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Quaternary Salts of E2020 Analogues as Acetylcholinesterase Inhibitors for the Reversal of Neuromuscular Block

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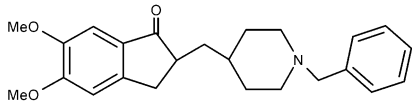
Abstract—A series benzylpiperidinium and benzylpyridinium quaternary salts have been synthesised and tested for inhibition of acetylcholinesterase and reversal of neuromuscular block induced by vecuronium. Several potent reversal agents have been identified and their haemodynamic effects measured.

Despite the continued widespread use of neostigmine as a reversal agent for neuromuscular block in surgical anaesthesia, there exists a need for an equally effective reversal agent devoid of cardiovascular (CV) side effects associated with neostigmine.[1] As a part of our programme[2] to develop water soluble reversal agents with reduced cardiovascular side effects, we were interested in quaternary amines as acetylcholinesterase (AChE) inhibitors. The advantages of such a permanently charged quaternary amino compound are that it is more water soluble and less likely to penetrate to the CNS than the corresponding tertiary amine. We started our lead finding by comparing a diverse range of known AChE inhibitors[3] with their *N*-methyl quaternised analogues. As shown in Table 1, all *N*-methyl quaternary derivatives are more potent than their corresponding tertiary amine counterparts at reversing vecuronium-induced neuromuscular block in anaesthetised cats[4] (*N*-methyl huperazine A was not tested due to the lack of materials). Ease of synthesis and greater scope for optimization led to the the *N*-methyl derivative of E2020 (aricept, donepezil)[5] being chosen for further investigations.[6] In this paper, we report the lead optimization that has resulted in several potent and water-soluble reversal agents.



E 2020

En-route to E2020, we were able to isolate the intermediate aldol **1** which proved to be a potent reversal agent against vecuronium-induced neuromuscular block in anaesthetised cats (ED_{50} 0.21 μmol/kg, maximum reversal 109% at 1.28 μmol/kg) with the following CV side effects (maximum changes in MAP −22%, HR −12%, Vagus +18%). However, when tested in anaesthetized monkeys, **1** gave only partial reversal against vecuronium.

Due to its cleaner CV profile, especially in vagal potentiation, than the lead *N*-methyl E2020 (Table 1), we further investigated the structure–activity relationship of the hydroxyl group in compound **1**. Separation of the diastereoisomers **2** and **3** demonstrated that the *anti* isomer **3** was slightly more potent than the *syn* isomer **2** (Table 2) in reversing vecuronium-induced block in-vitro in guinea pig (GP) hemi-diaphragm. Since the indanone chiral center is prone to racemization, no attempt was made to resolve further enantiomers. In addition to the two chiral centers in the indanone methanol part of the molecule, the piperidinium part also gives rise to two isomeric forms of the quaternary amine, in which *N*-benzyl favours the anti isomer (equatorial benzyl group) by ~9:1.

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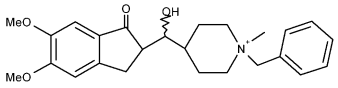
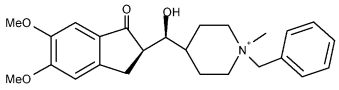
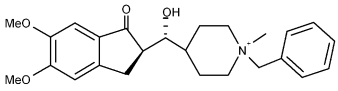
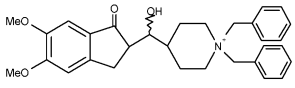
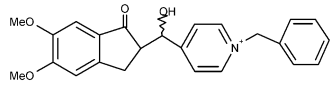
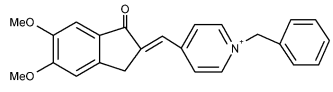
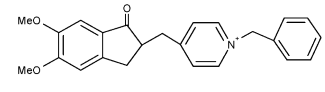
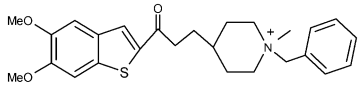
Table 1. Activities of some known AChE inhibitors and the corresponding *N*-methyl quaternary salts

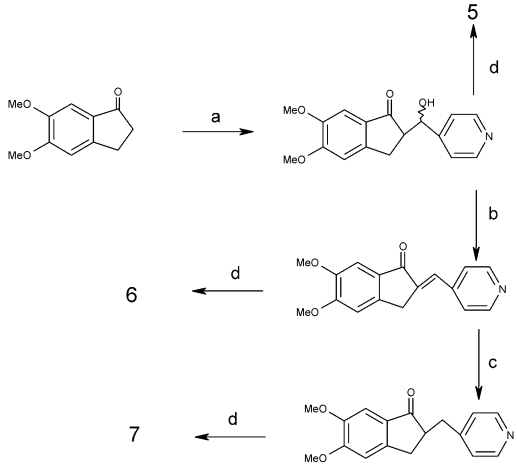
	AChE inhib[a] (IC_{50}, μM)	In-vitro GP[b] (EC_{50}, μM)	In-vivo cat[c] (ED_{50}, μmol/kg)	Max rev (%)	MAP[d] (Δ%)	HR[d] (Δ%)	Vagus[d] (Δ%)
E2020	0.018	0.043	0.53	106	−12	−12	+14
E2020 Quat	0.025	0.1	~0.06	131	−22	+11	+37
Gallanthamine	~0.5	1.1	n.t.	n.t.	n.t.	n.t.	n.t.
Gallanthamine Quat	~0.5	0.63	0.1	130	+16	−5	+80
Anseculin	0.085	1.45	9.7	61	−12	−9	−14
Anseculin Quat	<0.3	0.136	0.14	105	−20	−4	+25
Huperazine	0.03	0.019	0.08	156	−17	−8	+102
Huperazine Quat	>100	>10	n.t.	n.t.	n.t.	n.t.	n.t.
CP 118,954	<0.5	0.0047	0.12	140	−7	−6	+10
CP 118,954 Quat	<0.5	0.039	0.023	176	−11	−4	+15
TAK 147	<0.5	1.14	3.7	68	−11	−5	+8
TAK 147 Quat	<0.5	0.331	0.15	140	−6	+4	+10

Quaternary salts were prepared by reaction with methyl bromide or iodide (huperazine was di-methylated, then quaternised). n.t., not tested.
[a]Method according to Ellman et al.[7] using human recombinant AChE as enzyme source.
[b]GP diaphragm.
[c]Chloaralose anaesthetised cat.[4]
[d]% change in cardiovascular parameters at ED_{50}; MAP, mean arterial pressure; HR, heart rate; Vagus, change in response to vagal stimulation.

Table 2. Stereoselectivity and effects of reducing number of asymmetric centers in dimethoxyindanone series

	Structure	GP reversal (EC_{50}, μM)
1		1.44
2		2.7
3		0.89
4		43.5
5		1.88
6		0.012
7		0.1
8		0.019

The problem of quaternary amine isomers can be tackled by using identical alkyl groups or by replacing the piperidinium with pyridinium. When both piperidinium *N*-substituents are benzyl, the resulting compound **4** is a much weaker reversal agent, but when the piperidinium is replaced by pyridinium, the resulting compound **5** (Scheme 1) retains the reversal potency (Table 2).

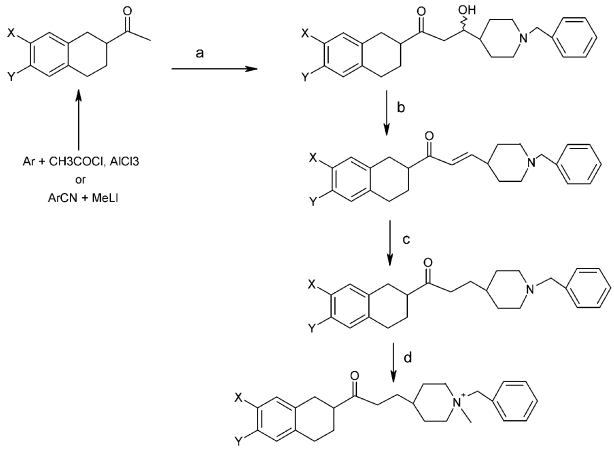


Scheme 1. Synthesis of dimethoxyindanone benzylpyridinium salts: (a) 4-pyridinecarboxaldehyde, LDA −78 °C; (b) HCl gas; (c) H_2, Pd; (d) benzyl bromide.

Dehydration of the aldol to the corresponding en-one derivative **6** resulted in a marked increase in in-vitro reversal potency. Hydrogenation of the exocyclic double bond **7**, however, reduced the in-vitro activity compared with **6**. The results of these structural manipulations seem to suggest that the indanone methanol moiety functions as a 'linker' to connect the two recognition fragments of dimethoxybenzene and piperidinium.

Literature data have already demonstrated the importance of the indanone carbonyl to the AChE inhibitory activities of E2020 analogues,[8] probably by forming a hydrogen bond within the enzyme active site. Since the indanone methanol moiety plays only the linker role to connect the dimethoxybenzene and piperidinium moieties, we decided to replace the indanone carbonyl by an exocyclic ketone, thus eliminating this chiral center.

A series of piperidiniums were synthesized from readily available methylarylketones (Scheme 2) by Aldol condensation with 1-benzylpiperidine-4-carboxaldehyde,



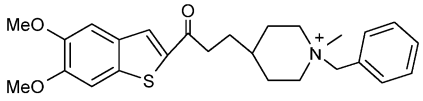
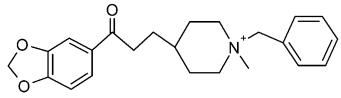
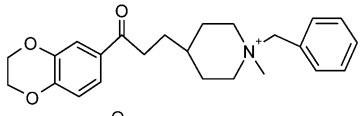
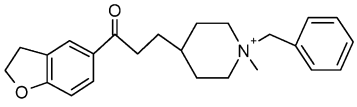
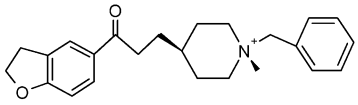
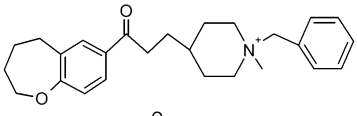
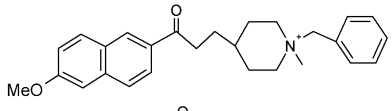
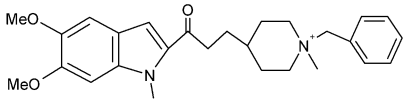
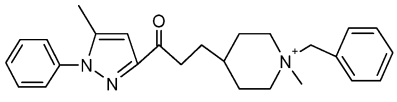
Scheme 2. Synthesis of benzylpiperidinium quaternary salts: (a) 1-benzyl-4-formylpiperidine, nBuLi −78 °C; (b) HCl gas; (c) H_2, Pd; (d) CH_3I or CH_3Br.

followed by dehydration, hydrogenation and quaternisation. From these piperidiniums with an exocyclic ketone group, the dimethoxybenzothiophene **8** was identified as a potent AChE inhibitor (IC_{50} 0.008 μM).

Our investigations were then extended to the synthesis of a variety of readily accessible aromatic components having an exocyclic ketone group in combination with the piperidinium salts linked by the three carbon chain unit (Table 3) (**9**–**16**) (Scheme 2) This series of compounds confirmed the general finding that those compounds with the saturated linker retained the best activity profiles. Water solubility was dependent on the lipophilicity of the aromatic component. Surprisingly, the di-oxygenated analogues **9**,**10** were less water soluble than the equivalent dihydrobenzofuran **11**. The minor syn quaternary isomer **12** was isolated by chromatography and found to be much more water soluble (>10.0 mg/mL vs 1.5), but with a 4-fold loss in AChE inhibition. As expected, the naphthalene derivative **14** was poorly water soluble, while the *N*-methyl indole **15** and the 1-phenylpyrazole **16** analogues showed modest water solubility while retaining good AChE inhibition (0.022 μM), in the case of **16**.

In conclusion, we have prepared a diverse series of benzylpiperidinium quaternary salts with nano molar inhibition of AChE and potent reversal of vecuronium-induced neuromuscular block (in-vitro GP, in-vivo anaesthetised GP, and in-vivo anaesthetised cat).

Table 3. Benzylpiperidinium quaternary salts with alternative aromatic substituents

	Structure	AChE inhib (IC_{50}, μM)	In-vitro GP (EC_{50}, μM)	H_2O solubility (mg/mL)
8		0.008	0.019	2.0
9		0.24	1.7	0.5
10		0.1	>3.0	<0.5
11		0.017	0.09	1.5
12		0.066	n.t.	>10.0
13		0.139	n.t.	5.0
14		0.05	0.53	<1.0
15		0.12	1.6	1.2
16		0.022	0.43	2.0

The dimethoxybenzthiophene derivative **8** remained the most promising lead and was subsequently studied in more detail, as reported in the following paper.[9]

Acknowledgements

We would like to thank K. Anderson, F. Hope and R. Mason for biological testing of the compounds and our colleagues in the Department of Analytical Chemistry for NMR, HPLC and MS data and interpretation.

References and Notes

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4. Female cats (1.9–3.0 kg) were anaesthetised with a mixture of α-chloralose (80 mg/kg) and sodium pentobarbitone (5 mg/kg) and neuromuscular block induced with a bolus dose of vecuronium, then maintained by infusion. The infusion rate was adjusted to provide stable 85–90% block of the tibialis anterior twitch height. After 10–15 min stable block, the infusion was switched off and the twitches allowed to recover. Two h after full recovery, stable block was again induced as above. Immediately after switching off the vecuronium infusion, the test substance was administered and the effects on recovery of NM function, heart rate, arterial pressure and autonomic vagal responses were recorded.
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