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IMINO 1,2,3,4-TETRAHYDROCYCLOPENT[B]INDOLE CARBAMATES AS DUAL INHIBITORS OF ACETYLCHOLINESTERASE AND MONOAMINE OXIDASE

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Abstract: A series of imino 1,2,3,4-tetrahydrocyclopent[b]indole carbamates was prepared and evaluated as dual acetylcholinesterase (AChE) and monoamine oxidase (MAO) inhibitors. Halogen substitution ortho to the carbamate functionality in the eight position resulted in a significant increase in binding affinity for both AChE and MAO-A.

Alzheimer's Disease (AD) is a neurodegenerative disorder that is the major cause of dementia amongst the elderly. This disease affects about 2% of the population over the age of 65, and up to 50% of the population over the age of 85.[1] As general health care improves and the proportion of elderly people in the population increases, the number of AD patients is anticipated to increase dramatically.[2]

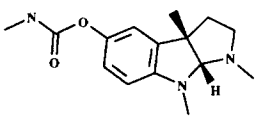
In the last five years significant progress has been made in identifying the processes underlying the deposition of the senile plaques and tangles which are characteristic of AD pathology. However, despite the efforts of the scientific community, the etiology of AD remains unknown.[3,4] In addition, no definitive procedures for the diagnosis of AD are available.[1] In light of the above difficulties, much current work concentrates on the numerous biochemical deficits that have been documented to be associated with the neuropathology of the disease.[5] The hallmark loss of cholinergic neurons found in the autopsied brains of AD patients led to the formulation of the cholinergic hypothesis.[6] A number of approaches to cholinergic enhancement have been investigated for the palliative treatment of AD.[1,5] The aminoacridine acetylcholinesterase inhibitor tacrine (Cognex) was recently approved by the FDA, however the aminoacridines suffer from dose-limiting side effects.[7]

Other neurotransmitter deficits are well documented in AD and may underlie or contribute to the cognitive and behavioral disorders associated with this disease. For example, numerous clinical findings have shown the loss of noradrenergic cell bodies of the locus ceruleus in the brain of AD patients.[8] This deficit may occur in one-half of the AD patient population. A prevalent noradrenergic deficit may be associated with early-onset or aggressive forms of AD.[9] In addition, the noradrenergic deficit correlates with the occurrence of depression in AD patients.[9,10]

Monoamine oxidase (MAO, EC1.4.3.4) is a flavoenzyme which is involved in the oxidative deamination of a variety of endogenous and exogenous amines. MAO exists in two isoenzymic forms, MAO-A and MAO-B. In the central nervous system MAO is responsible for the metabolism of the major monoamine neurotransmitters - norepinephrine, serotonin and dopamine. As such, MAO inhibitors have found use in the treatment of diseases such as depression and Parkinson's disease in which these neurotransmitters are affected. The more recent discovery of selective reversible MAO-A inhibitors (RIMA) such as moclobemide and brofaromine has spurred new research into the use of MAO inhibitors for the treatment of CNS disorders.[11,12]

Both moclobemide and L-deprenyl have shown utility in animal models of the cognitive deficits associated with Alzheimer's Disease (AD).[13] However, clinical trials with these two agents have not shown convincing improvement on cognition in patients with AD.[13,14] In contrast, preliminary data from small clinical trials combining the MAO inhibitor l-deprenyl and either tacrine or physostigmine suggest possible synergistic effects.[15,16]

Our initial strategy for the design of combined agents was to prepare hybrids of the known acetylcholinesterase inhibitor physostigmine (**1**) and known MAO inhibitors such as tranylcypromine (**2**.) This resulted in a series of dual inhibitors (e.g. **3**) which were irreversible MAO inhibitors,[17] due to the incorporation of an MAO pharmacophore such as the



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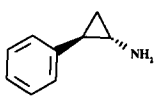

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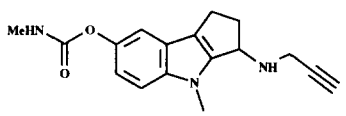
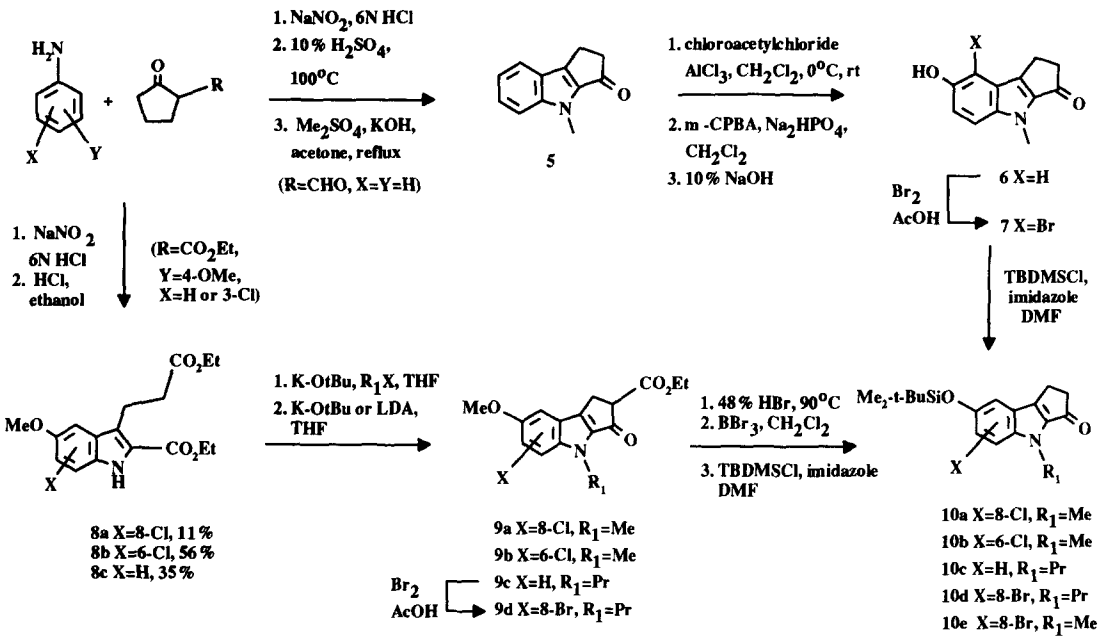


3

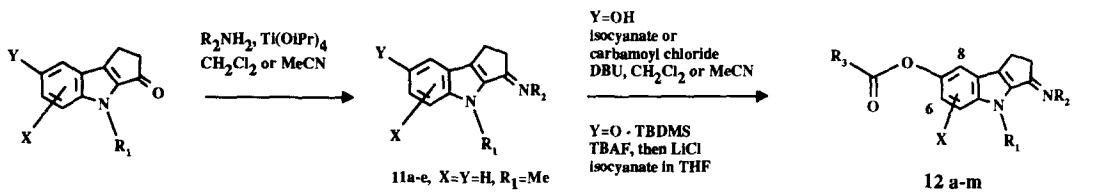
cyclopropyl amine of tranylcypromine of the propargyl amine of L-deprenyl . Biological testing of the imine precursors to these targets showed some weak combined activity, in which the MAO inhibitory activity appeared to be reversible.[18,19] In this paper, we report our initial findings on a series of imino 1,2,3,4-tetrahydrocyclopent[b]indole carbamates which are combined MAO and acetylcholinesterase inhibitors.

The targets were prepared as outlined in Schemes 1 and 2. Japp-Klingmann reaction of the diazonium salt of aniline with 2-formyl cyclopentanone followed by Fisher indolization as described by Elks,[20] and methylation gave 1,2,3,4-tetrahydrocyclopent[b]indol-3-one **5** in 29% yield. Attempted introduction of oxygen functionality by using an analogous route starting with *p*-anisidine was not synthetically useful. However, regioselective Friedel-Crafts acylation of the ketone **5** with chloroacetyl chloride followed by Bayer-Villager oxidation and hydrolysis gave the required phenol **6** in 58% yield.[21] Introduction of a bromine atom into the 8-position of the system was accomplished selectively in 68% yield by treatment of the phenol **6** with bromine in acetic acid. Introduction of a chlorine atom into the 6-position required a modified synthesis. Replacement of 2-formyl-cyclopentanone with 2-carboethoxy-cyclopentanone in the Japp-Klingmann reaction with 3-chloro-anisidine, and Fisher indolization provided a 5:1 mixture of the indoles **8a** and **8b** in 11 and 56% yield respectively.[22] In addition, in this sequence *p*-anisidine could be used as the aniline partner to give the 5-methoxy indole **8c** in 35% yield. Alkylation of the indole followed by Dieckmann condensation gave the desired ketones **9a-c**. Although this transformation could be carried out in one pot by sequential addition of potassium *tert*-butoxide and an alkylating reagent followed by addition of an excess potassium *tert*-butoxide, in practice it was preferable to perform the two steps separately. Ether demethylation and decarbomethoxylation and could also be carried out in one pot in refluxing 48% HBr, however superior yields were generally obtained in a two pot procedure [(a) 48% HBr, 90°C; (b) BBr_3]. The phenols were quite insoluble in the solvents used for imine formation. Therefore in later examples, they were reprotected as their *t*-butyldimethylsilyl ethers.

Scheme 1



Scheme 2



Imine formation was accomplished by treatment of ketones **5, 6, 7, and 10 a-e** with the appropriate amine and titanium tetraisopropoxide.[18,23,24] Reaction of the phenols with isocyanates or dimethylcarbamoyl chloride and DBU or desilylation/carbamylation of the silyl ethers (TBAF, LiCl, isocyanate)[25] provided the target carbamates **12a-m**.[26]

In vitro acetylcholinesterase inhibition (AChEI) was determined using the method of Ellman.[27] Monoamine oxidase (MAO) inhibition was determined via the method of Kindt.[28] IC_{50} values are reported in Tables 1 and 2. The effect of the imine substituent on MAO inhibition was initially explored with the aromatic ring unsubstituted (**11a-e**). In this series it was found that small unhindered alkyl groups (**11a-c**) provided the most potent and selective inhibition of MAO-A and B.

Table 1 ***In Vitro Monoamine Oxidase Inhibition of 11a-e***

compnd	R_1	R_2	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)
11a	Me	Me	0.92 (0.50-1.69)	317 (120-840)
b	Me	Et	2.12 (1.61-2.79)	77. 6 (48.1-125)
c	Me	Pr	1.61 (1.23-2.12)	>1000
d	Me	*i*-Pr	30.8 (21.9-43.2)	>1000
e	Me	4-pyridinylmethyl	113 (65.1-199)	>1000
brofaromine			0.18(0.13-0.24)	23.4(7.34-74.6)

Table 2 ***In Vitro Monoamine Oxidase Inhibition and Acetylcholinesterase Inhibition of 12a-m***

compd	R_1	R_2	X	R_3	MAO-A IC_{50} (μM)[a]	MAO-B IC_{50} (μM)	AChEI IC_{50} (μM)
12a	Me	Me	H	MeNH-	13.6(10.4-17.7)	>1000	3.36 (1.42-7.96)
b	Me	Et	H	MeNH-	3.74(2.18-6.44)	>1000	5.68 (4.27-7.54)
c	Me	Pr	H	MeNH-	25.4(14.7-43.9)	>1000	3.84 (2.90-5.08)
d	Me	phenethyl	H	MeNH-	>1000	>1000	0.16(0.126-0.20)
e	Me	Me	8-Br	MeNH-	0.65(0.32-1.32)	>1000	0.18 (0.11-0.28)
f	Me	Me	8-Cl	MeNH-	1.36 (0.69-2.70)	>1000	0.23 (0.06-0.80)
g	Me	Me	6-Cl	MeNH-	22.5 (13.2-38.3)	395 (221-705)	0.67 (0.49-0.91)
h	Me	Me	8-Br	BnNH-	5.47(4.05-7.39)	>1000	4.19 (1.46-12.07)
i	Me	Me	8-Br	BuNH-	0.75(0.30-1.87)	175 (68.2-449)	3.17 (2.53-3.97)
j	Me	Me	8-Br	Me_2N-	0.54(0.23-1.25)	51.0 (22.2-117)	0.06 (0.02-0.25)
k	Me	Et	8-Br	MeNH-	0.21(0.12-0.35)	>1000	0.14 (0.11-0.19)
l	Me	Et	8-Cl	MeNH-	0.10 (0.06-0.50)	>1000	0.16 (0.07-0.36)
m	Pr	Et	8-Br	MeNH-	>1000	>1000	0.23 (0.19-0.28)
heptylstigmine					--	--	0.009 (0.003-0.031)
brofaromine					0.18(0.13-0.24)	23.4(7.34-74.6)	--

(a) Values in parentheses are 95% confidence limits

Introduction of bulkier substituents such as isopropyl (**11d**) or pyridinylmethyl (**11e**) resulted in loss of enzyme affinity (but not selectivity). Introduction of carbamate functionality in the 5-position provided a compound (**12a**) which had 15 times weaker affinity for MAO-A than did the unsubstituted parent (**11a**). However, the compound was also a weak acetylcholinesterase inhibitor (IC_{50} = 3.4 μM).

The affect of the imine substituent was briefly explored (**12a-d**). Small alkyl groups had little influence on AChEI activity. A more significant effect was observed on MAO activity, with the ethyl imine being the most active compound. Interestingly, the introduction of a phenethyl substituent resulted in approximately a 20-fold increase in AChEI potency. However, consistent with the results in the unsubstituted series, this compound has little affinity for either MAO isoform.

In a series of isomeric physostigmine analogs, it was reported that introduction of a halogen atom ortho to the carbamate resulted in a 10- to 20-fold increase in acetylcholinesterase enzyme affinity.[29] Introduction of halogen (Br or Cl) in the 8-position of the present series led to a significant 14- to 40-fold increase in AChEI potency (**12 e, f, k, l** vs **12 a, b**). Introduction of a chlorine atom in the six position (**12g**) led to a more modest 5-fold increase in enzyme affinity. In addition to the influence on acetylcholinesterase inhibition, halogen substitution significantly affected MAO binding. Substitution at the

eight position resulted in about a 20-fold increase in affinity for MAO-A (**12e** vs **12a** and **12l** vs **12b**), while the 6-chloro isomer (**12g**) was about equipotent to **12a**. This compound was also less selective for MAO-A vs. MAO-B.

The carbamate substituent was varied in the methyl imine series. Larger groups (Bn and Bu, **12 h, i**) resulted in weaker affinity for acetylcholinesterase, while the dimethyl carbamate (**12j**) was the most potent AChEI in the series. The benzyl substituent also reduced MAO-A activity by a factor of 8. Finally, the effect of the indole nitrogen substituent was explored by replacement of the methyl group in **12k** with a propyl group **12m**. While this compound retained its affinity for acetylcholinesterase, the compound did not bind to either MAO isoform.

Table 3 Ex Vivo Inhibition of Acetylcholinesterase and Monoamine Oxidase[a]

compnd	MAO-A[a] % inhibition (± SEM @ time h)	MAO-B % inhibition (± SEM @ time h)	AChE % inhibition (± SEM @ time h)
12k	28.2 ± 3.7 @ 0.5**[b]	21.5 ± 2.4 @ 0.5*	15.1 ± 5.7 @ 0.5*
@ 50 mg/kg po	33.3 ± 1.7 @ 1**	17.2 ± 2.9 @ 1**	19.4 ± 1.7 @ 1**
	48.9 ± 1.4 @ 4**	25.9 ± 2.2 @ 4**	30.9 ± 3.6 @ 4**
	13.1 ± 2.6 @ 24**	17.8 ± 8.8 @ 24*	0.2 ± 0.2 @ 24
	11.7 ± 0.9 @ 48**	9.3 ± 3.2 @ 48	
12j	44.0 ± 1.9 @ 1**	20.4 ± 2.7 @ 1**	NA[c]
@ 50 mg/kg po	58.8 ± 2.0 @ 4**	25.9 ± 2..5 @ 4**	
	23.2 ± 1.1 @ 24**	6.6 ± 2.3 @ 24	
	22.3 ± 1.3 @ 48*	10.1 ± 1.3 @ 48*	
brofaromine	52.1 ± 6.7 @ 1**	29.6 ± 4.1 @ 1**	NT[d]
@ 10 mg/kg po	64.2 ± 3.3 @ 4**	42.6 ± 2.3 @ 4**	
	28.7 ± 4.4 @ 24**	22.2 ± 3.3 @ 24**	
	13.2 ± 1.1 @ 48*	18.3 ± 0.9 @ 48**	
heptylstigmine	NT	NT	33.2 ± 4.7 @ 0.5**
@ 10 mg/mg po			52.3 ± 3.7 @ 1**
			72.9 ± 3.5 @ 4**
			20.8 ± 1.4 @ 24**

(a) Ex vivo acetylcholinesterase inhibition was measured by a modification of Ellmans method [27] and ex-vivo MAOI by a modification of Kindt. [27] The compounds were administered as suspensions in water plus Tween 80. The assays were conducted using brain tissue (b) * $p<0.05$, ** $p<0.01$, Newman-Keuls (c) NA= not active, (d) NT- not tested

Several of the compounds were further tested orally for *ex vivo* inhibition of AChE and MAO (Table 3). These studies indicate that the compounds have low oral activity, possibly due to poor brain penetration or poor oral bioavailability. This is particularly evident in the AChEI assay, since carbamate acetylcholinesterase inhibitors with similar *in vitro* potency are active at much lower doses. In addition, at the high doses necessary to obtain good responses in the MAO assay selectivity for MAO-A versus MAO-B appears to be lost. Thus, the current series of compounds demonstrates the feasibility of combining acetylcholinesterase inhibition with monoamine oxidase inhibition. Although halogenation of the targets in the 8-position

resulted in a dramatic improvement in affinity for both AChE and for MAO-A, due to their low oral activity these compounds were unsuitable for further development.

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