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Novel Piperidinium and Pyridinium Agents as Water-Soluble Acetylcholinesterase Inhibitors for the Reversal of Neuromuscular Blockade

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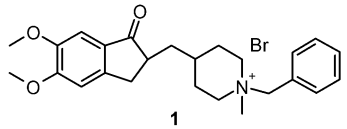
Abstract—A series of piperidinium and pyridinium agents containing a common structural fragment of 5,6-dimethoxybenzothiophene have been synthesised as water-soluble acetylcholinesterase inhibitors. Several compounds, for example **42** (AChE IC_{50} 0.03 μM) have been found to reverse the neuromuscular blockade induced by vecuronium bromide in vitro and in vivo. Coupled with their high water solubility (up to 30–60 mg/mL), these compounds are potentially useful as intravenous reversal agents of neuromuscular blocking agents in surgical anaesthesia.

Acetylcholinesterase (AChE) inhibitors[1] continue to have widespread use as therapeutic agents in disease areas[2] such as Alzheimer's disease, myasthnia gravis and glaucoma. In addition, they are also widely used in anaesthetic practice to reverse the skeletal muscle relaxation induced by non-depolarising neuromuscular blocking agents (NMBA).[3] By inhibiting the hydrolysis of acetylcholine (ACh), AChE inhibitors increase the levels of ACh in the neuromuscular junction facilitating cholinergic neurotransmission and hence recovery of muscle functions.



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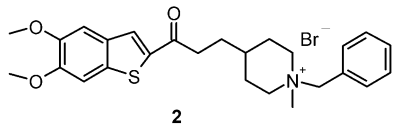

2

In our program to develop potent, water soluble reversal agents without cardiovascular liabilities, we have identified a series of piperidinium containing compounds, for example the quaternary salt of E2020 (**1**, $IC_{50} = 0.25$ μM) as lead compounds.[4] Subsequent optimisation of the dimethoxy-1-indanone moiety of **1** led to the discovery of benzothiophene **2** which had a marked improvement in AChE inhibition ($IC_{50} = 0.008$ μM) and one less asymmetric center than the lead **1**.

In this paper, we report our attempt to optimise **2** aiming at maintaining AChE inhibitory potency, increasing water solubility and removing the potential problems associated with stereoisomers of the quaternary salt.

The initial study began by optimising the linker unit (W-X-Y-Z) between the benzothiophene and the piperidinium ring (Table 1). The synthesis of benzothiophene analogues **2**, **6**, **7**, and **8** is outlined in Scheme 1. Standard aldol conditions with 2-acetyl-5,6-dimethoxy-1-benzothiophene **3** and the appropriate aldehyde afforded the aldol adducts **4**, which upon quaternisation with methyl bromide afforded **6** and **7**. Dehydration of the aldol products with HCl in methanol gave the alkenes, which under hydrogenation conditions afforded the saturated analogues **5**, which after quaternisation gave **2** and **8**. Quaternisation of these piperidines with methyl bromide afforded mixtures of isomers in favour of the syn methyl isomer (as determined by ^{1}H NMR). The

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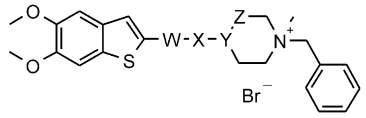
PII: S0960-894X(02)00483-3

Table 1. In vitro activity and water solubility of benzothiophene-containing piperidiniums



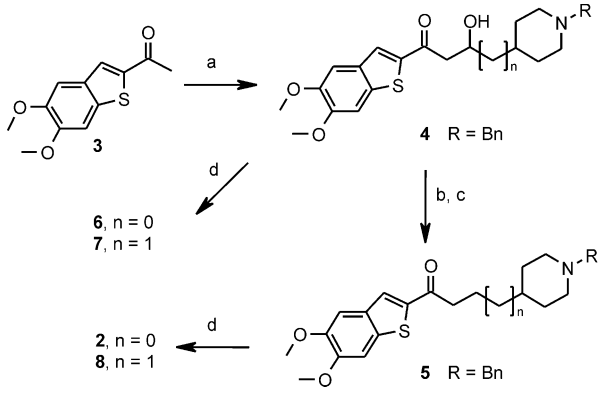
Compd	Quaternary isomer ratio (Me, *syn*/*anti*)	W	X-Y-Z	AChE inhibition IC_{50} (μM)[a]	Rev. EC_{50} (μM)	Solubility (mg/mL)[b]
2	95:5	CO	CH_2CH_2–CH–CH_2	0.008	0.019	2
6	97:3	CO	$CH_2C(OH)$–CH–CH_2	0.043	0.17	10
7	20:1	$C(O)CH_2$	$C(OH)CH_2CH_2$–CH–CH_2	0.38	6.6	2.5
8	95:5	$C(O)CH_2$	$CH_2CH_2CH_2$–CH–CH_2	0.11	>10	1
9	5:95	CO	$CH_2C(OH)$–CH–CH_2	0.19	—	10
10	85:15	CO	$CH_2C(OCH_3)$–CH–CH_2	0.12	0.65	1.5
11	95:5	CO	$CH_2C(OH)$–CH–CH_2 (+)-Isomer	0.219	No data	10
12	90:10	CO	$CH_2C(OH)$–CH–CH_2 (−)-Isomer	0.665	No data	8
14	95:5	CO	CH_2–C(OH)–CH_2	0.09	9.43	1.25
17	—	CO	HC=C–CH_2	0.52	7.67	25–30
18	—	CO	CH_2–CH=CH	0.75	>30	20–25
21	85:15	—	C(OH)–CH–CH_2	19.58	>30	2–3
23	—	—	HC=C–CH_2	2.67	>30	1–2
25	86:14	—	CH_2–CH–CH_2	10–50	>30	3

[a]Measured by the colorimetric method according to Ellman et al.[5] in 96-well microtitre plate format using ACh as the substrate and human recombinant AChE as the enzyme source. Values are means of three experiments.
[b]Measured in water.

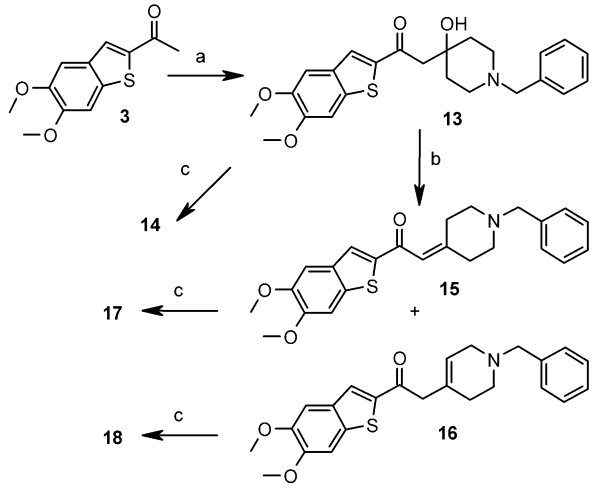
ratios of these quaternary salt isomers vary from compound to compound and are listed in Table 1.

To evaluate the difference between isomers of the quaternary salts in AChE inhibition, we prepared **9** enriched with the methyl anti isomer (Table 1). This was prepared in a similar manner as outlined in Scheme 1 but starting with 1-methyl-4-formyl piperidine (R = Me) as the aldehyde in the aldol reaction. After quaternisation with benzyl bromide, a 1:1 mixture of quaternary isomers was obtained. Extensive purification using silica gel chromatography followed by crystallisation from dichloromethane/ethanol gave **9**, which has a methyl *anti*/*syn* ratio of 95:5. Compound **9** showed approximately 4-fold decrease in AChE inhibition compared with **6** in which the *syn* methyl quaternary isomer is dominant, indicating the *syn* methyl isomer has the preferred conformation for AChE inhibition. As far as water solubility is concerned, the hydroxyl containing compounds **6** and **9** are more water soluble (10 mg/mL) than the alkane **2**, but >5–20-fold less active in AChE inhibition than **2**. Methylation of the hydroxyl resulted in **10** with even further decreased AChE inhibitory activity. The enantiomers of the aldol product (**11** and **12**) were separated by chiral HPLC and showed slight enantioselectivity in AChE inhibition in favour of the (+)-isomer **11**. However, compared with the alkane derivative **2**, the gain in water solubility in these OH-containing compounds is counterbalanced by the loss in their inhibitory potency against AChE.

The linker (W-X-Y-Z) was made shorter as outlined in Schemes 2 and 3. Treating the enolate of **3** with 1-benzyl-4-piperidone gave the spiro alcohol intermediate **13**, which upon quaternisation with methyl bromide afforded **14** (Scheme 2). Elimination of the hydroxyl unit resulted in two regioisomers, in which the double bond was either *exo* **15** or *endo*cyclic **16**. These were efficiently separated using silica gel chromatography and quaternised with methyl bromide to give **17** (*exo*) and **18** (*endo*).



Scheme 1. (a) Aldehyde, LDA, THF, DIPEA, −78 °C; (b) HCl gas, MeOH, 60 °C; (c) H_2, 10% Pd/C, 25 °C; (d) CH_3Br, CH_3CN, 25 °C.



Scheme 2. (a) 1-Benzyl-4-piperidone, LDA, THF, DIPEA, −78 °C; (b) HCl gas, MeOH, 60 °C; (c) CH_3Br, CH_3CN, 25 °C.

Compound **14** showed a 2-fold decrease in AChE inhibition compared to **6**. Both *exo* and *endo* compounds (**17** and **18**) were shown to have poor AChE inhibition (0.52 and 0.75 μM, respectively) but surprisingly greater water solubility.

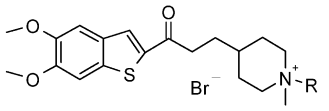
Further shortening the length of the linker (Scheme 3) by reacting the anion of 5,6-dimethoxy-1-benzothiophene **19** with 1-benzyl-4-piperidine acetaldehyde gave the secondary alcohol **20**, which upon quaternisation resulted in **21**. Elimination of the hydroxyl unit to the alkene **22** and subsequent quaternisation gave **23**. Reduction of the double bond gave **24**, which after quaternisation with methyl bromide resulted in **25**.

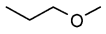
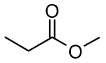
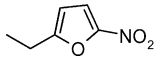
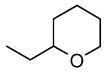
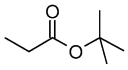
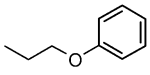
Shortening the linker further caused dramatic reduction in AChE inhibition, although lack of a carbonyl capable of forming hydrogen bond may also be a reason.[6] All these compounds are less active than **2** and **6**, suggesting the propanone is the optimum linker.

We went on to investigate the possibility of increasing water solubility by replacing the lipophilic benzyl at the quaternary nitrogen with smaller and/or more polar groups. Table 2 lists the results of some of these modifications.

All the groups investigated gave varying mixtures of quaternary isomers with the methyl predominately *syn*. Of the groups studied the *N*-methoxyethyl and *N*-carboxymethyl derivatives (**26** and **27**) gave excellent water solubility (60 mg/mL) whilst retaining reasonable AChE inhibitory potency, $IC_{50} = 0.054$ and 0.53 μM, respectively. The 2-nitrofuran **28** and tetrahydropyran **29** derivatives gave the best AChE inhibition ($IC_{50} = 0.032$ and 0.02 μM, respectively), although water solubility was less than 2 mg/mL in both cases. Phenoxyethyl derivative **31** and cyanomethyl derivative **32** showed decreased AChE inhibition and poor water solubility.

Table 2. In vitro activity and water solubility of various *N*-alkylated benzothiophene-containing piperidinium salts

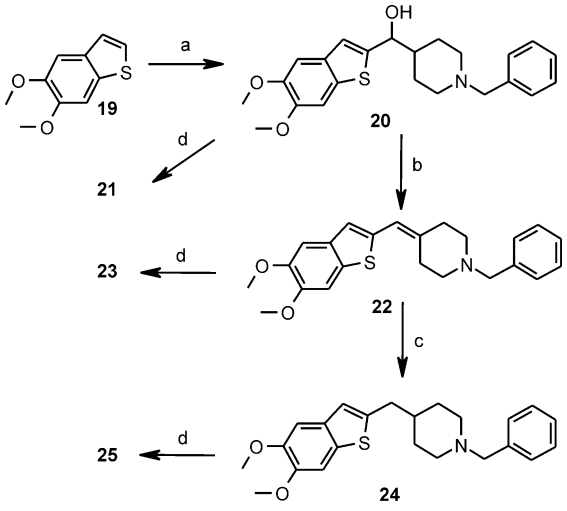


	Me Syn/Anti	R	AChE inhib[b] IC_{50} (μM)[a]	Solubility (mg/mL)[b]
26	2/1		0.053	60
27	54/46		0.054	60
28	1/1		0.032	0.6
29	65/35		0.028	1.5
30	5/1		0.079	1.5
31	3/2		0.39	0.9
32	70/30		>1.0	2

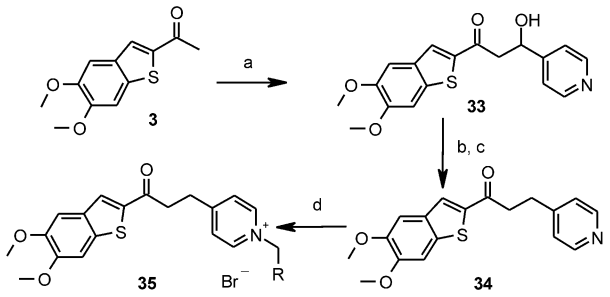
[a]Measured by the colorimetric method according to Ellman et al.[5] in 96-well microtitre plate format using ACh as the substrate and human recombinant AChE as the enzyme source. Values are means of three experiments.
[b]Measured in water.

We then decided to replace the piperidine moiety with a pyridinium group so to maintain the water solubility and avoid the difficulty in separating the quaternary isomers of piperidiniums.

By using different small and/or polar alkylating agents, we prepared a series of pyridinium salts (Table 3). The key intermediate 5,6-dimethoxy-2-(3-pyridin-4-yl)propionylbenzothiophene **34** was prepared in a similar way as described above for the piperidiniums but starting with pyridine-4-carboxaldehyde as the aldehyde in the aldol reaction with **3** (Scheme 4). The alcohol intermediate **33** was mesylated to facilitate the elimination of the hydroxyl unit. Subsequent reduction gave the key

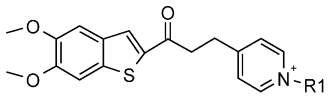


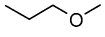
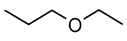
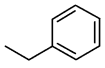
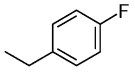
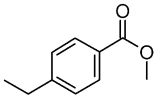
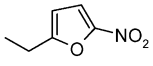
Scheme 3. (a) 1-Benzyl-4-piperidine acetoaldehyde, LDA, THF, DIPEA, −78 °C; (b) HCl gas, MeOH, 60 °C; (c) H_2, 10% Pd/C, 25 °C; (d) CH_3Br, CH_3CN, 25 °C.



Scheme 4. (a) Pyridine-4-carboxaldehyde, LDA, THF, DIPEA, −78 °C; (b) MsCl, Et_3N, CH_2Cl_2; (c) H_2, 10% Pd/C, MeOH-THF 25 °C; (d) RCH_2Br, CH_3CN, 60 °C.

Table 3. In vitro activity and water solubility of various *N*-alkylated benzothiophene-containing pyridinium salts



	R1	AChE inhib. IC_{50} (μM)[a]	Rev EC_{50} (μM)	Solubility (mg/mL)[b]
36	Me	0.9	2.5	3
37		0.28	>3.0	25
38		0.54	>3.0	25
39		2.57	No data	16
40		0.11	>3.0	15
41		0.04	1.72	6
42		0.03	0.29	33
43		0.007	No data	50
44		0.0046	0.099	2
45		0.0026	1.65	1–2
46		1.0	1.67	3.5
47		0.006	2.7	2
48		0.0045	2.67	35

[a]Measured by the colorimetric method according to Ellman et al.[5] in 96-well microtitre plate format using ACh as the substrate and human recombinant AChE as the enzyme source. Values are means of three experiments.
[b]Measured in water.

intermediate **34**. Quaternisation was conducted in acetonitrile at 60 °C to afford the pyridinium salt **35**.

As shown in Table 3, simple alkyl groups such as methyl **36**, ethyl **37** and allyl **38** have decreased AChE inhibition and in particular the in vitro reversal activity against vecuronium-induced block in guinea pig hemi-diaphragm. Introduction of a choline motif, for example, **42** and **43**, improved the AChE inhibition (IC_{50} = 0.03 μM and 0.007 μM, respectively) and dramatically increased the water solubility (33 and 50 mg/mL, respectively). The in vitro reversal potency of **42** (only measured for this compound) was also improved in comparison with the alkyl analogues (**36**–**41**).

N-Benzyl pyridinium **44** (the equivalent of **2** in the piperidinium series) showed high potency in AChE inhibition (IC_{50} = 0.0046 μM) and excellent in vitro reversal activity against vecuronium-induced block in isolated guinea pig hemi-diaphragm (EC_{50} = 0.099 μM). However, its water solubility is low at 2 mg/mL. Para-substitution on the benzene ring with a fluorine **45** enhanced the AChE inhibition but reduced the in vitro reversal activity probably due to low water solubility. Para-carboxyl substitution **46** improved slightly the water solubility but reduced both AChE inhibitory and in vitro reversal activities. Heteroaromatics such as the thiophene **47** and 2-nitrofuran **48** gave excellent AChE inhibition (IC_{50} = 0.006 and 0.007 μM, respectively), but their in vitro reversal potency proved disappointing.

In conclusion, we have identified a series of novel piperidiniums and pyridiniums that are potent inhibitors of AChE and as such reverse vecuronium-induced neuromuscular block in vitro and in vivo. Several of these compounds have good levels of water solubility (up to 30–50 mg/mL) and are compatible with intravenous formulation. The pyridiniums, specially those with a choline motif at the pyridine nitrogen, for example, **42** and **43**, have the additional advantage of being achiral in comparison with the piperidiniums. They are therefore devoid of potential difficulties of isomeric purification and are feasible for large scale production.

Acknowledgements

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References and Notes

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