_2CH_2OPhenyl(4-COOH)$	0.86
7	$-CH_2CH_2SPhenyl(4-COOH)$	2.5
14	$-CH_2CH_2NHPhenyl(4-COOH)$	5.4
15	$-CH_2CH_2N(CH_3)Phenyl(4-COOH)$	2.9
17	$-CH_2CH_2CH_2Phenyl(4-COOH)$	2.1
18	$-CH_2CH_2CH_2OPhenyl(4-COOH)$	3.3
19	$-CH_2CH_2CH_2CH_2OPhenyl(4-COOH)$	1.5
20	$-CH_2CH_2OPhenyl(3-OCH_3)(4-COOH)$	2.8
21	$-CH_2CH_2OPhenyl(3-OH)(4-COOH)$	2.3
22	$-CH_2CH_2OPhenyl(3-Cl)(4-COOH)$	2.9
23	$-CH_2CH_2OPhenyl(2-OCH_3)(4-COOH)$	4.9
24	$-CH_2CH_2OPhenyl(2-OH)(4-COOH)$	5.2
25	$-CH_2CH_2OPhenyl(2-Cl)(4-COOH)$	0.52
26	$-CH_2CH_2OPhenyl(2-F)(4-COOH)$	0.44
27	$-CH_2CH_2OPhenyl(2-CH_3)(4-COOH)$	0.57
28	$-CH_2CH_2OPhenyl(2-NO_2)(4-COOH)$	3.0
29	$-CH_2CH_2OPhenyl(2-Cl,6-Cl)(4-COOH)$	1.8
30	$-CH_2CH_2OPhenyl(2-F,6-F)(4-COOH)$	0.64
31	$-CH_2CH_2OPhenyl(2-CH_3,6-CH_3)(4-COOH)$	3.6
AR-C73346XX		0.24

[a]Values are the means of at least two independent determinations; errors are within ±20%; in case of **4**: $n=5$, standard deviation ±0.1 μM.

in the second *meta*-position (position 6 of the phenyl ring) did not have an additive effect, but resulted in a decrease of inhibition.

In summary, the structural variations performed led to a reduction of enzyme inhibition in most cases. However, none of the synthesized compounds was found to be inactive. Beneficial effects could be obtained with the introduction of a lipophilic methyl or chloro substituent or a fluoro moiety in *meta*-position to the carboxylic acid moiety of the benzoic acid residue. With an IC_{50} of 0.44 μM the fluoro derivative **26** was about twice as potent as the starting compound **4** and half as potent as the reference AR-C73346XX (4-{2-oxo-3-[4-(4-phenylbutylthio)phenoxy]propoxy}benzoic acid),[23] which belongs to the most potent $cPLA_2$ inhibitors known today.

References and Notes

1. Serhan, C. N.; Haeggström, J. Z.; Leslie, C. C. *FASEB J.* **1996**, *10*, 1147.
2. Huang, Y. H.; Schafer-Elinder, L.; Wu, R.; Lesson, H. E.; Frostegard, J. *Clin. Exp. Immunol.* **1999**, *116*, 326.
3. Ryborg, A. K.; Deleuran, B.; Sogaard, H. R.; Kragballe, K. *Acta Derm. Venereol.* **2000**, *80*, 242.
4. Connolly, S.; Robinson, D. H. *Expert Opin. Ther. Pat.* **1993**, *3*, 1141.
5. Connolly, S.; Robinson, D. H. *Expert Opin. Ther. Pat.* **1995**, *5*, 673.
6. Tibes, U.; Friebe, W. G. *Expert Opin. Invest. Drugs* **1997**, *6*, 279.
7. Mayer, R. J.; Marshall, L. A. *Emerging Drugs* **1998**, *3*, 333.
8. Six, D. A.; Dennis, E. A. *Biochim. Biophys. Acta* **2000**, *1488*, 1.
9. Lin, L. L.; Lin, A. Y.; Knopf, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6147.
10. Uozumi, N.; Kume, K.; Nagase, T.; Nakatani, N.; Ishii, S.; Tashiro, F.; Komagata, Y.; Maki, K.; Ikuta, K.; Ouchi, Y.; Miyazaki, J.; Shimizu, T. *Nature* **1997**, *390*, 618.
11. Bonventre, J. V.; Huang, Z.; Taheri, M. R.; O'Leary, E.; Li, E.; Moskowitz, M. A.; Sapirstein, A. *Nature* **1997**, *390*, 622.
12. Gijon, M. A.; Spencer, D. M.; Siddiqi, A. R.; Bonventre, J. V.; Leslie, C. C. *J. Biol. Chem.* **2000**, *275*, 20146.
13. Lehr, M. *J. Med. Chem.* **1997**, *40*, 2694.
14. Tries, S.; Neher, K.; Laufer, S.; Abraham, W. M.; Lehr, M. *Mediat. Inflamm.* **1999**, *8* (Suppl. 1), S123.
15. Tries, S.; Neupert, W.; Albrecht, W.; Lehr, M.; Laufer, S. *Abstract of Papers*, 10th National Meeting of the Inflammation Research Association, Hot Springs, USA, 2000.
16. Lehr, M.; Schulze Elfringhoff, A. *Arch. Pharm. Pharm. Med. Chem.* **2000**, *333*, 312.
17. Horner, L.; Lindel, H. *Phosphorus Sulfur* **1983**, *15*, 1.
18. Dave, M. P.; Patel, J. M.; Langalia, N. A.; Shah, S. R.; Thaker, K. A. *J. Indian Chem. Soc.* **1985**, *62*, 386.
19. Bicking, J. B.; Robb, C. M.; Cragoe, E. J.; Blaine, E. H.; Watson, L. S.; Dunlay, M. C. *J. Med. Chem.* **1983**, *26*, 335.
20. General procedure for the alkylation of 3-dodecanoylindole-2-carboxylic acid (**5**) using KOH/crown ether: To the mixture of **5** (372 mg, 1 mmol), powdered KOH (85%) (66 mg, 1 mmol) and 18-crown-6 ether (0.15 mmol, 40 mg) was added dry benzene (10 mL). After removing about half of the volume of the benzene by evaporation the mixture was refluxed for 30 min. The solution of the appropriate 4-(2-bromoethoxy) benzoic acid ester (1.2 mmol) in dry benzene (5 mL) was added and heating at reflux was continued for 5–12 h. To the cooled reaction mixture kieselguhr was added. After filtration, the solution was concentrated and chromatographed on silica gel using petroleum ether–ethyl acetate as eluent. The ester intermediate was hydrolyzed with KOH according to published procedures.
21. HPLC analysis of the arachidonic acid: internal standard 3-(4-decyloxyphenyl)propanoic acid (Diczfalusy, E.; Ferno, O.; Fex, H.; Hogberg, B. *Acta Chem. Scand.* **1963**, *17*, 2536); column: multospher 100 RP18—3 μM (125×3.0 mm) with a pre-column multospher 100 RP18—5 μM (20×3.0 mm) (CS-chromatographie service, Langerwehe, Germany); injection volume: 300 μL; mobile phase: acetonitrile/10 mM $(NH_4)_2HPO_4$ buffer adjusted to pH 7.4 with *ortho*-phosphoric acid (50:50, v/v); flow rate: 0.33 mL/min, detection wavelength 200 nm applying a Waters 2487 UV detector.
22. Lehr, M. *Arch. Pharm. Pharm. Med. Chem.* **1996**, *329*, 386.
23. Mete, A.; Austin, R.; Bennion, C.; Bernstein, M.; Connolly, S.; Jackson, C. G.; King, S.; Lawrence, L.; Lewis, R.; Robinson, D. H.; Stein, L.; Walters, I.; Withnall, W. J. *Abstract of Papers*, 15th EFMC Int. Symp. Med. Chem., Edinburgh, 1998; P250.