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Carbamate analogues of (–)-physostigmine: in vitro inhibition of acetyl- and butyrylcholinesterase

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Reaction of (–)-eseroline (**1**) with alkyl, aryl and aralkylisocyanates afforded a series of carbamate analogues of (–)-physostigmine (**2**) which were assayed for inhibition of acetyl- and butyrylcholinesterase (AChE and BChE, respectively) in vitro. Included in this study were two N-alkyl-substituted carbamates **9** and **14** obtained from (–)-eseroline (**1**) with dialkylcarbamoyl chlorides, and allophanates **12** and **13** obtained as by-products in the reaction of **1** and benzylcarbamoyl eseroline (**8**) with benzyl isocyanate. Whereas none of the analogues studied was more potent than **2** against electric eel AChE, and carbamates **6**, **7** and **8** were all more than 3 times more potent against human plasma BChE than **2**.

Carbamate analogue; Enzyme inhibition; Acetylcholinesterase; Butyrylcholinesterase

1. INTRODUCTION

(–)-Physostigmine (**2**), also named (–)-eserine, is a carbamate ester alkaloid from Calabar beans [1]. It is a potent inhibitor of acetylcholinesterase (AChE) as well as butyrylcholinesterase (BChE) and has been used clinically in the treatment of glaucoma, atropine intoxication, myasthenia gravis [2] and more recently, in experimental trials in Alzheimer's disease [3]. Although first isolated in 1864 by Jobst and Hesse [4], relatively little attention has been given to the relationship between structure and activity of (–)-physostigmine with the aim of obtaining compounds that might be non-clinically more useful (i.e. longer biological half-life, better specificity for AChE rather than BChE) than (–)-physostigmine.

With the discovery of a practical synthesis of (–)-eseroline (**1**) [5] and an easy route to **1** from commercially available (–)-physostigmine (**2**) [6] the preparation of carbamate analogues of **2** became feasible. In the present report, a number of analogues of (–)-physostigmine were prepared and their potencies (IC_{50}) were evaluated in vitro against electric eel AChE and human plasma BChE.

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2. MATERIALS AND METHODS

The starting material, (–)-eseroline (**1**), was prepared from (–)-physostigmine (**2**, Fluka or Aldrich) by the published procedure [6] and stored as fumarate salt. The preparation of the two carbamates **6** and **8** given below is representative of the preparation of the carbamates listed in table 1. Preparation of benzyl carbamate **8** afforded at the same time the less polar allophanate **12** as a by-product. Reaction of **1** with diethylcarbamoyl chloride afforded **14** by the procedure elaborated for its *N*-methyl analogue **9** [6]. Most carbamates and allophanates were obtained as oils with the exception of **3**, **4**, **5** and **9** which were crystalline. Compounds **3** and **4** were characterized, as fumarate salts. The purity of the compounds was assessed by TLC (silica gel, $CHCl_3$/MeOH = 10:1). Optical rotations were measured in chloroform solution and the molecular composition confirmed by mass spectra (CI-MS) and elemental analysis.

2.1. *(–)-Octylcarbamoyleseroline (6)*

(–)-Eseroline (**1**, 150 mg, 0.68 mmol) was dissolved in anhydrous ether (15 ml) and a small piece of sodium (~5 mg) added. After stirring for 2 min in a nitrogen atmosphere, octylisocyanate (130 mg, 0.83 mmol) was added and the remaining sodium removed. The solvent was evaporated under vacuum and the residue flash-chromatographed on a silica gel column (15 g) with 500 ml CH_2Cl_2/MeOH (100:1) followed by 500 ml CH_2Cl/MeOH (100:4) to afford, after evaporation of

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the solvent, the carbamate **6** as an oil (180 mg, 70.9%): $[\alpha]_D$ −58.4° (c = 0.6, $CHCl_3$); MS (CI) m/z 374 ($M^+ + 1$). Anal. Calc. for $C_{22}H_{35}N_3O_2$: C, 70.78; H, 9.45; N, 11.26. Found: C, 70.54; H, 9.48; N, 11.18.

2.2. *(−)-Benzylcarbamoyleseroline (8) and dibenzylallophanate (13)*

(−)-Eseroline (**1**, 300 mg, 1.37 mmol) was dissolved in anhydrous ether (15 ml) and a small piece of sodium (~5 mg) added. After stirring for 2 min in a nitrogen atmosphere, benzylisocyanate (272 mg, 2.02 mmol) was added and the remaining sodium removed with a pincer. The reaction mixture was stirred at room temperature for 20 min in a nitrogen atmosphere, the solvent removed in vacuum and the residue flash-chromatographed on a silica gel column (15 g) with 500 ml CH_2Cl_2/MeOH = 100:1 followed by 500 ml CH_2Cl_2/MeOH = 100:2 affording two products. The less polar material **13** was obtained as a gum (291 mg, 43.8%): $[\alpha]_D$ −51.6° (c = 1.5, $CHCl_3$); MS(CI) m/z 485 ($M^+ + 1$). Anal. Calc. for $C_{29}H_{32}N_4O_3$: C, 71.81; H, 6.66; N, 11.56. Found: C, 71.93; H, 6.69; N, 11.56. The more polar fraction, also obtained as a gum, was the benzylcarbamate **8** (239 mg, 49.6%): $[\alpha]_D$ −66.4° (c = 0.7, $CHCl_3$); MS(CI) m/z 352 ($M^+ + 1$). Anal. Calc. for $C_{21}H_{25}N_3O_2$: C, 71.76; H, 7.17; N, 11.96. Found: C, 71.62; H, 7.22; N, 11.90.

Similar procedures were employed to synthesize the other compounds shown in table 1.

2.3. *Experimental*

AChE and BChE activities were assayed (final incubation volume = 1.0 ml) in 50 μl aliquots of electric eel AChE (type III, 1000 units/mg protein, diluted 1:40000; Sigma, St. Louis, MO) and 50 μl human plasma (diluted 1:200), respectively, using the spectrophotometric method of Ellman [7]. 0.5 mM acetylmethylthiocholine and 0.5 mM butyrylthiocholine were used as specific substrates for AChE and BChE, respectively. The production of the yellow 5-thio-2-nitrobenzoate anion (produced by the reaction of 5:5-dithiobis-2-nitrobenzoic acid with thiocholine released by the enzymatic hydrolysis of either acetylmethylthiocholine or butyrylthiocholine) was followed at a wavelength of 412 nm.

AChE and BChE activities were determined in the presence of varying concentrations of each compound (10^{-10}–10^{-4} M) and were expressed as a percentage of the activity observed in the absence of test compound (table 1).

3. RESULTS AND DISCUSSION

3.1. *Acetylcholinesterase inhibition*

None of the (−)-physostigmine derivatives studied was as potent against electric eel AChE as (−)-physostigmine itself (IC_{50} = 61 ± 18 nM). Extension of the carbamoyl side chain by the addition of either an octyl or butyl group resulted in com-

Table 1

IC_{50} of (−)-physostigmine and carbamate analogues versus electric eel AChE and human plasma BChE

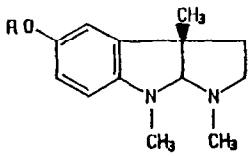
Compounds		Electric eel AChE		Plasma BChE		IC_{50} BChE/ IC_{50} AChE
No.	Name	IC_{50} (nM)	Relative potency (%)	IC_{50} (nM)	Relative potency (%)	
2	(−)-physostigmine	61 ± 18	100	14 ± 6	100	0.23
6	octylcarbamoyl eseroline	110 ± 11	55	3.6 ± 1.0	389	0.03
7	butylcarbamoyl eseroline	152 ± 9	40	4.1 ± 1.3	341	0.03
3	*N*-phenylcarbamoyl eseroline	350 ± 90	17	1300 ± 400	1	3.71
8	benzylcarbamoyl eseroline	460 ± 80	13	2.7 ± 1.6	520	0.01
9	*N*-methyl physostigmine	970 ± 260	6	420 ± 120	3	0.43
4	methoxyphenylcarbamoyl eseroline	1680 ± 380	4	28 ± 2	50	0.02
5	chlorophenylcarbamoyl eseroline	1800 ± 125	3	4000 ± 1290	<1	2.22
12	*N*-benzyl-*N*-methylallophanyl eseroline	6300	1	500 ± 150	3	0.08
10	(*R*)(+)-α-methylbenzylcarbamoyl eseroline	9900	<1	160 ± 90	9	0.02
11	(*S*)(−)-α-methylbenzylcarbamoyl eseroline	11000	<1	1600	<1	0.15
13	*N*-benzyl-*N*-benzylallophanyl eseroline	>10000	<1	1600	<1	<0.16
15	*t*-butylcarbamoyl eseroline	>10000	<1	2000	<1	<0.2
16	*N*-isopropylcarbamoyl eseroline	35000	<1	>100000	<1	>28.6
14	*N*-diethylcarbamoyl eseroline	40000	<1	40000	<1	1.0
1	eseroline	>10000	<1	10000	<1	<1.0

Values shown are mean ± standard deviation of 3–5 different assays. The ratio of IC_{50} BChE: IC_{50} AChE is an index of the relative selectivity of a compound for AChE or BChE: values >1 mean the compound is more potent against AChE than BChE whereas values <1 indicate that the compound is more potent against BChE than AChE

pounds, octylcarbamoyl eseroline (**6**) and butylcarbamoyl eseroline (**7**), approximately half as potent as (−)-physostigmine (IC_{50} and relative potencies, 110 ± 11 nM, 55% and 152 ± 9 nM, 40%, respectively), suggesting that lengthening of the carbamoyl side chain does not greatly reduce the ability of the carbonyl group to interact with the esteratic site of electric eel AChE. However, the addition of either a *N*-phenyl or benzyl group reduces the potency of the resulting compounds (*N*-phenylcarbamoyl eseroline (**3**) and benzylcarbamoyl eseroline (**8**)) much more (IC_{50} and relative potencies, 350 ± 90 nM, 17% and 460 ± 80 nM, 13%, respectively) indicating that the bulk of these groups reduces the interaction between the carbonyl group and the esteratic site. Indeed, when the bulk of the phenyl group is further increased by the addition of either a chlorine atom or a methoxy group, the potency of the resulting compounds (chlorophenylcarbamoyl eseroline (**5**) and methoxyphenylcarbamoyl eseroline (**4**), respectively) is reduced approx. 5-fold compared to phenylcarbamoyl eseroline (**3**) and to less than 5% of (−)-physostigmine. Compounds with very bulky carbamoyl side chain additions, such as *N*-benzyl-*N*-methylallophanyl eseroline (**12**) and *N*-dibenzylallophanyl eseroline (**13**), showed low anti-AChE potencies (relative potency <1%).

Methylation of the carbamoyl nitrogen resulted in a compound (*N*-methylphysostigmine (**9**)) which was less potent (IC_{50} = 970 ± 260 nM, relative potency: 6%) than (−)-physostigmine (**2**). This result differs from an earlier report in which *N*-methylphysostigmine (**9**) was reported to be approx. 20-times more potent than (−)-physostigmine against electric eel AChE [8].

Since (−)-physostigmine is an inhibitor by virtue of its carbamoyl group, it is not surprising that



Compound no.	R	MW	$[\alpha]_D$ (in CH_3Cl_3)	Properties
1	H	218.29	−109.7° (*c* = 1, MeOH)	m.p. 125–126°C[a]
2	$CONHCH_3$	275.34	−76° (*c* = 1.3)	m.p. 105–106°C[b]
3	CONHPh	337.41	−78.5° (*c* = 2, EtOH)	m.p. 148–149°C[c] Fumarate: m.p. 167–168°C $[\alpha]_D$ −97.8° (*c* = 0.1, MeOH)
4	CONHPh-OMe(p)	367.44	−73.2° (*c* = 0.5)	m.p. 129–130°C Fumarate: m.p. 180–183°C $[\alpha]_D$ −67.5° (*c* = 1, MeOH)
5	CONHPh-Cl(P)	371.86	−71.8° (*c* = 0.1)	m.p. 186–188°C
6	$CONH(CH_2)_7CH_3$	373.31	−58.4° (*c* = 0.6)	oil
7	$CONH(CH_2)_3CH_3$	317.42	−74.3° (*c* = 0.3)	oil
8	CONHBn	351.44	−66.4° (*c* = 0.7)	gum
9	$CON(CH_3)_2$	289.37	−74.2° (*c* = 1)	m.p. 73.5–74.0°C
10	(S)PhCHNHCO CH_3	365.47	−104° (*c* = 0.7)	gum
11	(R)PhCHNHCO CH_3	365.47	+3.3° (*c* = 1)	gum
12	$CONCH_3CONHBn$	408.50	−186.9° (*c* = 1)	gum
13	CONBnCONHBn	484.59	−51.6° (*c* = 1.5)	gum
14	$CON(CH_2CH_3)_2$	317.43	−63.6° (*c* = 1)	oil
15	$CONHC(CH_3)_3$	317.43	−55.8° (*c* = 1.2)	gum
16	$CONHCH(CH_3)_2$	303.40	−65.5° (*c* = 2)	gum

[a] Ref. [6]
[b] Merck Index, p.7267, 10th edition
[c] Beilstein, 1954, p.333, EII23

when this bond is hydrolyzed the resulting compound, eseroline, has, as reported previously, very little anti-AChE action (IC_{50} > 10000 nM) [8].

3.2. *Butyrylcholinesterase inhibition*

Unlike electric eel AChE, several of the (−)-physostigmine derivatives were more potent against human plasma BChE than (−)-physostigmine itself. Thus, the octyl-, butyl- and benzyl-carbamoyl eseroline derivatives (**6**, **7** and **8**) were more potent against plasma BChE (IC_{50} and relative potencies 3.6 ± 1.0 nm, 389%; 4.1 ± 1.3 nM, 341% and 2.7 ± 1.6 nM, 520%, respectively) than (−)-physostigmine (IC_{50} = 14 ± 6 nM). Interestingly, whereas the benzyl- and *N*-phenylcarbamoyl derivatives (**8** and **3**, respectively) were approximately equipotent against AChE, benzylcarbamoyl eseroline (**8**) was approximately 500 times more potent against plasma BChE (IC_{50} = 2.7 ± 1.6 nM, relative potency 520%) than *N*-phenylcarbamoyl eseroline (**3**) (IC_{50} = 1300 ± 400 nM, relative potency 1%). However, the addition of a methoxy group to the phenyl group in *N*-phenylcarbamoyl eseroline increased the potency approximately 50-fold (IC_{50} methoxyphenylcarbamoyl eseroline (**4**) = 28 ± 2 nM, relative potency 50%), but caused a 5-fold decrease in potency against electric eel AChE. Addition of a chlorine atom to the phenyl group reduces the potency of phenylcarbamoyl eseroline still further (IC_{50} chlorophenylcarbamoyl eseroline (**5**) = 4000 ± 1290 nM, relative potency <1%).

As with AChE, the addition of increasingly bulky side groups resulted in a decrease in the potency of the compounds against plasma BChE, and eseroline itself was a poor inhibitor (IC_{50} > 10000 nM, relative potency <1%).

4. DISCUSSION

Although (−)-physostigmine and the majority of the (−)-physostigmine derivatives were more potent against human plasma BChE than electric eel AChE, suggesting that these compounds might be more selective for BChE than AChE, it should be emphasized that there are interspecies variations in the inhibitory properties of AChE and BChE [9]. Consequently, it is uncertain whether the differences in potencies towards AChE and BChE observed in the present report are due to the compounds themselves or are merely a consequence of interspecies variability (i.e. is this same pattern of inhibitory properties seen in AChE and BChE derived from the same species?).

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