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Novel α-Amino-Acid Phenolic Ester Derivatives with Intravenous Anaesthetic Activity

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Abstract—A novel series of α-amino-acid phenolic ester derivatives containing sulphide, sulphoxide, sulphone, ester and amide side chains were prepared and shown to display potent intravenous anaesthetic activity.

Propofol (2,6-diisopropylphenol) is a widely used intravenous (iv) 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] We recently reported the SAR and general anaesthetic activity in mice of a series of α-amino acid phenolic ester derivatives, and Org 25435 **2** was tested in volunteers (Fig. 1).[5] The lead optimisation of compound **2** focussed mainly on structural modifications to the phenolic and amino moieties. Limited alkyl side-chain modifications (methyl to butyl) were explored and led to an increase in anaesthetic activity. Our aim was to further optimise the anaesthetic potency of compound **2** by incorporation of heteroatoms into the alkyl side chain.

In this paper we describe a series of α-amino-acid phenolic ester derivatives containing sulphide, sulphoxide and sulphone side chains **3**, and ester and amide side chains **4** with intravenous anaesthetic activity (Fig. 2).

The sulphide, sulphone and sulphoxide analogues **3** were synthesised as shown in Scheme 1. DL-Methionine **5** was diazotised in aqueous hydrobromic acid with either 1.0 equiv or 3.2 equiv of sodium nitrite giving the α-bromo acid derivatives **6** (sulphide side chain) and **7** (sulphone side chain). The acid chlorides were then prepared using oxalyl chloride in dichloromethane with catalytic pyridine and coupled with either 2,6-dimethoxyphenol or 2,6-dimethoxy-4-methylphenol to give the intermediates **8** and **9**. Sulphide **8** was converted to sulphoxide **10** using *meta*-chloroperbenzoic acid in dichloromethane. α-Bromophenolic ester intermediates **8**, **9** and **10** were converted to final compounds **11**–**18**, **19**–**23** and **24**–**25**, respectively, by reaction with selected amines (NHR'_2) at room or elevated temperatures.[6]

The ester and amide analogues **4** were synthesised as shown in Scheme 2. Maleic anhydride **26** was reacted with selected amines (NR''_2) in dichloromethane to give substituted maleic acid **27**, or reacted with methanol at 50 °C to give substituted maleic acid **28**. Reaction of **27** and **28** with selected secondary amines (NR'''_2) in methanol (at room or elevated temperatures) followed by cation exchange with sodium hydroxide gave amino acid derivatives **29** and **30**. Coupling of **29** and **30** with 2,6-dimethoxyphenol or 2,6-dimethoxy-4-methylphenol using Et_3N, DMAP, EDCI in dichloromethane or chloroform gave final products **31**–**40** and **41**–**50** respectively.[7]

The anaesthetic potency of compounds was determined upon their iv administration to mice.[8] 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

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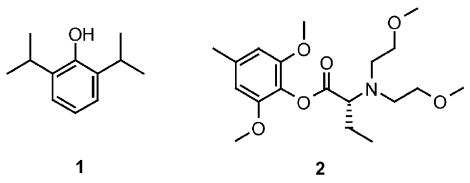


Figure 1. Propofol **1** and Org 25435 **2**.

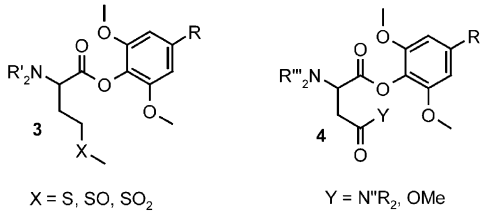


Figure 2. Sulphides, sulphones, sulphoxides **3** and esters and amides **4**.

injection over 10 s 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 **11–25**, **31–40**, and **41–50** (free base or hydrochloride salts) were administered as 5–10 mg mL^{-1} solutions in distilled water or suitable vehicle; all were tested as racemates. The anaesthetic activity of each series is shown in order of activity in Tables 1–3. Propofol **1** and the racemate of **2** are given for comparison.

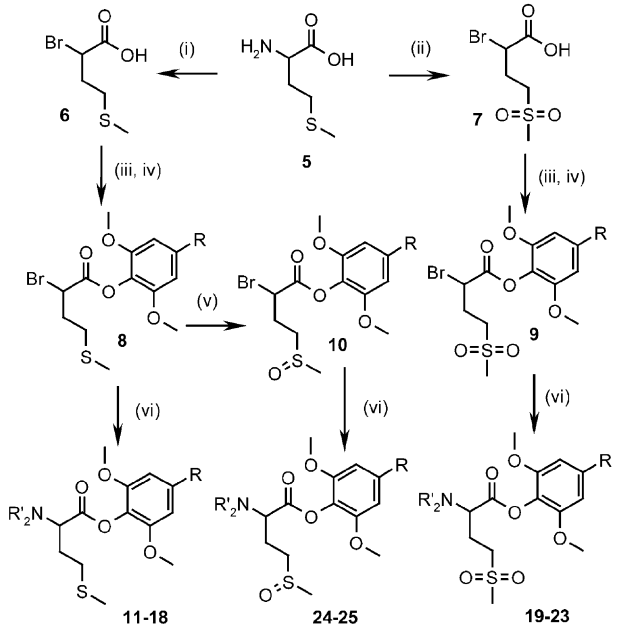
The in vivo anaesthetic potency of compounds with a sulphur containing side-chain are shown in Table 1. Of these the suphides were the most potent and compared favourably with propofol **1** (HD_{50} = 68 μmol kg^{-1}) and the racemate of **2** (HD_{50} = 21.7 μmol kg^{-1}). All sulphide analogues **11–18** had HD_{50} < 41 μmol kg^{-1}, the most potent containing a homopiperidine ring **11** (HD_{50} = 18.2 μmol kg^{-1}). The more polar sulphoxide and sulphone analogues displayed weaker activity, with sulphone **19** being the most potent (HD_{50} = 39.3 μmol kg^{-1}).

Table 1. Anaesthetic activity of sulphide **11–18**, sulphone **19–23** and sulphoxide **24–25** analogues in mice

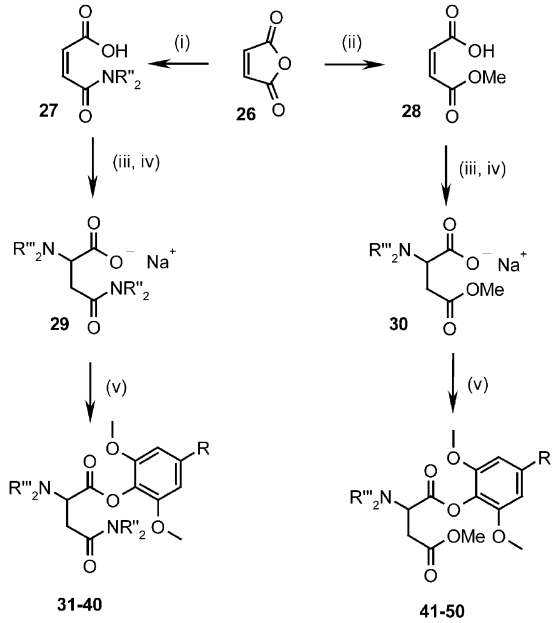
Compd	NR'_2	R	HD_{50} (μmol.kg^{-1})[a]
Propofol, **1**	N/A[b]	N/A[b]	68.0
Racemic **2**	N/A[b]	N/A[b]	21.7
11	Homopiperidine	H	18.2
12	Bismethoxyethylamine	Me	22.6
13	Piperidine	H	<25
14	Morpholine	H	26.1
15	Pyrrolidine	Me	31.4
16	Morpholine	Me	34.8
17	Heptamethyleneimine	H	35
18	Pyrrolidine	H	40
19	Tetrahydropyridine	Me	39.3
20	Bismethoxyethylamine	Me	77
21	Morpholine	H	99
22	Bismethoxyethylamine	H	>106
23	Morpholine	Me	108
24	Pyrrolidine	Me	73
25	Morpholine	Me	299

[a]Male MF-1 mice were used in all studies ($n \geq 5$).
[b]N/A = not applicable.

The in vivo anaesthetic potency of compounds with an ester containing side chain is shown in Table 2. Compounds **31–33** all showed good potency while compounds **34–38** had potencies comparable to propofol. The most potent analogue **31** (HD_{50} = 22.9 μmol kg^{-1}) again contained a homopiperidine ring.



Scheme 1. Reagents and conditions: (i) 1.0 equiv $NaNO_2$, HBr (aq), 0 °C, (ii) 3.2 equiv $NaNO_2$, HBr (aq), 0 °C, (iii) Oxalyl chloride, DCM, pyridine (cat.), (iv) 2,6-dimethoxyphenol or 2,6-dimethoxy-4-methylphenol, Et_3N, DCM, (v) *m*-CPBA, DCM, 0° C, (vi) selected amines (NHR'_2), Δ.



Scheme 2. (i) 1 Equiv selected amine (NR''_2), DCM, 0 °C, (ii) MeOH, 50 °C, (iii) 3–4 equiv selected amine (NR'''_2), MeOH, Δ, (iv) 1 equiv NaOH, MeOH, (v) 1.3 equiv 2,6-dimethoxyphenol or 2,6-dimethoxy-4-methylphenol, CH_2Cl_2 or $CHCl_3$, 2 equiv Et_3N, 1 eq. DMAP, 1.3 equiv EDCI.

Table 2. Anaesthetic activity of ester analogues **31–40** in mice

Compd	NR'''_2	R	HD_{50} (μmol kg^{-1})[a]
31	Homopiperidine	H	22.9
32	Homopiperidine	Me	26
33	Piperidine	H	26.4
34	Morpholine	Me	46
35	Bismethoxyethylamine	H	48
36	Bismethoxyethylamine	Me	48
37	Piperidine	Me	48
38	Pyrrolidine	Me	52
39	Pyrrolidine	H	77
40	Morpholine	H	79

[a]Male MF-1 mice were used in all studies ($n \geq 5$).

The in vivo anaesthetic potency of compounds with an amide containing side-chain is shown in Table 3. Compounds **41–44** all showed good potency ($HD_{50} = 34$–39 μmol kg^{-1}). The most active analogue **41** ($HD_{50} = 34$ μmol kg^{-1}) is again a homopiperidinyl derivative, though in this case a change from R = H to R = Me **45** halves the potency ($HD_{50} = 67$ μmol kg^{-1}). This is unusual, as in most cases the R group does not make a large difference to the potency of the compounds.

The in vitro effect of selected 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.[8] In each case the concentration of drug required to inhibit 50% binding of this radioligand was determined (TBPS IC_{50}). The in vitro results of selected active compounds, propofol **1** and the racemate of **2** are shown in Table 4. Some of the more potent intravenous anaesthetics also showed good inhibition of [^{35}S]TBPS binding to rat whole brain membranes, which suggests the in vivo anaesthetic activity may be mediated in part by a GABAergic mechanism.

Table 3. Anaesthetic activity of amide analogues **41–50** in mice

Compd	NR''_2	NR'''_2	R	HD_{50} (μmol kg^{-1})[a]
41	Pyrrolidine	Homopiperidine	H	34
42	Piperidine	Piperidine	Me	37.5
43	Diethylamine	Morpholine	Me	38
44	Piperidine	Piperidine	H	39
45	Pyrrolidine	Homopiperidine	Me	67
46	Pyrrolidine	Pyrrolidine	Me	81
47	Pyrrolidine	Pyrrolidine	H	102
48	Diethylamine	Morpholine	H	117
49	Morpholine	Morpholine	Me	> 218
50	Morpholine	Morpholine	H	> 225

[a]Male MF-1 mice were used in all studies ($n \geq 5$).

Table 4. $GABA_A$ receptor modulatory effects of selected compounds

Compd	Series	TBPS IC_{50} μM[a]	HD_{50} (μmol kg^{-1})[b]
Propofol, **1**	N/A[c]	18	68.0
Racemic **2**	N/A[c]	29.3	21.7
11	Sulphide	9.7	18.2
12	Sulphide	6.9	22.6
13	Sulphide	4.0	< 25
15	Sulphide	2.7	31.4
19	Sulphone	6.2	39.3
31	Ester	4.2	22.9
33	Ester	11.7	26.4
37	Ester	14.7	48

[a]Values are means of three experiments.
[b]Male MF-1 mice were used in all studies ($n \geq 5$).
[c]N/A = not applicable.

Conclusion

Many of the heteroatom side-chain containing compounds described in this paper display potent intravenous anaesthetic activity, which may be mediated by a GABAergic mechanism. The most potent compounds synthesised belonged to the sulphide **11** ($HD_{50} = 18.2$ μmol kg^{-1}) and ester **31** ($HD_{50} = 22.9$ μmol kg^{-1}) series and compared favourably with propofol **1** ($HD_{50} = 68$ μmol kg^{-1}) and the racemate of **2** ($HD_{50} = 21.7$ μmol kg^{-1}). Further optimisation of this series of α-amino-acid phenolic ester derivatives is ongoing and will be reported in the future.

Acknowledgements

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6. Example of sulphide synthesis: butanoic, 2-(1-piperidinyl)-4-(methylthio), 1-(2,6-dimethoxyphenyl)ester hydrochloride **13**. To a stirred solution of DL-methionine (85.4 g) in water (792 mL) and hydrobromic acid (47% aq, 528 mL) at 0 °C was added a solution of sodium nitrite (39.5 g) in water (100 mL). Stirring was continued for 2 h at 0 °C and then allowed to warm to room temperature and stand for 24 h. The aqueous phase was extracted with ethyl acetate (500 mL), dried (sodium sulphate), filtered and concentrated under reduced pressure to give butanoic acid, 2-bromo-4-(methylthio)-**6** (33 g, 27%) as an orange oil. To a solution of **6** (33 g) and pyridine (0.5 mL) in dichloromethane (50 mL) was added a solution of oxalyl chloride (27 mL) in dichloromethane (50 mL). The reaction mixture was stirred for 24 h and concentrated under reduced pressure. To the residue was added dichloromethane (100 mL), 2,6-dimethoxyphenol (23.9 g) and the solution was cooled to 0 °C. A solution of triethylamine (43 mL) in dichloromethane (50 mL) was then added dropwise and, after complete addition, the reaction mixture was allowed to warm to room temperature and stir for 2 h. Concentration under reduced pressure and chromatography on silica gel gave butanoic acid, 2-bromo-4-(methylthio), 1-(2,6-dimethoxy-

phenyl)ester (25.8 g, 48%) as an orange oil. Butanoic acid, 2-bromo-4-(methylthio), 1-(2,6-dimethoxyphenyl)ester (5 g) and piperidine (5.2 mL) were stirred together for 1 h. Chromatography on silica gel gave butanoic acid, 2-(1-piperidinyl)-4-(methylthio), 1-(2,6-dimethoxyphenyl)ester (1.3 g, 26%) as a solid. 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 off to give the title compound **13** as a white solid. Positive ion ESI $(M+H)^+$ 353.8. ^1H NMR ($CDCl_3$ + sodium carbonate); δ 1.42–1.51 (2H, m), 1.52–1.70 (4H, m), 2.01–2.11 (1H, m), 2.15 (3H, s), 2.17–2.26 (1H, m), 2.60–2.77 (4H, m), 2.80–2.88 (2H, m), 3.61 (1H, t), 3.81 (6H, s), 6.61 (2H, d), 7.12 (1H, t). Sulphides **11–12** and **14–18**, sulphones **19–23** and sulphoxides **24–25** were synthesised in a similar manner.

7. Example of ester synthesis: butanedioic acid, 1-(2,6-dimethoxyphenyl)ester, 2-(4-morpholinyl)-, 4-methyl ester hydrochloride **33**. A solution of maleic anhydride (25.3 g) in methanol (100 mL) was heated at reflux for 0.5 h with stirring, then cooled to 0 °C with an ice/acetone bath. Piperidine (67.5 mL) was added and the reaction mixture was heated to 50 °C for 1 h. Sodium hydroxide (9.2 g) was added and the resulting suspension was stirred for 1 h. The reaction mixture was concentrated under reduced pressure and the resulting solid was filtered off and washed with ethyl acetate (300 mL) to give butanedioic acid, 2-(1-piperidinyl)-, 4-methyl ester, sodium salt (43 g, 79%) as a white solid. To this material (10 g) was added 2,6-dimethoxyphenol (8.5 g), dichloromethane (100 mL), 4-dimethylamino pyridine (5.2 g), triethylamine (11.7 mL) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (10.5 g). The resulting suspension was stirred overnight then chromatographed on silica gel. The resulting solid was washed with diethyl ether (100 mL) to give butanedioic acid, 1-(2,6-dimethoxyphenyl)ester, 2-(1-piperidinyl)-, 4-methyl ester (3.3 g, 22%) as a white solid. 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 **33**. Positive ion ESI $(M+H)^+$ 353.8. ^1H NMR ($CDCl_3$ + sodium carbonate); δ 2.68–2.83 (3H, m), 2.90–3.01 (3H, m), 3.64–3.78 (7H, m), 3.82 (6H, s), 4.08 (1H, t), 6.62 (2H, d), 7.15 (1H, t). Esters **31–32** and **34–40** and amides **41–49** were synthesised in a similar manner.

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