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Synthesis and Anti-Acetylcholinesterase Activity of 1-Benzyl-4-[(5,6-dimethoxy-1-indanon-2-yl)methyl]piperidine hydrochloride (E2020) and Related Compounds

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In the previous paper,[1] 1-benzyl-4-{2-[N-(4'-benzylsulfonyl)benzoyl-N-methylamino]-ethyl}piperidine hydrochloride (**1**) was shown to be a potent acetylcholinesterase (AChE) inhibitor. The structure-activity relationship (SAR) of the rigid compounds, 1-benzyl-4-[2-(N-phthalimid-1-yl)ethyl]piperidine hydrochlorides (**2**) has been reported.[2] These results show that the amide and N-benzylpiperidine moieties are required for biological activity. Our findings also indicate that a variety of atoms (X=N, C) is tolerated at the amide nitrogen position. In order to explore further these structural requirements, indanone derivatives (**3**, X=C) were synthesized (Fig. 1). The results of our study on the synthesis, SAR and pharmacology of these are described herein.

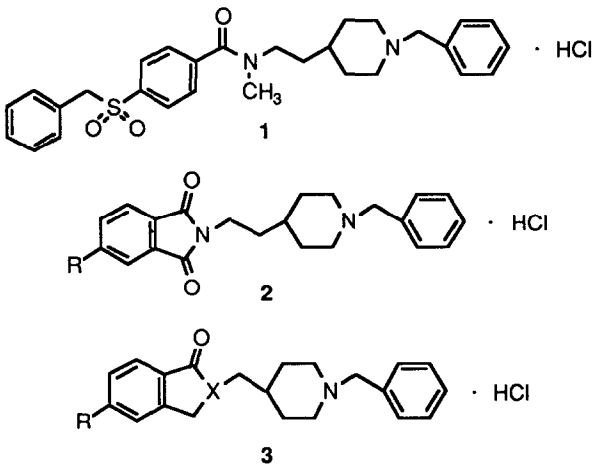
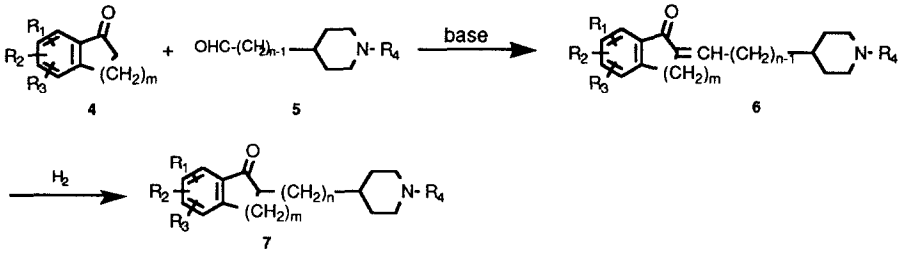


Figure 1

Chemistry: The general synthetic route to the indanone derivatives is shown in Scheme 1.[3] Compounds can be prepared by the following process. Diisopropylamine and n-butyllithium/hexane were added to tetrahydrofuran. A substituted 1-indanone (**4**) and hexamethylphosphoramide were added thereto at a temperature of about -80°C. The resulting enolate was reacted with 1-substituted-4-(ω-formyl alkyl)piperidines (**5**). The crude reaction mixture was subjected to dehydration, thereby producing compounds **6.** This compound was hydrogenated over 10% palladium carbon, at room temperature, at atmospheric pressure, to give compounds **7**.

Scheme 1

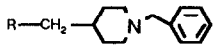


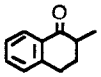
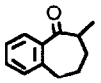
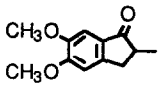
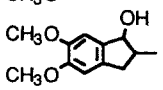
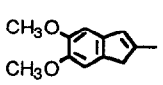
Structure-Activity Relationships: A new series of indanone derivatives and related compounds was tested for biological activity in vitro and ex vivo. A mouse brain homogenate was used as the AChE source and AChE activity was determined according to the method of Ellman et al.[4]

The effect of replacement of the indanone moiety in (**8**) with α-tetralone (**9**), 1-benzosuberone (**10**), 1-indanol (**11**) and 1-indene (**12**) was examined. Ring expansion of the cyclic ketone, compounds **9** and **10**, decreased anti-AChE activity. Introduction of the methoxy group at the 5 and 6-position on the indanone moiety (E2020) in compound **8** was found to greatly increase the activity. But the 5, 6-dimethoxy-1-indanol derivative (**11**) and 5, 6-dimethoxy indene derivative (**12**) poss had decreased potency (Table 1).

Table 2 shows that the anti-AChE activity of

Table 1 Anti-AChE Activity of 1-Benzyl-4-(1-substituted methyl)-piperidine derivatives



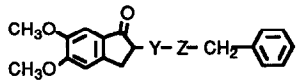
No	R	Inhibition of AChE IC50 (nM)
8		150
9		2100
10		15000
E2020		5.7
11		300
12		4400

5, 6-dimethoxy-1-indanone derivatives. The effects of modification of the linking group (Y) between the 5, 6-dimethoxy-1-indanone and piperidine moiety (Z) in E2020 were then examined, and it was found that their direct combination results in reduced potency (**13**). Introduction of an ethylene group (**17**) or a double bond (**16** or **18**) into the linking group also led to a reduction in potency. The nitrogen atom on the piperidine moiety is best located distal to at the point of attachment to the indanone ring, since compound **14** showed very low potency. Replacement of the piperidine with a piperazine ring in the E2020 structure was also found to decrease the potency (**15**).

The positional effect of methyl or nitro groups in the benzyl moiety in E2020 was examined, and it was found that the 3-position substituted benzyl derivatives (**20** and **23**) showed the highest potency, among the 2-, 3- and 4-substituted isomers. These compounds showed nearly equal potency to that of E2020 in the anti-AChE assay. The basicity of the nitrogen atom in the piperidine ring appears to have an important role in the degree of activity, since N-benzoylpiperidine (**25**) was almost inactive. Replacement of R_4 with hydrogen (**26**) caused a great reduction in the potency, but anti-AChE activity was retained after replacement with cyclohexylmethyl group (**27**). Replacement of R_4 with phenethyl group (**28**) resulted in decreased potency (Table 3).

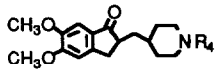
QSAR analyses and molecular shape analyses of E2020 have recently been reported. [5,6]

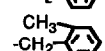
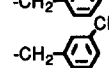
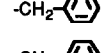
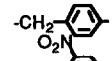
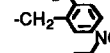
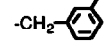
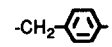
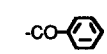
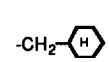
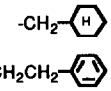
Table 2 Anti-AChE Activity of 5,6-Dimethoxy-1-indanone derivatives



No	Y	Z	Inhibition of AChE IC_{50} (nM)
13			3300
E2020	$-CH_2-$		5.7
14	$-CH_2-$		480
15	$-CH_2-$		94
16	$=CH-$		13
17	$-CH_2CH_2-$		30
18	$=CHCH_2-$		150

Table 3 Anti-AChE Activity of 1-Substituted-4-[(5,6-dimethoxy-1-indanon-2-yl)methyl]piperidine derivatives



No	R_4	Inhibition of AChE IC_{50} (nM)
E2020		5.7
19		10
20		2.7
21		40
22		160
23		4.0
24		100
25		>10000
26	H	5400
27		8.9
28		180

Pharmacology: The inhibitory effects of E2020 on the cholinesterase (ChE) activity in vitro and ex vivo were compared with those of two other cholinesterase inhibitors, physostigmine and tetrahydroaminoacridine (tacrine) with respect to their selectivity and the duration of action. The first experiment with this drug was designed to determine the relative inhibitory effects of E2020 on the activity of AChE and butyrylcholinesterase (BuChE; pseudocholinesterase), in comparison with other ChE inhibitors. In these experiments, rat brain homogenate was used as the source of AChE and rat plasma served as the source of BuChE. ChE activity was determined according to the method of Ellman et al.[4]. Both enzymes were incubated with different concentrations of each inhibitor.

As shown in Table 4, the IC_{50} of E2020 for AChE was 5.7 nM. The anti-AChE activity of E2020 was 14 times more potent than that of tacrine but 8 time less potent than that of physostigmine. In contrast, the IC_{50} of E2020 for BuChE was 7138 nM. Among the cholinesterase inhibitors tested E2020 was the least potent against BuChE. The selectivity of the drugs for the two enzymes is shown as a "ratio of IC_{50}" in Table 4. The ratio of IC_{50} of E2020 for BuChE : AChE (1252) is much greater than that of physostigmine (11.9) or tacrine (0.9). These results indicate that E2020 is a highly selective inhibitor of AChE compared with physostigmine and tacrine.

Table 4 Inhibitory Effects of E2020 and Reference Compounds on Rat Brain AChE and Rat Plasma BuChE In Vitro .

Compound	IC_{50} (nM)		Ratio of IC_{50} (BuChE / AChE)
	AChE Activity	BuChE Activity	
E2020	5.7 ± 0.2	7138 ± 133	1252
Physostigmine	0.68 ± 0.02	8.1 ± 0.3	11.9
Tacrine	80.6 ± 2.5	73.0 ± 0.9	0.9

Values represent the mean ± S.E. from 4 dose-response curves for each test drug.

The second experiment was designed to compare the relative duration of the anti-AChE action of E2020, physostigmine and tacrine. Test compounds were given orally to rats and the animals were sacrificed at various intervals after administration. AChE activity in the brain was determined as in the first experiment. The results indicate that 5 mg/kg of E2020 effectively inhibited AChE for at least 4 hours after treatment, and 10 mg/kg of E2020 inhibited AChE for at least 8 hours after administration (Fig. 2). In contrast, AChE activity after treatment with 5 mg/kg physostigmine returned to control levels by 2 hours (Fig. 3). Tacrine at 10 mg/kg did not inhibit AChE activity at any time point. At 30 mg/kg, tacrine exhibited a slight but significant inhibitory effect for 12 hours. These results indicate that at equal doses, E2020 has a longer duration of action than physostigmine. As the inhibitory action of tacrine

was weaker, it was difficult to compare the duration of action of tacrine with that of E2020 and physostigmine.

From these two experiments, it is concluded that E2020 is a highly selective inhibitor of AChE and exhibits a long duration of action in comparison with the other ChE inhibitors.

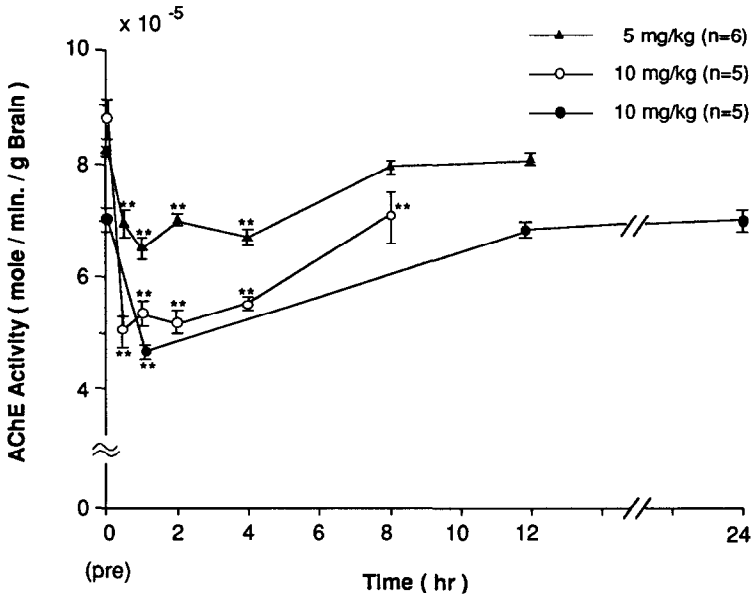


Fig. 2 Time Course of the Effect of Oral Administration of E2020 on ex vivo Brain AChE Activity in Rats

**: $p < 0.01$ vs pre (saline control) (Dunnett's t-test)

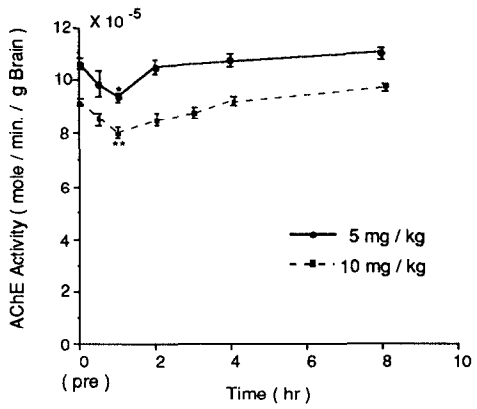


Fig.3 Time Couese of the Effect of Oral Administration of Physostigmine on Brain AChE Activity in Rats

*, **:p<0.05, p < 0.01 respectively vs "pre" (saline control) (Dunnett's t-test)

References and Notes

1. Sugimoto, H.; Tsuchiya, Y.; Sugumi, H.; Higurashi, K.; Karibe, N.; Iimura, Y.; Sasaki, A.; Kawakami, Y.; Nakamura, T.; Araki. S.; Yamanishi, Y.; Yamatsu, K. *J. Med. Chem.* **1990**, 33, 1880.

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