9-Amino-1,2,3,4-tetrahydroacridin-1-ols: Synthesis and Evaluation as Potential Alzheimer's Disease Therapeutics†

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The synthesis of a series of 9-amino-1,2,3,4-tetrahydroacridin-1-ols is reported. These compounds are related to 1,2,3,4-tetrahydro-9-acridinamine (THA, tacrine). They inhibit acetylcholinesterase in vitro and are active in a model that may be predictive of activity in Alzheimer's disease—the scopolamine-induced impairment of 24-h memory of a passive dark-avoidance paradigm in mice. Two compounds, (±)-9-amino-1,2,3,4-tetrahydroacridin-1-ol maleate (**1a**, HP-029) and (±)-9-(benzylamino)-1,2,3,4-tetrahydroacridin-1-ol maleate (**1p**, HP-128), were also active in reversing the deficit in 72-h retention of a one-trial dark-avoidance task in rats, induced by ibotenic acid lesions in the nucleus basalis magnocellularis. In addition, compound **1p** showed potent in vitro inhibition of the uptake of radiolabeled noradrenaline and dopamine (IC_{50} = 0.070 and 0.30 μM, respectively). Compounds **1a** and **1p**, which showed less acute toxicity in both rats and mice than THA, are in phase II and phase I clinical trials, respectively, for Alzheimer's disease.

Alzheimer's disease (AD) has been recognized as one of the most disabling conditions affecting the aged and is the major cause of dementia among elderly people.[1] As general health care has improved and the proportion of elderly in the population has continued to increase, diseases affecting cognition in the aged have assumed ever-increasing importance.[2] As a result, a wide-ranging, multidisciplinary effort—taking place in clinics, in academic research laboratories, and in the pharmaceutical industry—is currently under way, with the goal of finding effective treatments for AD and related cognitive disorders. The problems faced by research in this area are formidable and many, ranging from the lack of an unequivocal diagnostic procedure to conflicting data on the etiology and pathogenesis of AD and to the unavailability of appropriate animal models or proven efficacious drugs.[3] In light of these difficulties, much contemporary research is being directed toward gaining an increased understanding of the biochemical and neurochemical basis of AD, in the hope of ascertaining deficits in specific biochemical systems or neurochemical pathways. Such knowledge might enable the rational design of therapeutic agents that exert their effects through established mechanisms of action.

Numerous theories exist regarding the biochemical basis of AD; a role has been suggested for many of the endogenous chemical transmitters that control brain function, including the monoamines, the amino acids, the peptide hormones, and acetylcholine (ACh). The role that deficits in norepinephrine (NE), serotonin (5-HT), and, to a lesser extent, dopamine (DA) may play in the development of cognitive disorders has been recently reviewed,[4] and attempts to alleviate AD by influencing these systems with agonists[5] or monoamine oxidase inhibitors[6] have been discussed. A role has also been postulated for cortical glutamatergic neurones in AD, and diminished glutamate binding has been demonstrated.[7] Antagonists of *N*-methyl-D-aspartate have shown some ability to protect against glutamate toxicity, but have not as yet shown any promise in behavioral models.[8] On the other hand, some benzodiazepine-related compounds that may affect the GABA receptor complex have improved memory-related functioning in animal models.[9] There continue to be reports of somatostatin (S) and vasopressin (VP) deficits in AD[10] and there may be some future potential in the treatment of AD with analogues of S or VP.[11] Although each of the foregoing endogenous chemical systems continues to receive consideration as the putative neurochemical deficit in AD, the hypothesis that the cholinergic system is defective in AD has received the most attention in regard to a biochemical rationale that has supported a therapeutic strategy.[12]

Initial impetus for the cholinergic hypothesis came from postmortem examinations that indicated a severe loss of cholinergic neurons in AD,[13] suggesting that pharmacological manipulation of the cholinergic system in AD patients might be a viable approach to this disorder. While the different approaches to cholinergic enhancement—the use of muscarinic agonists, cholinergic releasing agents, or acetylcholinesterase (AChE) inhibitors—have their theo-

† Dedicated to Professor Dr. rer. nat. Hansgeorg Gareis in honor of his 60th birthday.

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(1) For recent reviews on Alzheimer's disease, see: (a) Armstrong, W. *Recent Trends in Research on Alzheimer's Disease*; PJB Publications Ltd.: Surrey, UK, 1986. (b) Katzmann, R. *N. Engl. J. Med.* **1986**, *314*, 964.
(2) Crook, T. *Clin. Gerontologist* **1986**, *5*, 489.
(3) Hershenson, F. M.; Moos, W. H. *J. Med. Chem.* **1986**, *29*, 1125.
(4) Mann, D. M.; Yates, P. O. *Human Neurobiol.* **1986**, *5*, 147.
(5) Arnsten, A. F. T.; Goldman-Rakic, P. S. In *Alzheimer's Disease: Advances in Basic Research and Therapies*; Wurtman, R. J., Corkin, S., Growdon, J., Eds.; Ctr. for Brain Sci. and Metab. Charitable Trust: Cambridge, MA, 1987; p 275.
(6) Tariot, P. N.; Sunderland, T.; Weingartner, H.; Murphy, D. L.; Welkowitz, J. A.; Thompson, K.; Cohen, R. M. *Psychopharmacol.* **1987**, *91*, 489.
(7) Greenamyre, J. T.; Penney, J. B.; Young, A. B.; D'Amato, C. J.; Hicks, S. P.; Shoulson, I. *Science* **1985**, *227*, 1496.
(8) Morris, R. G. M.; Anderson, E.; Lynch, G. S.; Baudry, M. *Nature* **1986**, *319*, 774.
(9) Venault, P.; Chapouthier, G.; deCarvalho, L. P.; Simiand, J.; Morre, M.; Dodd, R. H.; Rossier, J. *Nature* **1986**, *321*, 864.
(10) (a) Beal, M. F.; Mazurek, M. F.; Tran, V. T.; Chattha, G.; Bird, E. D.; Martin, J. B. *Science* **1985**, *229*, 289. (b) Beal, M. F.; Martin, J. B. *Ann. Neurol.* **1986**, *20*, 547.
(11) Quirion, R.; Martel, J. C.; Robitaille, Y.; Etienne, P.; Wood, P.; Nair, N. P. V.; Gautheir, S. *Can. J. Neurol. Sci.* **1986**, *13*, 503.
(12) (a) Kopelman, M. D. *Q. J. Exp. Psychol.* **1986**, *38A*, 535. (b) Bartus, R. T.; Dean, R. L.; Beer, B.; Lippa, A. S. *Science* **1982**, *217*, 408.
(13) (a) Davies, P.; Maloney, A. J. F. *Lancet* **1976**, ii, 1403. (b) Bowen, D. M.; Smith, C. B.; White, P.; Davison, A. N. *Brain* **1976**, *99*, 459. (c) Perry, E. K.; Gibson, P. H.; Blessed, G.; Perry, R. H.; Tomlinson, B. E. *J. Neurol. Sci.* **1977**, *34*, 247.

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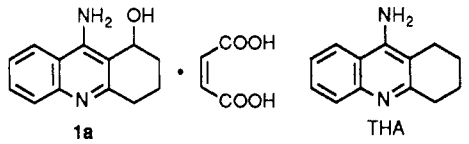
retical drawbacks (releasing agents and AChE inhibitors rely on a supply of endogenous ACh and the effects of agonists might be tonic, not mimicking the phasic effects of endogenously released ACh[14,15]) there are reports of success with each approach in AD patients.[16] Although the usefulness of an AChE inhibitor in AD might be limited for the reason given above, a recent report that the plaques observed in the brains of AD patients contain increased amounts of AChE[17] may suggest a firmer rationale for the treatment of AD with AChE inhibitors.

We recently reported some preliminary results with a new AChE inhibitor, (±)-9-amino-1,2,3,4-tetrahydroacridin-1-ol maleate (**1a**, HP-029), which is currently in



1a THA

phase II clinical trials for AD[18] and which is related to 1,2,3,4-tetrahydro-9-acridinamine (THA, tacrine).

Of the AChE inhibitors that have been tested in AD patients, THA attracted our interest as a synthetic model because of the unique mechanism by which it had been shown to inhibit AChE.[19] Many AChE inhibitors have been shown to interact with the catalytic surface of the enzyme at two points—at an anionic site and at an esteratic site, presumably the hydroxyl group of serine.[20] Physostigmine, for example, binds to the anionic site in its cationic, protonated-amine form and carbamoylates the esteratic site, while an inhibitor like edrophonium binds to the anionic site at its quaternary ammonium head and a suitably placed phenolic hydroxyl forms a hydrogen bond to the esteratic site.[21] We felt that THA had the advantage of binding reversibly to AChE through lipophilic and hydrogen-bonding interactions rather than irreversibly through a covalent bond. THA had, in our view, the additional advantage of being a molecule that could cross the blood–brain barrier in its uncharged, neutral form, and yet interact with AChE in its charged form. The primary disadvantage of THA was its reported toxicity.[22]

At the time of our initial publication,[18] THA had attracted considerable attention because of a report of its clinical efficacy,[23] resulting in the initiation of large-scale clinical trials. These trials had been temporarily suspended at that time because eight out of 41 patients showed signs of liver damage.[24] The clinical trials with THA have subsequently been restarted at lower doses,[25] and interest in this compound remains high. Our communication described an approach to synthesizing a less-toxic AChE inhibitor by incorporating the 1-hydroxy group into the molecule as a "handle" by which glucuronide conjugation and subsequent elimination would be facilitated.[26] It has been suggested[19] that THA binds to the anionic site of AChE at the ring nitrogen, with the 9-amino oriented away from the esteratic site. Thus, the 1-position was chosen so that the hydroxyl group would not interfere with binding at the ring nitrogen and so that if hydrogen bonding with the 9-amino group was taking place at another site on the enzyme, the hydroxyl group might provide additional opportunities for bonding. In addition, it was hoped that by placing the hydroxyl "handle" in a position where *intramolecular* hydrogen bonding to the 9-amino group could occur, some of the polarity of the oxygen

Scheme I

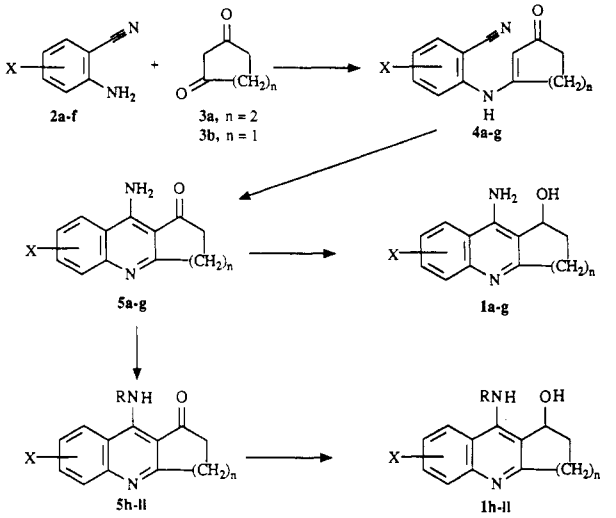
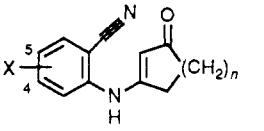


Table I. Data for *N*-(3-Oxocyclohexen-1-yl)-2-aminobenzonitriles

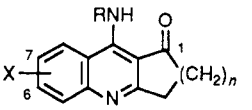


compd	X[a]	recrystn solv[b]	mp, °C[c]	% yield[d]	formula[e]
4a	H	I-M	188–190	92	$C_{13}H_{12}N_2O$
4b	5-Cl	*f*	*f*	*f*	*f*
4c	4-Cl	G	229 dec	96	$C_{13}H_{11}ClN_2O\cdot HCl$
4d	4-CH_3O	*f*	*f*	*f*	*f*
4e	4-CF_3	A–C	171–173	88	$C_{14}H_{11}F_3N_2O$
4f	4-F	C–F	169–171	74	$C_{13}H_{11}FN_2O$
4g	H (n = 1)	D	194–197	91	$C_{12}H_{10}N_2O$

[a] n = 2 for all compounds except the one indicated case (compound **4g**). [b] A = methanol, B = ethanol, C = 2-propanol, D = ethyl acetate, E = acetone, F = diethyl ether, G = tetrahydrofuran, H = isopropyl ether, I = dichloromethane, J = benzene, K = toluene, L = cyclohexane, M = hexane. [c] Melting points are uncorrected. [d] Yields were not optimized. [e] Where formulas are indicated, compounds were analyzed for C, H, and N within 0.40% of the calculated values, except where indicated. [f] These compounds were taken to the next reaction without purification.

(14) Palacios, J. M.; Spiegel, R. In *Progress in Brain Research*; Swaab, D. F., Fliers, E., Mirmiran, M., VanGool, W. A., Van Haaren, F., Eds.; Elsevier: Amsterdam, 1986; Vol. 70, pp 485–498.
(15) Bruno, G.; Mohr, E.; Gillespie, M.; Fedio, P.; Chase, T. N. *Arch Neurol.* **1986**, *43*, 659.
(16) (a) Harbaugh, R. E.; Roberts, D. W.; Coombs, D. W.; Saunders, R. L.; Reeder, T. M. *Neurosurgery* **1984**, *15*, 514. (b) Wesseling, H.; Agoston, S.; Van Dam, G. B. P.; Pasma, J.; DeWit, D. J.; Havinga, H. *N. Engl. J. Med.* **1984**, *310*, 988. (c) Mohs, R. C.; Davis, B. M.; Johns, C. A.; Mathe, A. A.; Greenwald, B. S.; Horvath, T. B.; Davis, K. L. *Am. J. Psychiatry* **1985**, *142*, 28.
(17) Mesulam, M. M.; Geula, C.; Moran, M. A. *Ann. Neurol.* **1987**, *22*, 683.
(18) Shutske, G. M.; Pierrat, F. A.; Cornfeldt, M. L.; Szewczak, M. R.; Huger, F. P.; Bores, G. M.; Haroutunian, V.; Davis, K. L. *J. Med. Chem.* **1988**, *31*, 1278.
(19) Steinberg, G. M.; Mednick, M. L.; Maddox, J.; Rice, R.; Cramer, J. *J. Med. Chem.* **1975**, *18*, 1056.
(20) Beckett, A. H. *Ann. N.Y. Acad. Sci.* **1967**, *144*, 675.
(21) Cannon, J. G. In *Burger's Medicinal Chemistry, 4th ed.*; Wolff, M. E., Ed.; Wiley: New York, 1981; Part III, pp 339–360.
(22) Summers, W. K.; Kaufman, K. R.; Altman, F., Jr.; Fischer, J. M. *Clin. Toxicol.* **1980**, *16*, 269.
(23) Summers, W. K.; Majovski, L. V.; Marsh, G. M.; Tachiki, K.; Kling, A. *N. Engl. J. Med.* **1986**, *315*, 1241.
(24) Marx, J. T. *Science* **1987**, *238*, 1041.
(25) *Scrip* **1988**, January 29, p 19.
(26) For a full discussion of this principle, see: Low, L. K.; Castagnoli, N., Jr. In *Burger's Medicinal Chemistry, 4th ed.*; Wolff, M. E., Ed.; Wiley: New York, 1981; Part I, pp 107–226.

Table II. Data for 9-Amino-1,2,3,4-tetrahydroacridin-1-ones



compd	X[a]	R	recrystn solv[b]	mp, °C[c]	% yield[d]	formula[e]
5a	H	H	I	236 dec	59	$C_{13}H_{12}N_2O$
5b	7-Cl	H	C-K	260 dec	4	$C_{13}H_{11}ClN_2O$
5c	6-Cl	H	D	285–287	47	$C_{13}H_{11}ClN_2O$
5d	6-CH_3O	H	A	244–246	36	$C_{14}H_{14}N_2O_2$
5e	6-CF_3	H	C-I	209–210	38	$C_{14}H_{11}F_3N_2O{\cdot}C_4H_4O_4$
5f	6-F	H	G	266–269	93	$C_{13}H_{11}FN_2O$
5g	H (n = 1)	H	A-F	191–193	38	$C_{12}H_{10}N_2O{\cdot}C_4H_4O_4$
5h	H	CH_3	I-M	116–117	70	$C_{14}H_{14}N_2O$
5i	H	n-C_3H_7	F-I	145–147	63	$C_{16}H_{18}N_2O$
5j	H	$(CH_2)_2N(CH_3)_2$	D	141–143	20	$C_{17}H_{21}N_3O$
5k	H	$(CH_2)_2C_6H_5$	I-M	129–132	24	$C_{21}H_{20}N_2O$
5l	H	$(CH_2)_3OC_6H_5$	I-M	104–106	27	$C_{22}H_{22}N_2O_2$
5m	H	$(CH_2)_3CH(C_6H_5)_2$	F-M	86–88	8	$C_{29}H_{28}N_2O$
5n	H	$(CH_2)_3CH(4\text{-}FC_6H_4)_2$	C	162–165	39	$C_{29}H_{26}F_2N_2O{\cdot}C_4H_4O_4$
5o	H	$(CH_2)_3CH(3\text{-}FC_6H_4)_2$	L	86–89	65	$C_{29}H_{26}F_2N_2O$
5p	H	$CH_2C_6H_5$	I-M	162–163	41	$C_{20}H_{18}N_2O$
5q	H	$CH_2(2\text{-}ClC_6H_4)$	A	163–165	53	$C_{20}H_{17}ClN_2O$
5r	H	$CH_2(3\text{-}ClC_6H_4)$	K-M	139–141	47	$C_{20}H_{17}ClN_2O$
5s	H	$CH_2(4\text{-}ClC_6H_4)$	A	163–165	73	$C_{20}H_{17}ClN_2O$
5t	H	$CH_2(2\text{-}FC_6H_4)$	D-K	163–164	54	$C_{20}H_{17}FN_2O$
5u	H	$CH_2(3\text{-}FC_6H_4)$	I-M	156–157	59	$C_{20}H_{17}FN_2O$[f]
5v	H	$CH_2(4\text{-}FC_6H_4)$	D-M	158–159	49	$C_{20}H_{17}FN_2O$
5w	H	$CH_2(2\text{-}CH_3OC_6H_4)$	J-M	111–112	87	$C_{21}H_{20}N_2O_2{\cdot}0.5H_2O$
5x	H	$CH_2(3\text{-}CH_3OC_6H_4)$	F-I	85–87	72	$C_{21}H_{20}N_2O_2$
5y	H	$CH_2(4\text{-}CH_3OC_6H_4)$	J-M	115–117	49	$C_{21}H_{20}N_2O_2$
5z	H	$CH_2(2\text{-}CH_3C_6H_4)$	J-M	163–165	63	$C_{21}H_{20}N_2O$[g]
5aa	H	$CH_2(3\text{-}CH_3C_6H_4)$	L	134–136	60	$C_{21}H_{20}N_2O$
5bb	H	$CH_2(4\text{-}CH_3C_6H_4)$	J-M	143–144	71	$C_{21}H_{20}N_2O$
5cc	H	$CH_2(2\text{-}CF_3C_6H_4)$	D-M	167–169	46	$C_{21}H_{17}F_3N_2O$
5dd	H	$CH_2(3\text{-}CF_3C_6H_4)$	I-M	147–149	42	$C_{21}H_{17}F_3N_2O$
5ee	H	$CH_2(4\text{-}CF_3C_6H_4)$	I-M	174–176	28	$C_{21}H_{17}F_3N_2O$
5ff	H	$CH_2C_6F_5$	D-M	122–124	42	$C_{20}H_{13}F_5N_2O$
5gg	H	$CH_2(2\text{-}C_4H_3S)$	A-C	174–175	73	$C_{18}H_{16}N_2OS{\cdot}C_4H_4O_4$
5hh	6-Cl	$CH_2C_6H_5$	I-M	141–143	50	$C_{20}H_{17}ClN_2O$
5ii	6-Cl	$CH_2(4\text{-}FC_6H_4)$	I-M	156–158	47	$C_{20}H_{16}FClN_2O$
5jj	6-F	$CH_2C_6H_5$	A-C	190–192	70	$C_{20}H_{17}FN_2O{\cdot}C_4H_4O_4$
5kk	6-F	$CH_2(2\text{-}CF_3C_6H_4)$	D	149–152	80	$C_{21}H_{16}F_4N_2O$
5ll	6-CF_3	$CH_2C_6H_5$	H	136–138	58	$C_{21}H_{17}F_3N_2O$

[a–e] See footnotes *a–e*, respectively, in Table I. $C_4H_4O_4$ = maleic acid salt. [f] C: calcd, 74.98; found, 75.49. [g] C: calcd, 79.71; found, 80.17.

would be offset so that the lipophilicity of the molecule and its concomitant ability to cross the blood–brain barrier would not be unfavorably diminished. Compound **1a** was indeed shown to have less acute toxicity than THA upon oral administration in rats and mice and, more importantly, displayed a favorable profile in phase I clinical investigation. In view of the encouraging results with **1a**, we present here the data on the complete series of compounds which led not only to the selection of **1a** but also to other compounds that differ in profile from THA and yet have potential as AD therapeutics as well.

Chemistry. The synthesis of these compounds is outlined in Scheme I. The properties of the intermediate enamines (**4a–g**) and ketones (**5a–ll**) are described in Tables I and II, respectively, and the properties of the targets (**1a–ll**) are described in Table III. The synthesis of **1a** from anthranilonitrile (**2a**) and cyclohexane-1,3-dione (**3a**) has already been outlined in our first report;[18] the other primary amines (**1b–f**) were prepared in analogous fashion from appropriately substituted anthranilonitriles (**2b–f**). The cyclopentyl analogue (**1g**) was prepared from **2a** and cyclopentane-1,3-dione (**3b**). The use of potassium carbonate and a copper catalyst for the cyclization of the intermediate enamino ketones (**4a–g**) was suggested by literature reports of additions to nitriles catalyzed by transition metals.[27] The presence of a catalyst is absolutely essential—the reaction does not proceed in the presence of potassium carbonate alone.

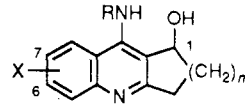
The compounds that were substituted on the 9-amino group were prepared by alkylating the corresponding ketones under phase-transfer conditions and then reducing the alkylated ketones to the alkylated alcohols. Because of the known propensity of aminoacridines and THA to alkylate at the ring nitrogen,[28] we felt that it was necessary to positively determine that the alkylation had occurred on the 9-amino group. This was accomplished by ^1H NMR: the exchange of the proton on the 9-amino group was slow enough so that the adjacent alkyl group and the NH proton coupled to each other with a coupling constant of about 5 Hz. This splitting was abolished upon D_2O exchange.

Pharmacology. It has been suggested that the screening of new therapeutic agents for the treatment of

(27) (a) Basato, M.; Corain, B.; Veronese, A. C.; D'Angeli, F.; Valle, G.; Zanotti, G. *J. Org. Chem.* **1984**, *24*, 4696. (b) Basato, M.; Corain, B.; Cofler, M.; Veronese, A. C.; Zanotti, G. *Chem. Commun.* **1984**, 1593.

(28) (a) Adcock, B. In *The Chemistry of Heterocyclic Compounds*; Weissberger, A., Taylor, E. C., Eds.; Wiley: New York, 1973; Acridines; Acheson, R. M., Ed.; p 122. (b) Selby, I. A. *Ibid.* p 477.

Table III. Data for 9-Amino-1,2,3,4-tetrahydroacridin-1-ols



compd	X[a]	R	recrystn solv[b]	mp, °C[c]	% yield[d]	formula[e]
1a	H	H	A	171–173	82	$C_{13}H_{14}N_2O \cdot C_4H_4O_4$
1b	7-Cl	H	A–D	200 dec	75	$C_{13}H_{13}ClN_2O \cdot C_4H_4O_4$
1c	6-Cl	H	A–D	190–191	58	$C_{13}H_{13}ClN_2O \cdot C_4H_4O_4$
1d	6-CH_3O	H	C–F	179–180	74	$C_{14}H_{16}N_2O_2 \cdot C_4H_4O_4$
1e	6-CF_3	H	G–M	225 dec	48	$C_{14}H_{13}F_3N_2O$
1f	6-F	H	G–M	207 dec	78	$C_{13}H_{13}FN_2O$
1g	H (n = 1)	H	A–F–G	160–162	29	$C_{12}H_{12}N_2O \cdot C_4H_4O_4$
1h	H	CH_3	G–M	160–161	73	$C_{14}H_{16}N_2O$
1i	H	n-C_3H_7	D–I	164–166	75	$C_{16}H_{20}N_2O$
1j	H	$(CH_2)_2N(CH_3)_2$	E–M	135–138	32	$C_{17}H_{23}N_3O$
1k	H	$(CH_2)_2C_6H_5$	A–D	169–170	34	$C_{21}H_{22}N_2O \cdot C_4H_4O_4$
1l	H	$(CH_2)_3OC_6H_5$	I–M	138–140	88	$C_{22}H_{24}N_2O_2$
1m	H	$(CH_2)_3CH(C_6H_5)_2$	E	146–148	74	$C_{29}H_{30}N_2O$
1n	H	$(CH_2)_3CH(4\text{-}FC_6H_4)_2$	A–H	157–159	51	$C_{29}H_{28}F_2N_2O$
1o	H	$(CH_2)_3CH(3\text{-}FC_6H_4)_2$	B–F	180 dec	39	$C_{29}H_{28}F_2N_2O \cdot 0.5C_4H_4O_4$[f]
1p	H	$CH_2C_6H_5$	A–D	142–144	67	$C_{20}H_{20}N_2O \cdot C_4H_4O_4$
1q	H	$CH_2(2\text{-}ClC_6H_4)$	D	141–143	68	$C_{20}H_{19}ClN_2O$
1r	H	$CH_2(3\text{-}ClC_6H_4)$	D	140–142	76	$C_{20}H_{19}ClN_2O$
1s	H	$CH_2(4\text{-}ClC_6H_4)$	D	176–178	79	$C_{20}H_{19}ClN_2O$
1t	H	$CH_2(2\text{-}FC_6H_4)$	A–D	151–152	81	$C_{20}H_{19}FN_2O \cdot C_4H_4O_4$
1u	H	$CH_2(3\text{-}FC_6H_4)$	I–M	128–129	83	$C_{20}H_{19}FN_2O$
1v	H	$CH_2(4\text{-}FC_6H_4)$	I–M	169–170	82	$C_{20}H_{19}FN_2O$
1w	H	$CH_2(2\text{-}CH_3OC_6H_4)$	D–M	115–117	72	$C_{21}H_{22}N_2O_2$
1x	H	$CH_2(3\text{-}CH_3OC_6H_4)$	F–M	123–125	55	$C_{21}H_{22}N_2O_2$
1y	H	$CH_2(4\text{-}CH_3OC_6H_4)$	I–M	163–165	71	$C_{21}H_{22}N_2O_2$
1z	H	$CH_2(2\text{-}CH_3C_6H_4)$	D	167–169	82	$C_{21}H_{22}N_2O$
1aa	H	$CH_2(3\text{-}CH_3C_6H_4)$	D–M	133–134	87	$C_{21}H_{22}N_2O$
1bb	H	$CH_2(4\text{-}CH_3C_6H_4)$	I–M	176–178	83	$C_{21}H_{22}N_2O$
1cc	H	$CH_2(2\text{-}CF_3C_6H_4)$	I–M	158–160	79	$C_{21}H_{19}F_3N_2O$
1dd	H	$CH_2(3\text{-}CF_3C_6H_4)$	I–M	154–155	80	$C_{21}H_{19}F_3N_2O$
1ee	H	$CH_2(4\text{-}CF_3C_6H_4)$	I–M	174–176	76	$C_{21}H_{19}F_3N_2O$
1ff	H	$CH_2C_6F_5$	A–B	214 dec	43	$C_{20}H_{15}F_5N_2O \cdot C_4H_4O_4$[f]
1gg	H	$CH_2(2\text{-}C_4H_3S)$	A–C	183–184	47	$C_{18}H_{18}N_2OS \cdot C_4H_4O_4$[f]
1hh	6-Cl	$CH_2C_6H_5$	F–G	163–164	73	$C_{20}H_{19}ClN_2O$
1ii	6-Cl	$CH_2(4\text{-}FC_6H_4)$	G	187–188	78	$C_{20}H_{18}ClFN_2O$
1jj	6-F	$CH_2C_6H_5$	B–F	164–166	49	$C_{20}H_{19}FN_2O$
1kk	6-F	$CH_2(2\text{-}CF_3C_6H_4)$	B–F	149–152	54	$C_{21}H_{18}F_4N_2O$
1ll	6-CF_3	$CH_2C_6H_5$	I	156–158	57	$C_{21}H_{19}F_3N_2O$

[a–e] See footnotes *a–e*, respectively, in Table I. $C_4H_4O_4$ = maleic acid salt, except where indicated. [f] Fumaric acid salt.

AD and related disorders incorporate biochemical studies in addition to behavioral testing. In vitro testing provides "high testing throughput and minimal complicating pharmacokinetic and metabolic factors" and serves as a means for selecting compounds for the more time consuming behavioral testing.[3] The present compounds were tested in vitro for their ability to inhibit AChE and to inhibit the reuptake of NE, 5-HT, and DA. It was recognized that, although potent AChE inhibition (AChEI) might be necessary for AD activity, it was not sufficient—additional efficacy in a behavioral assay was required in order to confirm that the compounds were being absorbed, were crossing the blood–brain barrier, and were actually exerting an effect on the central nervous system (CNS). Thus, compounds that showed promise in the in vitro assays were further evaluated in a paradigm in which dementia was mimicked in mice by inducing a functional cholinergic impairment through the use of an antimuscarinic drug—the reversal of scopolamine-induced impairment of 24-h memory in passive dark avoidance in mice (scopolamine dementia, SD). The most promising compounds were also tested in a model in which dementia was mimicked in rats by creating an actual lesion in an important cholinergic pathway through the use of a stereotaxically applied excitotoxin—the reversal of the deficit in 72-h retention of a one-trial dark-avoidance task in rats, induced by ibotenic acid lesions in the nucleus basalis magnocellularis (nbm).

As we and others[3] have pointed out, animal models of AD are, at best, imperfect. The scopolamine-induced dark-avoidance model, for example, gives positive test results not only with cholinergic enhancers, but also with analgesics, motor stimulants and so-called "nootropic" compounds.[29] Thus, emphasis was placed on compounds that were active *both* in vitro as AChE inhibitors and in vivo in reversing scopolamine dementia over a broad dose range as well as displaying low acute toxicity. Caution was exercised, however, because of the realization that the in vitro AChEI assay may not be strictly reflective of the effect of a given inhibitor in vivo, where AChE is membrane bound, conditions of saturation are never reached, and transient state kinetics apply.[30] In such a dynamic situation, the effect of an AChE inhibitor may not bear a direct relationship to the in vitro situation in which equilibrium is attained. Therefore compounds with weak to moderate AChEI were considered as well as the most potent AChE inhibitors.

There were other reasons for considering compounds

(29) Bammer, G. *Neurosci. Biobehav. Rev.* **1982**, *6*, 274.
(30) Main, A. R. In *Biology of Cholinergic Function*; Goldberg, A. M., Hanin, I., Eds.; Raven Press: New York, 1976; pp 274–275.

Table IV. Biological Data for the 9-Amino-1,2,3,4-tetrahydroacridin-1-ols

compd	acute toxicity in rats: LD_{50}, mg/kg ip[a]	acetylcholinesterase inhibition: IC_{50}, μM[b]	reversal of scopolamine-induced memory impairment: % > cutoff, (mg/kg sc)[c]						inhibition of amine reuptake: IC_{50}, μM[d]		
			0.16	0.31	0.63	1.25	2.5	5.0	NE	5-HT	DA
1a	<80 > 40	4.0 (3.14–5.02)	14	**20**	**33**	**20**	**31**	**33**	>20	>20	>20
1b	~80	24.7 (11.2–54.2)	NT[e]						>10		
1c	<10 > 5	0.0117 (0.0074–0.0185)	7	7	0	7	0	16	>20	>20	>20
1d	>80	2.00 (1.56–2.58)	NT[e]								
1e	<80 > 40	1.77 (1.35–2.33)	**40**	**26**	**71**	**33**	**21**	**33**	NT[e]		
1f	~40	0.292 (0.207–0.410)	**73**	**29**	**20**	**20**	**33**	**40**	>10		
1g	>80	5.97 (2.52–14.16)	13	13	0	0	7	7	NT[e]		
1h	<80 > 40	1.76 (1.34–2.30)	0	7	0	7	7	7	>20	>20	>20
1i	<80 > 40	13.3 (7.30–24.5)	NT[e]						>20	>20	>20
1j	>80	7.68 (3.20–18.45)	NT[e]								
1k	>80	3.39 (2.51–4.59)	7	**20**	0	**23**	0	0	1.4	7.7	4.7
1l	<80 > 40	3.98 (2.77–5.73)	0	0	0	13	7	–	NT[e]		
1m	>80	49.3 (34.7–70.2)	**20**	6	6	13	0	6	1.5	5.7	2.1
1n	>80	87.8 (60.0–128.6)	NT[e]								
1o	>80	63.0 (20.2–196.5)	NT[e]								
1p	<60 > 50	32.2 (10.4–99.5)	**27**	**50**	**20**	13	**21**	**36**	0.070	11	0.30
1q	>80	10.3 (7.8–13.5)	0	**27**	7	**27**	13	7	0.043	9.3	0.13
1r	>80	13.3 (10.4–16.9)	NT[e]								
1s	>80	27.2 (21.7–34.0)	NT[e]						1.1	5.7	9.8
1t	<80 > 40	41.3 (31.5–54.1)	7	14	**33**	7	20	7	0.11	17	0.41
1u	<80 > 40	13.1 (2.4–72.9)	**33**	**23**	7	0	14	**21**	0.12	6.2	0.30
1v	<80 > 40	16.7 (7.9–35.0)	7	**20**	**25**	**27**	**33**	6	1.8	12	2.9
1w	~80	36.0 (28.0–46.1)	**33**	7	**20**	7	13	**27**	4.8	9.8	0.72
1x	>80	21.1 (17.0–26.2)	0	0	0	0	0	0	1.5	6.2	2.1
1y	>80	10.9 (8.0–14.9)	NT[e]						2.5	5.0	5.3
1z	<80 > 40	5.89 (1.43–24.3)	0	7	7	13	7	0	0.053	9.0	0.25
1aa	<80 > 40	24.5 (19.1–31.5)	7	7	**20**	**20**	13	**20**	0.22	5.5	0.51
1bb	>80	21.0 (15.9–27.6)	NT[e]						1.1	8.0	2.2
1cc	~80	13.3 (9.8–18.0)	**38**	**23**	13	**20**	13	**33**	0.081	3.8	0.95
1dd	<80 > 40	14.2 (10.8–18.8)	0	0	0	7	0	0	0.93	2.3	2.4
1ee	>80	18.1 (14.3–23.0)	**22**	8	7	15	0	**20**	0.27	6.6	2.4
1ff	>80	12.6 (9.4–16.9)	7	7	7	13	0	**20**	11		
1gg	<80 > 40	15.3 (11.6–20.2)	NT[e]						0.29	20	2.4
1hh	>80	1.30 (1.00–1.69)	0	13	7	7	7	0	0.82	15	1.3
1ii	>80	0.823 (0.161–1.10)	13	7	7	7	0	0	2.2	6.6	4.1
1jj	<80 > 40	8.63 (3.69–20.17)	13	0	0	0	0	0	0.20		
1kk	>80	20.1 (15.5–26.1)	0	**20**	7	0	0	13	0.54	20	0.31
1ll	>80	68.2 (47.3–98.3)	NT[e]								
THA	<20 > 10	0.319 (0.245–0.416)	7	7	**33**	14	**31**	**33**	11	19	22
desipramine									13.5	>20	7.8

[a] Refer to the Experimental Section for test procedure and method of reporting results. [b] Values in parentheses are 95% confidence intervals. [c] A cutoff was defined for the scopolamine-vehicle group (see Experimental Section) as the value for the animal with the second longest latency time. Results are reported as the percent of animals in the scopolamine-drug group that exhibited latencies greater than the cutoff time. The boldface values are those that met the empirical positive-activity criterion of 20% of the animals with latencies greater than the cutoff. [d] See the Experimental Section for test procedures. [e] NT = not tested.

displaying the whole range of potencies in AChEI. For example, THA has recently shown to have ion-channel directed effects that may serve to increase the release of endogenous ACh.[31] It has also been shown to interact with the phencyclidine receptor site,[32] to inhibit potassium-evoked release of GABA,[33] to bind to nicotinic and muscarinic receptors,[34] and to inhibit *N*-methyl-D-aspartate neurotoxicity.[35] Such effects could contribute to the usefulness of THA-like compounds in AD while being separate from their activity as AChE inhibitors. Furthermore, by looking at NE, 5-HT, and DA reuptake, the compounds were examined for their potential to influence other endogenous chemical systems that have been implicated in the development of cognitive disorders (vide supra).

The results for the SD assay are reported as the percent of animals in the scopolamine-drug group that had latencies greater than a cutoff value that was determined for each group of animals (see the Experimental Section). Experiments with a variety of standards repeated under a number of environmental conditions led to the development of the empirical positive activity criterion of 20% of the animals with latencies greater than cutoff.

The acute toxicity of all compounds was evaluated in rats. The results are reported as approximate LD_{50}s in groups of four rats. Testing was begun at 80 mg/kg ip and lowered to 40, 20, 10, and 5 mg/kg ip, if necessary, until two or fewer deaths were observed. For the most promising compounds, an LD_{50} was determined in a large group of mice.

Biological Results and Discussion

The biological results for compounds **1a–ll** are given in Table IV. The compounds that were unsubstituted at the

(31) (a) Drukarch, B.; Kits, K. S.; Van der Meer, E. G.; Lodder, J. C.; Stoof, J. C. *Eur. J. Pharmacol.* **1987**, *141*, 153. (b) Freeman, S. E.; Lau, W.-M.; Szilagyi, M. *Eur. J. Pharmacol.* **1988**, *154*, 59. (c) Osterrieder, W. *Br. J. Pharmacol.* **1987**, *92*, 521. (d) Rogawski, M. A. *Eur. J. Pharmacol.* **1987**, *142*, 169. (e) Schauf, C. L.; Sattin, A. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 609. (f) Stevens, D. R.; Cotman, C. W. *Neurosci. Lett.* **1987**, *79*, 301.
(32) Albin, R. L.; Young, A. B.; Penney, J. B. *Neurosci. Lett.* **1988**, *88*, 303.
(33) deBelleroche, J.; Gardiner, I. M. *Br. J. Pharmacol.* **1988**, *94*, 1017.
(34) Perry, E. K.; Smith, C. J.; Court, J. A.; Bonham, J. R.; Rodway, M.; Atack, J. R. *Neurosci. Lett.* **1988**, *91*, 211.
(35) Davenport, C. J.; Monyer, H.; Choi, D. W. *Eur. J. Pharmacol.* **1988**, *154*, 73.

9-amino group (**1a–g**) exhibited, for the most part, good AChEI. Of these, the unsubstituted parent (**1a**) was moderately potent as an AChE inhibitor (4.0 μM, approximately 10 times less potent than THA), while the 7-chloro derivative (**1b**) was 6 times less potent than **1a**. This was in marked contrast to the 6-chloro derivative (**1c**), which showed the strongest inhibition at 0.012 μM (almost 30 times as potent as THA). Some other 6-substituents of varying electronic nature and hydrophobicity were examined (**1d–f**), and of these the 6-methoxy and 6-trifluoromethyl compounds (**1d** and **1e**) also had moderate activity in the range of **1a**, while the 6-fluoro compound (**1f**) showed good AChEI (0.29 μM, almost identical with THA), although it was not as potent as **1c**. The analogue with one less methylene group in the saturated ring (**1g**) also had moderate AChEI potency (6.0 μM) in the range of its homologue, **1a**. On the basis of the data with compounds **1a–f**, the AChE enzyme tends to tolerate a certain amount of variation in the area of the 6-position, while substitution at the 7-position seems to be detrimental to AChEI potency. Although more analogues would need to be tested in order to establish a true SAR, it seems doubtful that the activity of **1c** and **1f** can be ascribed to a simple increase in lipophilicity—**1b**, **1d**, and **1e** should have been equally potent in that case. One explanation that could account for the results with **1c** and **1f** would be the existence of a specific, as yet undertermined, receptor interaction with the region of the molecules in the area of the 6-position. Of the compounds that showed good to moderate AChEI (**1a**, **1c–g**), **1a**, **1e**, and **1f** met our criterion for activity in SD. The striking lack of SD activity in the potent AChE inhibitor **1c** may be due to poor absorption and/or distribution to the CNS, although recent results with THA indicate that compounds of this type have a favorable distribution into the brain.[36] Alternatively, the lack of SD activity of **1c** could be an example of the lack of a direct relationship between in vitro and in vivo AChEI activity, or an example of a case where the effect of **1c** on other neural pathways may be coming into play (vide supra). It is also possible that **1c** influences other factors that have a bearing on the SD model (e.g. pain sensitivity or locomotor activity). These considerations, along with the inherent variability of animal models such as SD,[29] could account for the lack of effect of **1c** in SD.

In addition to the effects of substitution on the acridine nucleus mentioned above, there would seem to be a limit to the size of substituent at the 9-amino group that can be accommodated by the AChE enzyme. While the methyl-substituted analogue (**1h**) retained good AChEI activity (1.8 μM), extending the chain length to *n*-propyl (**1i**) resulted in diminished AChEI (13 μM). The (dimethylamino)ethyl derivative (**1j**) had potency between that of **1h** and **1i** (7.7 μM)—an additional interaction with the aliphatic amino group may, to a certain extent, overcome the steric effect. Of these derivatives, the one with the best AChEI (**1h**) was inactive in SD (see the discussion of **1c** above) and the other analogues were not tested because of toxicity and poorer AChEI.

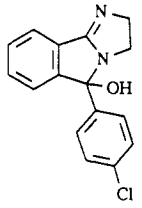
The addition of arylalkyl substituents on the 9-amino group gave, in certain cases, desirable combinations of AChEI and SD activity. When the aryl groups were attached to the 9-amino function by alkyl chains longer than CH_2 (**1k–o**), two compounds—the phenethyl (**1k**) and the phenoxypropyl (**1l**) analogues—had good AChEI activity (3.4 and 4.0 μM, respectively) and compound **1k** was active in SD. One compound (**1m**) that had weaker activity as a cholinesterase inhibitor was also effective in SD, albeit at only one dose. These results are similar to results that have been reported with THA analogues substituted on the 9-amino group.[19] This previous work postulated an additional, conformationally flexible, hydrophobic binding site on the enzyme near the area of the anionic subsite and it may be that such an interaction is responsible for the good activity seen with **1k** and **1l**.

When aryl groups were attached to the 9-amino function by a CH_2 linkage (i.e., benzyl groups), a number of compounds were obtained which combined AChEI with activity in SD. A series of benzyl derivatives was synthesized (**1p–gg**), consisting of the unsubstituted compound **1p** and derivatives substituted at the ortho, meta, and para positions of the benzyl groups with chloro (**1q–s**), fluoro (**1t–v**), methoxy (**1w–y**), methyl (**1z–bb**), and trifluoromethyl (**1cc–ee**) substituents, as well as compounds substituted at the 9-amino group by pentafluorobenzyl (**1ff**) and 2-thienylmethyl (**1gg**) substituents. The unsubstituted prototype **1p** was moderately active in AChEI and satisfied our criterion for a statistically significant effect in SD. The remaining analogues showed moderate AChEI in the range of 10–30 μM. The *o*-fluoro (**1t**) and *o*-methoxy (**1w**) analogues were slightly less potent (in the 30–40 μM range) while the *o*-methyl analogue (**1z**) was slightly more potent as an AChE inhibitor in vitro (5.9 μM). The *o*-chloro analogue (**1q**), in addition to showing moderate cholinesterase inhibition, also met the criterion for activity in SD, showing good reversal of the scopolamine effect at two doses. The *o*-, *m*-, and *p*-fluoro analogues (**1t–v**) were also active in SD, although **1t**, which was less potent in AChEI, was only active at a single dose in SD. Of the methoxy compounds, only the *o*-methoxy entry (**1w**) demonstrated AChEI and SD activity, while, among the methyl compounds, only the meta-substituted derivative (**1aa**) was active in both ACHEI and SD. The *o*- and *p*-trifluoromethyl derivatives (**1cc** and **1ee**) were active in both AChEI and SD. The pentafluorobenzyl (**1ff**) and 2-thienylmethyl (**1gg**) derivatives were, like their counterparts **1o–ee**, moderately potent inhibitors of AChE; compound **1ff** was also active in SD, although only at one dose. Effects similar to those seen with the primary amines were observed among these compounds also: even within closely related analogues with good AChEI, there were differences in SD activity (**1dd** and **1ee**, for example) and the most potent AChE inhibitor (**1z**) was inactive in SD. Possible explanations for these data have already been discussed.

One of the most interesting effects of substitution in this series was the increase in synaptosomal uptake inhibition brought about by aralkyl substituents on the 9-amino group. THA has recently been shown to inhibit uptake of NE, DA, and 5-HT in the range of 1–10 μM,[37] and our own experiments gave values in the 10–20 μM range, using higher ^{3}H-labeled-ligand concentrations (see the Experimental Section). Virtually no uptake inhibition was seen with the primary amines **1a–g** or the compounds bearing small alkyl groups (**1h–j**). When the 9-amino group was substituted with phenethyl (**1k**) and diphenylbutyl (**1m**) groups, however, uptake inhibition in the 1–5 μM range was seen, and when the benzyl analogues (**1p–1gg**) were examined, NE uptake inhibition at the μM level and below was discovered, with many of the derivatives more potent than the standard, desipramine. No derivative showed serotonin-uptake inhibition below 2.3 μM, but the un-

(36) Liston, D.; Russo, L.; Mena, E. E.; Williams, I. H. *Alzheimer's Dis. Assoc. Disord.* **1988**, *2*, 219.

(37) Drukarch, B.; Leysen, J. E.; Stoof, J. C. *Life Sci.* **1988**, *42*, 1011.



6

Figure 1.

substituted parent (**1p**) and several substituted analogues (**1q**, **1t**, **1u**, **1z**, **1aa**, and **1cc**) inhibited NE uptake at concentrations of 0.22 μM and below and inhibited DA uptake at concentrations of 0.95 μM and below. Two ortho-substituted analogues, the chloro (**1q**) and methyl (**1z**) compounds, displayed particularly good NE (<0.06 μM) and DA (<0.3 μM) uptake inhibition, more than 100 times as potent as desipramine in the whole-brain preparation. Two other ortho-substituted derivatives, the fluoro (**1t**) and trifluoromethyl (**1cc**) compounds, also showed good NE- and DA-uptake inhibition, but, like the *m*-fluoro (**1u**) and *m*-methyl (**1aa**) analogues, not quite at the level of **1q** and **1z**. These data would seem to indicate a crucial role for the aryl group, its distance from the amino groups, and its conformation relative to the acridine ring.

It is widely held that derivatives of phenethylamine (NE and DA) interact with the neuronal-uptake system in the fully extended or *antiperiplanar* configuration.[38] Preliminary studies with Dreiding models suggest that, if the ring nitrogen and benzyl phenyl group of analogues **1p–gg** can interact respectively with the same site as the nitrogen and the phenyl group of the phenethylamines, an accessible configuration of **1p–gg** would be a reasonable fit to the fully extended phenethylamines. Amine-uptake inhibitors that incorporate an imino tertiary nitrogen into a ring are known (i.e. mazindole (**6**), Figure 1), suggesting that the interaction of the ring nitrogen of **1p–gg** with the phenethylamine site is a reasonable hypothesis. Furthermore, viewing the central portion of **1p–gg** as a unit that overlaps the DA- or NE-uptake site could explain the good inhibition shown by the ortho-substituted compounds. Such substitution may be responsible for a steric interaction that induces a favorable torsion angle in the phenyl group of the benzyl moiety, relative to the plane that contains the nitrogen atom: the *o*-chloro (**1q**), *o*-methyl (**1z**), and *o*-trifluoromethyl (**1cc**) analogues were the most potent in terms of combined NE- and DA-uptake inhibition. Further studies of favored conformations and fit to the extended configuration of NE and DA using molecular modeling are under way and will be published later.

The pattern of uptake inhibition bears comment. It has been shown that NE- and DA-uptake inhibition often parallel each other, due to the similarity of the conformations in which each of these transmitters interacts with its respective uptake system, while good inhibitors of NE and DA uptake tend to be less-potent inhibitors of 5-HT uptake.[39] There was a tendency toward this pattern in the compounds of this series, as evidenced by the fact that the three most potent inhibitors of NE uptake (**1p**, **1q**, and **1z** <0.08 μM) were also the three most potent inhibitors of DA uptake (<0.30 μM). The uptake of 5-HT, on the other hand, tended to be inhibited at least 10–100 times the concentration required for NE. Although brain levels of 5-HT have been shown to be decreased in AD,[4] making 5-HT-uptake inhibition a desirable quality in an AD therapeutic, reports that 5-HT may exert an inhibitory influence on learning and memory[40] suggest that the pattern of uptake inhibition seen with these compounds may be advantageous.

Table V. Acute Toxicity of Selected Compounds

compd	acute toxicity in mice: LD_{50}, mg/kg ip[a]
1a	162 (131–201)
1p	94 (83–107)
THA	29.1 (24.0–35.2)

[a] See the Experimental Section. Values in parentheses are 95% confidence limits.

In an attempt to combine enhanced AChEI activity with good uptake inhibition, five compounds were synthesized that combined substituents on the 6-position of the acridine ring with benzyl groups on the 9-amino group (**1hh–ll**). In this series, some of the trends noted above were continued—substitution by chlorine or fluorine in the 6-position gave, in three cases, compounds that were more active inhibitors of AChE than the unsubstituted parent (compare **1hh** with **1p**, **1ii** with **1v**, and **1jj** with **1p**)—but other trends were not—the *o*-(trifluoromethyl)benzyl analogue (**1kk**) was not a better inhibitor of NE uptake than the corresponding unsubstituted compound (**1jj**), for example. Activity in SD was lost in all but **1kk**, which was only active at a single dose.

Two compounds, **1a** and **1p** (HP-128), were selected for further testing in the nbm lesion model and for further toxicological evaluation. Of the primary amines (**1a–g**), while **1a**, **1e**, and **1f** satisfied the requirement for activity in AChEI and SD, **1a** appeared less toxic (no deaths at 40 mg/g ip).[41] Upon testing in the nbm model, **1a** reversed the retention deficit in the lesioned animals and enhanced learning in the sham-operated animals.[18] At a dose of 2.5 mg/kg, the deficit in the lesioned animals was not only reversed, but performance was actually improved above the level of normal sham-operated controls. The LD_{50} of **1a** in mice was also considerably higher than THA (Table V). Because it was a racemate, **1a** was resolved into its (+) and (−) forms by repeated recrystallization of the dibenzoyltartaric acid salts ($[\alpha]^{20}_{D}$ −161.5° and +158.5° (*c* = 1, DMSO)). Some in vitro differences between the enantiomers were found (most of the AChEI activity appears to reside in the (−) isomer, for example), but racemization in dilute acid was found to be so rapid, due to the benzylic carbinol nature of the molecule, that comparative pharmacological testing was deemed meaningless.

Of the secondary amines, the selection of a compound for testing in the nbm model was based not only on AChE inhibition and SD activity but also on NE- and DA-uptake inhibition, for the reasons given above. Of the compounds that displayed acceptable activity in AChEI and SD, **1p** and **1q** showed particularly potent inhibition of NE and DA uptake, while being more than 100 times less potent in the inhibition of 5-HT uptake. In addition to the advantages of such a profile given above, it was recognized that such compounds had potential antidepressant activity. Thus, they were also screened for the reversal of tetrabenazine ptosis (TBZ) in mice.[42] Several compounds with

(38) Rutledge, C. O.; Walters, D. E.; Grunewald, G. L. In *Handbook of Stereoisomers: Drugs in Psychopharmacology*; Smith, D. F., Ed.; CRC: Boca Raton, FL, 1984; pp 79–116.
(39) Bogeso, K. P.; Christensen, A. V.; Hyttel, J.; Liljefors, T. *J. Med. Chem.* **1985**, *28*, 1817.
(40) Altman, H. J.; Normilie, H. J. *Psychopharmacol.* **1986**, *90*, 24.
(41) Our studies indicate that the route of administration for SD (subcutaneous) and acute toxicity (intraperitoneal) are bioequivalent (unpublished results).

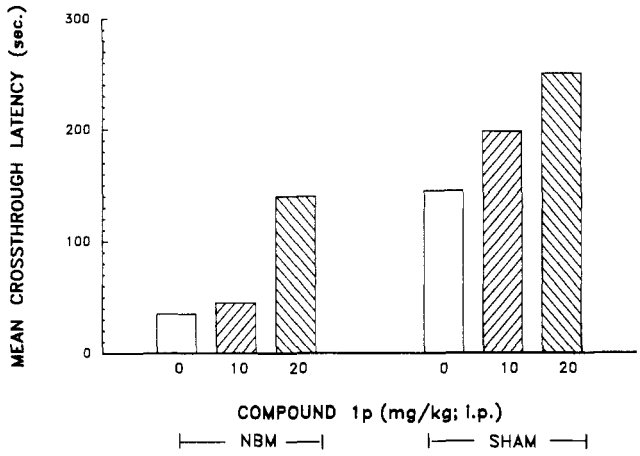


Figure 2. Effect of **1p** on the 72-h retention of passive avoidance in NBM lesioned rats. The procedures for this test are described in ref 51. Groups of 10 rats were used per dose; the zero mg/kg dose corresponds to an injection of pure saline. There was a statistically significant enhancement of retention by **1p** in both lesioned and sham-operated rats (ANOVA $F > 3.8$, $P < 0.025$). In the lesioned rats, **1p** exerted its greatest effect at the 20 mg/kg dose.

good NE- and DA-uptake inhibition also had moderate TBZ activity (**1p**, ED_{50} = 27.4 mg/kg ip; **1z**, ED_{50} = 25.3 mg/kg ip; and **1cc**, ED_{50} = 16.2 mg/kg ip), but only the unsubstituted-benzyl analogue **1p** combined AChEI activity with the ability to reverse SD and TBZ. For this reason, **1p** was chosen for testing in the nbm model. In this paradigm, **1p** was active at 20 mg/kg and, like **1a**, reversed the retention deficit in the lesioned animals and enhanced learning in the sham-operated animals (Figure 2). The acute LD_{50} of compound **1p** in mice was 94 mg/kg ip (Table V).

Clinical trials have been initiated with **1a** and with **1p**. As reported in our preliminary communication,[18] phase I has been completed with **1a** and an acceptable dose range for outpatient evaluation was defined. Neither laboratory nor clinical evidence of drug-induced hepatotoxicity was reported in 1396 subject days of exposure (normal young and elderly volunteers, as well as in AD patients). Subsequent phase II trials with **1a** were temporarily halted when two cases of reversibly elevated liver enzymes were found among 49 patients; these studies have recently been restarted at the same doses as called for in the original protocol.[43] Further studies to establish the safety and efficacy of **1a** and **1p** in AD are under way.

Experimental Section

The structures of all compounds were supported by their IR (Perkin-Elmer 547) and ^{1}H NMR (Varian XL-200) spectra. Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Mass spectra data were determined by direct insertion with a Finnigan 4000 GC-MS equipped with a INCOS data system. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements (performed by Micro-Tech Laboratories, Skokie, IL) were within ±0.4% of theoretical values.

The syntheses of 5-chloroanthranilonitrile (**2b**), as well as 4-chloro- (**2c**), 4-methoxy- (**2d**), 4-(trifluoromethyl)- (**2e**), and 4-fluoroanthranilonitrile (**2f**) have been described in the literature.[44-48]

Chemistry. *N*-(3-Oxocyclohexen-1-yl)-2-aminobenzonitrile (4a). A suspension of anthranilonitrile (200.0 g, 1.69 mol) in 1 L of toluene containing 1,3-cyclohexanedione (208.5 g, 1.86 mol) and *p*-toluenesulfonic acid monohydrate (10.3 g, 0.054 mol) was refluxed for 2 h, and the water was collected in a Dean–Stark water separator. At the end of this time, the reaction mixture was chilled to ca. 0 °C and the product was filtered off and washed with cold toluene. The crude product obtained in this manner was slurried with 1 L of water and stirred for 1 h, after which it was filtered off and dried under reduced pressure to give 329.8 g (91.9%) of **4a**: mp 188–190 °C (The melting point was unchanged upon recrystallization of the analytical sample from CH_2Cl_2–hexane.); IR ($CHCl_3$) 2250, 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.10 (2 H, quintet, J = 7 Hz, 5′-CH_2), 2.40 (2 H, t, J = 7 Hz, 4′-CH_2), 2.60 (2 H, t, J = 7 Hz, 6′-CH_2), 5.59 (1 H, s, vinylic H), 7.0 (1 H, br s, exchanges with D_2O, NH), 7.25 (1 H, t, J = 8 Hz, ArH), 7.45–7.70 (3 H, m, ArH); MS m/e 212 (M^+). Anal. ($C_{13}H_{12}N_2O$) C, H, N.

The other compounds **4b–g** were prepared from the appropriately substituted anthranilonitriles and diketones in a similar manner (Table I).

9-Amino-3,4-dihydroacridin-1(2*H*)-one (5a). In 4 L of THF was suspended **4a** (182.1 g, 0.8579 mol), anhydrous K_2CO_3 (250 g, 0.155 mol), and CuCl (3.0 g, 0.030 mol). The reaction mixture was refluxed for 5 h and then the hot mixture was filtered into 4 L of hexane. The precipitated **5a** was filtered off, washed with water, and dried to give 113.5 g (62.3%), mp 237–239 °C. An analytical sample was recrystallized from *i*-PrOH: mp 236–238 °C; IR ($CHCl_3$) 1640, 1605 cm^{-1}; ^{1}H NMR ($CDCl_3$, DMSO-d_6) δ 2.18 (2 H, quintet, J = 7 Hz, 3-CH_2), 2.74 (2 H, t, J = 7 Hz, 2-CH_2), 3.10 (2 H, t, J = 7 Hz, 4-CH_2), 7.40 (1 H, dt, J_o = 8 Hz, J_m = 1 Hz, ArH), 7.70 (1 H, dt, J_o = 8 Hz, J_m = 1 Hz, ArH), 7.6–7.8 (1 H, br s, exchanges with D_2O, NH), 7.80 (dd, J_o = 8 Hz, J_m = 1 Hz, ArH), 8.20 (dd, J_o = 8 Hz, J_m = 1 Hz, ArH), 10.2 (1 H, br s, exchanges with D_2O, hydrogen bonded NH); MS m/e 212 (M^+). Anal. ($C_{13}H_{12}N_2O$) C, H, N.

The other ketones **5b–g** were prepared from the analogous enaminones **4b–g** in a similar manner (Table II).

9-Amino-1,2,3,4-tetrahydroacridin-1(2*H*)-ol Maleate (1a). Ketone **5a** (5.00 g, 0.024 mol) was suspended in 100 mL of dry THF and a solution of lithium aluminum hydride in Et_2O (22 mL of 1.1 M, 0.024 mol) was added dropwise over 15 min. The reaction was allowed to stir for 2 h and then quenched by the careful addition of 20 mL of 10% HCl. This solution was made strongly basic with 30% NaOH, enabling the crude **1a** free base to be filtered off. The crude free base isolated in this manner was converted to the maleate in *i*-PrOH to give, after recrystallization from MeOH–EtOAc, 6.4 g (82%) of **1a**: mp 171–173 °C; ^{1}H NMR (DMSO-d_6) δ 2.7–3.1 (4 H, m, 2- and 3-CH_2), 2.8–3.0 (2 H, m, 4-CH_2), 4.90 (1 H, s, H-1), 5.35 (1 H, br s, exchanges with D_2O, OH), 6.10 (2 H, s, maleic acid), 7.64 (1 H, t, J_o = 8 Hz, ArH), 7.80 (1 H, d, J_o = 8 Hz, ArH), 7.92 (1 H, t, J_o = 8 Hz, ArH), 8.52 (1 H, d, J_o = 8 Hz, ArH), 8.6 (2 H, br s, exchanges with D_2O, NH_2), 13.5 (2 H, br s, exchanges with D_2O, COO*H*); MS m/e 214 (M^+). Anal. ($C_{13}H_{14}N_2O{\cdot}C_4H_4O_4$) C, H, N.

The other hydroxy compounds **1b–g** were prepared from the analogous ketones **5b–g** in a similar fashion (Table III).

9-(Benzylamino)-3,4-dihydroacridin-1(2*H*)-one (5p). Ketone **5a** (4.00 g, 0.019 mol) was dissolved in a biphasic mixture of 150 mL of CH_2Cl_2 and 100 mL of 50% NaOH to which tetra-*n*-butylammonium hydrogen sulfate (0.96 g, 0.015 mol) had been added. Benzyl bromide (3.56 g, 0.021 mol) was then added and the reaction was stirred vigorously for 4 h. At the end of this time the organic phase was separated, washed with water, and evaporated to a solid. Two recrystallizations from CH_2Cl_2–hexane gave 2.35 g (41%) of **5p**: mp 162–163 °C; IR ($CHCl_3$) 1620, 1580 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.16 (2 H, quintet, J = 7 Hz, 3-CH_2),

(42) Benesova, O.; Nahunek, K. *Psychopharmacologia* **1971**, *20*, 337.
(43) *Scrip* **1988**, May 11, p 27.
(44) Grivsky, E. M.; Hitchings, G. H. *Ind. Chim. Belge* **1974**, *39*, 490.
(45) McKee, R. L.; McKee, M. K.; Bost, R. W. *J. Am. Chem. Soc.* **1947**, *69*, 940.
(46) Cook, A. H.; Heilbron, I. M.; Reed, K. J.; Strachan, M. N. *J. Chem. Soc.* **1945**, 861.
(47) U.S. Patent 3 254 094, May 31, 1966.
(48) Hunziker, F.; Fischer, R.; Kipfer, P.; Schmutz, J.; Bürki, H. R.; Eichenberger, E.; White, T. G. *Eur. J. Med. Chem.* **1981**, *16*, 391.

2.74 (2 H, t, J = 7 Hz, 2-CH_2), 3.12 (2 H, t, J = 7 Hz, 4-CH_2), 5.08 (2 H, d, J = 6 Hz, collapses to s upon D_2O exchange, benzyl CH_2), 7.2–7.5 (6 H, m, benzyl C_6H_5 + acridine ArH), 7.68 (1 H, t, J = 8 Hz, ArH), 7.86 (1 H, d, J = 8 Hz, ArH), 8.22 (1 H, d, J = 8 Hz, ArH), 11.75 (1 H, br t, exchanges with D_2O); MS, m/e 302 (M^+). Anal. ($C_{20}H_{18}N_2O$) C, H, N.

The other alkylated ketones **5h–o** and **5q–ll** were prepared in similar fashion from ketones **5a–f** (Table II).

9-(Benzylamino)-1,2,3,4-tetrahydroacridin-1-ol Maleate (1p). Ketone **5p** (3.81 g, 0.013 mol) was reduced in 75 mL of dry THF with 1.1 M lithium aluminum hydride in Et_2O (5.9 mL, 0.0065 mol) as in the preparation of **1a** above. The maleate was formed after isolating the crude free base, giving 4.10 g (75%) of **1p** after recrystallization from MeOH–EtOAc: mp 142–144 °C; ^1H NMR (DMSO-d_6) δ 1.7–2.1 (4 H, m, 2- and 3-CH_2), 2.8–3.1 (2 H, m, 4-CH_2), 4.90 (1 H, br s, H-1), 5.28 (2 H, ddd, J_{gem} = 14 Hz, J_{vic} = 6 Hz, collapses to dd upon D_2O exchange, benzyl CH_2), 5.74 (1 H, br s, exchanges with D_2O, OH), 6.05 (2 H, s, maleic acid), 7.3–7.6 (6 H, m, benzyl C_6H_5 + acridine ArH), 7.8–8.0 (2 H, m, ArH), 8.45 (1 H, d, J_o = 8 Hz, ArH), 8.78 (1 H, br t, exchanges with D_2O, NH), 13.5 (2 H, br s, exchanges with D_2O, COO*H*); CIMS m/e 305 (MH^+). Anal. ($C_{20}H_{20}N_2O \cdot C_4H_4O_4$) C, H, N.

The other compounds **1h–o** and **1q–ll** were prepared in similar fashion from the ketones **5h–o** and **5q–ll** (Table III).

Biological Methods. Acute Toxicity. For acute toxicity in rats, groups of four male Wistar rats were used. Dosing was begun at 80 mg/kg ip, and if fewer than two deaths occurred within 24 h, the LD_{50} was recorded as >80 mg/kg. If two deaths occurred, the LD_{50} was recorded as ~80 mg/kg. If more than two deaths occurred, the dose was lowered as necessary to 40, 20, 10, and 5 mg/kg until two or fewer deaths occurred within 24 h and the LD_{50} was recorded accordingly. For acute toxicity in mice, groups of 10 mice were tested at four doses, and LD_{50}s were determined by a modification of the method of Bliss.[49]

Acetylcholinesterase Inhibition. The method of Ellman[50] was used with a rat striatal preparation. Acetylthiocholine was employed as substrate, at a concentration of 5.0 mM. Determinations were done at four different concentrations and an IC_{50} was calculated with a log-probit analysis.

Reversal of Scopolamine Dementia. Three groups of 15 male CFW mice were used—a vehicle/vehicle control group, a scopolamine/vehicle group, and a scopolamine/drug group. Thirty minutes prior to training, the vehicle/vehicle control group received normal saline sc, and the scopolamine/vehicle and scopolamine/drug groups received scopolamine sc (3.0 mg/kg, administered as scopolamine hydrobromide). Five minutes prior to training, the vehicle/vehicle control and scopolamine/vehicle groups received distilled water and the scopolamine/drug group received the test compound in distilled water.

The training/testing apparatus consisted of a Plexiglas box approximately 48 cm long, 30 cm high and tapering from 26 cm wide at the top to 3 cm wide at the bottom. The interior of the box was divided equally by a vertical barrier into a light compartment (illuminated by a 25-W reflector lamp suspended 30 cm from the floor) and a dark compartment (covered). There was a hole at the bottom of the barrier 2.5 cm wide and 6 cm tall and a trap door which could be dropped to prevent an animal from passing between the two compartments. A Coulbourn Instruments small-animal shocker was attached to two metal plates which ran the entire length of the apparatus, and a photocell was placed in the dark compartment 7.5 cm from the vertical barrier and 2 cm off the floor. The behavioral session was controlled by a PDP 11/34 minicomputer.

At the end of the pretreatment interval, an animal was placed in the light chamber directly under the light fixture and facing away from the door to the dark chamber. The apparatus was then covered and the system was activated. If the mouse passed through the barrier to the dark compartment and broke the photocell beam within 180 s, the trap door dropped to block escape to the light compartment and an electric shock was administered at an intensity of 0.4 mA for 3 s. The animal was then immediately removed from the dark compartment and placed in its home cage. If the animal failed to break the photocell beam within 180 s, it was discarded. The latency in seconds for each mouse was recorded.

Twenty-four hours later, the animals were again tested in the same apparatus except that no injections were made and the mice did not receive a shock. The test-day latency in seconds for each animal was recorded, and the animals were then discarded.

The high degree of variability (due to season of the year, housing conditions, and handling) found in one-trial passive-avoidance paradigms is well-known (see ref 29). To control for this fact, individual cutoff values were determined for each test, compensating for intertest variability. Additionally, it was found that 5–7% of the mice in the scopolamine/vehicle control groups were insensitive to scopolamine at 3 mg/kg sc. Thus, the cutoff value was defined as the second-highest latency time in the control group to more accurately reflect the 1/15 expected control responders in each test group. Experiments with a variety of standards repeated under a number of environmental conditions led to the development of the following empirical criteria: for a valid test, the cutoff value had to be less than 120 s and the vehicle/vehicle control group had to have a least 5/15 animals with latencies greater than cutoff. For a compound to be considered active the scopolamine/compound group had to have at least 3/15 mice with latencies greater than cutoff.

Reversal of NBM Lesion. The procedures for this test are described in ref 51.

Inhibition of Amine Reuptake. Male Wistar rats were decapitated and the brains were rapidly removed. Whole brains minus cerebella were used for NE and 5-HT uptake and corpora striata were used for DA uptake. The labeled compounds used were *l*-[2,5,6-^{3}H]NE (40–50 Ci/mmol), 5-[1,2-^{3}H(N)]HT (20–30 Ci/mmol), and [8-^{3}H(N)]DA (4–34 Ci/mmol), supplied by New England Nuclear. The uptake of ^{3}H-labeled ligand was measured at a final concentration of 50 nM as previously described[52] in the presence of seven concentrations of test compound and an IC_{50} value was derived from log–probit analysis.

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(49) Bliss, C. I. *Q. J. Pharm. Pharmacol.* **1938**, *11*, 192.
(50) Ellman, G. L.; Courtney, K. D.; Andres, V., Jr.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.
(51) Haroutunian, V.; Kanof, P.; Davis, K. L. *Life Sci.* **1985**, *37*, 945.
(52) (a) Snyder, S. H.; Coyle, J. T. *J. Pharmacol. Exp. Ther.* **1969**, *165*, 78. (b) Horn, A. S. *J. Neurochem.* **1973**, *21*, 883.