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Water-soluble salts of aminoacid esters of the anaesthetic agent Propofol

Giuseppe Trapani [a,*], Andrea Latrofa [a], Massimo Franco [a], Angela Lopedota [a], Elisabetta Maciocco [b], Gaetano Liso [a]

[a] *Dipartimento Farmaco-Chimico, Facoltà di Farmacia, Università degli Studi di Bari, Via Orabona 4, 70125 Bari, Italy*
[b] *Dipartimento di Biologia Sperimentale, Cattedra di Farmacologia, Università di Cagliari, Via Palabanda 12, 09123 Cagliari, Italy*

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Abstract

The glycinates **4**, **5**, acetates **6**, **7**, **10**, propionate **8**, butyrate **9** and carbonate **11** were synthesized and evaluated as potential water-soluble prodrugs of Propofol (2,6-diisopropylphenol) **1** suitable for parenteral administration. The **4–9** · HCl salts were also prepared and some of them (i.e. **4** · HCl and **6** · HCl) were found sufficiently soluble in aqueous solutions. The kinetics of hydrolysis of the esters **4–11** and **4–9** · HCl salts were studied in 0.05 M phosphate buffer pH 7.4, and a number of derivatives (**4**, **6**, **7**, and corresponding HCl salts) were examined for their stability in human plasma and brain homogenate. Our results indicated that the salts **4** · HCl and **6** · HCl, sufficiently soluble in water, are relatively stable in physiological media. Most of the examined compounds, in particular compound **6**, were found to inhibit the binding of [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]TBPS) demonstrating to possess affinity for the Propofol recognition site on $GABA_A$ receptors. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Propofol (2,6-diisopropylphenol) **1** (Chart 1) is an intravenous anaesthetic agent, structurally unrelated to other anaesthetics, which is gaining increasing popularity in anaesthesia. Induction of anaesthesia with Propofol is rapid, and maintenance can be achieved by continuous infusion or by intermittent bolus doses (Langley and Heel, 1988). A number of studies have shown that γ-aminobutyric acid ($GABA_A$) receptors represent a sensitive target for the action of this intravenous anaesthetic (Collins, 1988; Concas et al., 1991;

* Corresponding author. Tel.: +39 80 5442764; fax: +39 80 5442724; e-mail: trapani@farmchim.uniba.it

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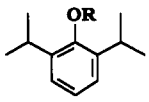
Chart 1



1 R = H Propofol

4 R = $COCH_2N(C_2H_5)_2$

5 R = $COCH_2N(C_3H_7)_2$

6 R = $COCH_2$—N

7 R = $COCH_2$—N O

8 R = $COCH(CH_3)$—N O

9 R = $COCH(C_2H_5)$—N O

10 R = $COCH_2NHCH_2CH(OCH_3)_2$

11 R = $COOC_2H_5$

Hales and Lambert, 1991) as well as other intravenous and volatile anaesthetics (Franks and Lie, 1994).

The development of a safe and useful parenteral dosage form of Propofol has been impaired by the very low aqueous solubility of the drug. The anaesthetic agent is at present formulated as 1% w/v of oil/water emulsion of soya bean oil, glycerol and purified egg phosphatide (Diprivan®). Unfortunately, lipid-based emulsions suffer from several limitations including poor physical stability, the potential for embolism, and pain on injection (Prankerd and Stella, 1990). Inclusion complexes of Propofol and hydroxypropyl-β-cyclodextrin were examined as alternative aqueous formulations suitable for intravenous administration (Viernstein et al., 1993; Trapani et al., 1996, 1998b). In an attempt to develop a potential aqueous formulation for Propofol, we have considered the prodrug approach which has been successfully applied to overcome aqueous solubility problems for a wide variety of drugs (Bundgaard, 1991; Jensen and Bundegaard, 1991; Pop et al., 1996a,b). The prodrug strategy consists of a transient modification of the physicochemical properties of a given compound through its chemical derivatization. Such temporary chemical modification is usually designed to alter aqueous solubility and biodistribution while the release of the parent drug follows in vivo hydrolysis. Furthermore, the prodrug approach, as applied to **1**, should allow both to reduce side effects, such as those at cardiovascular level, and to prolong the drug action. An attempt to address these last two points by the dihydropyridine-pyridinium salt redox delivery system has been recently described by Bodor's group (Pop et al., 1992).

In this paper, we report the synthesis and physicochemical properties of the *N,N*-disubstituted-α-aminoacid esters (**4–9**), and their corresponding hydrochlorides, the *N*-monosubstituted α-aminoacid ester (**10**), and the carbonate ester (**11**) of Propofol (Chart 1) as potential water soluble prodrugs. Their in vitro chemical stability as well as the effect of them on the binding of [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]TBPS), a very sensitive tool for studying the function of the $GABA_A$ receptor, are reported.

2. Materials and methods

2.1. Chemicals

Propofol, dicyclohexylcarbodiimide, α-chloroacetic, α-chloropropionic, α-chlorobutyric acids and the appropriate amines were purchased from Aldrich. Reagents used for the preparation of the buffers were of analytical grade. Fresh deionized water from all glass apparatus was used in the preparation of all the solutions. High-performance liquid chromatography (HPLC) mobile phase was prepared from HPLC-grade methanol.

2.2. Apparatus

Melting points were determined by the capillary method on a Büchi apparatus and are uncorrected. Infrared (IR) spectra were recorded as Nujol films for liquids and KBr pellets for solids on a Perkin-Elmer 283 spectrophotometer. ^{1}H NMR spectra were taken on a Varian EM 390 spectrometer operating at 90 MHz. Chemical shifts are given in δ values downfield from te-

tramethylsilane as internal standard. Mass spectra were recorded on a Hewlett-Packard 5995c Gas chromatography-mass spectroscopy (GC-MS) low resolution spectrometer operating in electron impact mode. Elemental analyses were performed on a Hewlett-Packard 185 C, H, N analyzer and agreed with theoretical values within $\pm 0.40\%$. HPLC analyses were performed with a Water Associates Model 600 pump equipped with a Water 990 variable wavelength UV detector and a 20 μl loop injection valve (U6K). For analysis, a reversed phase μ Bondapack C_{18} (30 cm × 3.9 mm; 10 μm particles) or a reversed phase Simmetry (25 cm × 3.9 mm; 5 μm particles) column in conjunction with Guard-Pak precolumn module with μ Bondapack C_{18} insert was eluted with mixtures of methanol and deionized water. The flow rate of 1 ml/min was maintained. The column effluent was monitored continuously at 260 nm. Quantification of the compounds was carried out by measuring the peak areas or peak heights in relation to those of standards chromatographed under the same conditions. Stability studies were carried out at controlled temperature at 37°C (± 0.2°C) in a water bath.

2.3. Synthesis of Propofol derivatives

2,6-Diisopropylphenyl chloroacetate. To a stirred solution of 2,6-diisopropylphenol **1** (0.8 g, 3.27 mmol), monochloroacetic acid (0.4 g, 4.23 mmol), and dimethylaminopyridine (0.1 g) in dry dichloromethane (15 ml), a solution of dicyclohexylcarbodiimide (6.3 mmol) in dry dichloromethane (10 ml) was added dropwise for 10 min. Stirring was continued at room temperature for 24 h and then the resulting precipitate was removed. The solution was evaporated under reduced pressure to give a residue which was purified by column chromatography on silica gel (petroleum ether–ethyl acetate 98:2 v/v as eluent) to give the (2,6-diisopropyl)phenyl-chloroacetate in 72% yield. Similarly by using the α-chloropropionic or α-chlorobutyric acid instead of monochloroacetic acid the corresponding α-chloropropionate or α-chlorobutyrate of 2,6-diisopropylphenol were prepared, respectively.

2.3.1. Reaction of chloroacyl derivatives 3 with amines

A mixture of **3** (15 mmol) and the appropriate dialkyl or cycloalkyl amine (22.4 mmol) was heated at 150°C in a sealed tube for 20 h in all cases except when pyrrolidine was used. In this case the reaction was carried out in THF (15 ml) under stirring at room temperature for 24 h. Then, the solvent was evaporated under reduced pressure. The resulting residue, as well as the crude reaction mixture obtained in all other cases, was purified by column chromatography on silica gel (petroleum ether–ethyl acetate 98:2 v/v as eluent) to give compounds **4–10**. Physical and spectral data of compounds **4–7**, and **10** are reported elsewhere (Trapani et al., 1998a). Herein we report the physical characteristics of the **4–7** hydrochlorides as well as the physical and spectral data of the compounds **8** and **9**. The **4–9** HCl salts were prepared by bubbling gaseous HCl into ethereal solutions of the corresponding bases **4–9** and collecting the precipitate formed. Attempts to prepare in such a way **10** · HCl salt were unsuccessful.**4** · HCl: m.p. 136–139°C; **5** · HCl: m.p. 150–153°C; **6** · HCl: m.p. 243–245°C; **7** · HCl: m.p. 220–224°C.

2.3.2. (2,6-Diisopropylphenyl)(morpholin-1-yl)-propionate (8):

Yield 70%, m.p. 53–55°C; IR 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20 (d, 12H, CH_3), 1.50 (d, 3H, CH_3), 2.7–2.8 (m, 4H, CH_2), 2.9 (m, 2H, CH), 3.60 (q, 1H, CH); 3.6–3.7 (m, 4H, CH_2), 7.1–7.2 (m, 3H, arom); MS *m/z* 319 (M^+) 114. **8** · HCl: m.p. 174–177°C.

2.3.3. (2,6-Diisopropylphenyl)(morpholin-1-yl)-butyrate (9):

Yield 30%, oil; ^1H NMR($CDCl_3$) δ 1.05 (t, 3H, CH_3), 1.20 (d, 12H, CH_3), 1.7–2.0 (m, 2H, CH_2), 2.7–2.8 (m, 4H, CH_2), 2.8–3.0 (m, 2H, CH), 3.3–3.5 (m, 1H, CH), 3.6–3.7 (m, 4H, CH_2), 7.1–7.2 (m, 3H, arom); MS *m/z* 331 (M^+) 128. **9** · HCl: m.p. 157–160°C.

2.3.4. Reaction of Propofol 1 with ethyl chloroformate

To a solution of 2,6-diisopropylphenol **1** (3.46 g, 19.4 mmol) in dry pyridine (10 ml), ethyl

chloroformate (9.12 g, 84 mmol) was added dropwise for 10 min. Stirring was continued at room temperature for 0.5 h and then the resulting white precipitate was removed. The solution treated with water and extracted with chloroform (3 × 30 ml) dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography on silica gel (petroleum ether–ethyl acetate 95:5 v/v as eluent) to give the pure compound **11**.

*2.3.5. (2,6-Diisopropylphenyl)ethyl carbonate (**11**):* Yield 18%, oil. IR 1750 cm^{-1}; ^1H NMR($CDCl_3$) δ 1.20 (d, 12H, CH_3), 1.33 (t, 3H, CH_2CH_3), 2.8–3.2 (m, 2H, CH), 4.30 (q, 2H, OCH_2), 7.28 (br s, 3H, CHAr); MS m/z 250 (M^+).

2.4. Determination of solubility

The aqueous solubility of esters **4–11** was determined in 0.05 M phosphate buffer (pH = 7.4) at 25°C by adding excess amount of compound to 2–4 ml of buffer in screw-capped test tube. The resulting mixture was vortexed for 10 min and kept in a bath at 25°C under stirring for 36 h. Then, an aliquot of aqueous phase of each mixture was transferred to a 10 ml glass syringe preheated at the appropriate temperature and filtered through a 0.45 μm membrane filter (Millipore®, cellulose acetate) in thermostated test tubes. Next, about 0.5 ml of the clear filtrate were collected in a test tube, diluted (1: 5) with buffer, and allowed to stand in bath at 25°C until analyzed by HPLC. The injection volume was 20 μl. All of the manipulations were made without removal of the test tubes from the water bath, using thermostated pipettes, syringes, and buffer solution. Similarly were determined the solubilities of **4-9**.HCl in 0.05 M phosphate buffer (pH = 7.4), 0.05 M acetate buffer (pH = 4.5), and deionized water (pH = 6).

2.5. Determination of lipophilicity parameters

The lipophilicity of these Propofol derivatives was evaluated by means of reversed-phase HPLC capacity factors. In this method, the capacity factor (k') of a solute was taken as a measure of the relative lipophilicity and was calculated as: $k' = (t_R - t_0)/t_0$ were t_R is the retention time of the solute and t_0 is the elution time of the solvent. For analysis, a reversed phase Simmetry (25 cm × 3.9 mm; 5 μm particles) column in conjunction with Guard-Pak precolumn module was eluted. The k' values were determined using methanol/water in 85/15 ratio. The flow rate was maintained at 1 ml/min and column effluent was monitored at 260 nm.

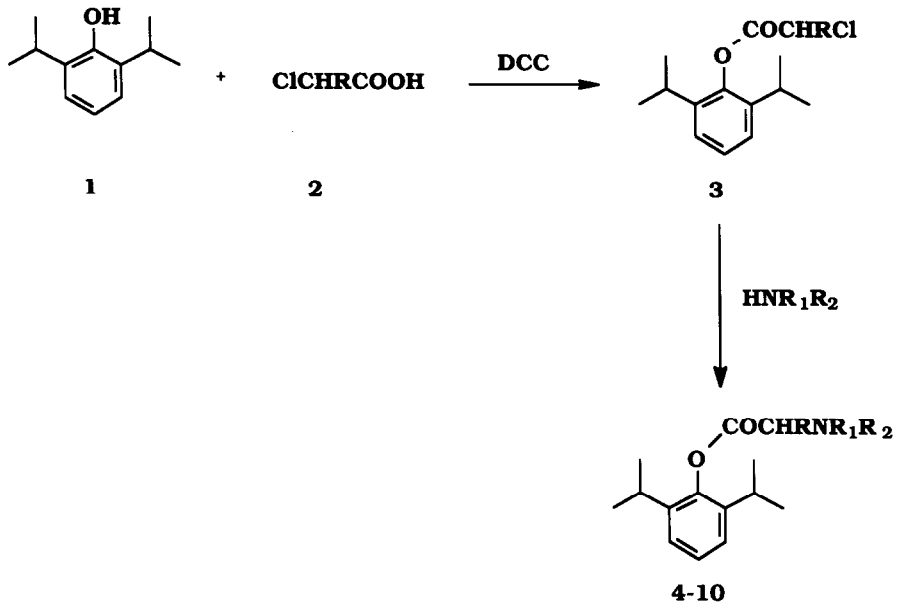
2.6. Chemical hydrolysis

The hydrolysis of the esters **4–11** and HCl salts was studied at pH 7.4 in 0.05 M phosphate buffer at 37°C. The reaction was carried out by adding to 20–50 ml of the buffer solution preheated at 37°C 100 μl of a stock solution of the ester (2–4 mg) in methanol in screw-capped test tubes. The final concentration of the compounds was about 2.0×10^{-4} M. The solutions were maintained in a water bath at constant temperature, and aliquots of 20 μl were removed at appropriate intervals and analyzed by HPLC. Pseudo-first-order rate constants for the hydrolysis of the derivatives were determined from the slopes of linear plots of the logarithms of residual derivative against time.

2.7. Stability in physiological media

The hydrolysis of the esters **4**, **6**, and **7** and corresponding HCl salts was studied at 37°C in 0.05 M phosphate buffer at pH 7.4 containing 80% of human serum. The reaction was carried out by adding 50 μl of the stock solution of compound [(30 and 31 mg, respectively) in methanol (5 ml)] to 2 ml of preheated plasma solution and the mixture was maintained in water bath at 37°C. Aliquots of 100 μl were withdrawn at appropriate intervals and added to 500 μl of cold acetonitrile in order to deproteinize the plasma (the final concentration of the compound being 8×10^{-5} and 9×10^{-5} M, respectively). After mixing and centrifugation for 10 min at 4000 rpm, 20 μl of the clear supernatant were filtered through 0.2 μm membrane filter (Waters, PTFE 0.2 mm) and analyzed by HPLC.

The hydrolysis of the esters **4**, **6**, and **7** and corresponding HCl salts was also studied in 0.05 M Tris citrate buffer at pH 7.4 at 25°C in the



Scheme 1.

presence of brain homogenate. The reaction was carried out by adding to 427 μl of Tris citrate buffer, 12 μl of NaCl 0.2 N, 50 μl of brain homogenate and 10 μl of the stock solution of compound above reported diluted 1:1 (v:v) with 0.05 M Tris citrate buffer. The mixtures were maintained in water bath at 25°C. Samples of 500 μl were withdrawn at below reported intervals and added to 500 μl of cold acetonitrile in order to deproteinize the mixtures. After mixing and centrifugation for 10 min at 4000 rpm, 20 μl of the clear supernatant were filtered through μm membrane filter (Millipore, Millex-FG_{13} 0.2 μm) and analyzed by HPLC. Four time points (0, 30, 90, and 3600 min) were considered in this study; zero-time determinations were performed by extracting the solution with acetonitrile immediately following the solution preparation.

2.8. *In vitro [^{35}S]TBPS binding assay*

Male Sprague-Dawley CD^R rats (Charles River, Como, Italy) weighing 180–200 g were used. The animals were kept on a controlled light-dark cycle (light period between 8:00 and 20:00 h) in a room with constant temperature (22 ± 2°C) and humidity (65%). Upon arrival at the animal facilities there was a minimum of 7 days of acclimation during which the animals had free access to food and water. Rats were killed by decapitation and their brains were rapidly removed The cerebral cortex was dissected out and homogenized in 50 volumes of ice-cold 50 mM Tris-citrate buffer (pH 7.4 at 25°C) containing 100 mM $CaCl_2$ using a Polytron PT 10 (setting 5, for 20 s) and centrifuged at 20000 × *g* for 20 min. The resulting pellet was resuspended in 50 volumes of 50 mM Tris-citrate buffer (pH 7.4 at 25°C) and used for the assay. [^{35}S]TBPS binding was determined in a final volume of 500 μl consisting of: 200 μl of tissue homogenate (0.20–0.25 mg protein), 50 μl of [^{35}S]TBPS (final assay concentration, 1 nM), 50 μl 2 M NaCl, 50 μl of drugs or solvent and buffer to volume. Incubations (25°C) were initiated by addition of tissue and terminated 90 min later by rapid filtration through glass-fiber filter strips (Whatman GF/B, Clifton, NJ), which were rinsed twice with a 4-ml portion of ice-cold Tris-citrate buffer using a Cell Harvester filtration manifold (model M-24m Brandel, Gaithersburg, MD). Filter bound radioactivity was quantitated by liquid scintillation spectrometry. Nonspecific binding

Table 1
Solubiliy (S, mg/ml), lipophilicity indexes ($\log k'$) of aminoacid esters and ethyl carbonate of Propofol and corresponding HCl salts[a]

Compound	S (phosphate buffer pH 7.4)	$\log k'$	$\log P$[b]	Compound	S (phosphate buffer pH 7.4)	S (acetate buffer pH 4.5)	S (deionized water pH 6)
1	0.154 ± 0.010	0.14	4.33				
4	0.019 ± 0.002	0.52	5.14	**4** · HCl	0.064[c]	4.670 ± 0.250	5.300 ± 0.010
5	<0.0058[c]	0.89	6.20	**5** · HCl	[d]	[d]	[d]
6	0.111 ± 0.015	0.36	5.04	**6** · HCl	0.735 ± 0.000	6.920 ± 0.400	3.660 ± 0.250
7	0.218 ± 0.003	0.15	4.33	**7** · HCl	0.213 ± 0.008	0.350 ± 0.010	[d]
8	0.052 ± 0.003	0.28	4.64	**8** · HCl	0.190 ± 0.005	[d]	[d]
9	0.014 ± 0.002	0.49	5.17	**9** · HCl	0.158 ± 0.013	[d]	[d]
10	0.569 ± 0.025	0.13	3.17				
11	<0.021[c]	0.36	4.65				

[a] Data are means ± S.D. of three determinations, unless otherwise reported.
[b] Estimated by CLOGP program.
[c] One determination.
[d] Not determined.

Table 2
Half-lives for the hydrolysis of aminoacid esters and ethyl carbonate of Propofol and corresponding HCl salts in 0.05 M phosphate buffer (pH = 7.4) at 37°C[a]

Compound	$t_{1/2}$ (h) in phosphate buffer 0.05 M (pH 7.4)	Compound	$t_{1/2}$ (h) in phosphate buffer 0.05 M (pH 7.4)
4	165	**4** · HCl	139
5	159	**5** · HCl	126
6	60	**6** · HCl	57
7	[b]	**7** · HCl	[c]
8	[d]	**8** · HCl	[d]
9	1176	**9** · HCl	960
10	32		
11	71		

[a] Data are means of two determinations (less than 10% of difference).
[b] Hydrolyzed in 10% after 39 days.
[c] Hydrolyzed in 29% after 39 days.
[d] Stable after 30 days.

was defined as binding in the presence of 100 μM picrotoxin and represented about 10% of total binding. Proteins were assayed with the method of Lowry et al. (1951) using bovine serum albumin as standard.

3. Results and discussion

3.1. Solubility, lipophilicity, and chemical and enzymatic stability studies of aminoacid esters of Propofol

According to Scheme 1, the *N*-mono- or *N,N*-disubstituted α-aminoacid esters **4–10** were prepared in two steps: (i) acylation of **1** using the required α-chlorocarboxylic acid **2** and dicyclohexylcarbodiimide (DCC) as dehydrating agent; (ii) reaction of the intermediate chloroesters **3** with the appropriate amines. Compound **11** was obtained by the reaction of **1** with ethyl chloroformate. By these synthetic procedures compounds **4–11** were prepared from moderate to good yield.

The synthesized esters and hydrochlorides were soluble in the systems examined from negligible to sufficient extent (Table 1). The highest solubilities were observed under acidic conditions for compounds **4** · HCl and **6** · HCl (4.67 and 6.92 mg/ml, respectively). Furthermore, it can be seen from Table 1 that in phosphate buffer at pH 7.4, the *N*-monosubstituted derivative **10** was more soluble than Propofol and all of *N,N*-disubstituted compounds. In the same conditions the glycinates **4**, **5**, and carbonate **11** were less soluble than Propofol. The lipophilicity of the esters **4–11** was assessed by measuring capacity factors (k') in reversed-phase HPLC. Log k' values, listed in Table 1, are linearly correlated ($r = 0.90$) with log P values calculated by the fragmental method of Hansch and Leo (1979), using the computer program CLOGP (version 3.54). The molar solubilities of compounds **4**, **6–10** are also linearly correlated ($r = 0.96$) with the calculated log P values.

The chemical stability of Propofol derivatives under examination was evaluated by studying their hydrolysis kinetics in aqueous solution at physiological pH (0.05 M phosphate buffer, pH 7.4) and temperature (37°C). The half-lives of the esters, or alternatively the percent of **1** formed after a long time (more than 1 month) for the highly stable derivatives, were measured and reported in Table 2. The results showed that no difference exists between the behaviour of esters and their corresponding HCl salts, and that the investigated esters are quite resistent to chemical hydrolysis, the least stable compound **10** having an half-life of more than 30 h. This notable stability most likely depends on the steric protection of the C(O)–O bond by bulky flanking diisopropyl groups on the aromatic ring.

Three derivatives, namely **4**, **6**, **7**, were finally selected for an examination of the biolability of aminoacid esters of Propofol. Selection was made taking into account the water-solubility particularly under acidic conditions. Indeed, compound **6** showed the highest solubility in aqueous solution within the whole series, whereas compound **4** was more soluble than its higher homologue **5**, and compound **7** was the most soluble among the morpholino-derivatives. Compound **10** was excluded due to the lack of the corresponding HCl salt.

The in vitro stability in 80% human plasma (pH 7.4) at 37°C and in brain homogenate in 0.05 M Tris citrate buffer at pH 7.4 at 25°C (i.e. the conditions used for receptor binding measurements) was determined by measuring the percent of hydrolyzed aminoacid ester after 24 h of incubation. The results, summarized in Table 3, indicated that the esters under examination, are stable also in physiological media, since more than 50% of intact derivatives of Propofol is detected after 24 h of incubation.

Hydrochloride salts showed behaviours similar to their corresponding aminoacid esters, whereas a comparison between the data of degradation in plasma and brain homogenate with the hydrolytic rate data in buffer solution alone reveals that compounds **4** and **6** are subjected to hydrolysis catalyzed by esterases. The highest acceleration in the hydrolysis was observed for compound **4**. Moreover, the rank order of stability in brain homogenate is the same as that observed in buffer solution (i.e. **7** > **4** > **6**). In contrast, the stability in human plasma does not parallel the chemical stability and appears inversely related to lipophilicity, as assessed by log k' and CLOGP. In fact, the most hydrophilic morpholino-derivative **7** was the most stable in plasma, whereas the most lipophilic compound **4** was hydrolyzed by plasma esterases up to ca. 40% within 24 h. The above observations indicated that, within the limits of the property space examined, the hydrophobicity of aminoacid moieties plays a role in modulating the degradation rate in plasma.

However, our results showed that the examined derivatives, possessing both adequate solubility and chemically stability, cannot be considered as prodrugs, since they are not readily converted to the parent drug in physiological conditions.

Table 3
Stability of compounds **4**, **6**, **7** and corresponding HCl salts, in human plasma and in the presence of brain homogenate

Compound	Human plasma	Rat brain homogenate
4	40	7
6	23	44
7	0	0
4 · HCl	35	10
6 · HCl	13	39
7 · HCl	0	16

[a] Data (% of hydrolyzed compound after 24 h) are means of two determinations (less than 15% of difference).

Table 4
Effect of in vitro addition of Propofol and compounds **4–11** on the [^{3}H]TBPS binding in unwashed rat brain

	IC_{50} μM[a]
1	3.95
4	21.80
5	[b]
6	6.52
7	[b]
8	[b]
9	12.63
10	15.60
11	22.30

[a] Data are means of two determinations (less than 10% of difference).
[b] No displacement.

3.2. Receptor binding studies

The binding of [^{35}S]TBPS, a cage convulsant which binds in close proximity to the chloride channel portion of the $GABA_A$ receptor at level of the picrotoxin binding site, constitutes a very sensitive tool for studying the function of the $GABA_A$ receptor complex. Propofol, mimicking the action of other general anaesthetics such as alphaxalone and pentobarbital, reduces [^{35}S]TBPS binding in a concentration-dependent manner (Concas et al., 1991). The ability of the compounds **4–11** to interact with [^{35}S]TBPS binding sites was evaluated for assessing their potential

use as active analogues of Propofol. Affinity data, summarized in Table 4, showed that most of Propofol derivatives examined in this study, like to Propofol itself, are able to reduce the [^{35}S]TBPS binding. Due to their observed chemical and biological stabilities, it is reasonable to ascribe the binding affinity for $GABA_A$ receptors to the intact derivatives rather than to Propofol released by hydrolysis in the assay medium. While attempts to derive quantitative structure–affinity relationships by regression analysis, due to the limited number of acyl derivatives tested so far, were inconclusive, pyrrolidinoacetyl ester **6** emerged as a promising active derivative, its IC_{50} value being very close to that of Propofol.

4. Conclusions

This study showed that, among the aminoacid esters of the anaesthetic agent Propofol, the *N,N*-diethylglycinate and pyrrolidinoacetate as hydrochloride salts (**4** · HCl and **6** · HCl) are satisfactory soluble in water solution under acidic conditions. Their stability in physiological media, both in plasma and brain homogenate, led us to conclude that they cannot be considered as classical prodrugs. But, a preliminary evaluation of structure–stability relationships suggested that the degradation rate in plasma should increase as a function of the acyl chain lipophilicity. Interestingly, we demonstrated that most of the Propofol aminoacid esters bind as such to $GABA_A$ receptors. Among them the pyrrolidinoacetyl ester **6**, which displayed an in vitro IC_{50} value comparable to that of Propofol, can be selected as a candidate for in vivo pharmacological evaluation, whereas, on the other hand, it could helpfully allow further insights into pharmacophore, and represent a lead for further optimization studies.

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