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ACETYLCHOLINE-RELEASING AGENTS AS COGNITION ENHANCERS. STRUCTURE-ACTIVITY RELATIONSHIPS OF PYRIDINYL PENDANT GROUPS ON SELECTED CORE STRUCTURES.

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Abstract: A number of analogs of the cognition enhancing agent DuP 996 were prepared by varying the core structure and pendant groups in an independent fashion. The SAR of 2-, 3-, and 4-pyridinylmethyl groups as pendant groups on selected cores was examined.

Numerous neurochemical studies have demonstrated that cholinergic, noradrenergic, and serotonergic neurotransmitter substances are severely depleted in dementia and in particular Alzheimer's Disease (AD).[1,2] Although these neurotransmitter deficits provide a basis for the symptomatology of the disease, they are secondary to neuronal loss caused by the disease process itself. While neurotransmitter deficits may not represent the primary lesion in the pathology of AD, neurotransmitter deficit hypotheses have provided a pragmatic approach to the development of treatments for AD. Most efforts at modulation of neurotransmitter systems have focused on the cholinergic system since acetylcholine depletion has been most often associated with the severity of dementia.[3-6] Various animal experiments also support the role of acetylcholine in cognition and learning.[7-9]

A strategy towards enhancement of cholinergic function is the use of drugs which increase endogenous stimulus-induced ACh release, which would result in elevated levels of ACh solely when its release is triggered by excitation of the cholinergic neuron. Such action should result in an improvement of the signal-to-noise ratio of ACh during transmission of cholinergic function, diminishing the potential of ACh overload toxicity that is typical of cholinesterase inhibitors, and without the distortion of temporal patterns in cholinergic transmission, caused by direct cholinergic agonists.

In the course of our program to evaluate compounds which might reduce cholinergic system dysfunction by increasing acetylcholine (ACh) levels in the brain and enhancing

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cognitive function, DuP 996 (AVIVA™, linopirdine) was identified to have activity both *in vivo* and *in vitro*.[10,11] Although the mechanism of action responsible for this ability to enhance ACh release is not totally understood, DuP 996 is not an acetylcholinesterase inhibitior, and does not bind to either muscarinic or nicotinic receptors, suggesting that it does not act as a direct cholinergic agonist.[12]

In order to examine the SAR of acetylcholine release enhancement, we prepared analogs of DuP 996 by varying the core structure and pendant groups independently. Scheme 1 graphically shows this strategy. This communication describes the SAR of 2-, 3-, and 4-pyridinylmethyl groups as pendants on various core structures. The compounds under discussion are listed in Figure 1.

Scheme 1

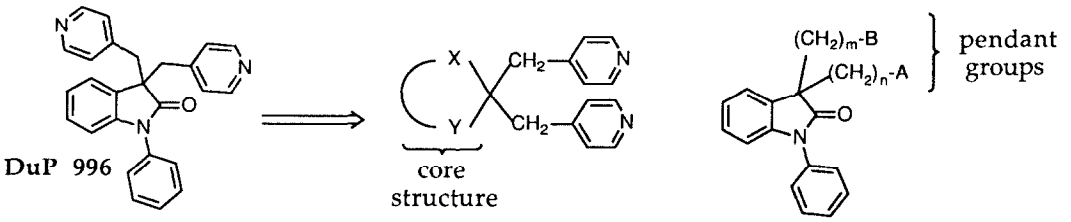
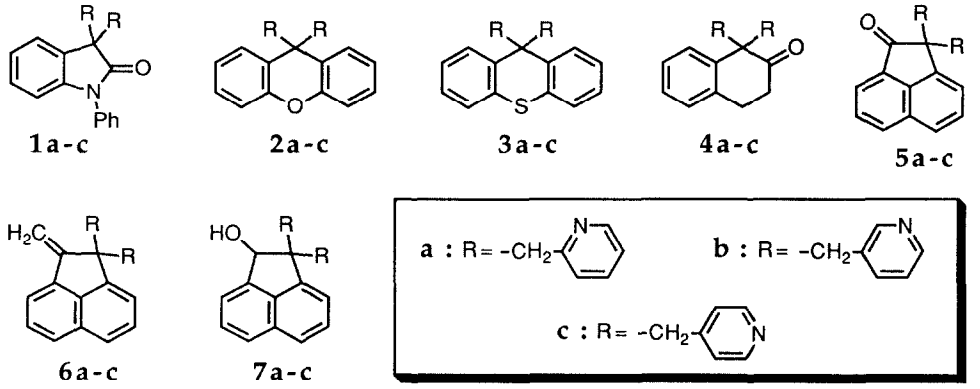


Figure 1



Chemistry[13]

The xanthene (**2**, R = H), thioxanthene (**3**, R = H), and 2-tetralone (**4**, R = H) starting materials are commercially available. N-Phenyl oxindole[14] (**1**, R = H) and acenaphthenone[15] (**5**, R = H) were prepared according to the literature. The alkylating agents 2-, 3-, 4-picolyl chlorides are commercially available as their hydrochlorides and are converted to the free bases if necessary. The core structures were dialkyated by one of three methods: (1) LDA in THF (**2a-c**, **3a-c**); (2) NaH in THF (**4b-c**); (3) phase-transfer catalyzed dialkylation (**1a-c**, **4a**, **5-c**).[16] Compounds **6a-c** were prepared by Wittig olefination of **5a-c** (CH_3PPh_3Br, n-BuLi, THF, r.t., 18 h.). Reduction of **5a-c** provided alcohols **7a-c** ($LiAlH_4$, THF, r.t. to 50°C, 1 h.).

Biology

Compounds were evaluated for their ability to enhance the release of acetylcholine (ACh) *in vitro* in a brain slice superfusion system.[10] In this system, rat brain slices (0.25 mm x 0.25 mm) were loaded with ^{3}H-choline and subsequently superfused with oxygenated Krebs-Ringer medium. Release of ^{3}H-ACh is evoked once in the absence of the test compound with 25 mM KCl-containing Krebs-Ringer medium (S1) and once in the presence of 10μM of the test compound (S2). The ratio of S1/S2 (as compared to controls where no compound is present during S2) is a measure of the ability of the compound to enhance depolarization-induced ACh release. The ability of a drug to enhance this release of ACh is expressed as a per cent increase of control (control = 100). The data used in this report reflect the average of duplicate analyses where the standard deviation (s.d.) was less than 10%. This number is then converted into a value which adjusts it against a positive control (DuP 996, 10μM) being run on the same day. This value allows for easier comparison of compounds tested at different times, and corrects for the variability between tissue slices. The equation used for this value is : %ACh = [(release of experimental compound - 100)/(release of DuP 996 - 100)] X 100. These values are then normalized so that % ACh of DuP 996 = 100, and are shown in Table 1. The accompanying graph plots the % ACh values of the compounds in Figure 1, grouped by pyridine isomer.

Discussion

We have previously shown that there is a correlation between the potency (EC_{100}) of a compound to enhance ACh release and its affinity for the [^{3}H]DuP 996 binding site.[12] Indeed, binding studies performed on compounds **1a-c**, **2a-c** and **5a-c** showed excellent correlation between % ACh and affinity for this site.[17] However, we consider K^+-induced ACh release, rather than binding site affinity our primary screen for this class of compounds is , because we feel the release assay is a better indicator of the potential therapeutic use of the compounds. One can, however, make assumptions about how structure influences the binding of compounds based on ACh release, due to this good correlation between these two properties.

As can be seen from the graph, for a given core structure, the % ACh generally increases across the series 2-pyridyl < 3-pyridyl < 4-pyridyl. Since these pendants are very similar in size (molar refractivity, MR), lipophilicity (logP), and basicity (pK_a), the topography of the pyridine nitrogens as the molecule interacts with its binding site must be a major factor in the differences in ACh release. The reason that these subtle structural differences have an influence on release is not fully understood, and is currently under investigation.

For core structures **4** (tetralones) and **5** (acenaphthenones), the order of release activity is 3-pyridyl < 2-pyridyl < 4-pyridyl. Perhaps the ketone in these structures is interfering with the binding of the 3-pyridyl groups to the binding site more so than the 2- or the 4- isomers. The acenaphenol core **7** shows an inverse ordering of activity of the pyridyl isomers, this is most likely due to some as yet uninvestigated effect of the hydroxy group at the binding site.

In regard to the comparison of core structures, **1**, **2**, **3**, and **6** are the most active. This

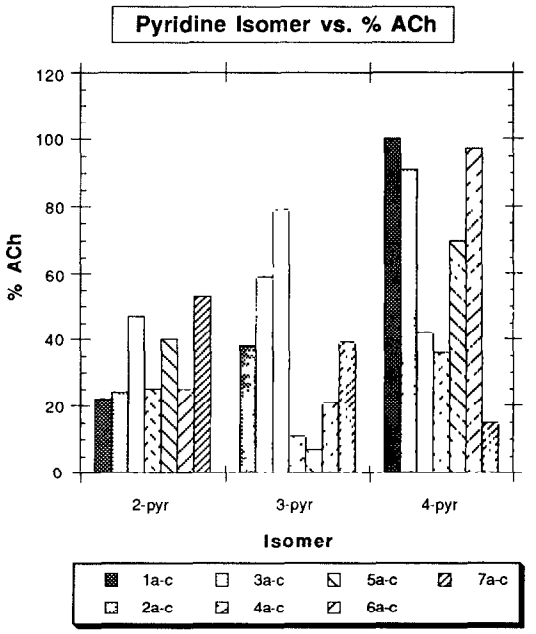


Table 1

Cmpd.	% ACh		
	a	**b**	**c**
1	22	38	100
2	24	59	91
3	47	79	42
4	25	11	36
5	40	7	70
6	25	21	97
7	53	39	15

may be due to extra binding stabilization of the second aryl group in **1**, **2**, and **3**, or in the case of **6**, the *exo* methylene group.

Other analogs of DuP 996 are being prepared and tested according to the strategy outlined in Scheme 1. Additional results of this approach will be reported in future publications.

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