Potential prodrugs of non-steroidal anti-inflammatory agents for targeted drug delivery to the CNS

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · SEPTEMBER 2004

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2004.05.006 · Source: PubMed

Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 39 (2004) 715–727
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

Potential prodrugs of non-steroidal anti-inflammatory agents for targeted drug delivery to the CNS

Luana Perioli *, Valeria Ambrogi, Claudia Bernardini, Giuliano Grandolini, Maurizio Ricci, Stefano Giovagnoli, Carlo Rossi

Dipartimento di Chimica e Tecnologia del Farmaco, Via del Liceo 1, Università degli Studi di Perugia, 06123 Perugia, Italy

Received 27 January 2004; received in revised form 29 April 2004; accepted 10 May 2004

Abstract

Recently non-steroidal anti-inflammatory drugs (NSAIDs) have been proposed to prevent or to cure Alzheimer's disease. In this respect, we synthesized new potential prodrugs of several NSAIDs in order to increase their access to the brain. The carboxylic group of NSAIDs was attached to the 1,4-dihydro-1-methylpyridine-3-carboxylate moiety, which acts as a carrier, via an amino alcohol bridge, according to the chemical delivery approach developed by Bodor. The lipophilicity of potential prodrugs was evaluated both via traditional experimental parameters, such as partition coefficient and chromatographic R_m value, and by predictive computational methods. From experimental parameters, all prodrugs were more lipophilic when compared to their corresponding parent compounds and consequently a better blood brain barrier (BBB) penetration is hypothesised. Prodrug lipophilicity was correlated with a calculated log *P* value according to Kowwin's method. The correlation between experimental R_m^0 and calculated log *P*, determined by PLS analysis, was good for all compounds with the exception of compound **7i**. In addition the BBB permeation profile of our synthesized compounds was predicted using the BBB VolSurf model and seven of the synthesized prodrugs resulted in good candidates for BBB penetration.

Keywords: Prodrugs; NSAIDs; Alzheimer's disease; Lipophilicity; Predicting blood brain barrier permeation

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia [1]. Although at present the aetiology of AD is not well understood, many neurobiological and environmental factors contributing to the pathogenesis of AD have been described. Recently, it has been revealed that there is a presence of an inflammatory component in the pathogenesis of AD [1,2]. In fact, immunochemistry completed on post-mortem AD brains revealed that numerous inflammatory components are associated with neuritic plaques [3] and epidemiological studies have shown that therapy with anti-inflammatory drugs reduces the risk of developing AD [4–8].

In order to understand the mechanisms by which NSAIDs can protect the nervous system from the ravages caused by AD, extended clinical observations at the cellular level have been carried out. Numerous studies indicate the involvement of immune and chronic inflammatory mechanisms in AD [9,10].

If the evidence for an inflammatory reaction in AD is clear, it is still unclear whether this inflammatory reaction is the cause of AD or only a secondary event that develops the degenerative process [1]. All these data indicate that an anti-inflammatory therapy [11,12] reduces the risk of developing AD in subjects without genetic predisposition, delays the onset of AD in genetically vulnerable individuals and delays the progressive cognitive deterioration in AD patients.

The aim of this study is to synthesize and predict the permeation profiles of a few NSAID derivatives designed to increase their access to the brain.

Some potential prodrugs of several NSAIDs, such as diclofenac (DIK), ibuprofen (IBU), ketoprofen (KET), tiaprofenic acid (TIAP) and tolmetin (TOLM), were synthesized using as a carrier, the 1,4-dihydro-1-methylpyridine-3-carboxylate, which was attached to the drug via an amino

* Corresponding author. Tel.: +39-75-585-5133; fax: +39-75-585-5163.
E-mail address: luanaper@unipg.it (L. Perioli).

doi:10.1016/j.ejmech.2004.05.006

alcohol bridge. Furthermore, in order to evaluate the effect of the bridge on prodrug lipophilicity, different amino alcoholic chains were introduced.

The lipophilicity of all potential prodrugs was evaluated using both traditional experimental parameters, such as partition coefficient and chromatographic R_m value, and predictive computational methods. CNS penetration was predicted using a BBB model [13] as described in the VolSurf procedure [14].

2. Results and discussion

2.1. Chemical delivery system

Several techniques to obtain improved access of drugs to the brain were developed. Among them, the chemical delivery system (CDS) approach developed by Bodor [15] is one of the most interesting procedures to deliver drugs in a sustained and specific manner to the CNS. It involves the release of active species from a lipophilic prodrug through a multistep conversion.

The CDS approach is based on a dihydropyridine pyridium salt equilibrium type redox molecular carrier, similar to the endogenous NADH/NAD^+ coenzyme system.

The CDS consists of a drug, a carrier (dihydropyridine) and a linkage between them. A non-steroidal antiinflammatory drug containing a carboxylic group can be attached to the carrier via an amino alcohol bridge. By intravenous administration, the dihydropyridine form of the CDS is rapidly distributed throughout the body, including CNS. Next, the dihydropyridine moiety is oxidized to the pyridinium salt. This hydrophilic form is sequestrated in the CNS, whereas it is rapidly eliminated from the periphery. By enzymatic hydrolysis, the active drug is released into the brain and can exert its action, whilst the small carrier molecule (pyridinium salt) is actively transported out of the CNS. This kind of drug delivery, sustained and specific, cannot be achieved by the use of simple lipophilic drugs.

2.2. Chemistry

The synthetic route for these prodrugs **7a–q** is shown in Scheme 1. This procedure involves the formation of an ester group between the carboxylic group of the drug and the alcoholic function of the pyridine derivative **1–4**, the successive quaternization of the pyridine nitrogen and eventually their reduction to 1,4-dihydroderivatives.

The hydroxyalkylnicotinamides **1–4** were prepared by reacting of the appropriate amino alcohol with the ethyl ester of nicotinic acid at room temperature [16]. The formation of esters **5a–q** was achieved by reacting the drug with the appropriate hydroxyalkyl nicotinamides **1–4** in acetonitrile at room temperature in the presence of dicyclohexylcarbodiimide and 4-dimethylaminopyridine.

Compounds **5a–q** were *N*-methylated by a solution of methyl iodide in nitromethane, to give the quaternary ammonium salts **6a–q**. These compounds were subsequently reduced, in dilute alkaline sodium bicarbonate solution, by sodium dithionite, a selective reduction agent, into the 1,4-dihydropyridine ring. This reaction was performed at room temperature in a biphasic system of water and ethyl acetate, in the absence of oxygen and keeping the pH of the medium above 7.

All compounds were characterized by elemental analyses and ^{1}H nuclear magnetic resonance. UV spectra were recorded for compounds **7a–q** and revealed the formation of 1,4-dihydropyridine derivatives by their typical UV maximum absorption at ca. λ 360 nm. The ^{1}H-NMR spectra of **7a–q** showed the disappearance of typical signals due to the 3-substituted pyridine ring and revealed the presence of appropriate signals due to the 1,4-dihydropyridine moiety.

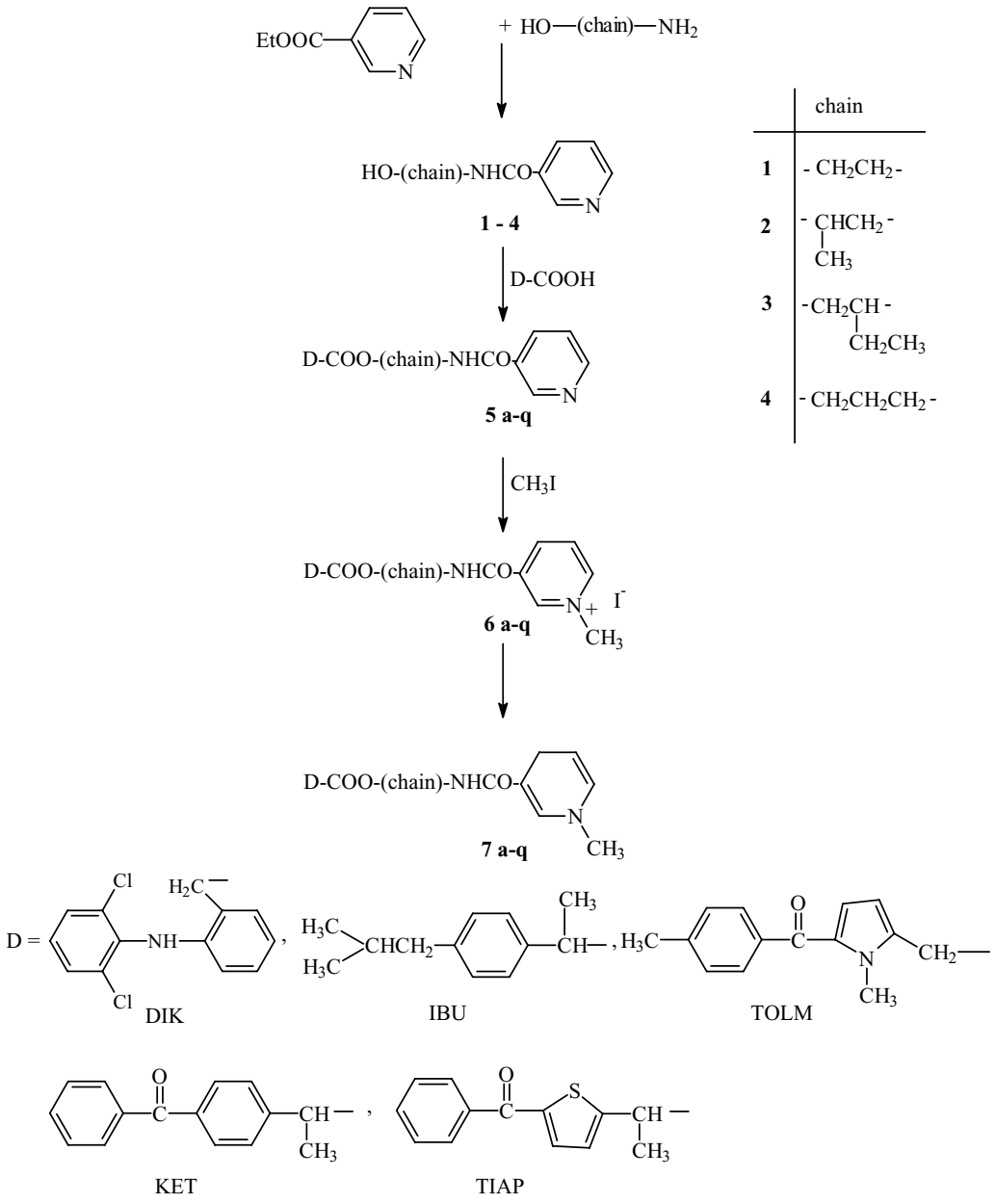
2.3. Lipophilicity

Lipophilicity is an essential feature for the penetration of a molecule through the BBB. Thus, the lipophilicity of compounds **7a–q** was determined and was compared to that of the parent drugs. First the determination of lipophilicity was performed using the shake-flask method between an aqueous buffer at pH 7.5 and *n*-octanol, followed by UV prodrug determination after shaking. In the case of prodrugs, this method failed because their lipophilicity increased to such an extent that the molecule concentrations in the aqueous phase could not be determined. Thus, the chromatographic R_m values, related to the partition coefficient between the mobile and stationary phase of a chromatographic system, were determined and correlated to the lipophilicity of the compounds [17,18].

The R_m values of each drug and the corresponding prodrugs were determined using the formula $R_m = \log[(1/R_f) - 1]$. The R_f values were measured by means of a reverse-phase TLC method with various concentrations of acetone and aqueous phosphate buffer at pH 7 as a mobile phase. The R_m values were plotted as a function of acetone concentrations and eventually the R_m values, corresponding to a mobile phase of 0% acetone (R_m^0), were determined by extrapolation of the linear part of R_m value curves to a mobile phase of 0% acetone (Table 1). R_m^0 values were considered as lipophilic indexes.

All the prodrugs were found much more lipophilic when compared to their corresponding drugs. Lipophilicity increased significantly by introducing a branched substituent (methyl or ethyl) in the ethylic bridge and by increasing the length of the alkyl bridge (propyl > ethyl). Compounds **7g** and **7m** resulted the most lipophilic compounds.

From the obtained R_m^0 values, for all synthesized prodrugs a better BBB penetration than the parent compounds may be hypothesised. The lipophilicity of all prodrugs was also correlated to the calculated log *P* value by means of the Kowwin's method [19]. The correlation between experimental



Scheme 1. Synthesis of prodrugs **7a–q**.

R_m^0 and calculated log *P* was determined by PLS analysis [20] (Table 1). Fig. 1 shows the correlation between R_m^0 variations and calculated log *P* variations and makes clear that a good correlation does exist, with the exception of compound **7i** (not reported in Fig. 1), which can be considered an outlier.

2.4. Predicting BBB permeation studies

The BBB is a complex cellular system and its function is to maintain the homeostasis of the CNS by separating the brain from the systemic blood circulation.

Crivori et al. [13] reported a model for predicting BBB permeation of molecules based on literature data. The study was conducted to demonstrate values of descriptors, derived from 3D-molecular fields to estimate the BBB permeation for a large set of compounds.

The model distinguished well between the BBB+ (penetrating) and BBB– (non-penetrating) compounds, and was mainly driven by a passive diffusion mechanism. The model correctly assigned the corresponding BBB profile to more than 90% of the compounds. Since the prediction error (SDEP) of the discriminant PLS was 0.6 units, a confidence interval was built in the t_1–t_2 space between the BBB+ and BBB– regions. In this interval, the BBB prediction given by the model were considered borderline and doubtful.

The BBB VolSurf model was used in this work to predict the permeation profile of our synthesized prodrugs. All 17 compounds were built in 3D coordinates (see Section 4) and then projected in the VolSurf model. The results of the

Table 1
Log *P*, R_m^0 and PLS data of drugs and compounds **7a–q**

Compound	Drug	Chain	Log *P* (Kowwin)	R_m^0 (*r*) [a]	PLS
	IBU	–	3.79	1.025 (0.998)	1.025
	DIK	–	4.02	1.281 (0.997)	1.28
	KET	–	3.0	0.6179 (0.990)	–
	TIAP	–	2.82	0.458 (0.993)	–
	TOLM	–	2.02	–	–
7a	DIK	CH_2CH_2	4.61	2.380 (0.995)	2.38
7b	IBU	CH_2CH_2	4.38	3.663 (0.997)	3.66
7c	TOLM	CH_2CH_2	2.61	–	–
7d	KET	CH_2CH_2	3.59	–	–
7e	TIAP	CH_2CH_2	3.41	–	–
7f	DIK	$CH(CH_3)CH_2$	5.02	3.89 (0.991)	4.04
7g	IBU	$CH(CH_3)CH_2$	4.80	4.972(0.994)	4.97
7h	KET	$CH(CH_3)CH_2$	4.01	3.89 (0.992)	–
7i	TIAP	$CH(CH_3)CH_2$	3.83	4.57 (0.993)	4.0
7j	DIK	$CH_2CH(CH_2CH_3)$	5.51	4.52 (0.991)	4.52
7k	IBU	$CH_2CH(CH_2CH_3)$	5.29	4.269 (0.996)	4.27
7l	KET	$CH_2CH(CH_2CH_3)$	4.50	3.92 (0.998)	3.93
7m	TIAP	$CH_2CH(CH_2CH_3)$	4.32	4.96 (0.997)	4.26
7n	DIK	$CH_2CH_2CH_2$	5.10	3.841 (0.997)	3.84
7o	IBU	$CH_2CH_2CH_2$	4.87	4.082 (0.994)	4.08
7p	KET	$CH_2CH_2CH_2$	4.08	4.122 (0.991)	2.88
7q	TIAP	$CH_2CH_2CH_2$	3.90	2.91 (0.991)	2.58

[a] *r* = correlation coefficient.

projection are reported in Fig. 2, which shows the BBB profile as predicted by VolSurf. All prodrugs are located in the BBB+ region of the plot. However, from a statistical point of view, the seven compounds coded with **a, b, g, j, k, n, o** are better candidates for BBB penetration than the other compounds located in the borderline regions of the plot.

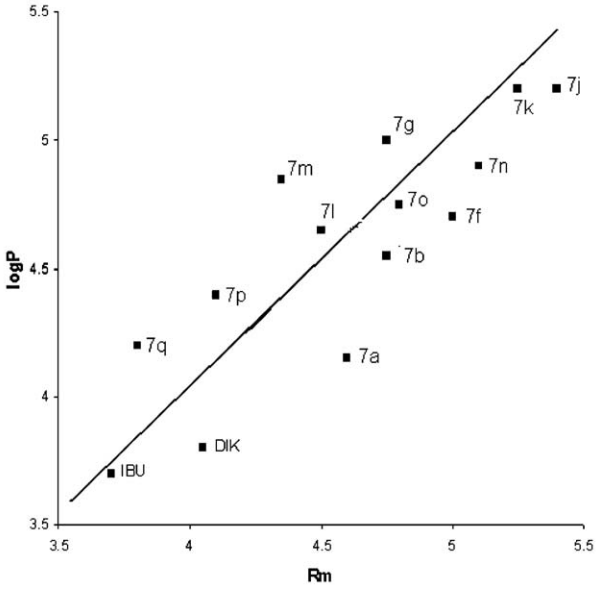


Fig. 1. The line represents the PLS regression model between the computed log P and the experimental R^0_m values for compounds **7a–q**.

3. Conclusions

All prodrugs were successfully synthesized and showed a higher lipophilicity when compared to their parent drugs. In particular lipophilicity increased significantly by introducing a branched substituent (methyl or ethyl) in the ethyl bridge or by increasing the length of the alkyl bridge (propyl > ethyl) as in the case of compound **7j**, a prodrug of diclofenac. All compounds, with the exception of **7i**, showed good correlation between R_m^0 parameters and calculated log *P*.

Predictive BBB permeation studies demonstrated that all potential prodrugs are located in the BBB+ region of the plot. In particular compounds **7a, 7b, 7g, 7j, 7k, 7n**, and **7o** are the best candidates for BBB penetration. They have two things in common, first they are prodrugs of ibuprofen and diclofenac, and second, they all contain either a propylic or a branched chain bridge.

4. Experimental

4.1. Chemistry

4.1.1. Materials and methods

Diclofenac sodium salt was purchased from Sigma Chemical Company, (Milano, Italy), ibuprofen (acid), tolmetin (acid), tiaprofenic acid and ketoprofen (acid) were kindly supplied by Francesco Angelini S.p.A. (Roma, Italy), Cilag (Schaffhausen, Switzerland), Camillo Corvi (Milan, Italy) and Rhone-Poulenc Rorer (Origgio, Italy), respectively. All starting materials were of reagent grade.

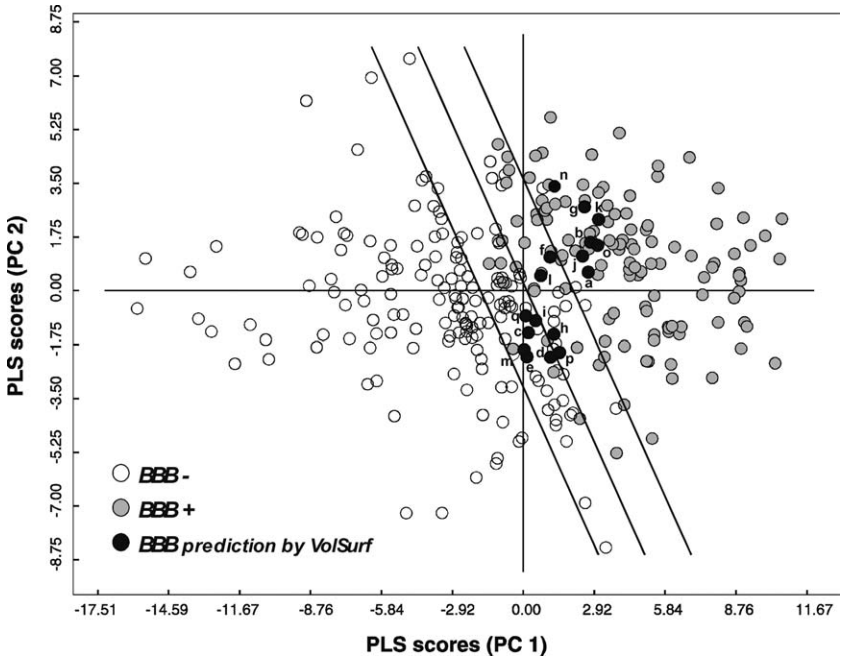


Fig. 2. BBB profile as predicted by Volsurf. a = **7a**, b = **7b**, c = **7c**, d = **7d**, e = **7e**, f = **7f**, g = **7g**, h = **7h**, i = **7i**, j = **7j**, k = **7k**, l = **7l**, m = **7m**, n = **7n**, o = **7o**, p = **7p**, q = **7q**.

Melting points were obtained using a Kofler-hot stage apparatus and are uncorrected. Mass spectra were recorded in a Varian MAT instrument. Proton nuclear magnetic resonance spectra (^{1}H-NMR) were recorded with a Bruker AC 200 (200 MHz) spectrometer, using the solvents indicated and chemical shifts (δ) were reported in ppm relative to an internal standard (tetramethylsilane). Elemental analyses (C, H, N), performed by a Carlo Erba 1106 elemental analyser, resulted within ± 0.4% of theoretical values. UV spectra were performed in ethanol using a Jasco V-520 (Tokyo) spectrophotometer. The purity of compounds was checked by TLC (pre-coated plates, silica gel Kieselgel 60 F254 (Merck) and DC-Alufolien Aluminium oxide 60 F254 neutral, Typ.E (Merck)).

4.1.2. Synthesis of N-*(2-hydroxyethyl)-3-nicotinamide* ***1***

This compound was synthesized as described [16].

4.1.3. Synthesis of N-*(2-hydroxyalkyl)-3-nicotinamide* ***2–4***

Mixtures of ethyl nicotinate (0.02 mmol) and appropriate amino alcohols (0.02 mmol) were stirred at 50 °C for 30 min, and then left stirring at room temperature for 20–25 days. The reaction mixtures were dried in vacuo to give compounds [**2–4**] in quantitative yields. The crude oily products were employed as such since they showed single spots by TLC ($CHCl_3$/MeOH 95:5).

2: ^{1}H-NMR ($CDCl_3$): 1.22 (d, *J* = 6 Hz, 3H, –CH_3), 3.10–3.35 (br s, 1H, –OH), 3.55–3.75 (m, 1H, –CH–), 3.92–4.05 (m, 1H, C_1–H), 7.28–7.38 (m, C_5–H, pyridine), 7.45 (br s, 1H, –NH–), 8.10 (dd, 8.1 and 2.1 Hz, 1H, C_4–H, pyridine), 8.60 (dd, *J* = 4.8, 1.6 Hz, 1H, C_6–H pyridine), 9.00 (d, *J* = 2.0 Hz, 1H, C_2–H pyridine).

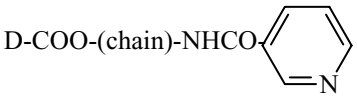
3: ^{1}H-NMR ($CDCl_3$): 1.00 (t, *J* = 7 Hz, 3H, –CH_2CH_3), 1.21–1.80 (m, 3H, –CH–CH_2CH_3), 3.10 (s, 1H, OH), 4.00–4.28 (m, 2H, HOCH_2CH–), 7.24–7.38 (m, C_5–H, pyridine) 7.42 (br s, 1H, –NH–), 8.10 (dd, *J* = 7.8 and 2.2 Hz, 1H, C_4–H pyridine), 9.00 (d, *J* = 2.2 Hz, 1H, C_2–H pyridine).

4: ^{1}H-NMR ($CDCl_3$): 1.92–2.18 (m, 2H, –$CH_2CH_2CH_2$–), 3.15 (s, 1H, –OH), 3.40–3.65 (m, 2H, $OCH_2CH_2CH_2$NH), 4.26–4.38 (m, 2H, O$CH_2CH_2CH_2$NH), 7.25–7.37 (m, C_5–H, pyridine), 7.48 (br s, 1H, –NH–), 8.08 (dd, *J* = 7.9, 2.0 Hz, 1H, C_4–H pyridine), 8.98 (d, *J* = 2.0 Hz, 1H, C_2–H pyridine).

4.1.4. General procedure for the synthesis of ***5a–q*** *(Table 2)*

Suitable hydroxyalkylnicotinamides **1–4** (16 mmol), dicyclohexylcarbodiimide (16 mmol) and fine 4-dimethylaminopyridine (1.5 mmol) were added to a solution of the drug (in acidic form) (15 mmol) in anhydrous acetonitrile (150 ml). The mixtures were filtered and filtrates were dried in vacuo. The residues were dissolved in $CHCl_3$ and washed first with a diluted $NaHCO_3$ solution, then with diluted HCl and eventually with water. The chloroform solutions were dried over anhydrous Na_2SO_4 and the solvent was evaporated in vacuo. The oily residues yielded pure compounds **5g** and **5k**, which were used for the successive reaction without further purification. In the case of compound **5c**, the residue was induced to crystallize by adding few drops of diethyl ether. The resulting solid was recrystallized by ethanol/hexane (1:3). In all other cases the oily residues were purified by flash chromatography using $CHCl_3$ as eluant, with the exception of compound **5e** where the eluant used was $CHCl_3$/MeOH (99:1).

Table 2
Physical and chemical data of compounds **5a-q**



5 a-q

Compound	Drug	Chain	m.p. °C (solvent)	Yield (%)	Formula (MW)	^{1}H NMR δ
5a	DIK	CH_2CH_2	92–94 (EtAc)	45	$C_{22}H_{19}N_3O_3Cl_2$ (444.3)	3.72–3.82 (m, 2H, C$\underline{H}_2$NH), 3.85 (s, 2H, $\underline{CH}_2$COO), 4.37 (t, *J* = 7.0 Hz, 2H, $CH_2COO\underline{CH}_2$), 6.41–7.38 (m, 10H, 7 aromatic + 2NH + C_5–H pyridine), 7.88–8.05 (m, 1H, C_4–H pyridine), 8.70 (dd, *J* = 1.5, 5.2 Hz, 1H, C_6–H, pyridine), 8.90 (d, *J* = 2.3 Hz, 1H, C_2–H, pyridine) ($CDCl_3$)
5b	IBU	CH_2CH_2	72–75 (EtAc)	80	$C_{21}H_{26}N_2O_3$ (354.5)	0.79 (d, *J* = 9.0 Hz, 6H, CH($\underline{CH}_3$)$_2$), 1.32 (d, *J* = 10.0 Hz, 3H, CH($\underline{CH}_3$), 1.59–1.80 (m, 1H, $\underline{CH}(CH_3)_2$), 2.32 (d, *J* = 10.0 Hz, 2H, CH$\underline{CH}_2$), 3.48–3.73 (m, 2H, $CH_2\underline{CH}_2$), 3.75 (q, *J* = 10.0 Hz, 1H, $\underline{CH}(CH_3)$, 4.02–4.30 (m, 2H, CH_2CH_2), 7.00, 7.15 (A_2B_2 system, *J* = 10.0 Hz, 4H, C_6H_4), 7.50–7.62 (m, 1H, C_5-H pyridine), 8.18 (dd, 1H, C_4-H pyridine), 8.65–8.88 (m, 1H, C_6–H pyridine), 9.00 (br s, 1H, C_2–H pyridine) (DMSO–d_6)
5c	TOLM	CH_2CH_2	115–118 (EtOH/hexane; 1:3)	68	$C_{23}H_{23}N_3O_4$ (405.5)	2.35 (s, 3H, C_6H_4–CH_3), 3.45–3.60 (m, 2H, CH_2CH_2), 3.82 (s, 3H, NCH_3), 3.87 (s, 2H, CH_2COO), 4.20–4.35 (m, 2H, CH_2CH_2), 6.10, 6.53 (AB system, *J* = 8 Hz, 2H, pyrrole), 7.25, 7.58 (A_2B_2 system, *J* = 12.0 Hz, 4H, C_6H_4), 7.45–7.50 (m, 1H, C_5–H pyridine), 8.13–8.25 (m, 1H, C_4–H pyridine), 8.67–8.75 (m, 1H, C_6–H pyridine), 8.80 (br s, 1H, NH), 8.95 (d, 1H, C_2–H pyridine) (DMSO-d_6)
5d	KET	CH_2CH_2	Oil	77	$C_{24}H_{22}N_2O_4$ (402.4)	1.55 (d, *J* = 10.0 Hz, 3H, CH_3), 3.60–3.78 (m, 2H, CH_2), 3.83 (q, 1H, CH), 4.20–4.46 (m, 2H, CH_2), 6.70 (br s, 1H, NH), 7.20–7.60 (m, 9H, C_6H_4 + C_6H_5), 7.70–7.80 (m, 1H, C_5–H pyridine), 7.90 (dd, 1H, C_4–H pyridine), 8.62 (d, 1H, C_6–H pyridine), 8.81 (s, 1H, C_2–H pyridine) ($CDCl_3$)
5e	TIAP	CH_2CH_2	Oil	67	$C_{22}H_{20}N_2O_4S$ (408.5)	1.66 (d, *J* = 7.0 Hz, 3H, CH_3), 3.63–3.87 (m, 2H, CH_2), 4.10 (q, *J* = 7.0 Hz, 1H, CH), 4.26–4.48 (m, 2H, CH_2), 6.45 (br s, 1H, NH), 6.98–7.90 (m, 8H, 7 aromatic + C_5–H pyridine), 8.02 (d, 1H, C_4–H pyridine), 8.70 (d, 1H, C_6–H pyridine), 8.92 (s, 1H, C_2–H pyridine) ($CDCl_3$)
5f	DIK	$CH(CH_3)CH_2$	Oil	45	$C_{23}H_{21}N_3O_3Cl_2$ (458.3)	0.99 (d, *J* = 6.2 Hz, CH_3), 2.99 (m, 2H, CH_2), 3.80 (s, 2H, CH_2COO), 4.73–4.86 (m, 1H, CH), 6.15–7.48 (m, 10H, 7 aromatic + N$\underline{H}CH_2$ + C_5–H and C_4–H pyridine), 8.25–8.38 (m, 2H, C_6–H and C_2–H pyridine) (DMSO-d_6)
5g	IBU	$CH(CH_3)CH_2$	Oil	Quantitative	$C_{22}H_{28}N_2O_3$ (368.5)	0.8 (d, *J* = 6.6 Hz, 6H $(CH_3)_2CH$), 1.17–1.30 (m, 3H, C$\underline{H}_3$CHO), 1.45 (d, *J* = 7.2 Hz, 3H, $\underline{CH}_3$CHCO), 1.55–1.82 (m, 1H, $(CH_3)_2\underline{CH}CH_2$), 2.29–2.58 (m, 2H, $(CH_3)_2CH\underline{CH}_2$), 3.25–3.73 (m + q superimposed, 3H, CHC$\underline{H}_2$NH and $CH_3C\underline{H}COO$), 5.0–5.2 (m, 1H, OC$\underline{H}CH_2$), 6.02 (br s, 1H, NH), 6.90–7.35 (m, 5H aromatic + C_5–H pyridine), 7.70–7.80 (m, 1H, C_4–H pyridine), 8.63–8.72 (m, 1H, C_6–H pyridine), 8.83 (dd, *J* = 2.1 Hz, 1H, C_2–H pyridine) ($CDCl_3$)
5h	KET	$CH(CH_3)CH_2$	Oil	71	$C_{25}H_{24}N_2O_4$ (416.5)	1.32 (d, *J* = 7.6 Hz, 3H, $\underline{CH}_3CHCH_2$), 1.58 (d, *J* = 7.1 Hz, 3H, $\underline{CH}_3CHC_6H_4$), 3.35–3.75 (m, 2H, CH_2NH), 3.80 (q, *J* = 7.1 Hz, 1H, $CH_3CHC_6H_4$), 5.05–5.20 (m, 1H, $CH_3\underline{CH}CH_2$), 6.48 (br s, 1H, NH), 7.20–7.89 (m, 11H, 9 aromatic + C_5–H pyridine + C_4–H pyridine), 8.53–8.77 (m, 2H, C_6–H and C_2–H pyridine) ($CDCl_3$)
5i	TIAP	$CH(CH_3)CH_2$	Oil	48	$C_{23}H_{22}N_2O_4S$ (422.5)	1.52–1.85 (2d superimposed, 6H, $\underline{CH}_3CHCO$ + $OCH(\underline{CH}_3)CH_2$), 3.20–3.45 (m, 2H, $CH\underline{CH}_2NH$), 3.48–3.67 (m, 1H, $CH_3\underline{CH}CO$), 5.21–5.38 (m, 1H, $CH_3\underline{CH}CH_2$), 6.65 (br s, 1H, NHCO), 6.95–7.85 (m, 8H, 7 aromatic + C_5–H pyridine), 7.86–7.91 (m, 1H, C_4–H pyridine), 8.62–8.70 (m, 1H, C_6–H pyridine), 9.01 (s, 1H, C_2–H pyridine) (DMSO-d_6)
5j	DIK	$CH_2CH(CH_2CH_3)$	Oil	62	$C_{24}H_{23}N_3O_3Cl_2$ (471.4)	0.80–1.10 (m, 3H, $\underline{CH}_3CH_2$), 1.44–1.78 (m, 2H, $CH_3\underline{CH}_2$), 3.85 (s, 2H, CH_2COO), 4.18–4.49 (m, 3H, $O\underline{CH}_2CH$ + $CH_2\underline{CH}NH$), 6.25–7.45 (m, 8H, 7 aromatic + C_5–H pyridine), 7.93 (d, *J* = 7.2 Hz, 1H, C_4–H pyridine), 8.65 (s, 1H, C_6–H pyridine), 8.9 (s, 1H, C_2–H pyridine) ($CDCl_3$)

(continued on next page)

Table 2
(continued)

Compound	Drug	Chain	m.p. °C (solvent)	Yield (%)	Formula (MW)	^{1}H NMR δ
5k	IBU	$CH_2CH(CH_2CH_3)$	Oil	67	$C_{23}H_{30}N_2O_3$ (382.5)	0.80–1.00 (t + 2d superimposed, 9H, $(CH_3)_2CH$ + CH_3CH_2), 1.38–1.53 (m, 3H, CH_3CHCO), 1.70–1.90 (m, 3H, $(CH_3)_2CH$ + CH_2CH_3)), 2.22–2.45 (m, 2H, $CHCH_2C_6H_4$), 3.68 (q, *J* = 7.2 Hz, 1H, CHC_6H_4), 4.02–4.34 (m, 3H, OCH_2CHNH), 6.40 (t, *J* = 8.2 Hz, 1H, NHCO), 6.90–7.18 (m, 4H, aromatic), 7.26–7.33 (m, 1H, C_5–H pyridine), 7.86–7.96 (m, 1H, C_4–H pyridine), 8.64 (dd, *J* = 6.5 and 1.7 Hz, 1H, C_6–H pyridine), 8.79–8.84 (m, 1H, C_2–H pyridine) ($CDCl_3$)
5l	KET	$CH_2CH(CH_2CH_3)$	Oil	70	$C_{26}H_{26}N_2O_4$ (430.5)	0.89–1.00 (m, 3H, CH_3CH_2), 1.41–1.70 (m, 5H, CH_3CH_2 + CH_3CH), 3.95 (q, *J* = 5.5 Hz, 1H, CHNH), 4.18–4.45 (m, 3H, $CHCOOCH_2$), 6.43 (br s, 1H, NH), 7.30–7.80 (m, 10H, 9 aromatic + C_5–H pyridine), 7.90–8.10 (m, 1H, C_4–H pyridine), 8.70 (br s, 1H, C_6–H pyridine), 8.90 (br s, 1H, C_2–H pyridine) ($CDCl_3$)
5m	TIAP	$CH_2CH(CH_2CH_3)$	Oil	72	$C_{24}H_{24}N_2O_4S$ (436.5)	0.75–0.92 (m, 3H, CH_3CH_2), 1.35–1.80 (m, 5H, CH_3CH + CH_3CH_2), 3.35–3.50 (m, 1H, $CHCH_3$), 4.10–4.30 (m, 1H, CHNH), 6.80 (br s, 1H, NH), 7.10–7.90 (m, 7H, aromatic), 8.10–8.20 (m, 1H, C_5–H pyridine), 8.70–8.90 (m, 1H, C_4–H pyridine), 9.00 (s, 1H, C_6–H pyridine), 9.10–9.27 (m, 1H, C_2–H pyridine) (DMSO-d_6)
5n	DIK	$CH_2CH_2CH_2$	Oil	50	$C_{23}H_{21}N_3O_3Cl_2$ (458.3)	1.90–2.10 (m, 2H, $CH_2CH_2CH_2$), 3.47 (q, *J* = 6.3 Hz, 2H, $CH_2CH_2CH_2NH$), 3.85 (s, 2H, CH_2COO), 4.31 (t, *J* = 6.0 Hz, 2H, $COOCH_2CH_2CH_2$), 6.48–7.40 (m, 9H, 7 aromatic + C_5–H pyridine, NH), 8.1 (dd, *J* = 7.9 and 1.9 Hz, 1H, C_4–H pyridine), 8.7 (dd, *J* = 4.8 and 1.5 Hz, 1H, C_6–H pyridine), 9.0 (s, 1H, C_2–H pyridine) ($CDCl_3$)
5o	IBU	$CH_2CH_2CH_2$	Oil	77	$C_{22}H_{28}N_2O_3$ (368.5)	0.85 (d, *J* = 6.6 Hz, 6H, $CH(CH_3)_2$), 1.47 (d, *J* = 7.2 Hz, 3H, CH_3CHCO), 1.70–1.97 (m, 3H, $CH_2CH_2CH_2NH$ + $CH(CH_3)_2$), 2.40 (d, *J* = 7.2 Hz, 2H, $CH_2C_6H_4$), 3.18–3.42 (m, 2H, $CH_2CH_2CH_2NH$), 4.15 (t, *J* = 6.4 Hz, 2H, $CH_2CH_2CH_2NH$), 6.78 (br s, 1H, NH), 6.98–7.38 (m, 5H, 4 aromatic + C_5–H pyridine), 8.00–8.08 (m, 1H, C_4–H pyridine), 8.65 (dd, *J* = 4.8 and 1.7 Hz, 1H, C_6–H pyridine), 8.85 (d, *J* = 1.8 Hz, 1H, C_2–H pyridine) ($CDCl_3$)
5p	KET	$CH_2CH_2CH_2$	Oil	65	$C_{25}H_{24}N_2O_4$ (416.5)	1.50 (d, *J* = 7.1 Hz, 3H, CH_3), 1.80-2.00 (m, 2H, $CH_2CH_2CH_2$), 3.20-3.48 (m, 2H, $CH_2CH_2CH_2NH$), 3.75-3.85 (d, *J* = 7.1 Hz, 1H, CH), 4.07-4.30 (m, 2H, $CH_2CH_2CH_2NH$), 7.22-7.80 (m, 10H, 9 aromatic + C_5-H pyridine), 8.05-8.12 (m, 1H, C_4-H pyridine), 8.60 (dd, *J* = 5.2 and 2.3 Hz, 1H, C_6-H), 8.95 (d, *J* = 1.1 Hz, 1H, C_2-H pyridine) ($CDCl_3$)
5q	TIAP	$CH_2CH_2CH_2$	Oil	30	$C_{23}H_{22}N_2O_4S$ (422.5)	1.60 (d, *J* = 7.2 Hz, 3H, CH_3), 1.85–2.05 (m, 2H, $CH_2CH_2CH_2$), 2.08–2.40 (br s, 1H, NH), 3.40–3.60 (m, 2H, $CH_2CH_2CH_2NH$), 4.00–4.15 (m, 1H, CH), 4.18–4.30 (m, 2H, $CH_2CH_2CH_2NH$), 6.80–7.85 (m, 8H, 7 aromatic + C_5–H pyridine), 8.10 (dd, *J* = 8.2 and 2.2 Hz, 1H, C_4–H pyridine), 8.72 (d, *J* = 4.4 Hz, 1H, C_6–H pyridine), 9.00 (s, 1H, C_2–H pyridine) ($CDCl_3$)

*4.1.5. General procedure for the synthesis of **6a–q** (Table 3)*

Solutions of (**5a–q**) (1 mmol) and CH_3I (3.3 mmol) in CH_2Cl_2/MeCN (3:1) (10 ml) were stirred in a closed system at room temperature for 1 day for compounds (**6a**), (**6d**) and (**6e**) and 6 days for all the others. The reaction mixtures were brought down to dryness in vacuo. The oily residues, if pure by TLC, were used in the successive reaction without further purification, otherwise were purified by flash chromatography, using $CHCl_3$ as eluant for compounds (**6e**) and (**6l**) or induced to crystallize with ethyl diether for (**6b**) or MeOH (**6c**).

*4.1.6. General procedure for the synthesis of **7a–q** (Table 4)*

$NaHCO_3$ (10 mmol) and $Na_2S_2O_4$ (2.2 mmol) in an aqueous solution (150 ml) and ethyl acetate (30 ml) were added to an ice-cooled solution of methyl iodide pyridinium derivatives (**6a–q**) in degassed water (150 ml) under N_2 atmosphere. The reaction mixtures were stirred for 15 min at 0 °C under N_2, maintaining the pH at approximately 7 by addition of $NaHCO_3$. The organic phases were washed with water several times, dried over Na_2SO_4, filtered and brought to dryness in vacuo to afford 1,4-dihydropyridine derivatives **7a–q**.

4.2. Lipophilicity measurements

4.2.1. R_m determination

R_m values were determined by TLC using pre-coated glass plates, C8 Whatman, 200 μm thickness and 20 μm particle size with fluorescent indicator. The compounds were dissolved in acetone and the solutions were applied along a line at about 2.5 cm from the bottom of the plate. The mobile phases consisted of a mixture (200 ml) of various concentrations of phosphate buffer (pH 7) and acetone. The phosphate buffer was prepared according to Farmacopea Ufficiale della Repubblica italiana XI ed. (F.U. XI) and then was diluted 1:10 (0.0067 M) with water. The mobile phase was allowed to run 10 cm from the origin spots. The developed plates were dried and detected by UV light.

R_m values vs. acetone concentrations were plotted and the theoretical values of R_m at 0% of acetone in the mobile phase were calculated by the least-squares method.

4.2.2. Log P computation

Log *P* was estimated using the Kowwin computer program, which applies atom/fragment contribution as described [19].

4.2.3. Partial least square (PLS) discriminant analysis

The multivariate PLS projections to latent structure method [20] was used to delineate the relationship between R_m^0 values (linear regression) and calculated log *P*. PLS is a chemiometric tool for extracting and rationalizing the information from any multivariate description of a biological system. Complexity reduction and data simplification are two of the most important features of such a tool. PLS condenses the overall information into two smaller matrixes, namely the score plot (which shows the pattern of compounds) and the loading plot (which shows the pattern of descriptors). Since the chemical interpretation of score and loading plots is simple and straightforward, PLS is usually preferred to other non-linear methods, especially when the noise is relatively high.

Score and loading plots are interconnected so that any descriptor change in the loading plot is reflected by changes in the position of compounds in the score plot. Pair wise comparison can be made directly with interactive plots as developed in the VolSurf program [14], and the relative contributions to the property under study are shown in the related descriptors space.

4.2.4. Predicting BBB permeation study using VolSurf

This study was conducted by a new predictive computerized method [13] using descriptors derived from 3D molecular fields in estimating the BBB permeation of a large set of compounds. The computational approach consists of four main steps and implies the VolSurf procedure able to compress the relevant information present in 3D maps into a few descriptors, simple to handle, to interpret, and correlate them with experimental permeation. Three-dimensional molecular fields in general contain large amount of data some of which are redundant or not relevant for a given problem. Therefore, novel tools are required to extract useful descriptors from images of 3D molecular interactions fields (MIFs) and to link experimental observations with molecular structures.

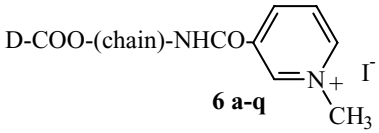
VolSurf automatically converts 3D molecular fields into physico-chemically relevant molecular descriptors. In the standard procedure, interaction fields with a water probe and a hydrophobic probe are calculated for all the molecules in the data set. However, grid maps produced by other probes (ionic probes etc,) or by various molecular mechanics or semi empirical approaches (e.g., electrostatic potential) can also be used. Molecular recognition is achieved using image analysis software coupled with external chemical knowledge. Within this context, VolSurf selects the most appropriate descriptors and parameterization according to the type of 3D maps under study.

The molecular descriptors obtained refer to molecular size and shape, to size and shape of both hydrophilic and hydrophobic regions and also to the balance between them [13].

Acknowledgements

We thank Professor Gabriele Cruciani for helpful discussion on the VolSurf procedure.

Table 3
Physical and chemical data of compounds **6a–q**



6 a-q

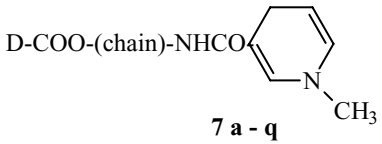
Compound	Drug	Chain	m.p. °C (solvent)	Yield (%)	Formula (MW)	^{1}H NMR δ ($CDCl_3$)
6a	DIK	CH_2CH_2	Oil	88	$C_{23}H_{22}N_3O_3Cl_2I$ (586.3)	3.70–3.85 (m, 2H, $CH_2\underline{CH_2}NH$), 3.92 (s, 2H, $\underline{CH_2}CO$), 4.35–4.44 (m, 2H, $\underline{CH_2}CH_2NH$), 4.48 (s, 3H, $N^+\underline{CH_3}$), 6.38–7.35 (m, 8H, 7 aromatic + NH), 7.90–8.00 (m, 1H, C_5–H pyridinium), 8.60 (br s, 1H, C_4–H pyridinium), 8.85–9.00 (m, 1H, C_6–H pyridinium) ($CDCl_3$)
6b	IBU	CH_2CH_2	144–6 (EtOH)	90	$C_{22}H_{29}N_2O_3I$ (496.4)	0.80 (d, J = 9 Hz, 6H, $CH(\underline{CH_3})_2$), 1.32 (d, J = 10 Hz, 3H, $CH\underline{CH_3}$), 1.60–1.88 (m, 1H, $\underline{CH}(CH_3)_2$), 2.35 (d, J = 10 Hz, $\underline{CH_2}C_6H_4$), 3.43–3.80 (m; 3H, $CH_2\underline{CH_2}NH$ + $C_6H_4\underline{CH}CH_3$), 4.02–4.28 (m, 2H, $O\underline{CH_2}CH_2$), 4.40 (s, 3H, $N^+\underline{CH_3}$), 7.05, 7.15 (AB system, 4H, C_6H_4), 8.19–8.31 (m, 1H, C_5–H pyridinium), 8.82 (d, J = 7.7 Hz, 1H, C_4–H pyridinium), 9.05–9.28 (m, 2H, NH + C_6–H pyridinium), 9.36 (s, 1H, C_2–H pyridinium) (DMSO-d_6)
6c	TOLM	CH_2CH_2	107–111 (MeOH)	79	$C_{24}H_{26}N_3O_4I$ (547.4)	2.35 (s, 3H, $C_6H_4\underline{CH_3}$), 3.40–3.65 (m, 2H, $CH_2\underline{CH_2}NH$), 3.75 (s, 3H, $N^+\underline{CH_3}$), 3.85 (s, 2H, $\underline{CH_2}COO$), 4.18–4.30 (m, 2H, $\underline{CH_2}CH_2NH$), 6.05, 6.50 (AB system, J = 8.0 Hz, 2H, pyrrole), 7.25, 7.58 (AB system, J = 7.7 Hz, 4H, C_6H_4) 8.10 (m, 1H, C_4–H pyridinium), 8.60 (m, 1H, C_6–H pyridinium), 8.85 (s, 1H, NH), 8.98 (s, 1H, C_2–H pyridinium) (DMSO-d_6)
6d	KET	CH_2CH_2	Oil	52	$C_{25}H_{25}N_2O_4I$ (544.4)	1.55 (d, J = 6.0 Hz, 3H, $CH\underline{CH_3}$), 3.60–3.80 (m, 3H, $CH_2\underline{CH_2}NH$ + NH), 3.98 (q, 1H, $\underline{CH}CH_3$), 4.25–4.45 (m, 2H, $\underline{CH_2}CH_2NH$), 4.60 (s, 3H, $N^+\underline{CH_3}$), 7.33–7.80 (m, 9H, aromatic), 7.90–8.08 (m, 1H, C_5–H pyridinium), 8.78 (d, J = 7.2 Hz, 1H, C_4–H pyridinium), 8.85–9.00 (m, 1H, C_6–H pyridinium), 9.99 (s, 1H, C_2–H pyridinium) ($CDCl_3$)
6e	TIAP	CH_2CH_2	Oil	55	$C_{23}H_{23}N_2O_4SI$ (550.4)	1.50 (d, J = 7.1 Hz, 3H, CH_3), 3.45–3.75 (m, 2H, $CH_2\underline{CH_2}NH$), 4.18–4.45 (m and s superimposed, 6H, $\underline{CH_2}CH_2NH$ + $\underline{CH}CH_3$ + $N^+\underline{CH_3}$), 6.85 (s, 1H, NH), 6.90–8.35 (m, 7H, aromatic), 8.70–8.90 (m, 1H, C_5–H pyridinium), 9.10 (d, J = 6.0 Hz, 1H, C_4–H pyridinium), 9.15–9.30 (m, 1H, C_6–H pyridinium), 9.38 (s, 1H, C_2–H pyridinium) (DMSO-d_6)
6f	DIK	$CH(CH_3)CH_2$	151–3 (EtAc)	65	$C_{24}H_{24}N_3O_3Cl_2I$ (600.3)	1.27 (d, J = 6.3 Hz, 3H, CH_3), 3.40–3.70 (m, 2H, $\underline{CH_2}NH$), 3.80 (s, 2H, $C_6H_4\underline{CH_2}CO$), 4.38 (s, 3H, $N^+\underline{CH_3}$), 4.99–5.19 (m, 1H, CH), 6.11–7.22 (m, 5H, aromatic), 7.49 (d, J = 8.0 Hz, 2H, C_3–H and C_5–H, dichlorophenyl), 8.16 (t, J = 7.3, 1H, C_5–H pyridinium), 8.8 (d, J = 8.1 Hz, 1H, C_4–H pyridinium), 9.07 (d, J = 5.8 Hz, 1H, C_6–H pyridinium), 9.13–9.24 (m, 1H, C_2–H pyridinium), 9.32 (s, 1H, C_6H_3–$\underline{NH}$–C_6H_4) (DMSO-d_6)
6g	IBU	$CH(CH_3)CH_2$	Oil	89	$C_{23}H_{31}N_2O_3I$ (510.4)	0.70–0.85 (m, 6H, $CH(\underline{CH_3})_2$), 1.08–1.38 (m, 6H, $\underline{CH_3}CHO$ + $\underline{CH_3}CHCO$), 1.50–1.80 (m, 1H, $\underline{CH}(CH_3)_2$), 2.20–2.37 (m, 2H, CH_2N), 3.60 (q, J = 7.0 Hz, 1H, $CH_3\underline{CH}$), 4.39 (s, 3H, $N^+\underline{CH_3}$), 4.90–5.07 (m, 1H, $\underline{CH}CH_3$), 6.88–7.27 (m, 4H, aromatic), 8.10–8.28 (m, 1H, C_5–H piridinium), 8.65–8.90 (m, 1H, C_4–H pyridinium), 9.01–9.20 (m, 2H, C_6–H pyridinium + NH), 9.25–9.35 (m, 1H, C_2–H pyridinium) (DMSO-d_6)
6h	KET	$CH(CH_3)CH_2$	Oil	80	$C_{26}H_{27}N_2O_4I$ (558.4)	1.20 (d, J = 6.3 Hz, 3H, $\underline{CH_3}CH$), 1.40 (d, J = 6.8 Hz, 3H, $CH(\underline{CH_3})$), 3.15 (d, J = 5.2, 2H, $\underline{CH_2}NH$), 3.85 (q, J = 6.3 Hz, 1H, $CH_3\underline{CH}$), 4.10 (q, J = 6.8 Hz, 1H, $\underline{CH}CH_3$), 4.38 (s, 3H, $N^+\underline{CH_3}$), 7.33–7.73 (m, 9H, aromatic), 8.12–8.27 (m, 1H, C_5–H piridinium), 8.64 (d, J = 7.9 Hz, 1H, C_4–H pyridinium), 8.98–9.10 (m, 2H, C_6–H pyridinium + NHCO), 9.25 (s, 1H, C_2–H, pyridinium) (DMSO-d_6)
6i	TIAP	$CH(CH_3)CH_2$	Oil	68	$C_{24}H_{25}N_2O_4SI$ (564.4)	1.13–1.40 (m, 6H, $\underline{CH_3}CHO$ + $\underline{CH_3}CHCH_2$), 3.10–3.60 (m, 3H, $\underline{CH_2}NH$ + $CH_3\underline{CH}CO$), 4.40 (s, 3H, $N^+\underline{CH_3}$), 4.89-5.22 (m, 1H, $CH_3\underline{CH}CH_2$), 6.50–7.85 (m, 7H, C_6H_4 + thiophene), 8.00–8.30 (m, 1H, C_5–H pyridinium), 8.60–9.45 (m, 3H, C_4–H, C_6–H, C_2–H pyridinium) (DMSO-d_6)

(continued on next page)

Table 3
(continued)

Compound	Drug	Chain	m.p. °C (solvent)	Yield (%)	Formula (MW)	^{1}H NMR δ
6j	DIK	$CH_2CH(CH_2CH_3)$	Oil	95	$C_{25}H_{26}N_3O_3ClI$ (614.2)	0.83–1.12 (m, 3H, $\underline{CH_3}CH_2$), 1.40–1.75 (m, 2H, $CH_3\underline{CH_2}$), 3.82 (s, 2H, $\underline{CH_2}COO$), 4.10-4.45 (m, $CH_2\underline{CH}NH$ + $\underline{CH_2}CHNH$), 4.47 (s, 3H, $N^+\underline{CH_3}$), 6.25–7.42 (m, 7H, 6 aromatic + C_5–H pyridinium), 7.85–8.00 (m, 1H, C_4–H pyridinium), 8.55–8.75 (m, 1H, C_6–H pyridinium), 8.90 (s, 1H, C_2–H pyridinium) ($CDCl_3$)
6k	IBU	$CH_2CH(CH_2CH_3)$	Oil	Quantitative	$C_{24}H_{33}N_2O_3I$ (524.2)	0.80–1.05 (m, 9H, $CH(\underline{CH_3})_2$ + $\underline{CH_3}CH_2$), 1.45–1.53 (m, 3H, $\underline{CH_3}CHCO$), 1.60–1.90 (m, 3H, $(CH_3)_2\underline{CH}$ + $CH\underline{CH_2}CH_3$), 2.33, 2.37 (2d, J = 7.09 Hz, J = 7.14 Hz, 2H, $\underline{CH_2}C_6H_4$), 2.39–2.48 (m, 1H, $\underline{CH}NH$), 3.68–3.92 (m, 1H, $\underline{CH}CO$), 4.20–4.40 (m, 2H, $O\underline{CH_2}$), 4.55 (s, 3H, $N^+\underline{CH_3}$), 6.95-7.35 (m, 4H, aromatic), 7.94–8.08 (m, 1H, C_5–H pyridiniuim), 8.40–8.53 (m, 1H, C_4–H pyridinium), 8.78 (br s, 1H, NH), 8.85–8.93 (m, 1H, C_6–H pyridinium), 9.90 (s, 1H, C_2–H pyridinium) ($CDCl_3$)
6l	KET	$CH_2CH(CH_2CH_3)$	Oil	45	$C_{27}H_{29}N_2O_4I$ (572.1)	0.75–0.95 (m, 3H, $CH_2\underline{CH_3}$), 1.30–1.70 (m, 5H $CH\underline{CH_3}$ + $\underline{CH_2}CH_3$), 3.85–4.00 (s, 1H, $\underline{CH}CH_3$), 4.03–4.22 (m, 2H, $O\underline{CH_2}$), 4.40 (s, 3H, $N^+\underline{CH_3}$), 7.30–7.80 (m, 9H, aromatic), 8.10–8.25 (m, 1H, C_5–H pyridinium), 8.70–8.82 (m, 2H, NH + C_4–H pyridinium), 9.00–9.12 (m, 1H, C_6–H pyridinium), 9.25 (s, 1H, C_2–H pyridinium) (DMSO-d_6)
6m	TIAP	$CH_2CH(CH_2CH_3)$	Oil	62	$C_{25}H_{27}N_2O_4SI$ (578.1)	0.78–0.95 (m, 3H, $\underline{CH_3}CH_2$), 1.35–1.75 (m, 5H, $\underline{CH_3}CH$ + $CH_3\underline{CH_2}$), 3.40–3.50 (m, 1H, $\underline{CH}CH_3$), 4.10–4.32 (m, 1H, $\underline{CH}NH$); 4.40 (s, 3H, $N^+\underline{CH_3}$), 7.00 (br s, 1H, NH), 7.12–7.85 (m, 7H, aromatic), 8.10–8.18 (m, 1H, C_5–H pyridinium), 8.70–8.90 (m, 1H, C_4–H pyridinium), 9.10 (s, 1H, C_6–H pyridinium), 9.26–9.40 (m, 1H, C_2–H pyridinium) (DMSO-d_6)
6n	DIK	$CH_2CH_2CH_2$	Oil	80	$C_{24}H_{24}N_3O_3Cl_2I$ (559.0)	1.79–1.98 (m, 2H, $CH_2\underline{CH_2}CH_2NH$), 3.25–3.50 (q, J = 6.4 Hz, 2H, $CH_2CH_2\underline{CH_2}NH$), 3.80 (s, 2H, $\underline{CH_2}COO$), 4.10–4.20 (t, J = 6.3 Hz, 2H, $\underline{CH_2}CH_2CH_2NH$), 4.38 (s, 2H, N^+CH3), 6.25 (d, J = 7.9, 1H, NH), 6.80–7.55 (m, 7H, aromatic), 8.20–8.35 (m, 1H, C_5-H pyridinium), 8.85 (d, J = 8.2 Hz, 1H, C_4–H pyridinium), 9.05 (d, J = 5.1 Hz, 1H, C_6–H pyridinium), 9.33 (s, 1H, C_2-H pyridinium) (DMSO-d_6)
6o	IBU	$CH_2CH_2CH_2$	Oil	Quantitative	$C_{23}H_{31}N_2O_3I$ (510.1)	1.85 (d, J = 6.6 Hz, 3H, $CH(\underline{CH_3})_2$), 1.45 (d, J = 7.2 Hz, 3H, $CH\underline{CH_3}$), 1.60–1.90 (m, 1H, $\underline{CH}(CH_3)_2$), 1.95–2.10 (m, 2H, $CH_2CH_2\underline{CH_2}NH$), 2.40 (d, J = 7.1 Hz, 2H, $CH_2C_6H_4$), 3.45–3.57 (m, 2H, $CH_2CH_2\underline{CH_2}NH$), 3.75 (q, J = 7.3 Hz, 1H, $\underline{CH}CH_3$), 4.00–4.30 (m, 2H, $\underline{CH_2}CH_2CH_2NH$), 4.60 (s, 3H, N^+CH3), 7.00–7.25 (m, 4H, aromatics), 8.01–8.10 (m, 1H, C_5–H pyridinium), 8.65–8.80 (m, 2H, NH + C_4–H pyridinium), 8.95 (d, J = 5.1 Hz, 1H, C_6–H pyridinium), 10.15 (s, 1H, C_2–H pyridinium) (DMSO-d_6)
6p	KET	$CH_2CH_2CH_2$	Oil	68	$C_{24}H_{25}N_2O_4I$ (532.1)	1.40 (d, J = 7.13 Hz, 3H, $CH\underline{CH_3}$), 1.75–1.90 (m, 2H, $CH_2\underline{CH_2}CH_2NH$), 3.09–3.18 (m, 2H, $CH_2CH_2\underline{CH_2}NH$), 3.85–4.00 (q, 1H, $CH_3\underline{CH}$), 4.05–4.20 (m, 2H, $CH_2CH_2CH_2NH$), 4.40 (s, 3H, $N^+\underline{CH_3}$), 7.40–7.80 (m, 9H, aromatic), 8.18-8.28 (m, 1H, C_5-H pyridinium), 8.85 (d, J = 8.2 Hz, 1H, C_4–H pyridinium), 8.90–9.09 (m + s superimposed, 2H, C_6–H pyridinium + NHCO), 9.22 (s, 1H, C_2-H pyridinium) (DMSO-d_6)
6q	TIAP	$CH_2CH_2CH_2$	Oil	80	$C_{24}H_{25}N_2O_4SI$ (564.1)	1.65–1.80 (m, 2H, $CH_2\underline{CH_2}CH_2$), 1.82–1.90 (m, 3H, $CH\underline{CH_3}$), 3.09–3.18 (m, 2H, $CH_2CH_2\underline{CH_2}NH$), 4.02 (q, J = 8.1 Hz, $\underline{CH}CH_3$), 4.10–4.28 (m, 2H, $\underline{CH_2}CH_2CH_2NH$), 4.37 (s, 3H, $N^+\underline{CH_3}$), 7.45–7.85 (m, 7H, aromatic), 8.08-8.18 (m, 1H, C_5-H pyridinium), 8.85-8.95 (m, 1H, C_4-H pyridinium), 9.04–9.12 (m and s superimposed, 2H, NH + C_6–H pyridinium), 9.30 (s, 1H, C_2–H pyridinium) (DMSO-d_6)

Table 4
Physical and chemical data of compounds **7a–q** graphique



7 a - q

Compound	Drug	Chain	Yield (%)	Formula (MW)	^{1}H NMR δ ($CDCl_3$)
7a	DIK	CH_2CH_2	Quantitative	$C_{23}H_{23}N_3O_3Cl_2$ (459.1)	2.80 (br s, 2H, CH_2 dihydropyridine), 3.00 (s, 3H, NCH_3), 3.72–3.85 (m, 2H, $\underline{CH_2}NH$), 3.95 (s, 2H, $\underline{CH_2}COOCH_2$), 4.15–4.26 (m, 2H, $CH_2COO\underline{CH_2}$), 4.58–4.75 (m, 1H, C_5–H dihydropyridine), 5.20 (br s, 1H, NH), 5.55 (m, 1H, C_6–H dihydropyridine), 6.90 (s, 1H, C_2–H dihydropyridine), 6.35–7.34 (m, 8H, 7 aromatic + NH) ($CDCl_3$)
7b	IBU	CH_2CH_2	Quantitative	$C_{22}H_{30}N_2O_3$ (370.2)	0.90 (d, *J* = 6.6 Hz, 6H, $CH(\underline{CH_3})_2$, 1.45 (d, *J* = 7.1 Hz, 3H, $CH\underline{CH_3}$), 1.75–1.95 (m, 1H $\underline{CH}(CH_3)_2$), 2.45 (d, *J* = 7.5 Hz, 2H, $\underline{CH_2}C_6H_4$), 2.88 (br s, 2H CH_2 dihydropyridine), 2.95 (s, 3H, NCH_3), 3.47–3.58 (m, 2H, $CH_2\underline{CH_2}NH$), 3.68–3.85 (m, 1H, $C_6H_4\underline{CH}CH_3$), 4.08–4.27 (m, 2H, $\underline{CH_2}CH_2NH$), 4.65–4.73 (m, 1H, C_5–H dihydropyridine), 5.23 (br s, 1H, NH), 5.65–5.70 (m, 1H, C_6–H dihydropyridine), 6.95 (s, 1H, C_2–H dihydropyridine), 7.04–7.20 (A_2B_2 system, 4H, C_6H_4) ($CDCl_3$)
7c	TOLM	CH_2CH_2	Quantitative	$C_{24}H_{27}N_3O_4$ (421.2)	2.35 (s, 3H, $C_6H_4\underline{CH_3}$), 2.90 (br s, 2H, CH_2 dihydropyridine), 3.40–3.65 (m, 2H, $\underline{CH_2}CH_2$), 3.85 (s, 3H, NCH_3), 3.90 (s, 2H, CH_2COO), 4.22–4.38 (m, 2H, $CH_2\underline{CH_2}$), 4.58–4.70 (m, 1H, C_5–H dihydropyridine), 6.08, 6.50 (AB system, *J* = 8 Hz, 2H, pyrrole), 7.00 (s, 1H, C_2–H dihydropyridine), 7.23, 7.58 (A_2B_2 system, *J* = 12.0 Hz, 4H, aromatic), 8.95 (s, 1H, NH) (DMSO-d_6)
7d	KET	CH_2CH_2	83	$C_{25}H_{26}N_2O_4$ (418.2)	1.53 (d, *J* = 9.8 Hz, 3H, CH_3), 2.90 (2s superimposed, 5H, NCH_3 + CH_2 dihydropyridine), 3.48–3.51 (m, 2H, $CH_2\underline{CH_2}NH$), 3.83 (q, *J* = 9.8 Hz, 1H, $\underline{CH}CH_3$), 4.08–4.20 (m, 2H, $\underline{CH_2}CH_2NH$), 4.55–4.68 (m, 1H, C_5–H dihydropyridine), 5.28 (br s, 1H, NH), 5.57–5.68 (m, 1H, C_6–H dihydropyridine), 6.95 (s, 1H, C_2–H dihydropyridine), 7.35–7.85 (m, 9H, aromatic) ($CDCl_3$)
7e	TIAP	CH_2CH_2	52	$C_{23}H_{24}N_2O_4S$ (424.1)	1.56 (d, *J* = 7.6 Hz, 3H, $CH\underline{CH_3}$), 2.83 (s, 3H, NCH_3), 2.95 (br s, 2H, CH_2 dihydropyridine), 3.50–3.70 (m, 2H, $CH_2\underline{CH_2}NH$), 4.18–4.39 (m, 2H, $\underline{CH_2}CH_2NH$), 4.51–4.68 (m, 1H, C_5–H dihydropyridine), 5.40–5.62 (m, 2H, NH + C_6–H dihydropyridine), 6.90 (br s, 1H, C_2–H dihydropyridine), 7.23–7.81 (m, 7H, aromatic) ($CDCl_3$)
7f	DIK	$CH(CH_3)CH_2$	57	$C_{24}H_{25}N_3O_3Cl_2$ (473.1)	0.85–1.02 (m, 3H, $CH\underline{CH_3}$), 2.85 (br s, 2H, CH_2 dihydropyridine), 2.90 (s, 3H, NCH_3), 3.52–3.80 (m, 2H, $\underline{CH_2}NH$), 4.43 (s, 2H, CH_2COO), 4.52–4.68 (m, 1H, C_5–H dihydropyridine), 5.10 (br s, 1H, NHC_6H_4), 5.20–5.45 (m, 1H, $O\underline{CH}CH_3$), 5.50–5.66 (m, 1H, C_6–H dihydropyridine), 6.80 (s, 1H, C_2–H dihydropyridine), 6.90–7.48 (m, 7H, aromatic), 10.10 (br s, 1H, *NH*CO) ($CDCl_3$)
7g	IBU	$CH(CH_3)CH_2$	68	$C_{23}H_{32}N_2O_3$ (384.2)	0.90 (d, *J* = 6.6 Hz, 6H, $CH(\underline{CH_3})_2$), 1.15–1.25 (m, 3H, $OCH\underline{CH_3}$), 1.40–1.50 (m, 3H, $OCH\underline{CH_3}$), 1.70–1.97 (m, 1H, $(CH_3)_2\underline{CH}$), 2.38–2.49 (d, *J* = 7.2 Hz, 2H, $\underline{CH_2}C_6H_4$), 2.65 (br s, H, CH_2 dihydropyridine), 2.90 (s, 3H, NCH_3), 3.20–3.75 (m, 3H, $CH_3\underline{CH}CO$ + $\underline{CH_2}NH$), 4.50–4.70 (m, 1H, $O\underline{CH}CH_3$), 4.90–5.05 (m, 1H, C_5–H dihydropyridine), 5.57–5.70 (m, 1H, C_6–H dihydropyridine), 6.90 (s, 1H, C_2–H dihydropyridine), 7.00–7.23 (m, 4H, aromatic) ($CDCl_3$)
7h	KET	$CH(CH_3)CH_2$	60	$C_{26}H_{28}N_2O_4$ (432.2)	1.25 (d, *J* = 8.08 Hz, 3H, $CH\underline{CH_3}$), 1.50 (d, 7.2 Hz. 3H, $CHCH_3$), 2.66 (s, 2H, C_4–H dihydropyridine), 2.86 (s, 3H, NCH_3), 3.23–3.29 (m, 2H, $CH_2\underline{NH}$), 3.70–3.85 (q, *J* = 8.1 Hz, 1H, $\underline{CH}CH_3$), 4.48–4.59 (m, 1H, C_5–H dihydropyridine), 4.90–5.15 (m, 2H, $\underline{CH}CH_3$ + NH), 6.88 (s, 1H, C_2–H dihydropyridine), 7.31–7.77 (m, 9H, aromatic) ($CDCl_3$)
7i	TIAP	$CH(CH_3)CH_2$	78	$C_{24}H_{26}N_2O_4S$ (438.2)	1.30–1.50 (2d superimposed, 6H, $OCH\underline{CH_3}$ + $\underline{CH_3}CHCO$), 2.85 (s, 3H, NCH_3), 2.92 (s, 2H, CH_2 dihydropyridine), 3.55–3.80 (m, 1H, $CH_3\underline{CH}CO$), 4.50–4.72 (m, 1H, C_5–H dihydropyridine), 5.05–5.25 (m, 1H, $O\underline{CH}CH_3$), 5.50–5.72 (m, 1H, C_6–H dihydropyridine), 6.85 (s, 1H, C_2–H dihydropyridine), 7.10–7.92 (m, 7H, aromatic) ($CDCl_3$)
7j	DIK	$CH_2CH(CH_2CH_3)$	50	$C_{25}H_{27}N_3O_3Cl_2$ (487.1)	0.85–1.12 (m, 3H, $\underline{CH_3}CH_2$), 1.48–1.80 (m, 2H, $CH_3\underline{CH_2}$), 2.85 (s, 3H, NCH_3), 2.92 (br s, 2H, CH_2 dihydropyridine), 3.80 (s, 2H, $C_6H_4\underline{CH_2}CO$), 4.15–4.35 (m, 3H, $O\underline{CH_2}CH$ + $CH_2\underline{CH}NH$), 4.45–4.68 (m, 1H, C_5-H dihydropyridine), 5.12 (br s, 1H, NHC_6H_4), 5.50–5.65 (m, 1H, C_6–H dihydropyridine), 6.25–7.49 (m, 8H, 7 aromatic + C_2–H dihydropyridine), 9.95 (br s, 1H, NHCO) ($CDCl_3$)

(continued on next page)

Table 4
(continued)

Compound	Drug	Chain	Yield (%)	Formula (MW)	^{1}H NMR δ ($CDCl_3$)
7k	IBU	$CH_2CH(CH_2CH_3)$	67	$C_{24}H_{33}N_2O_3$ (397.5)	0.80–0.95 (m, 9H, $CH(\underline{CH_3})_2$ + $CH_2\underline{CH_3}$), 1.40–1.54 (m, 3H, $\underline{CH_3}CHCO$), 1.60–2.00 (m, 3H, $(CH_3)_2\underline{CH}$ + $\underline{CH_2}CH_3$), 2.40–2.50 (m, 2H, $\underline{CH_2}C_6H_4$), 2.90 (2 s superimposed, 5H, NCH_3, CH_2 dihydropyridine), 3.60–3.80 (s, 1H, $\underline{CH}CH_3$), 4.08–4.23 (m, 3H, $\underline{CH_2}CHN$), 4.82–5.05 (m, 1H, C_5–H dihydropyridine), 5.62–5.70 (m, 1H, C_6–H dihydropyridine), 6.94 (s, 1H, C_2–H dihydropyridine), 7.00–7.30 (m, 4H, aromatic) ($CDCl_3$)
7l	KET	$CH_2CH(CH_2CH_3)$	82	$C_{27}H_{30}N_2O_4$ (446.5)	0.89 (t, *J* = 7.3 Hz, 3H, $CH_2\underline{CH_3}$), 1.40–1.53 (m and d superimposed, *J* = 7.2 Hz, 5H, $\underline{CH_2}CH_3$ + $CH\underline{CH_3}$), 2.85 (s, 2H, CH_2 dihydropyridine), 2.90 (s, 2H, NCH_3), 3.70–3.85 (q, *J* = 7.2 Hz, 1H, $\underline{CH}CH_3$), 3.98–4.15 (m, 1H, $OCH_2\underline{CH}$), 4.55–4.70 (m, 3H, $O\underline{CH_2}CH$ + C_5–H dihydropyridine), 5.00 (br s, 1H, NH), 5.50–5.55 (m, 1H, C_6–H dihydropyridine), 6.90 (s, 1H, C_2–H dihydropyridine), 7.30–7.80 (m, 9H, aromatic) ($CDCl_3$)
7m	TIAP	$CH_2CH(CH_2CH_3)$	47	$C_{25}H_{28}N_2O_4S$ (452.6)	0.80–0.95 (m, 3H, $\underline{CH_3}CH_2$), 1.38–1.91 (m, 5H, $\underline{CH_3}CH$ + $CH_3\underline{CH_2}$), 2.90 (s, 3H, NCH_3), 2.96 (m, 2H, CH_2 dihydropyridine), 3.41–3.55 (m, 1H, $\underline{CH}CH_3$), 4.05–4.25 (m, 1H, $\underline{CH}NH$), 4.57–4.72 (m, 1H, C_5–H dihydropyridine), 5.40–5.58 (m, 1H, C_6–H dihydropyridine), 6.75 (br s, 1H, NH), 6.95 (br s, 1H, C_2–H dihydropyridine), 7.15–8.00 (m, 7H, aromatic) ($CDCl_3$)
7n	DIK	$CH_2CH_2CH_2$	78	$C_{24}H_{25}N_3O_3Cl_2$ (474.4)	1.79–2.05 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.85 (br s, 2H, CH_2 dihydropyridine), 2.92 (s, 3H, NCH_3), 3.40–3.56 (m, 2H, $CH_2CH_2\underline{CH_2}NH$), 3.91 (s, 2H, $\underline{CH_2}COO$), 4.28 (t, *J* = 6.3 Hz, 2H, $\underline{CH_2}CH_2CH_2NH$), 4.55–4.68 (m, 1H, C_5–H dihydropyridine), 5.60–5.72 (m, 1H, C_6-H dihydropyridine), 6.45–7.38 (m, 9H, 7 aromatic + C_2-H dihydropyridine + NH) ($CDCl_3$)
7o	IBU	$CH_2CH_2CH_2$	65	$C_{23}H_{32}N_2O_3$ (384.5)	0.80 (d, *J* = 7.0 Hz, 6H, $CH(\underline{CH_3})_2$, 1.45 (d, *J* = 7.2 Hz, 3H, $\underline{CH_3}CHO$), 1.65–1.88 (m, 3H, $CH_2\underline{CH_2}CH_2NH$ + $\underline{CH}(CH_3)_2$, 2.45 (d, *J* = 7.2 Hz, 2H, $\underline{CH_2}C_6H_4$), 2.90 (br s, 2H, CH_2 dihydropyridine), 2.96 (s, 3H, NCH_3), 3.16–3.38 (m, 2H, $CH_2CH_2\underline{CH_2}NH$), 4.10 (t, *J* = 6.4 Hz, 2H $\underline{CH_2}CH_2CH_2NH$), 4.60–4.69 (m, 1H, C_5–H dihydropyridine), 5.60–5.68 (m, 1H, C_6–H dihydropyridine), 6.78–7.38 (m, 6H, 4 aromatic, NH, C_2–H dihydropyridine) ($CDCl_3$)
7p	KET	$CH_2CH_2CH_2$	68	$C_{26}H_{28}N_2O_4$ (432.5)	1.55 (d, *J* = 7.2 Hz, 3H, $\underline{CH_3}CH$), 1.67–1.90 (m, 2H, $CH_2\underline{CH_2}CH_2NH$), 2.90 (s, 3H, N^+CH_3), 3.10 (br s, 2H, CH_2 dihydropyridine), 3.15–3.38 (m, 2H, $CH_2\underline{CH_2}CH_2NH$), 3.80 (q, *J* = 7.2 Hz, 1H, $\underline{CH}CH_3$), 4.00–4.20 (m, 2H, $\underline{CH_2}CH_2CH_2NH$), 4.60–4.75 (m, 1H, C_5–H dihydropyridine), 5.48 (br s, 1H, NHCO), 5.60–5.70 (m, 1H, C_6–H dihydropyridine), 6.95 (s, 1H, C_2–H dihydropyridine), 7.35–7.84 (m, 9H, aromatic) ($CDCl_3$)
7q	TIAP	$CH_2CH_2CH_2$	72	$C_{24}H_{26}N_2O_4S$ (438.5)	1.85 (d, *J* = 6.4 Hz, 3H, $\underline{CH_3}CH$), 1.90–2.02 (m, 2H, $CH_2\underline{CH_2}CH_2NH$), 2.90 (s, 3H, NCH_3), 3.10 (br s, 2H, CH_2 dihydropyridine), 3.30–3.45 (m, 2H, $CH_2CH_2\underline{CH_2}NH$), 4.28–4.40 (m, 2H, $\underline{CH_2}CH_2CH_2NH$), 4.60–4.76 (m, 1H, C_5–H dihydropyridine), 5.55 (br s, 1H, NHCO), 5.60–5.70 (m, 1H, C_6–H dihydropyridine), 6.98 (s, 1H, C_2–H dihydropyridine), 6.93–7.90 (m, 7H, aromatic) ($CDCl_3$)

References

[1] R. Diaz Brinton, R.S. Yamazaki, Pharm. Res. 15 (1998) 386–398.

[2] P. Eikelenboom, A.J.M. Rozemuller, J.J.M. Hoozemans, R. Veerhuis, W.A. Van Gool, Alz. Dis. Assoc. Dis. 14 (2000) S54–S61.

[3] M.A.A. Blom, M.G.H. van Twillert, S.C. de Vries, F. Engels, C.E. Finch, R. Veerhuis, P. Eikelenboom, Brain Res. 777 (1997) 210–218.

[4] K. Anderson, L.J. Launer, A. Ott, A.W. Hoes, M.M. Breteler, A. Hofman, Neurology 45 (1995) 1441–1445.

[5] W.F. Stewart, C. Kawas, M. Corrada, E.J. Metter, Neurology 48 (1997) 626–632.

[6] B.L. Flynn, K.A. Theesen, Ann. Pharmacother. 33 (1999) 840–849.

[7] K. Sugaya, T. Uz, V. Kumar, H. Manev, Jpn. J. Pharmacol. 82 (2000) 85–94.

[8] P.L. McGeer, M. Schulzer, E.G. McGeer, in: J. Rogers (Ed.), Neuroinflammatory Mechanism in Alzheimer's Disease, Birkhaeuser Verlag, Switzerland, Basel, 2001, pp. 53–64.

[9] J.C.S. Breitner, Annu. Rev. Med. 47 (1996) 401–411.

[10] P.S. Aisen, K.L. Davis, Am. J. Psychiatr. 151 (1994) 1105–1113.

[11] P.S. Aisen, Drug Develop. Res. 56 (2001) 421–427.

[12] M. Hull, K. Lieb, B.L. Fiebich, Expert Opin. Inv. Drug 9 (2000) 671–683.

[13] P. Crivori, G. Cruciani, P.A. Carrupt, B. Testa, J. Med. Chem. 43 (2000) 2204–2216.

[14] G. Cruciani, P. Crivori, P.A. Carrupt, B. Testa, J. Mol, Struc-Theochem 503 (2000) 17–30.

[15] E. Pop, Curr. Med. Chem. 4 (1997) 279–294; N. Bodor, in: E.B. Roch (Ed.), Theory of Application of Bioreversible Carriers to Drug Design, Pergamon, New York, 1985, pp. 95–120; E. Pop, E. Shek, T. Murakami, N.S. Bodor, J. Pharm. Sci. 78 (1989) 609–616; E. Pop, W. Whei-Mei, E. Shek, N. Bodor, J. Med. Chem. 32 (1989) 1774–1781; E. Pop, W. Whei-Mei, N. Bodor, J. Med. Chem. 32 (1989) 1789–1795; E. Pop, W. Anderson, K. Prókai-Tátrai, M.E. Brewster, M. Fregly, N. Bodor, J. Med. Chem. 33 (1990) 2216–2221; M.J. Phelan, N. Bodor, Pharm. Res. 6 (1989) 667–676.

[16] T. Ogowa, K. Katayama, H. Maeda, Y. Kita, Chem. Pharm. Bull. 42 (1994) 1579–1589.

[17] C.B.C. Boyce, B.V. Milborrow, Nature 208 (1995) 537–539.

[18] G.L. Biagi, A.M. Barbaro, M.F. Gamba, M.C. Guerra, J. Chromatog. 41 (1969) 371–379.

[19] W.M. Meylan, P.H. Howard, J. Pharm. Sci. 84 (1995) 83–92.

[20] H. Wold, Research Papers in Statistics: Ferstschrift for J. Neyman, Wiley, New York, 1966.