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Synthesis, *in vitro* and *in vivo* characterization of novel ethyl dioxy phosphate prodrug of propofol

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ABSTRACT

A novel ethyl dioxy phosphate prodrug of propofol (**3**) was synthesized and characterized *in vitro* and *in vivo* as safer alternative for phosphonooxymethyl prodrugs. The synthesis of **3** was achieved via vinyl and 1-chloroethyl ether intermediates, followed by addition of phosphate group. Aqueous solubility and chemical stability of **3** was determined in buffer solutions and the bioconversion of **3** to propofol was determined *in vitro* and *in vivo*. The results show that **3** greatly enhanced the aqueous solubility of propofol (solubility over 10 mg/mL) and the stability in buffer solution ($t_{1/2} = 5.2 \pm 0.2$ days at pH 7.4, r.t.) was sufficient for i.v. administration. The enzymatic hydrolysis of **3** to propofol was extremely rapid *in vitro* ($t_{1/2} = 21 \pm 3$ s) and **3** was readily converted to propofol *in vivo* in rats. During bioconversion, **3** releases acetaldehyde, a less toxic compound than the formaldehyde released from the phosphonooxymethyl prodrug of propofol (Aquavan), currently undergoing clinical trials. The maximum plasma concentration of propofol, 3.0 ± 0.2 μg/mL, was reached within 2.1 ± 0.8 min after the i.v. administration of **3**. The present study indicates that ethyl dioxy phosphate represents a potentially useful water-soluble prodrug structure suitable for i.v. administration.

1. Introduction

Solubility limitations of drug molecules are an emerging challenge for the pharmaceutical industry with an objective to find pharmacologically more potent drug molecules. Since sufficient aqueous solubility is crucial in both i.v. and non-parenteral administration and because the possibilities to formulate the poorly water-soluble drug molecules are limited, prodrug technology has become a popular way to enhance the aqueous solubility of drug molecules.

Phosphates are probably the most extensively used water-soluble prodrugs for i.v. administration. Phosphates have been used as promoieties in hydroxyl or amine functionalities of the parent drug, either directly or via an oxymethyl spacer group. Oxymethyl spacers are generally used to increase the space around the enzymatically cleavable bond (phosphates, esters, carbonates) (Safadi et al., 1993; Varia et al., 1984) and they undergo rapid spontaneous chemical hydrolysis after enzymatic hydrolysis of the promoiety. One possible drawback of the oxymethyl linker structure is the systemic liberation of the toxic compound, formaldehyde, in the body, which in addition to its toxicity, may alter homeostasis within cells (such as induction of enzymes, metabolic switching and cell proliferation) (Heck et al., 1990). An alternative strategy to achieve sufficiently fast enzymatic hydrolysis rate and more favourable safety profile of prodrugs is to use an

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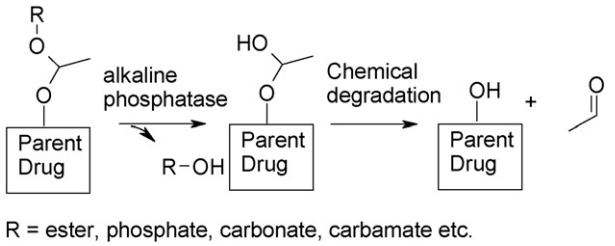

Fig. 1 – The hydrolysis of the ethyl dioxy phosphate prodrug of propofol to the parent drug, promoiety and acetaldehyde.

ethyl dioxy-spacer structure that liberates the much less toxic compound, acetaldehyde, from the prodrug structure (Fig. 1). Although acetaldehyde is not fully harmless, as it can cause gastrointestinal tract cancer after long-term chronic alcohol consumption (Seitz et al., 2005), it is substantially less toxic than formaldehyde.

The (acyloxy)alkyl group has been widely used as linkers between an ester, a carbonate or a carbamate promoiety and parent drugs containing a carboxylic acid functionality (Beaumont et al., 2003), in fact many such prodrugs are even marketed (e.g. candesartan cilexitil (Easthope and Jarvis, 2002), cefuroxime axetil (Scott et al., 2001), bacmecillinam (Josefsson et al., 1982)). By comparison, no ethyl dioxy linked phosphate prodrugs have been described in the literature. One possible reason is the challenge involved in the synthesis of these compounds, in fact there are no techniques for the synthesis of ethyl dioxy phosphates described in the literature (SciFinder Scholar, Beilstein Crossfire). This represented our starting point; we were interested in determining whether an ethyl dioxy linked phosphate could be synthesized and whether the ethyl dioxy spacer could serve as a safe and hydrolysable linker between the parent drug and the phosphate promoiety.

For this study, we choose an anesthetic drug propofol as our model drug. Propofol (Fig. 2) is a widely used anesthetic with a rapid onset, a short duration of action and minimal side effects (Baker and Naguib, 2005). Because of the high lipophilicity of propofol, it needs to be administered in oil-in-water emulsion. However, the extremely low water-solubility, high lipophilicity, inherent emulsion instability, pain on injection and hyperlipidemia on prolonged administration (Baker and Naguib, 2005) are major drawbacks, and thus have lead to the development of more water-soluble prodrugs. At least two phosphate prodrugs of propofol have been described in the literature (Fig. 2), one with the phosphate group attached directly to the hydroxyl functionality of propofol (Propofol Phosphate, PP)(Banaszczyk et al., 2002) and another with an oxymethyl spacer between the parent drug and the phosphate promoiety (**5**, GPI 15715, Aquavan®) (Gibiansky et al., 2005; Schywalsky et al., 2003; Struys et al., 2005). Both prodrugs enhance the water-solubility of propofol by many-fold, as would be predicted from their structures. The enzymatic hydrolysis of GPI 15715 (Schywalsky et al., 2003) is faster than that of PP (Banaszczyk et al., 2002), but on the other hand, PP does not liberate the toxic formaldehyde as a breakdown product to the body. We hoped

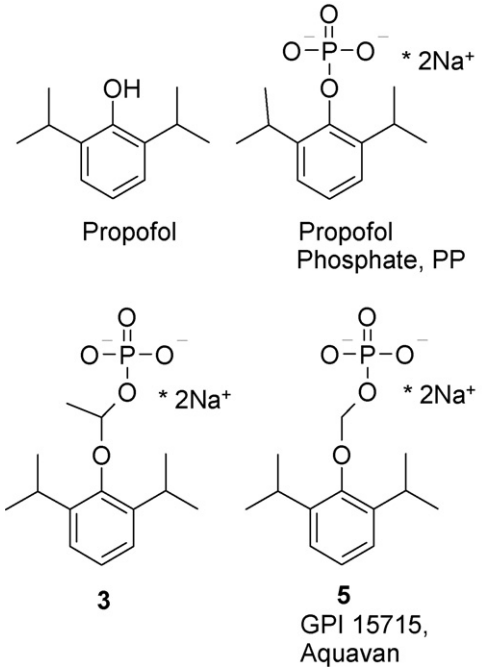

Fig. 2 – The structures of propofol and its phosphate-, phosphonooxymethyl- (5, GPI 15715, Aquavan®) and ethyl dioxy phosphate- (3) prodrugs.

to combine the properties of these two prodrugs, and therefore, we synthesized an ethyl dioxy linked phosphate prodrug **3** of propofol. Furthermore, we have extensively characterized the ethyl dioxy structure as a novel water-soluble phosphate prodrug structure *in vitro* and *in vivo* in rats.

2. Materials and methods

All materials, reagents and enzymes were obtained from commercial suppliers and were used without further purifications.

2.1. *Synthesis of compounds*

2.1.1. *General synthetic procedures*

All the described reactions were monitored by thin-layer chromatography using aluminum sheets precoated with Merck silica gel 60 F_{254}. Samples were visualized by UV-light. Column chromatography was executed on Merck silica gel 60 F_{254} (0.063–0.200 mm mesh). ^{1}H, ^{13}C and ^{31}P NMR-spectra were recorded on a Bruker Avance DRX500 spectrometer (Bruker, Rheinstetter, Germany) operating at 500 MHz, 125.76 and 202.46 MHz at 25 °C, respectively. TMS for $CDCl_3$-samples and TSP for D_2O samples were used as an internal reference. The splitting pattern abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, sep = septet. Electrospray ionization mass spectra were acquired by an LCQ quadrupole ion trap mass spectrometer with an electronspray ionization source (Finnigan MAT, San Jose, CA). Elemental analyses were carried out on a ThermoQuest CE Instruments EA 1110-CHNS-O elemental analyzer.

2.1.2. *2,6-Diisopropyl-2-vinyloxy-benzene (1)*

Anhydrous copper(II)acetate (3.67 g, 20.19 mmol) was added to a solution of 2,6-diisopropyl phenol (3 g, 16.83 mmol) in dry acetonitrile. The reaction flask was rinsed with O_2. Tetravinyltin (3.74 g, 16.48 mmol) was added to the reaction mixture via a syringe and the reaction was stirred for 1 h at room temperature. O_2-bubbling was replaced by a O_2-balloon and reaction was stirred at 60 °C for 16 h. The resulting brown mixture was poured into a 25% NH_4OAc-solution (30 mL) and stirred for 15 min. The reaction was extracted with Et_2O (150 mL). The organic layer was washed with saturated brine (2 × 40 mL), dried over Na_2SO_4 and evaporated to dryness. Flash chromatography on SiO_2 (2% ethyl acetate in petrol ether) gave **1** as a colorless oil (1.34 g, 6.56 mmol, 39%). ^{1}H NMR ($CDCl_3$) δ 7.18–7.10 (3H, m), 6.60 (1H, dd, J = 14.0 Hz and 6.5 Hz), 4.15–4.08 (2H, m), 3.18 (2H, sep, J = 6.9 Hz), 1.19 (12 H, d, J = 6.9 Hz).

2.1.3. *2-(1-Chloro-ethoxy)-1,3-diisopropyl-benzene (2)*

Ethyl acetate was saturated with dry HCl-gas by bubbling gaseous HCl through the solution for 10 min. **2** (1.04 g, 5.1 mmol) was dissolved in 10 mL of HCl-saturated ethyl acetate. The mixture was heated in a microwave-oven at 100 °C at 5 bar for 10 min and evaporated to dryness. The next reaction was continued immediately without any further purification.

2.1.4. *Phosphoric acid mono-[1-(2,6-diisopropyl-phenoxy)-ethyl] ester disodium salt (3)*

To a solution of tetrabutylammonium phosphate (0.4 M in acetonitrile, 30 mL, 12 mmol) was added **2** (5.1 mmol, theoretical maximum yield) in triethylamine (5 mL, 36 mmol) under argon. The reaction mixture was stirred for 18 h and the solvents were evaporated. Thirty milliliters of water was added and the residue was extracted with diethyl ether (3 × 50 mL). Solvents were evaporated, 10 mL of acetonitrile was added and the pH of the mixture was adjusted to 11 with saturated NaOH. After stirring for 10 min, the solvents were evaporated, the residue was purified with preparative HPLC on a reversed phase Kromasil 100 Å (C8) column by gradient elution using water and acetonitrile (40–80% ACN) as eluent and lyophilized to obtain **3** as a white solid (494 mg, 1,43 mmol, 28%). ^{1}H NMR (D_2O) δ 7.29–7.22 (3H, M), 5.54 (1H, q, J = 5.1 Hz), 3.42 (2H, m), 1.66 (3H, d, J = 5.1 Hz), 1.21 (12 H, d, J = 6.9 Hz). ^{13}C NMR (D_2O) δ 152.30, 146.45, 128.57, 127.08, 103.04, 29.28, 26.03 (d, J = 15.2 Hz), 24.97. ^{31}P NMR (D_2O) δ 0.03. ESI-MS: 301.3 (M−2Na + 1). Anal. Calcd for $C_{14}H_{21}Na_2O_5P{\cdot}0.7H_2O$: C, 46.85; H, 6.29. Found: C, 46.79; H, 6.29.

2.1.5. *2-Chloromethoxy-1,3-diisopropyl-benzene (4)*

Sodium hydroxide pellets (1.2 g, 30.0 mmol) and bromochloromethane (28.7 mL, 57.1 g, 441 mmol) were added to a solution of 2,6-diisopropyl phenol (2.7 g, 15.2 mmol) in dry tetrahydrofuran under nitrogen. The reaction mixture was heated at 64 °C for 2 h, allowed to cool to room temperature and filtered. The filtrate was evaporated to dryness to obtain **4** as a yellowish oil (3.2 g, 14.1 mmol, 93%). **4** was used in the next reaction without any further purification. ^{1}H NMR ($CDCl_3$) δ 7.20–7.09 (3H, m), 5.75 (2H, s), 3.35 (2H, m), 1.22 (12 H, d, J = 6.9 Hz).

2.1.6. *Phosphoric acid mono-(2,6-diisopropyl-phenoxymethyl) ester disodium salt (5)*

To a solution of triethylamine (13.03 mL, 9.46 g, 93.5 mmol) and 85% phosphoric acid (5.86 mL, 9.87 g, 101 mmol) was added **4** (3.2 g, 14.1 mmol) and the reaction mixture was heated at 64 °C for 2.5 h. The solvents were evaporated, 100 mL of water was added to the residue and the pH of the mixture was adjusted to 1.5 with 6 M HCl. The aqueous solution was extracted with diethyl ether (3 × 50 mL), washed with brine and evaporated to dryness. 50 mL of water was added to the residue and pH was adjusted to 9 with saturated NaOH-solution. The mixture was washed with toluene (2 × 30 mL) and concentrated under reduced pressure to half of the volume. 100 mL of isopropanol was added, the mixture was frozen, allowed to warm to 0 °C, filtered and dried in a vacuum oven to obtain **5** as a white solid (0.87 g, 3.0 mmol, 21%) ^{1}H NMR (D_2O) δ 7.32–7.24 (3H, m), 5.27 (2H, d, J = 7.5 Hz), 3.46 (2H, m), 1.22 (12 H, d, J = 6.9 Hz). ^{13}C NMR (D_2O) 152.96, 145.90, 128.63, 127.03, 96.11, 28.97, 25.95. ^{31}P NMR (D_2O) δ 2.02. ESI-MS: 287.1 (M−2Na + 1). Anal. Calcd for $C_{13}H_{19}Na_2O_5P{\cdot}0.2H_2O$: C, 46.49; H, 5.82. Found: C, 46.12; H, 5.82.

2.2. *HPLC analysis*

HPLC analysis of **3**, **5** and propofol was performed using an Agilent Technologies 1100 series gradient RP-HPLC system with UV detection (220 nm) and for rat whole blood samples the fluorescence detection (Ex = 276 nm, Em = 310). The HPLC system consisted of Agilent 1100 Series Binary Pump, 1100 Series Autosampler, 1100 Series Micro Vacuum Degasser, 1100 Series Thermostated Column Compartment, 1100 Series Fluorescence Detector, HP 1050 Variable Wavelenght Detector, 1100 Series Control Module and a Zorbax Eclipse XDB-C8 (4.6 mm × 150 mm, 5 μm) analytical column (Agilent Technologies Inc., Little Falls, Wilmington, DE). Loop injection volume was 20 μL. The isocratic elution was performed by using a mobile phase consisting of 90% (v/v) acetonitrile and 10 mM tetrabutylammonium dihydrogen phosphate at a ratio of 65:35 at a flow rate of 1.0 mL/min at 30 °C.

2.3. *Aqueous solubility*

The aqueous solubilities of **3** and propofol were determined at room temperature in Tris–HCl-buffer (50 mM) at pH 7.4. The pH of the mixtures was held constant during the study. Six milligrams of **3** or excess amounts of propofol were added to 0.5 mL of buffer, the mixtures were stirred for 1 h, filtered (0.45 μm Millipore) and analyzed by HPLC.

2.4. *Hydrolysis in aqueous solutions*

The rate of the chemical hydrolysis of **3** and **5** was determined in borate buffer (180 mM) and in Tris buffer (50 mM) at pH 7.4 at 37 °C and at r.t. An appropriate amount of **3** or **5** (concentration approx. 50 μM) was dissolved in preheated buffer solution or in buffer solution at room temperature and the solutions were placed in a thermostatically controlled water bath at 37 °C or to magnetic stirrer at r.t. Samples were taken at appropriate intervals and analyzed by HPLC. Pseudo-first-order half-lives ($t_{1/2}$) for the hydrolysis of prodrugs were

calculated from the slope of the linear portion of the plotted logarithm of the remaining prodrug versus time.

2.5. *Enzymatic hydrolysis in alkaline phosphatase solution*

Hydrolysis of **3** in alkaline phosphatase solution (type VII-S from bovine intestinal mucosa, Sigma–Aldrich) was determined at 37 °C. 4 μL (81.8 units) of alkaline phosphatase was added to a solution of **3** in 50 mM Tris–HCl buffer (pH 7.4, final concentration 50 μM) at 37 °C. In blank solutions, alkaline phosphatase was replaced with the same volume of water to ensure that the hydrolysis was only enzymatic. At predetermined time intervals, 200 μL samples were removed and 200 μL of ice-cold acetonitrile was added to each sample to stop the enzymatic hydrolysis. The samples were kept on ice, centrifuged for 10 min at 14,000 rpm, and the supernatant was analyzed by HPLC. Pseudo-first-order half-lives ($t_{1/2}$) for the hydrolysis of **3** were calculated from the slope of the linear portion of the plotted logarithm of the remaining prodrug versus time.

2.6. *Animal treatments*

Adult male Wistar rats weighing 250 ± 5 g were purchased from the National Laboratory Animal Centre (Kuopio, Finland). Rats were housed on a 12-h light/12-h dark cycle. All experiments were carried out during the light phase. The rats had free access to tap water and food pellets. All procedures were reviewed and approved by the Animal Ethics Committee at the University of Kuopio. Rats were anesthetized with ketamine (90 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.). The right jugular vein was exposed aseptically as described by Waynforth and Flecnell, (Waynforth and Fleckell, 1994) and cannulated with a PE-50 catheter filled with 100 IE/mL heparin. The catheter was exteriorised through a small incision made in the neck. The rats were allowed to recover until the following day.

The drug solutions were administered to the conscious rats as 30-s i.v. injections (including the rinsing of the catheter with 0.9% NaCl). For the determination of propofol, venous blood samples of 200 μL were collected immediately before and 1, 2, 4, 6, 10 and 30 min after the i.v. bolus administration, mixed with 20 μL of 3% EDTA in 0.7% NaCl-solution and frozen immediately. The drawn blood volume was replaced with 0.9% NaCl.

2.7. *Rat blood sample preparations*

For the assay of propofol in rat whole blood samples, the red blood cells were lysed with a total of three freeze/thaw cycles. 10 μL of 90 μg/mL thymol as internal standard was added to 100 μL of lysed whole blood, then 250 μL of acetonitrile was added to precipitate proteins and the samples were centrifuged for 3 min at 12,000 rpm. The supernatant was injected directly into the HPLC column. The quantity of propofol was determined using external, spiked standards of lysed whole blood and the concentration was estimated based on peak-area ratio of propofol to the internal standard (thymol). All concentration curves and pharmacokinetic parameters (T_{max}, C_{max}) after i.v. administration for each animal were calculated by standard pharmacokinetic methods using WinNonlin Nonlinear Estimation Program, Professional version 5.0.1 (Pharsight Corporation).

3. Results

3.1. *Synthesis*

The phosphonooxymethyl derivative of propofol (**5**, GPI 15715) was synthesized as previously described (Bonneville et al., 2003) from propofol with bromochloromethane and phosphoric acid (Fig. 3) so that chemical stability studies could be undertaken. The synthesis of ethyl dioxy linked prodrug **3** was more challenging. Our first attempt to develop a suitable synthesis route for ethyl dioxy linked prodrugs involved the method for the synthesis of 1-chloroethyl phosphates (Kumpulainen et al., 2005, 2006). It was thought that 1-chloroethyl phosphates might possibly be used as starting materials for the synthesis of ethyl dioxy linked phosphate

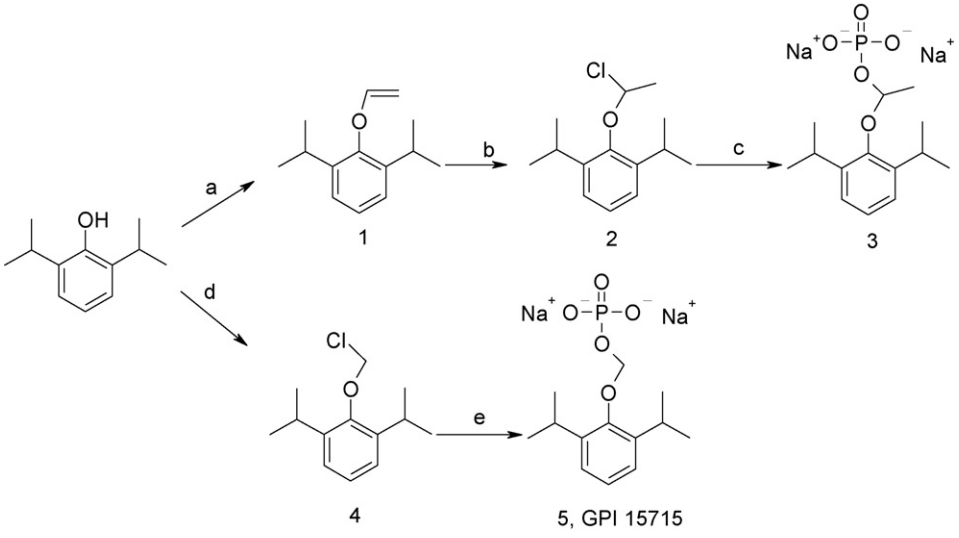

Fig. 3 – Synthesis of the ethyl dioxy phosphate prodrug 3 and phosphonooxymethyl prodrug 5, GPI 15715, of propofol.

Table 1 – The aqueous solubility of 3 and propofol in buffer solution, half-lives of 3 and 5 in buffer solutions and half-life of 3 in alkaline phosphatase solution and, all at pH 7.4 (mean ± S.D.; *n* = at least 3)

Compound	Solubility ($mg\,mL^{-1}$); Tris buffer r.t.[c]	Chemical stability, $t_{1/2}$; Tris buffer 37 °C	Chemical stability, $t_{1/2}$; borate buffer 37 °C	Chemical stability, $t_{1/2}$; borate buffer r.t.[c]	Enzymatic hydrolysis, $t_{1/2}$; alkaline phosphatase, 37 °C
3	>10	25.8 ± 3.3 h	21.5 ± 0.8 h	5.2 ± 0.2 days	21 ± 3 s
5	–[a]	–[a]	Stable[b]	–[a]	–[a]
Propofol	0.13 ± 0.002	–[a]	–[a]	–[a]	–[a]

[a] Not determined.
[b] No degradation was observed after three weeks.
[c] Room temperature.

prodrugs. However, probably because of the steric hindrance and the presence of a phenolic hydroxyl group which is a good leaving group, the 1-chloroethyl phosphates did not react with propofol. The second attempt was to use the same synthesis route to synthesize 1-chloroethyl ether of propofol via vinyl ether, which could be further derivatized to phosphate. We found that the only way to make vinyl ether of propofol was the use of tetravinyl tin and cuprous acetate in an oxygen atmosphere (Blouin and Frenette, 2001). The vinyl ether underwent fast microwave-assisted conversion to 1-chloroethyl ether in the presence of HCl-gas at 100 °C within 10 min.

Since the ethyl dioxy structure was found to be chemically acid-labile and since many protecting groups of phosphates require acidic conditions for their removal, the synthesis of an ethyl dioxy phosphate was carried out without any protecting groups being present. The synthesis was achieved by using tetrabutylammonium phosphate in acetonitrile with an excess of triethylamine under dry and basic conditions in organic solvent. The sodium salt **3** was obtained with the treatment of the product with sodium hydroxide and the final product was purified with preparative HPLC. Since most of the marketed (acyloxy) alkyl prodrugs are racemic mixtures, the enantiomers were not separated, but the racemic mixture was used in all assays which will be described.

3.2. *Aqueous solubility*

The aqueous solubility of propofol (130 ± 2 μg/mL) was greatly enhanced by the prodrug **3** since this compound has solubility over 10 mg/mL (Table 1). The maximum solubility of **3** was not determined due to the small amount of prodrug available. However, this limit was chosen since it has been estimated that in general a drug needs to have a solubility of at least 10 mg/mL to be suitable for i.v. administration (Stella, 2006). Due to the fast and complete dissolution and limited stability of **3**, the shaking time was reduced to 1 h from the conventional 72 h.

3.3. *Stability in buffer solutions*

The chemical hydrolysis rates of **3** and **5** were determined in 180 mM borate buffers at pH 7.4 at 37 °C and at room temperature (r.t.). The chemical degradation of both **3** and **5** followed first-order kinetics and the half-lives are presented in Table 1. The stability of **3** in buffer solution ($t_{1/2}$ = 5.2 ± 0.2 days at r.t. and $t_{1/2}$ = 25.8 ± 3.3 h at 37 °C, pH 7.4) was reasonably good if the drug were to be used for i.v. administration, although the chemical degradation was significantly faster than that of **5** (no degradation was observed after three weeks). There were no significant differences between the stabilities of **3** at 37 °C in different buffer solutions (Tris- and borate buffer).

3.4. *Enzymatic hydrolysis in alkaline phosphatase solution*

The enzymatic hydrolysis of **3** was determined in alkaline phosphatase solution at pH 7.4. Only the half-life of **3** but not the exact amount of formed propofol was determined due to the low solubility of propofol. The enzymatic hydrolysis of **3** to propofol was extremely rapid with a half-life of approximately 20 s (Table 1).

3.5. *Bioconversion of 3 to propofol in vivo*

The bioconversion of **3** to propofol was demonstrated *in vivo* in rats after a single i.v. bolus administration. One group of three adult male Wistar rats received a single bolus dose of 10 mg/kg of propofol (0.056 mmol/kg) and another group of three rats received 19.4 mg/kg of **3** (0.056 mmol/kg). The plasma concentration of propofol was determined from whole blood samples using thymol as an internal standard with the method described previously in the literature (Yeganeh and Ramzan, 1997). Only the amount of propofol, not the amount of prodrug, was determined and compared between the groups. The concentration curves of propofol after administration of **3** and propofol are presented in Fig. 4, in which each line shows the values of individual rats. After administration of prodrug or propofol, the pharmacokinetics revealed a fast initial decline and a slower elimination phase and these were best described by a two-compartmental model. The levels of propofol in whole blood (μg/mL) and the pharmacokinetic parameters (T_{max}, C_{max}) were determined using the above mentioned simulation and are shown in Table 2. The pharmacokinetic parameters of GPI 15715 and propofol phosphate are gathered from the publications of (Schywalsky et al., 2003) and (Banaszczyk et al., 2002), respectively.

Prodrug **3** was readily converted to propofol in each rat after i.v. administration. The maximum plasma concentration (C_{max}) of propofol, 3.0 ± 0.2 μg/mL, was reached within 2.1 ± 0.8 min (T_{max}) after the administration of **3**. Rats that received **3** were uncoordinated and had difficulties in maintaining their balance. In contrast, the dose of 10 mg/kg of propofol was sufficient to evoke sedation which

Table 2 – Estimated pharmacokinetic parameters (mean ± S.D.; $n = 3$) of propofol after single i.v. bolus dose of 3 (19.4 mg/kg, 0.056 mmol/kg) and propofol (10 mg/kg, 0.056 mmol/kg) into rats and referenced pharmacokinetic parameters of GPI 15715 and Propofol Phosphate (PP) from the literature

Compound	3	Propofol	GPI 15715[a]	PP[b]
Dose	19.4 mg/kg (0.056 mmol/kg)	10 mg/kg (0.056 mmol/kg)	40 mg/kg (0.120 mmol/kg)	130 mg/kg (0.499 mmol/kg)
C_{max} (μg/mL)	3.0 ± 0.2	14.3 ± 1.8	7.1 ± 1.6	9.6 ± 2.1
T_{max} (min)	2.1 ± 0.8	–[c]	3.7 ± 0.2	7.3 ± 3.8

C_{max} = maximum concentration of propofol. T_{max} = time to maximum concentration of propofol.

[a] From Schywalsky et al. (2003).

[b] From Banaszczyk et al. (2002).

[c] After i.v. bolus T_{max} = 0 min.

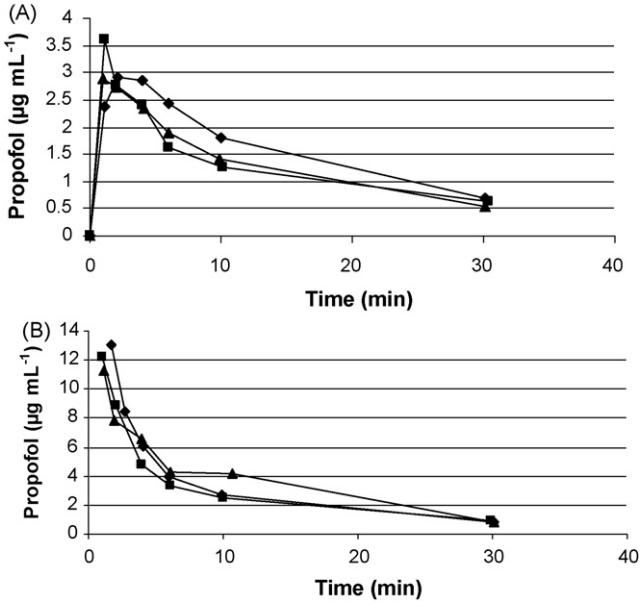

Fig. 4 – Propofol levels after single i.v. bolus dose of (A) 3 (19.4 mg/kg) and (B) propofol (10 mg/kg) in rats ($n = 3$). Each line shows the values of one rat.

occurred at approximately 13 s after the i.v. bolus. When compared to GPI 15715, the maximum concentration of propofol, 3.0 ± 0.2 μg/mL after administration of **3** (19.4 mg/kg, 0.056 mmol/kg), is approximately half of the concentration achieved after administration of GPI 15715 with over double the dose (7.1 ± 1.6 μg/mL after 40 mg/kg, 0.120 mmol/kg) (Schywalsky et al., 2003). The maximum concentration of propofol was also reached at approximately in the same time (2.1 ± 0.8 min and 3.7 ± 0.2 min for **3** and GPI 15715, respectively). The bioconversion of **3** to propofol was much faster and more effective, when compared to PP (Banaszczyk et al., 2002) (T_{max} of 7.3 ± 3.8 min and a C_{max} of 9.6 ± 2.1 μg/mL after i.v. administration of PP with a dose as great as 130 mg/kg (0.499 mmol/kg)).

4. Discussion

Ethyl dioxy phosphates are a promising strategy for the development of novel phosphate prodrugs with fast bioconversion and lack of the formaldehyde release. Ethyl dioxy phosphate of propofol (**3**) greatly enhanced the aqueous solubility of propofol, the solubility being over 10 mg/mL and sufficient for i.v. administration. Moreover, **3** was bioconverted to propofol as efficiently as the phosphonooxymethyl prodrug of propofol (**5**, GPI 15715), the latter being synthesized for the purpose of comparison of chemical stability of these two prodrugs. The faster chemical hydrolysis of **3** compared to that of **5** is probably due to chemical structure of the ethyl dioxy acetal. It is known that acetals with a good leaving group (such as phenol) and a neighbouring carboxylic acid group undergo intramolecular facilitated hydrolysis (Anderson and Fife, 1973; Fife et al., 1996). Therefore it can be postulated that the acidic groups of phosphate facilitate the hydrolysis of **3** by forming an oxocarbonium ion, which is internally greatly resonance-stabilized, leading to the breaking of the C—O bond and the release of propofol (Fig. 5). Nonetheless, the chemical stability of **3** is adequate for i.v. use, though it may require the reconstitution of the preparation prior to use.

The fast enzymatic hydrolysis of **3** suggests that ethyl dioxy linked prodrugs act in a similar manner as conventional phosphate and phosphonooxymethyl prodrugs. The difference between the concentrations of propofol after equimolar administration of **3** and propofol is similar to the results reported for GPI 15715 (Schywalsky et al., 2003) and is probably due to formulation-dependent pharmacokinetics of propofol.

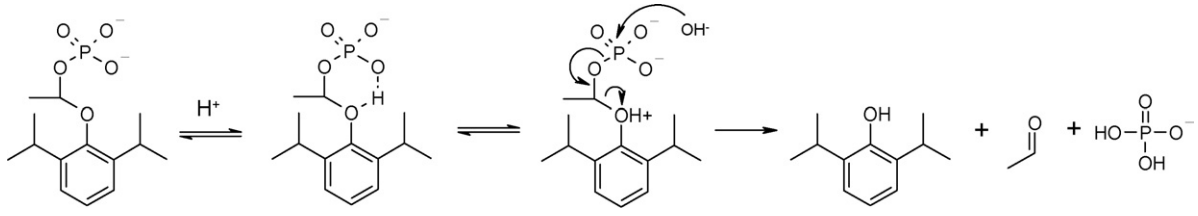

Fig. 5 – The chemical hydrolysis of the ethyl dioxy phosphate prodrug of propofol (3).

A lipid-free formulation of propofol has a greater volume of distribution and a greater elimination clearance, but a similar terminal half-life and larger distribution in the lungs when compared to the oil-in-water formulation (Dutta and Ebling, 1998b). The slow release of propofol from the lungs leads to decreased concentrations in the circulation (Dutta and Ebling, 1998a). **3**, like GPI 15715 (Schywalsky et al., 2003), probably acts like the lipid-free formulation, releasing the lower concentrations of propofol to the circulation than is seen after equimolar administration of propofol. **3** and GPI 15715 (Schywalsky et al., 2003) have approximately the same T_{max} and proportionate C_{max}. We therefore conclude that the bioconversion of **3** to propofol is as effective as that of GPI 15715 and much faster and more effective, when compared to PP (Banaszczyk et al., 2002).

The purpose of this study was to confirm the bioconversion of **3** to propofol *in vivo*, not to evoke complete sedation in the rats. The sedated behaviour of rats demonstrated that propofol had been released from **3**, but larger doses would be needed to achieve complete sedation. This effect is similar to that reported earlier in case of GPI 15715 (Schywalsky et al., 2003). Even though the pharmacokinetic parameters are not truly comparable between different studies due to the dose-dependent onset of the action and recovery of propofol from prodrugs, this study indicates that **3** is bioconverted to propofol in a similar manner as the phosphonooxymethyl prodrug GPI 15715. In this study, no evidence was observed of any abnormal pharmacokinetics that could be explained by chirality. However, since enantiomers often have different pharmaceutical and pharmacokinetic profiles (Hutt and Tan, 1996) and also differences in binding to serum albumin (Chuang and Otagiri, 2006), in the future it might be worthwhile to separate the enantiomers and to evaluate their individual pharmaceutical and pharmacokinetic behaviours. This is not a unique problem of **3**, as most of the (acyloxy) alkyl prodrugs are marketed as racemic mixtures.

In a conclusion, a novel ethyl dioxy phosphate prodrug of propofol (**3**) has been shown to increase the water-solubility of the poorly water-soluble drug propofol. The chemical stability of **3** is sufficient for i.v. administration, but is limited due to the presence of the more labile ethyl dioxy acetal. The enzymatic release of propofol from **3** is rapid both *in vitro* with alkaline phosphatase solution and *in vivo* in rats. The bioconversion of **3** to propofol is similar to that of GPI 15715, the phosphonooxymethyl prodrug of propofol, and much faster when compared to propofol phosphate. The results provide clear evidence that the ethyl dioxy linker is suitable for preparing phosphate prodrugs designed for i.v. administration by combining the properties of previously described phosphate and phosphonooxymethyl prodrugs (i.e. efficient bioconversion to propofol and lack of systemic release of formaldehyde). Further studies with different model drugs containing hydroxyl and amine functionalities are ongoing in order to explore the overall versatility of this novel phosphate prodrug structure.

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