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Water-Soluble Propofol Analogues with Intravenous Anaesthetic Activity

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Abstract—Propofol (2,6-diisopropylphenol) is a widely used intravenous anaesthetic that is formulated as an emulsion since it lacks water solubility. We report a range of water-soluble analogues of propofol, containing a *para*-alkylamino substituent, which retain good intravenous anaesthetic activity in rodents.

Introduction

Propofol (2,6-diisopropylphenol) is a widely used intravenous anaesthetic. Its mechanism of action involves the positive allosteric modulation of the neurotransmitter γ-aminobutyric acid (GABA) at $GABA_A$ receptors.[1] The main advantages of propofol are favourable operating conditions and a rapid recovery but disadvantages include cardiovascular side-effects and pain on injection.[2–4] A number of approaches have been used to overcome some of the drawbacks associated with the formulation of propofol as an emulsion, and these have been reviewed.[2] Recent evidence suggests that propofol analogues containing a *para* substituent still retain good activity at the $GABA_A$ receptor.[5,6] Our aim was to produce water-soluble analogues of propofol, containing a *para*-alkylamino substituent, which retained intravenous anaesthetic activity.

Experimental

Several propofol analogues were prepared containing a *para*-aminomethyl substituent to aid water-solubility. Reaction of propofol with aqueous formaldehyde and the secondary amine of choice in a refluxing ethanol/water mixture afforded the desired products (**1**–**5**; Fig. 1).[7]

Chain-extended *para*-aminoalkyl and aminoacyl analogues were also prepared as shown in Scheme 1. Compound **6** was prepared by a Friedel–Crafts acylation of propofol using aluminium trichloride and bromoacetylbromide in dichloromethane. Reaction of this α-bromoketone intermediate with the desired amine in dichloromethane using triethylamine as base afforded products **7** and **8**. The carbonyl group in compound **7** was reduced to the alcohol intermediate using lithium aluminium hydride in tetrahydrofuran. Subsequent removal of the alcohol with potassium borohydride and potassium hydroxide in an ethanol/water mixture afforded compound **9**.

Replacement of the phenolic hydroxyl group in compounds **1**–**3** with an aniline function gave compounds **10**–**12**. This was achieved by reaction of 2,6-diisopropylaniline with aqueous foramldehyde and the desired amine in a refluxing ethanol/water mixture for several days to afford the desired products (Fig. 2).[8]

A number of analogues of compound **10** were synthesised to explore SARs around the aniline moiety. Removal of the aniline function was achieved by diazotisation of compound **10** using sodium nitrite in hypophosphorus

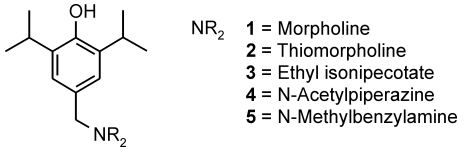

Figure 1.

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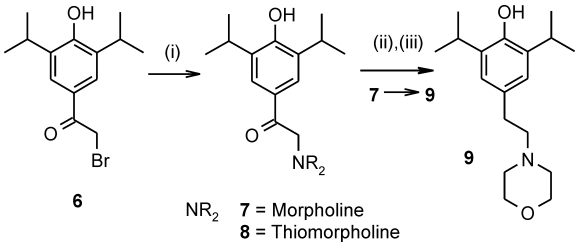
acid (50% aqueous) and gave compound **13**. Compound **10** was converted to acetamide **14** by reaction with acetyl chloride in dichloromethane using triethylamine as the base. The *N*,*N*-dimethyl aniline analogue **15** was prepared by reaction of aniline **10** with aqueous formaldehyde and formic acid. Conversion of aniline **10** to the *N*-methyl aniline analogue **16** was achieved by a two step process: firstly compound **10** was converted to the intermediate formamide by reaction with formic acid and acetic anhydride and this product was then reduced to compound **16** using lithium aluminium hydride in tetrahydrofuran (Scheme 2).

Results and Discussion

All final compounds (**1**–**5** and **8**–**15**) were converted to the hydrochloride salt by passing hydrogen chloride gas through a solution of the free amine in diethyl ether. The hydrochoride salts that precipitated were filtered to give water-soluble white solids, which were used for pharmacological testing.



Scheme 1. (i) Amine, Et_3N, CH_2Cl_2; (ii) $LiAlH_4$, THF; (iii) KBH_4, KOH, EtOH, H_2O.

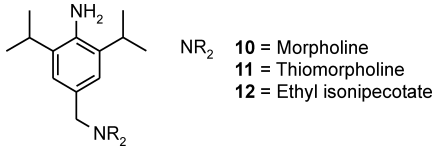
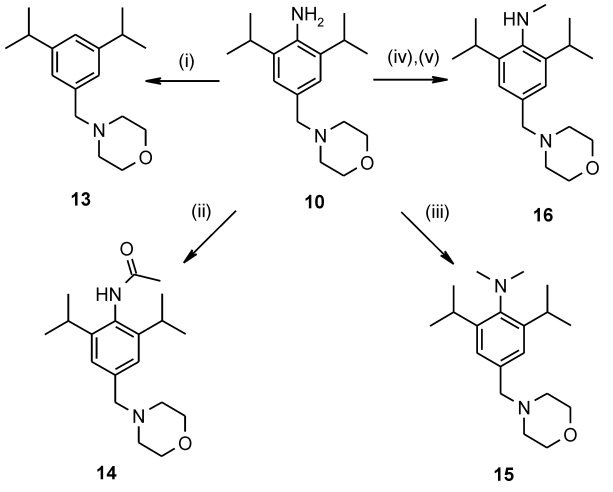


Figure 2.



Scheme 2. (i) Hypophosphorus acid (50% aqueous), $NaNO_2$; (ii) acetyl chloride, Et_3N, CH_2Cl_2; (iii) aqueous formaldehyde, formic acid; (iv) formic acid, acetic anhydride; (v) $LiAlH_4$, THF, reflux.

The anaesthetic potency of compounds was determined upon their intravenous administration to mice. In each case the dose required to cause a loss of righting reflex for a minimum period of 30 s in 50% of treated mice after iv injection over 10 seconds was determined by probit analysis. This dose is termed the HD_{50} (hypnotic $dose_{50}$). Propofol was injected as the commercial veterinary product (Rapinovet™, Schering-Plough Animal Health, UK) at 10 mg mL^{-1}. Compounds **1**–**5** and **8**–**16** (hydrochloride salts) were administered as 10 mg mL^{-1} solutions in distilled water. The in vitro effect of the compounds at $GABA_A$ receptors was also assessed, by determination of their ability to inhibit [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]TBPS) binding to rat whole brain membranes. In each case the concentration of drug required to inhibit 50% binding of this radioligand was determined (TBPS IC_{50}). The in vitro and in vivo results are shown in Table 1. All in vitro and in vivo studies were carried out as described by Anderson et al. except male MF-1 mice were used instead of male CFLP mice for the in vivo studies.[9]

The in vivo anaesthetic potency of compounds (**1**–**5** and **8**–**15**) was determined upon their intravenous administration to mice. Analogues containing a *para*-methylamino substituent (**1**–**5**) compared very favourably with propofol ($HD_{50} = 68$ μmol kg^{-1}), the most potent being compound **3** ($HD_{50} = 19$ μmol kg^{-1}). Surprisingly, this observation differs from the SAR for simple propofol-like phenols where introduction of a *para*-methyl or ethyl substituent resulted in lower hypnotic activity in mice.[10] To further explore the SAR around the *para*-substituent the chain extended analogue **9** was synthesised and was also found to retain good anaesthetic potency ($HD_{50} = 35.8$ μmol kg^{-1}).

Previous studies had reported the importance of the phenolic hydroxyl group for anaesthetic activity but replacement with an aniline moiety had never been investigated.[10] All the aniline derivatives **10**–**12** exhibited good hypnotic potency, especially compound **12** ($HD_{50} = 14.4$ μmol kg^{-1}). To explore how important the hydrogen bond donating moiety was for activity in this

Table 1. Anaesthetic activity and $GABA_A$ receptor modulatory effects of final compounds

Compound	HD_{50} μmol kg^{-1}[a]	TBPS IC_{50} (μM)[b]
Propofol	68.0	18
1	32.0	>100
2	25.5	27.6
3	19.0	31.0
4	38.8	>100
5	25.1	>10 ($n = 2$)
8	68.5	>10
9	35.8	>100
10	36.5	>100
11	22.9	>100
12	14.4	na
13	Convulsant	>30
14	>282	>10
15	83.0	>30
16	51.0	>100

[a]Male MF-1 mice were used in all studies ($n = 8$).
[b]Values are means of three experiments (na = not active).

aniline series, compounds **13**–**16** were synthesised. Complete removal of the aniline substituent gave compound **13**, which was convulsant. Conversion of the aniline to the amide **14**, which still contains a weak hydrogen bond donor, dramatically reduced potency. The mono-methyl aniline derivative **16**, which has a stronger hydrogen bond donor, retained some hypnotic activity ($HD_{50} = 51.0\,\mu mol\,kg^{-1}$), while the dimethylaniline derivative **15** was less active.

The in vitro effect of the compounds at $GABA_A$ receptors was assessed by determination of their ability to inhibit [^{35}S]TBPS binding to rat whole brain membranes. Most of the compounds were inactive or very weakly active in this assay suggesting that their in vivo anaesthetic activity is mediated by a non-GABAergic mechanism.

The anaesthetic effects of compound **1** and propofol were also studied in male Wistar rats by extraction of EEG parameters. Seventeen male Wistar rats (218–420 g at the time of injection), previously implanted with extradural electrodes, with a minimum of seven days recovery period, were injected intravenously (10 s) via a tail vein using a computer controlled infusion pump (Harvard 44, Harvard Apparatus). The animals were lightly restrained by hand during induction of anaesthesia. Six animals were injected with $51.0\,\mu mol\,kg^{-1}$ compound **1** (hydrochloride salt), five with $33.7\,\mu mol\,kg^{-1}$ propofol and the remaining six with $47.7\,\mu mol\,kg^{-1}$ propofol. The burst suppression ratio (BSR) was extracted from the EEG as previously described.[11] The following parameters were determined: the time to the first 0.25 s burst suppression epoch (onset of anaesthesia), time to the maximum BSR, time to 50% decrease in the BSR (offset time) and the time to gain of the righting reflex (recovery time). Compound **1** (hydrochloride salt) was dissolved in distilled water at $20\,mg\,mL^{-1}$. Propofol was injected as the commercial veterinary product (Rapinovet™, Schering-Plough Animal Health, UK) at $10\,mg\,mL^{-1}$. The BSR profiles for propofol ($33.7\,\mu mol\,kg^{-1}$) and compound **1** (hydrochloride salt, $51.0\,\mu mol\,kg^{-1}$) are shown in Figure 3. At these doses similar sleep times were observed (approximately 4 min) and the gain of righting reflex occurred around the same time as the BSR returned to pre-drug levels. However, compound **1** (hydrochloride salt) produced more burst suppression than propofol (55% and 39%, respectively). Another comparison is to have two doses that produced the same maximum burst suppression, unfortunately this did not occur. However, it can be achieved by estimating the dose producing 55% BSR for propofol based on linear interpolation between the two doses as shown in Table 2. Compared to propofol, compound **1** (hydrochloride salt) was slightly less potent and was slower in onset. However, recovery from what is a reasonable depth of anaesthesia was slightly faster with compound **1** (hydrochloride salt). Respiratory depression was a prominent feature during the induction phase with both compounds, but otherwise the sleeps were uneventful.

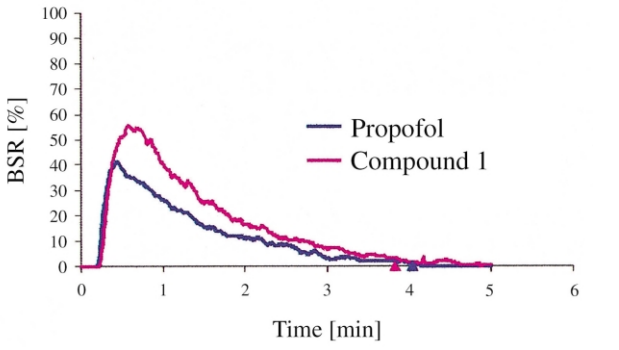


Figure 3. Burst suppression ratio (BSR) profiles for $33.7\,\mu mol\,kg^{-1}$ propofol and $51.1\,\mu mol\,kg^{-1}$ compound **1** (hydrochloride salt). The lines represent the means of five and six rats, respectively. The triangles represent the time to gain of the righting reflex (recovery time).

Table 2. Comparison between the anaesthetic profiles of compound **1** (hydrochloride salt) and propofol at 55% BSR[a]

Drug	Compound **1** (hydrochloride salt)	Propofol
Dose producing 55% BSR ($\mu mol\,kg^{-1}$)	51.0	39.2
Onset time (s)	16.2±0.9	13.2±0.3
Maximum BSR (%)	54.9±10.3	55
Time to max. BSR (s)	40±2.4	30±2
Offset time (min)	1.7±0.9	1.4±0.2
Recovery time (min)	3.8±0.9	5.2±0.4

[a]The values for propofol are estimates based on linear interpolation between the two doses tested. The values represent the mean and SEM.

Conclusion

A number of water-soluble derivatives of the intravenous anaesthetic propofol were prepared and found to retain good anaesthetic activity in mice. The anaesthetic potency did not correlate with the ability of the compounds to displace [^{35}S]TBPS binding to rat whole brain membranes, though compound **1** (hydrochloride salt) was active in an EEG study of anaesthesia in rats. While the anaesthetic activity may in part be related to the ability of the compounds to allosterically modulate $GABA_A$ receptors, the mechanism of action by which these compounds mediate their anaesthetic effects is not clear.

Acknowledgements

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7. Synthesis of 2,6-diisopropyl-4-(4-morpholinylmethyl)-phenol hydrochloride **1**: To a solution of 2,6-diisopropylphenol (17.83 g, 0.1 mol) in an ethanol (70 mL)/water (30 mL) mixture was added morpholine (8.72 mL, 0.1 mol) and formaldehyde (37 wt% in water, 8.12 g, 0.1 mol). The reaction mixture was heated to reflux for 2 h and allowed to cool to room temperature. The solution was partitioned between ethyl acetate and water. The organic layer was separated, dried (Na_2SO_4) and the solvent removed under reduced pressure. Chromatography of the crude product on silica gel (gradient eluent: dichloromethane to EtOAc) gave the free amine of the title compound as an orange oil. Conversion to the hydrochloride salt was achieved by passing hydrogen chloride gas through a solution of the free amine in diethyl ether. The hydrochoride salt that precipitated was filtered to give the title compound as a white solid (19 g, 61%). Positive ion ESI $(M+H)^+$ 278.5. ^1H NMR ($CDCl_3 + d_5$-pyridine); δ 1.27–1.29 (12H, d), 2.96–3.10 (4H, br s), 3.26–3.33 (2H, septet), 4.02–4.15 (6H, m), 7.24 (2H, s). Elemental analysis: (C H N Cl) Found; C 65.01, H 8.93, N 4.35, Cl 10.54%. Calculated; C 65.06, H 9.09, N 4.46, Cl 10.17% (0.9 mol Cl and 0.2 mol H_2O). Compounds **2**–**5** were synthesised in a similar manner.

8. Synthesis of 2,6-diisopropyl-4-(4-morpholinylmethyl)-aniline hydrochloride **10**: To a solution of 2,6-diisopropylaniline (21 mL, 0.1 mol) in an ethanol (90 mL)/water (50 mL) mixture was added morpholine (26.16 mL, 0.3 mol) and formaldehyde (37 wt% in water, 24.35 g, 0.3 mol). The reaction mixture was heated to reflux for 3 days and allowed to cool to room temperature. The solution was partitioned between ethyl acetate and water. The organic layer was separated, dried (Na_2SO_4) and the solvent removed under reduced pressure. Chromatography on silica gel (gradient eluent: 2:1 petroleum ether/EtOAc to EtOAc) gave the free amine of the title compound as a brown oil. Conversion to the hydrochloride salt was achieved by passing hydrogen chloride gas through a solution of the free amine in diethyl ether. The hydrochoride salt that precipitated was filtered to give the title compound as a white solid (20 g, 64%). Positive ion ESI $(M+H)^+$ 277.2. ^1H NMR ($CDCl_3$); δ 1.26–1.28 (12H, d), 2.42–2.46 (4H, m), 2.89–2.98 (2H, septet), 3.42 (2H, s), 3.69–3.73 (4H, m), 6.95 (2H, s). Elemental analysis: (C H N Cl) Found; C 57.67, H 8.61, N 7.61, Cl 18.25%. Calculated; C 57.86, H 8.81, N 7.94, Cl 18.59% (1.85 mol Cl and 0.5 mol H_2O). Compounds **11** and **12** were synthesised in a similar manner.

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