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Oxyaniliniums as Acetylcholinesterase Inhibitors for the Reversal of Neuromuscular Block

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Abstract—A series of oxyanilinium-based AChE inhibitors have been synthesised and tested for the reversal of vecuronium-induced neuromuscular block. Several compounds, for example 2-hydroxy- and 2-methoxy-*N*,*N*-dimethyl-*N*-allylanilinium bromide (**3** and **6**) showed comparable reversal potencies to edrophonium and clean in vivo cardiovascular profiles.

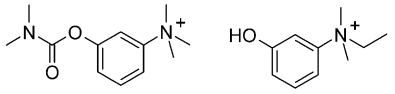
As well as being useful therapeutic agents for Alzheimer's disease, myasthenia gravis, glaucoma and so on, acetylcholinesterase (AChE) inhibitors are important drugs widely used in anaesthetic practice to reverse the skeletal muscle relaxation induced by nondepolarising neuromuscular blocking agents (NMBA).[1] By inhibiting the breakdown of acetylcholine (ACh), AChE inhibitors such as neostigmine (**1**) and edrophonium (**2**) (Fig. 1) increase the levels of ACh in the synaptic clefts so to facilitate cholinergic neurotransmission in the neuromuscular junction and hence the recovery of muscle function.

However, the use of AChE-based NMB reversal agents is often associated with bradycardiac side effects due to non-selective activation of muscarinic receptors in the cardiac parasympathetic pathway by the elevated ACh levels. In clinical practice, NMB reversal agents have to be used in combination with muscarinic receptor antagonists such as atropine and glycopyrrolate to minimise cardiovascular (CV) side effects. Ironically, muscarinic receptor antagonists themselves can cause CV side effects such as tachycardia.

Although many believe CV side effects of NMB reversal agents are inevitable because of their mechanism of action, there is compelling evidence suggesting that effects that are not related to AChE inhibition, for example, direct activation of cardiac muscarinic ACh receptors, are at least in part responsible for the more severe bradycardiac side effects of neostigmine (**1**).[2] Edrophonium (**2**), an AChE inhibitor with no direct effects on ACh receptors, has been found to cause much less CV side effects. Edrophonium is, however, less potent than neostigmine as a NMB reversal agent and therefore is less widely used.

Within our program of finding a cardiovascularly cleaner NMB reversal agent, we were interested in water-soluble, fast-onset AChE inhibitors with potency similar to or better than neostigmine but CV side effects at least not more than those of the combination of neostigmine and glycopyrrolate.

In screening a commercial library of 240 quaternary amines (Microsource), we found a lead **3** (Table 1) that showed an IC_{50} of 1.85 μM against human recombinant AChE in vitro and an ED_{50} of 0.50 μmol/kg in reversing vecuronium-induced neuromuscular block in cats in vivo. Although compound **3** is less potent than neostigmine in reversing vecuronium-induced block, it has a



1 **2**

Figure 1. Structures of neostigmine (**1**) and edrophonium (**2**).

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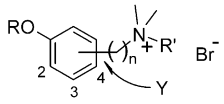
Table 1. In vitro AChE inhibitory activities of oxyphenyl- and oxybenzyl-ammonium bromides



Compd	Structure				AChE inhibition IC_{50} (μM)[a]	In vitro reversal GPHD, EC_{50} (μM)[b]
	R	R′	Y	*n*		
3	H	$CH_2CH{=}CH_2$	2	0	1.85	74
4	H	$CH_2CH{=}CH_2$	3	0	~3	13
5	H	$CH_2CH{=}CH_2$	4	0	>10	>3
6	CH_3	$CH_2CH{=}CH_2$	2	0	1–3	2.89
7	CH_3	$CH_2CH{=}CH_2$	3	0	>10	>3
8	CH_3	$CH_2CH{=}CH_2$	4	0	>10	>30
9	Ac	$CH_2CH{=}CH_2$	3	0	1–3	3.87
10	Ac	$CH_2CH{=}CH_2$	4	0	>10	>3
11	H	$CH_2C(CH_3){=}CH_2$	2	0	>10	>3
12	H	$CH_2C(CH_3){=}CH_2$	3	0	>10	>3
13	H	$CH_2C(CH_3){=}CH_2$	4	0	>10	>3
14	CH_3	$CH_2C(CH_3){=}CH_2$	2	0	>10	>3
15	CH_3	$CH_2C(CH_3){=}CH_2$	3	0	>10	>3
16	H	CH_2Ph	2	0	>10	>3
17	H	CH_2Ph	3	0	>10	>3
18	H	CH_2Ph	4	0	>10	>3
19	CH_3	CH_2Ph	2	0	>10	>3
20	CH_3	CH_2Ph	3	0	>10	>3
21	CH_3	CH_2Ph	4	0	>10	>3
22	Ac	CH_2Ph	2	0	>10	>3
23	Ac	CH_2Ph	3	0	>10	>3
24	Ac	CH_2Ph	4	0	>10	>3
25	H	$CH_2CH_2CH{=}CH_2$	2	0	~10	>10
26	H	$CH_2CH{=}CH_2$	3	1	>10	>3
27	CH_3	$CH_2CH{=}CH_2$	3	1	>10	>3
28	H	CH_2Ph	3	1	>10	n.t.
29	CH_3	CH_2Ph	3	1	>10	n.t.
30	H	CH_3	3	1	>10	>3
31	CH_3	CH_3	3	1	>10	n.t.
Edrophonium	H	CH_2CH_3	3	0	3.96	2.77
Neostigmine	$(CH_3)_2NCO$	CH_3	3	0	0.002	0.028

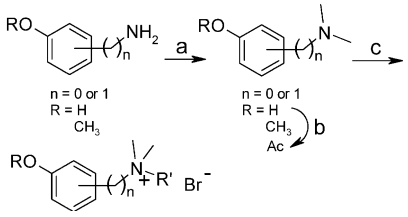
[a]Measured by the colorimetric method according to Ellman et al.[4] in 96-well microtitre plate formate using ACh as the substrate and human recombinant AChE as the enzyme source. Values are means of three experiments, standard deviation is given in parentheses.
[b]n.t., not tested.

much cleaner CV profile than neostigmine with maximum changes of <10% in all hemodynamic parameters. In this paper, we would like to report our attempt to optimise this lead aiming at increasing the reversal potency whilst maintaining its clean CV profile.

Compounds in Table 1 were synthesised by reductive methylation of a corresponding aniline or benzylamine, followed by quaternisation with an alkyl bromide (Scheme 1). Most of starting anilines and benzylamines are commercially available. 2-Methoxy-*N*,*N*-dimethylaniline, the intermediate for the preparation of compounds **6**, **14** and **19**, was prepared by direct amination of veratrole with lithium dimethylamide in THF.[3] The yield (not optimised) was about 22%. 3-Hydroxy-*N*,*N*-dimethylbenzylamine, the starting material for compounds **26**, **28** and **30**, was prepared by reductive amination of 3-hydroxybenzaldehyde with dimethylamine and $NaBH(OAc)_3$ (yield: ~96%).

As shown in Table 1, the SAR of this series is very tight and does not allow much structural manipulation. The only interesting compound of this series is **6** which exhibited similar AChE inhibitory potency to that of the lead **3** but showed higher in vitro potency in reversing vecuronium-induced block in guinea pig hemidiaphram.

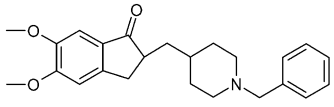
From the X-ray crystal structure of *Torpedo* AChE–edrophonium complex,[5] it was known that edrophonium only interacts with the bottom part of the 20-Å long active site gorge of AChE, that is the quaternary N interacting with the indole of Trp-84 and its *m*-hydroxy showing bifurcated H-bonding to two members of the catalytic triad, Ser-200 and His-440. The upper half of the active site gorge is not occupied by edrophonium. Since the structures of the current series are very similar



Scheme 1. (a) HCHO, $NaBH_4$, CH_3OH, rt, yields: 72–96%; (b) AcCl, Hunigs base, DCM, rt, yields: >90%; (c) R′Br, CH_3CN (for the phenols) or refluxing acetone or neat (for the rest), yields: 11–98%, all quats were isolated by simple filtration.

to that of edrophonium, it is not unreasonable to believe that they, too, bind to the bottom part of the enzyme active site. We reckoned that the AChE inhibitory activity could be increased if we could increase the size of the inhibitor so to have full occupancy of the active site.

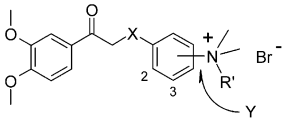
Donepezil (**32**) is one of the most potent AChE inhibitors in clinical use (IC_{50} ~6 nM). Several computer modelling studies have indicated that the molecule binds to the active site gorge in its extended conformation with full occupancy of the narrow active site gorge.[6] The protonated N of donepezil, like that of edrophonium, forms a cation–π interaction with Trp-84 at the bottom of the gorge and the indanone interacts with aromatic residues near the entrance of the gorge, for example Trp-279.[6] The two methoxyls of donepezil are exposed to water. This mode of interaction was later supported by the X-ray crystal structure of donepezil–AChE complex although Phe-330 seemed more important than Trp-84 in forming the interaction with the protonated N.[7]



32

We therefore further modified this series of oxyaniliniums by substituting the benzene ring with a group that is similar in length to the dimethoxy-indanone-2-methyl of donepezil, that is 3,4-dimethoxy-phenylethoxy or -phenylpropyl (**33**–**41**, Table 2). As illustrated in Schemes 2 and 3, these compounds were synthesised in two different ways. The ether derivatives **33**–**37** were prepared by alkylation of dimethylaminophenols with α-bromo-3,4-dimethoxy-acetophenone in acetone, followed by quaternisation (Scheme 2) and their methylene analogues **38**–**41** were synthesised by Aldol condensation between 3,4-dimethoxyacetophenone and an appropriately substituted benzaldehyde, followed by hydrogenation and quaternisation (Scheme 3).

Table 2. In vitro AChE inhibitory activities of extended anilinium bromides

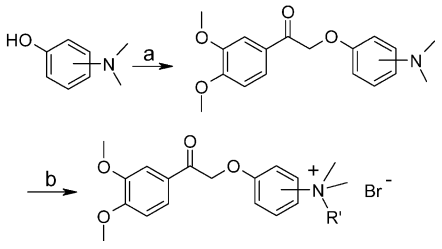


Compd	Structure			AChE inhib (IC_{50}, μM)	In vitro reversal (EC_{50}, μM)
	X	R′	Y		
33	O	CH_3	3	~10	7.08
34	O	CH_2Ph	3	0.33	1.69
35	O	CH_3	4	~10	9.8
36	O	CH_2Ph	4	~10	19.4
37	O	$CH_2CH{=}CH_2$	4	~1.0	0.5
38	CH_2	CH_3	3	>10	n.t.
39	CH_2	CH_2Ph	3	1.04±0.01	>3
40	CH_2	CH_3	4	3–10	2
41	CH_2	CH_2Ph	4	1.3±0.04	4.11

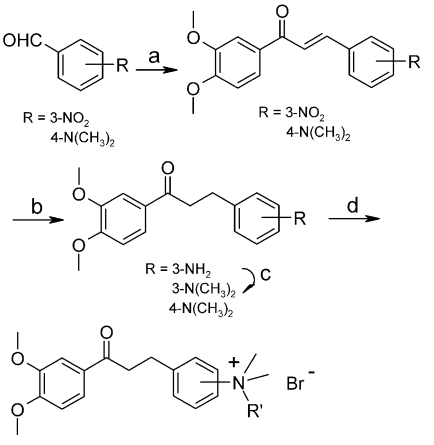
As shown in Table 2, most benzyl quaternised compounds are more active than methyl quaternised analogues, indicating the importance of molecular size. One compound **34** showed >5.5-fold increased AChE inhibitory potency as compared with the lead **3** and it was also slightly more potent than edrophonium in reversing vecuronium-induced block in guinea pig hemidiaphgram in vitro.

When tested in anaesthetised cats,[8] **6** showed slightly improved reversal potency (ED_{50} 0.22 μmol/kg) as compared to the lead **3** (ED_{50} 0.50 μmol/kg). More importantly, both **3** and **6**, at their maximum reversal doses (2.10 and 1.28 μmol/kg, respectively), caused much less changes in haemodynamic parameters than either the combination of neostigmine plus glycopyrrolate or edrophonium plus atropine (Table 3). Compound **34**, on the other hand caused more pronounced CV effects than the standard combinations at 2.56 μmol/kg, the dose that gave maximum reversal.

In conclusion, we have identified two quaternary aniliniums **3** and **6** that reverse vecuronium-induced neuromuscular block in vitro and in vivo via the inhibition of AChE. In the in vivo cat model, both compounds showed cleaner CV profiles than the standard combinations of AChE inhibitors plus mAChR antagonists, which suggest they may potentially be useful NMB reversal agents.



Scheme 2. (a) α-Bromo-3,4-dimethoxyacetophenone, K_2CO_3, acetone, reflux, 4 h, yields: 26–40%; (b) R′Br, CH_3CN, rt, overnight, yields: 40–88%.



Scheme 3. (a) 3,4-Dimethoxyacetophenone, aq NaOH, EtOH, rt, overnight, yields: 60–68%; (b) H_2, PtO_2, EtOH/THF, yields: 78–90%; (c) HCHO, $NaBH(OAc)_3$, THF, yield: 90%; (d) R′Br, CH_3CN, rt, overnight, yields: 11–37%.

Table 3. Haemodynamic effects in anaesthetised cats of the test compounds at doses producing full reversal of vecuronium-induced neuromuscular block

Compd	Dose (μmol/kg)	Haemodynamic changes (%)					
		MAP	HR	LVP	LV d*p*/d*t*	Vagus	Nic Memb
3 (4)	2.1±0.7	2±7	−1±1	12±5	8±3	5±11	−5±2
6 (1)	1.28	−24	−2	−9	−9	41	−2
34 (1)	2.56	56	8	63	82	5	34
Neo/Gly (2)	0.076/0.011	−22±1	4±5	−14±1	−12±1	62±2	−6±2
Edro/atr (3)	1.026/0.089	22±7/−12±6	2±2	23±6/−6±5	20±6/−5±4	−67±15	−7±1

Number of experiments in parentheses beside compound. Compounds **3**, **6** and **34** were administered by cumulative bolus dosing to produce complete reversal of neuromuscular block, dose increments being administered immediately following the maximum effects from the preceding dose. Neo/Gly and Edr/atr were administered as a single bolus dose sufficient to produce full reversal of neuromuscular block.

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8. Reversal experiments were carried out in female cats (1.9–3.0 kg) anaesthetised with a mixture of α-chloralose 80 mg kg^{-1} and sodium pentobarbitone 5 mg kg^{-1} ip. Neuromuscular block was induced using a bolus followed by an infusion of vecuronium. The infusion rate was adjusted to provide a stable 85–95% depression of tibialis anterior muscle twitch height. After 10–15 min stable block, the infusion was switched-off and the twitches allowed to recover. Two hours after full recovery, stable block was induced again as above. Immediately after switching-off the infusion, test drug was administered and the effects on neuromuscular (twitch), autonomic vagal and nictitating membrane, arterial pressure, heart rate, LV pressure and LV d*p*/d*t* responses were recorded.