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SYNTHESIS AND BIOLOGICAL ACTIVITY OF PUTATIVE *mono*-HYDROXYLATED METABOLITES OF VELNACRINE

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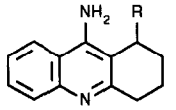
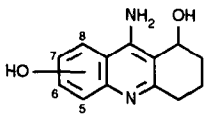
Abstract: Ten 9-amino-1,2,3,4-tetrahydroacridinediols were prepared as potential *mono*-hydroxy metabolites of velnacrine. They were tested for acute toxicity as well as for their ability to inhibit acetylcholinesterase *in vitro* and to reverse scopolamine-induced memory impairment in mice.

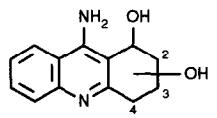
We recently reported the synthesis of a series of 9-amino-1,2,3,4-tetrahydro-1-acridinols.[1] These compounds were acetylcholinesterase inhibitors and were active in a dementia paradigm in mice, reversing the impairment of 24-h memory induced by scopolamine. Based on these data (and data from an nbM lesion model), one of these compounds (velnacrine maleate, **1a**) was chosen for clinical trials in Alzheimer's disease. The pharmacokinetic profile of **1a** in normal young and elderly volunteers is predictable,[2] and clinical trials in Alzheimer's patients have been highly encouraging.[3] Since oxidation by the cytochrome P-450 mixed function oxidase system is a fundamental means by which drugs are metabolized in mammals,[4] and since **1a** itself has been shown to be one of several hydroxylated metabolites of 9-amino-1,2,3,4-tetrahydroacridine (THA, tacrine, **1b**) in the rat,[5] hydroxylation was considered to be a likely pathway for the metabolism of **1a**. Thus, as the clinical trials with **1a** progressed, it was desirable to have the hydroxylated derivatives of **1a** in hand to be used as standards for chromatographic and spectral comparison with metabolites of **1a** isolated from biological fluids. This paper describes the synthesis of ten such acridinediols, four hydroxylated on the aromatic ring (**2a-d**) and six hydroxylated on the alicyclic ring (**3-5**, each consisting of two diastereomers), along with the results of the initial biological testing.



1a, R = OH (maleate)
1b, R = H

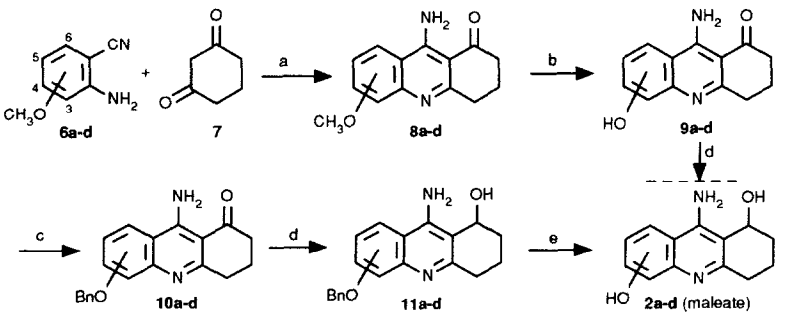


2a, 5-OH
2b, 6-OH
2c, 7-OH
2d, 8-OH



3a, cis2-OH **4a**, cis-3-OH
3b, trans-2-OH **4b**, trans-3-OH
5a, cis-4-OH
5b, trans-4-OH

Compounds **2a-d** were synthesized according to the same general method, shown in Scheme I. 3-, 4-, 5- and 6-Methoxyanthranilonitrile (**6a-d**)[6] were condensed with cyclohexane-1,3-dione (**7**) in the presence of $ZnCl_2$ to give the methoxy-substituted aminoacridinones **8a-d**. In the past, this type of condensation has been carried out in excess ketone,[10] but we found it convenient to use one equivalent of **7** and to run the reactions in nitrobenzene. These conditions gave zinc complexes of the products, which could be collected by filtration and then broken up by treatment with aqueous ammonia. Treatment of **8a-d** with BBr_3 gave the hydroxyketones **9a-d**, which did not prove to be suitable as the immediate precursors to the acridinediols **2a-d**: metal hydride reduction of **9a-d** did indeed give **2a-d**, as evidenced by ^1H nmr, but it proved

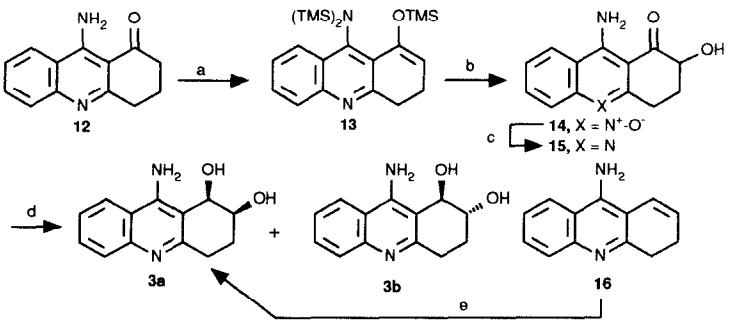


Scheme I

Reagents, conditions, and yields (for **2c**). (a) $ZnCl_2$, $C_6H_5NO_2$, 120 °C, then aq NH_3, 48%; (b) BBr_3, $(CH_2)_2Cl_2$, reflux, 88%; (c) BnBr, K_2CO_3, DMF, 100 °C, 86%, (d) $LiAlH_4$, THF, 75%, (e) H_2, 10% Pd/C, EtOH, 50 °C, 50 psi, then maleic acid, MeOH, 75%.

impossible to separate **2a-d** from the metal salts that were by-products of the reaction conditions. Thus, the hydroxyketones (**9a-d**) were converted to the benzyloxyketones (**10a-d**), which were then reduced to the benzyloxyacridinols (**11a-d**). The desired **2a-d** were then available by hydrogenolysis.

The synthesis of the two diastereomeric 1,2-diols (**3a** and **3b**) was achieved by the hydroxylation of **12**[1] (Scheme II). This was first attempted using 3-phenyl-2-(phenylsulfonyl)oxaziridine and potassium



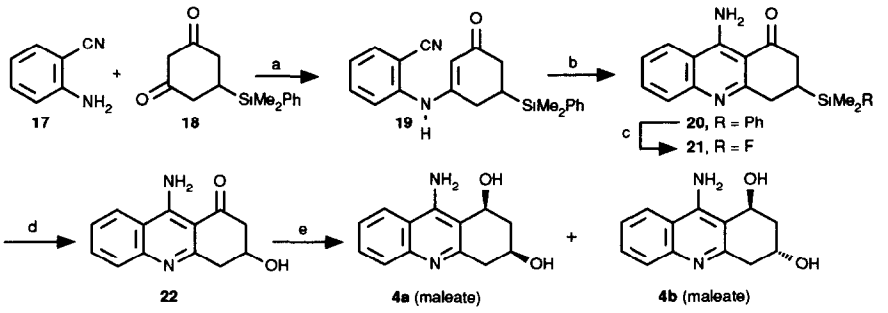
Scheme II

Reagents, conditions, and yields: (a) TMSCl, $LiN(TMS)_2$, THF, 78%, (b) mCPBA, THF, then aq HCl, THF, 86%, (c) $TiCl_3$, aq HCl, MeOH, 64%; (d) $LiAlH_4$, THF, chromatography, 20% **3a**, 34% **3b**; (e) $KMnO_4$, $C_6H_5CH_2(C_2H_5)_3N^+Cl^-$, CH_2Cl_2, 8%.

bis(trimethylsilyl)amide.[11] However, this only afforded small amounts of the hydroxyketone, even when a large excess of base was used. It proved to be more advantageous to use the indirect method shown in Scheme II. A mixture of **12** and an excess of TMS chloride was treated with excess lithium bis(trimethylsilyl)amide, giving the silyl enol ether in which the primary amino group had also been bis-silylated (**13**). Treatment of **13** with m-chloroperbenzoic acid and then with aqueous acid to remove the silyl groups gave the hydroxyketone 10-oxide (**14**). Compound **14** was treated with titanium trichloride to give hydroxyketone **15** which was reduced with $LiAlH_4$ to give a mixture of **3a** and **3b**. This mixture was separated by flash chromatography in a solvent system consisting of CH_2Cl_2–MeOH–Et_3N (8:1:1, **3a** eluted first). The *cis*-diol **3a** could also be obtained by the $KMnO_4$ oxidation of the olefin **16** under nonaqueous

conditions.[12] This reaction gave a low yield and was not preparatively useful but served to establish the *cis* stereochemistry of **3a**.

The use of a phenyldimethylsilyl group as a masked hydroxyl group was the key feature of the synthesis of the diastereomeric 1,3-diols (**4a** and **4b**), illustrated in Scheme III. Anthranilonitrile (**17**) was

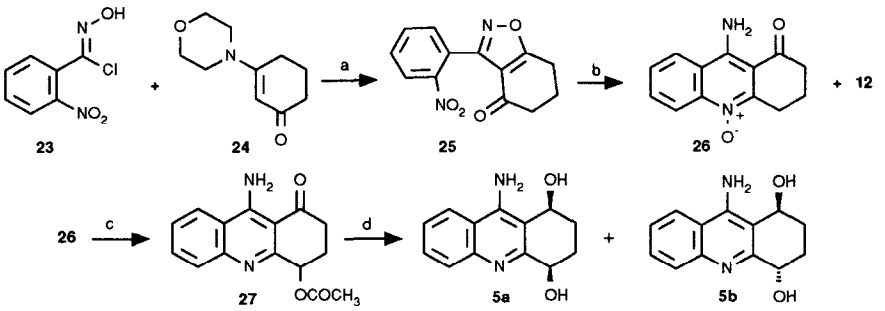


Scheme III

Reagents, conditions, and yields: (a) toluene, TsOH, reflux, 68%; (b) K_2CO_3, CuCl, THF, reflux, 80%; (c) $HBF_4 \cdot Et_2O$, CH_2Cl_2, 90%; (d) 30% H_2O_2, $NaHCO_3$, KF, MeOH, THF, 89%; (e) $Li(C_2H_5)_3BH$, THF, chromatography, then maleic acid, MeOH, 37% **4a**, 26% **4b**.

condensed with 5-(phenyldimethylsilyl)cyclohexane-1,3-dione (**18**)[13] to give an enaminoketone (**19**), which was subjected to conditions that were previously developed for the synthesis of **12**[1] to give an acridinone with a latent hydroxyl group at the 3-position (**20**). Treatment of **20** with tetrafluoroboric acid gave the fluorosilane (**21**) which, when exposed to basic peroxide in the presence of potassium fluoride, gave the hydroxyketone (**22**). Compound **22** was then reduced with lithium triethylborohydride to give a mixture of **4a** and **4b**, which was separated by flash chromatography in a solvent system consisting of CH_2Cl_2-EtOH-Et_3N (17:2:1, **4a** eluted first). The hydrogens on the alicyclic rings of **4a** and **4b** were well resolved at 200 MHz so that after D_2O exchange homonuclear decoupling of the carbinol protons made it possible to determine all the coupling constants by first-order analysis and hence to assign the relative stereochemistry of **4a** and **4b**.[14]

The approach to the synthesis of the diastereomeric 1,4-diols (Scheme IV) was suggested by a literature report which described the synthesis of a 1,2,3,4-tetrahydro-4-acridinol by a Polonovski rearrangement of the corresponding 10-oxide.[16] Primary amino groups are generally not compatible with the conditions for forming aromatic N-oxides[17] and, as expected, treatment of **12** with mCPBA gave only intractable mixtures of highly polar products. An alternative approach to the required 10-oxide was suggested, however, by some earlier work from our laboratory, in which isoxazolo[5,4,3-kl]acridine served as the precursor to ketone **12** (by hydrogenolysis of the N-O bond, the isoxazole nitrogen and oxygen became the amino and carbonyl groups, respectively, of **12**).[18] In the present case, the 1,3-dipolar addition of 2-nitrobenzonitrile oxide (derived from 2-nitrobenzohydroximino chloride, **23**[19]) and the morpholine enamine of cyclohexane-1,3-dione (**24**) gave the nitroketone (**25**) in good yield. Catalytic hydrogenation over a sulfer-poisoned platinum catalyst then gave the desired 10-oxide (**26**) by partial reduction of the nitro group and scission of the isoxazole N-O bond. The only by-product of this reaction was a small amount of **12**. The Polonovski rearrangement of **26** was carried out in acetic anhydride at room temperature, giving the



Scheme IV

Reagents, conditions, and yields: (a) Et_3N, THF, reflux, 66%; (b) H_2, 5% Pt/sulfided C (Aldrich), aq. HCl, THF, 50 psi, 80%; (c) Ac_2O, 82%; (d) $LiAlH_4$, THF, chromatography, 53% **5a**, 19% **5b**.

acetoxyketone, **27**. Reduction of **27** with $LiAlH_4$ gave a mixture of **5a** and **5b**, which was separated by flash chromatography in a solvent system consisting of CH_2Cl_2-MeOH/NH_3(sat.)-Et_3N (87:3:10, **5b** eluted first). In contrast to **4a** and **4b**, the 2- and 3-methylene groups of **5a** and **5b** were not well resolved at 200 MHz. The apparent coupling constants which were evident from the multiplicities of the carbinol methine resonances resulted in the tentative assignment of **5a** and **5b** as *cis* and *trans*, respectively; the *trans* configuration of **5b** was confirmed by X-ray crystallography (figure 1).[20]

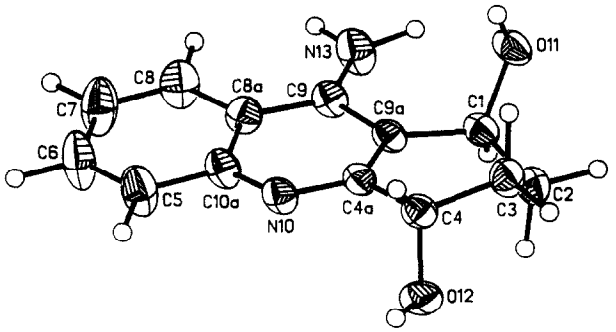


Figure 1. ORTEP drawing of **5b** derived from the X-ray coordinates

Compounds **2-5** were evaluated in the same initial battery of tests that has already been described for **1a** and **1b**;[1b] the results are listed in Table I. All of these compounds showed less acute toxicity in mice than **1b**, results that were consistent with those initially reported for **1a** in rats.[1b] Adding the second hydroxyl group made the acridinediols generally less potent acetylcholinesterase inhibitors than **1a**, particularly when the second hydroxyl group was on the aromatic ring (**2a-d**). It is interesting that while both of the 1,2-diols were very weak acetylcholinesterase inhibitors, the *cis*-1,3-diol (**4a**) and the *cis*-1,4-diol (**5a**) approached the potency of **1a**. It would seem that the region around the 1-hydroxyl group may be critical to interaction with the enzyme and that hydroxylation at the 2-position is not tolerated. Hydroxylation at the 3- and 4-positions, however, was not deleterious to acetylcholinesterase inhibition as

Table I. Physical and Biological Data for 9-Amino-1,2,3,4-tetrahydroacridinols

Compd.	mp °C[a]	acute toxicity in mice: LD_{50} mg/kg, sc[b,c]	acetylcholinesterase inhibition: IC_{50}, μM[b,d]	reversal of scopolamine-induced memory impairment in mice: active doses (mg/kg, sc)[b,e]
1a	ref 1b	>80	1.3 ± 0.15	0.31 - 5
1b	——	<40>20	0.23 ± 0.03	0.63 - 5
2a	180-181	>80	25.1 ± 2.9	NA (1 - 10)
2b	186-187	>80	64.4 ± 4.9	NA (0.16 - 5)
2c	223 dec	>80	10.6 ± 1.6	NA ((0.16 - 5)
2d	165-167	>80	27.4 ± 6.3	NA (1 - 10)
3a	237 dec	NT[f]	>100	NT[f]
3b	233 dec	NT[f]	>100	NT[f]
4a	179-180	>80	5.4 ± 1.1	1 - 10
4b	158-160	>80	14.7 ± 2.5	1 - 10
5a	200 dec	>80	4.6 ± 0.6	3
5b	182-184	>80	62.1 ± 5.5	NA (1 - 10)

[a]Melting points are uncorrected; compounds analyzed for C, H, and N within ±0.4% of the theoretical values. [b]The detailed procedures for these tests are described in ref. 1b. [c]The data for **1a** and **1b** differ slightly from ref. 1b, where this test was done in rats, ip. [d]The test compounds were incubated with enzyme at 37 °C, resulting in slightly lower IC_{50}'s than found in ref. 1b, where incubation was at 25 °C. [e]The test compounds were evaluated at doses of 0.16, 0.31, 0.63, 1.25, 2.5 and 5.0 mg/kg, sc or at doses of 1.0, 3.0 and 10 mg/kg, sc. [f]NT = not tested (compounds with acetylcholinesterase inhibition >100 μM were not tested further).

long as the hydroxy groups were on the same side of the alicyclic ring as the 1-hydroxyl group. This finding is consistent with previous work with the 1,4-methano- and 1,4-ethano analogs,[22] which would also indicate that potent acetylcholinesterase inhibition can be retained in the presence of substitution on a single side of the alicyclic ring. This is the first report, to our knowledge, of the deleterious effect of *trans* substitution on the alicyclic ring, particularly at the 4-position. For the most part, the more potent acetylcholinesterase inhibitors were active in reversing scopolamine-induced memory impairment in mice at the doses tested, the one exception being **2c**. The possible reasons for such an exception have already been discussed.[1b] These could include a lack of a direct relationship between *in vitro* and *in vivo* acetylcholinesterase inhibition as well as the presence of other pharmacological effects besides acetylcholinesterase inhibition. The cytotoxic potential of these compounds has been tested in cultured rat, dog and human hepatocytes, and these results are reported elsewhere.[23]

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6. Syntheses of **6b-d** have been described in the literature,[7] but **6a** was a new compound. It was synthesized from 3-methoxy-2-nitrobenzaldehyde, which was converted to the corresponding nitrile in standard fashion through the intermediacy of the oxime. All attempts to reduce the nitronitrile to **6a** under any of the usual conditions gave mostly 3-methoxyanthranilamide, a common by-product in these reductions, presumably arising from intramolecular oxygen transfer.[8] After an attempt to perform the $ZnCl_2$ condensation with the anthranilamide failed, the problem of the reduction of the nitronitrile to **6a** was solved by the use of $TiCl_3$ under acidic conditions.[9]
7. (a) For **6b** see: Cook, A. H.; Heilbron, I. M.; Reed, K. J.; Strachan, M. N. *J. Chem. Soc.* **1945**, 861. See also: Adachi, M.; Sugasawa, T. *Synth. Commun.* **1990**, *20*, 71. (b) **6c**: Campbell Jr., J. B.; Davenport, T. W. *Synth. Commun.* **1989**, *19*, 2255. (c) **6d**: Klaubert, D. H.; Sellstedt, J. H.; Guinosso, C. J.; Capetola, R. J.; Bell, S. C. *J. Med. Chem.* **1981**, *24*, 742.
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14. The following coupling constants were measured: **4a**: $J_{1\beta\text{-}2\beta} = 6.7$, $J_{1\beta\text{-}2\alpha} = 7.2$, $J_{2(gem)} = 13.1$, $J_{2\beta\text{-}3\beta} = 1.1$, $J_{2\alpha\text{-}3\beta} = 9.7$, $J_{3\beta\text{-}4\beta} = 4.2$, $J_{3\beta\text{-}4\alpha} = 8.0$, $J_{4(gem)} = 17.0$ Hz; **4b**: $J_{1\alpha\text{-}2\beta} = 4.1$, $J_{1\alpha\text{-}2\alpha} = 3.8$, $J_{2(gem)} = 12.6$, $J_{2\beta\text{-}3\beta} = 1.5$, $J_{2\alpha\text{-}3\beta} = 11.2$, $J_{3\beta\text{-}4\beta} = 4.5$, $J_{3\beta\text{-}4\alpha} = 8.6$, $J_{4(gem)} = 17.1$ Hz. The alicyclic ring in these molecules is somewhat flattened by fusion to the quinoline ring (see figure 1) and this is reflected in the coupling constants. It is apparent that the 3-hydroxyl group is quasi-equitorial in both **4a** and **4b** and that the 1-hydroxyl group is, respectively, quasi-equitorial and quasi-axial. These data are consistent with those reported for *cis*- and *trans*-3-methyl-1-tetralol.[15]
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20. Crystallographic supplementary material for **5b** has been deposited at the Cambridge Data Centre: $C_{13}H_{14}N_2O_2$, colorless plates by vapor diffusion of chloroform into a methanol solution, $M = 230.3$, monoclinic, space group $P2_1/n$, $a = 5.635(1)$, $b = 11.099(2)$, $c = 18.615(4)$ Å, $\beta = 94.92(2)$ °, $V = 1159.9(4)$ Å^3, $Z = 4$, $F(000) = 488$, $\mu = 0.85$ cm^{-1}, $D_c = 1.32$ g cm^{-3}, graphite monochromated (Mo-Kα)radiation. 2680 Reflections were collected on a Nicolet R3mE automated diffractometer in the range $3 < 2\Theta < 55$ °. 985 Unique reflections with $I > 2\sigma(I)$ were used for structure determination and refinement. The structure was solved by direct methods and refined by block-cascade least squares, minimizing $\Sigma w\Delta^2$ with 101 parameters in each full-matrix block. No corrections for absorption or extinction were required. Atomic scattering factors, including terms for anomalous scattering, were taken from Cromer and Waber.[21] The refinement was performed on a Data General Eclipse computer, using the SHELXTL program package by G. M. Sheldrick, Nicolet Instrument Corp, Madison, WI. The refinement was carried out to $R = 0.043$ and $R_w = 0.047$, where $w = k(\sigma^2(F_o) + 0.0008F_o^2)^{-1}$. All hydrogen positions were located on a difference map, but calculated positions were used in structure refinement for hydrogen atoms bonded to carbons, since the data set was too small to refine all hydrogen positions. The positional parameters for OH and NH_2 hydrogens were refined. A common isotropic thermal parameter for all hydrogen atoms refined to a value of $U = 0.052(3)$ Å^2. The largest peak on the final difference map was 0.2 eÅ^{-3}.
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