Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 2, pp. 157-162, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(96)00592-6

SYNTHESIS AND PRELIMINARY STRUCTURE-ACTIVITY RELATIONSHIPS OF 1-[(3-FLUORO-4-PYRIDINYL)AMINO]-3-METHYL-1H-INDOL-5-YL METHYL CARBAMATE (P10358), A NOVEL ACETYLCHOLINESTERASE INHIBITOR

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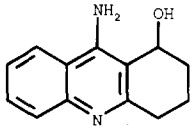
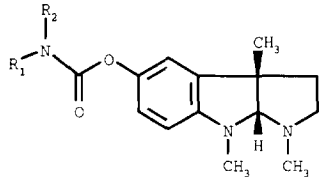
Abstract: A series of carbamate analogs of besipirdine (HP 749) was synthesized as potential agents with enhanced cholinomimetic properties for the treatment of Alzheimer's disease. Compound **5a** (P10358, 1-[3-fluoro-4-pyridinyl)amino]-3-methyl-1H-indol-5-yl methyl carbamate) emerged as a potent, reversible acetylcholinesterase inhibitor that significantly enhanced performance on oral or parenteral administration in learning and memory paradigms.

Alzheimer's disease (AD) is an age-related, chronic neurodegenerative disorder occurring in middle or late life, and is characterized by a progressive dementia that is associated with both a severe disability in performing the activities of everyday life and a reduced life expectancy. In 1993 AD was estimated to affect 3.75 million persons in the United States, and the prevalence of the disease was projected to rise to 9 million persons by 2040 as a greater proportion of the population reaches a longer life expectancy.[1] The well known cholinergic hypothesis[2] of AD was based on evidence suggesting that the breakdown of central cholinergic transmission is intimately associated with cognitive deficits and other symptoms of the disease, and resulted in efforts to treat AD with various cholinomimetic agents. Previous reports from this laboratory described our early work based on the cholinergic hypothesis of AD with the aminoacridine and carbamate-type acetylcholinesterase inhibitors velnacrine[3] (HP 029) and HP 290,[4] respectively. More recently we reported the structure-activity relationships and biological profile for N-propyl-N-(4-pyridinyl)-1H-indol-1-amine (HP 749, besipirdine).[5] This compound, which reverses scopolamine-induced behavioral deficits and passive avoidance deficits induced by combined



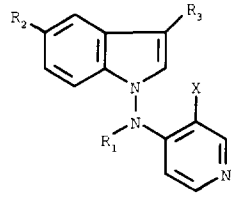
velnacrine



physostigmine R_1=CH_3, R_2=H

heptylphysostigmine R_1=n-C_7H_{15}, R_2=H

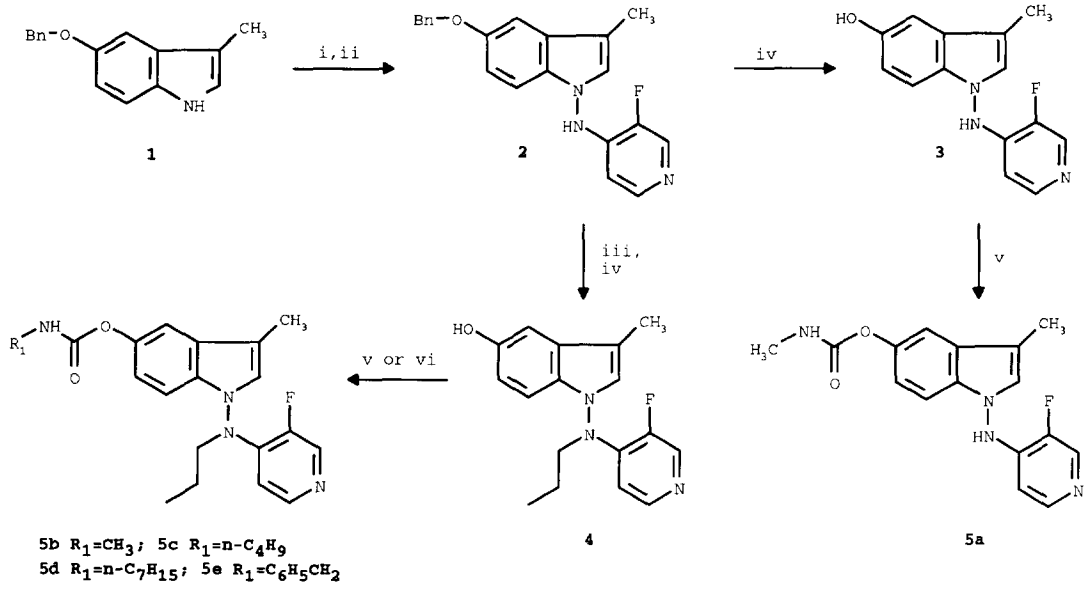
HP 290 R_1R_2N =



besipirdine R_1=n-C_3H_7
R_2=R_3=X=H

P10358 R_1=H, R_2=CH_3NHCOO
R_3=CH_3, X=F

cholinergic and noradrenergic lesions in rats, is both a potent inhibitor of synaptosomal norepinephrine reuptake and a potent alpha-2 adrenoceptor antagonist. However, besipirdine failed to inhibit rat brain acetylcholinesterase or stimulate muscarinic or nicotinic receptors.[5-11] Based on the structural relationship to the carbamate-type acetylcholinesterase inhibitors (e.g., physostigmine), a series of carbamate derivatives of besipirdine was investigated in order to introduce biochemically definitive cholinomimetic properties. A preliminary account of this work was reported,[12] and the compound P10358 (**5a**) subsequently emerged as a potent, centrally active, reversible acetylcholinesterase inhibitor that significantly enhances performance in learning and memory paradigms on parenteral or oral administration.



Reagents and conditions: (i) pwd KOH, N-methylpyrrolidine; then H_2NOSO_3H, <20 °C; (ii) 4-chloro-3-fluoropyridine HCl, N-methylpyrrolidine, 80 °C; (iii) NaH, DMF; then n-C_3H_7Br, DMF, rt; (iv) H_2, 10% Pd/C, EtOH, 50 psi, 50 °C; (v) K_2CO_3, THF; then R_1NCO, THF, rt; (vi) N,N'-carbonyldiimidazole, THF, 0 °C; then AcOH, n-$C_7H_{15}NH_2$, THF, 0 °C to rt

Scheme 1

The compounds were synthesized as depicted in Scheme 1. Treatment of 5-benzyloxy-3-methylindole[13] (**1**) with hydroxylamine-O-sulfonic acid provided 5-benzyloxy-N-aminoindole, which was treated with 4-chloro-3-fluoropyridine hydrochloride,[14] under conditions as previously described,[5] to afford **2**. Hydrogenolysis of **2** provided phenol **3**, which was converted to the carbamate analog **5a** with methyl isocyanate under alkaline

conditions. N-Propyl analog **4** was prepared by alkylation of **2** with propyl bromide and hydrogenolysis of the benzyloxy moiety. A series of carbamate analogs **5b-e** was prepared by treating **4** with an alkylisocyanate under alkaline conditions or with N,N'-carbonyldiimidazole and an alkylamine.

Structure-activity relationships for this preliminary series of carbamates are summarized in Table 1. Compound **5a** is the most interesting analog based on potent in vitro inhibition of brain (striatal) acetylcholinesterase (AChE); significant ex vivo inhibition of striatal AChE at 10 and 20 mg/kg orally (XChEI), with effects extending to 24 h at the 20 mg/kg dose; and significant activity in vivo at all doses evaluated in reversing scopolamine dementia dark avoidance (SDDA), suggesting positive effects on memory enhancement. The effect of introducing an alkyl moiety on the indole N-amino nitrogen (**5b**) and varying the substituents on the carbamate moiety (**5c-e**) was briefly explored. Although **5b** exhibited equally potent in vitro inhibition of AChE, the compound was significantly less robust than **5a** with respect to ex vivo AChE inhibition and in vivo reversal of SDDA. Increasing the carbamate N-alkyl moiety from methyl (**5b**) to butyl (**5c**) or heptyl (**5d**) led to significant loss of AChE inhibitory activity. Although the N-benzyl carbamate **5e** significantly inhibited AChE in vitro, the compound was not particularly robust with respect to ex vivo inhibition of brain acetylcholinesterase and appeared to enhance learning and memory only at higher doses in SDDA.

Human serum contains butyrylcholinesterase (BChE) and, although the biological function of BChE remains controversial,[15] BChE may be regarded as a potential site of loss for certain drugs including carbamate-type cholinesterase inhibitors. Although **5a** is approximately equipotent with respect to inhibition of both AChE and BChE, the reference compound heptylphysostigmine (HEP) exhibits a 13-fold greater selectivity for BChE. Although HEP is approximately 15-fold more potent in vitro as an AChE inhibitor than **5a**, the two compounds are approximately equipotent one hour after oral administration with respect to their ex vivo AChE inhibitory ED_{50} values of 10.1 and 8.3 mg/kg, respectively. These nearly equal ex vivo results indicate favorable CNS bioavailability for **5a**.

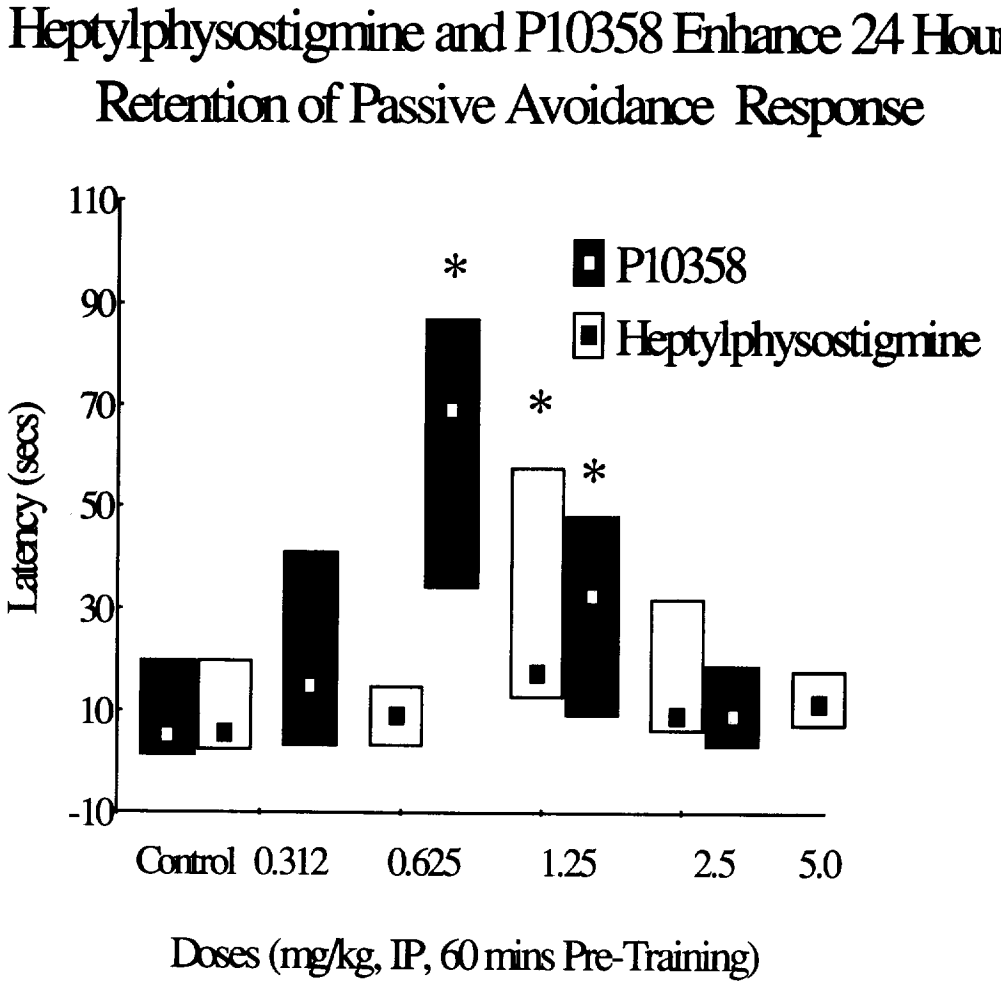
Given the significant number of false positives that can be identified by learning and memory paradigms,[16,17] it is desirable to have both mechanistic or biochemical support for compound effects and compound evaluation in a number of learning and memory models. Biochemically **5a** significantly inhibits AChE; however, the compound shows insignificant in vitro affinity for other receptors including alpha-1, alpha-2, D_2-dopaminergic, muscarinic, nicotinic, adenosine, 5-HT_{1A}, 5-HT_{2A}, $GABA_A$, and NMDA receptors, nor does the compound significantly inhibit biogenic amine reuptake (norepinephrine, serotonin, and dopamine). Compound **5a** thus appears to be a pure cholinesterase inhibitor and is devoid of adrenergic properties in contrast to the noncarbamate predecessor

Table 1 In Vitro, Ex Vivo and In Vivo Data for Compounds 5a-5e

Cmpd No	AChEI[a] IC_{50} (μM)	BChEI[b] IC_{50} (μM)	SDDA[c] A (Doses/Total Doses) Active dose (%response)	XChEI[d] Dose (mg/kg, po)	% Inhibition @ 1	4	6	24h
5a	0.13 (0.084-0.19)	0.19 (0.15-0.22)	A (3/3) 0.03 (40), 0.1 (21), 0.3 (28)	10 20	67 82	56 84	3 82	0 20
5b	0.058 (0.024-0.14)	0.031 (0.023-0.041)	A (1/6) 0.63 (27)	20	50	41		9
5c	4.0 (3.3-4.8)	0.92 (0.69-1.2)	A (2/3) 1.0 (33), 3.0 (33)	50		26		11
5d	43 (21-88)							
5e	0.043 (0.014-0.14)	0.027 (0.01-0.069)	A (4/9) 0.04 (20), 1.25 (20), 2.5 (20), 5 (20)	20	15	12	6	2
heptylphysostigmine	0.009 (0.0026-0.031)	0.00069 (0.00029-0.001)	A (5/7) 0.001 (40), 0.003 (53), 0.01 (45), 0.1 (33), 1.0 (20)	10	52	72	54	21

Footnotes to Table I

[a]Acetylcholinesterase inhibition (rat striatum) using acetylthiocholine as substrate. [b]Butyrylcholinesterase inhibition (human serum) using butyrylthiocholine as substrate. [c]Antagonism of scopolamine-induced behavioral deficits in mice (n = 15 per group) in the scopolamine dementia dark avoidance paradigm. A cutoff was defined for the scopolamine-vehicle group as the value for the animal with the second longest latency time. Results are reported as active (A) with the number of active (≥20% response is considered as positive activity based on experiments with a variety of standard drugs[3]) dosages versus the total number of dosages evaluated in parentheses. The second line of data represents the active doses (mg/kg, sc) with the percent response in parentheses (i.e., the percent of animals in the scopolamine-drug group with latencies greater than the cutoff time). [d]Percent ex vivo inhibition of rat striatal acetylcholinesterase at 1, 4, 6, and 24 h.



series. Compound **5a** was subsequently evaluated in a step-down passive avoidance paradigm, enhancement of passive avoidance (EPA), for effects on enhancing memory. Previous work indicated that drug-treated rats that show an increase in their median latency to step-down are considered to show an enhancement of learning.[18] Camacho et al.[19] demonstrated that AChE inhibitors (galanthamine, HEP, and tacrine) enhance the 24-hour latency, thus providing support for the involvement of brain acetylcholine in memory formation for this behavior. In EPA, using the procedure of Camacho et al.[19], **5a** proved to be the most potent AChE inhibitor evaluated in this procedure and exhibited maximal effects at 0.625 mg/kg, ip, in comparison with HEP, which exhibited a less robust maximal effect at 1.25 mg/kg, ip. In summary, **5a** appears to be a pure cholinesterase inhibitor with greater selectivity for AChE versus BChE than HEP, and the compound exhibits positive effects on memory

enhancement at significantly lower doses than HEP. Further evaluation of **5a** including safety and additional pharmacological studies are the subject of companion papers.[20, 21]

Acknowledgment:

The authors wish to thank Ms. Dianne Saumsiegle for assistance in preparation of the manuscript.

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(*Received in USA* 30 *September* 1996; *accepted* 5 *December* 1996)