residue was dissolved in petroleum ether, bp 60–80 °C. The crude phenol that precipitated was coollected by filtration and dissolved in DMF (30 mL), which was stirred under N_2 at 25 °C. NaH (0.7 g) was added, the mixture was warmed to 65 °C, and $Me_2NCH_2CH_2Cl$·HCl (0.75 g) was added in small portions over 30 min. After a further 30 min, the yellow color of the phenolate anion had discharged. The mixture was cooled in an ice bath, excess NaH was destroyed by the addition of 2-propanol (1 mL), and then the mixture was partitioned between Et_2O (100 mL) and H_2O (100 mL). The Et_2O solution was dried (Na_2SO_4) and concentrated. The residue was crystallized from petroleum ether, bp 80–100 °C to give crystals of **7** (681 mg, 58%): mp 125–126 °C; NMR ($CDCl_3$) δ_H 2.17 (quint, J = 7 Hz, 2, H-6), 2.35 (s, 6, NMe_2), 2.40 (t, J = 7 Hz, 2, H-7), 2.74 (t, J = 5.8 Hz, 2, CH_2N), 2.79 (t, J = 7 Hz, 2, H-5), 4.09 (t, J = 5.8 Hz, 2, OCH_2), 6.70 (dd, J = 2.6, 8.5 Hz, 1, H-2), 6.78 (d, J = 8.5 Hz, 1, H-1), 6.85 (d, J = 2.6 Hz, 1, H-4), 6.88–6.93 and 7.04–7.26 (m, 10, 2 Ph). Anal. ($C_{27}H_{29}NO$) C, H, N.

Binding-Affinity Studies.[15,21,22] Calf uterine cytosol was incubated at 18 °C for 30 min with 5×10^{-9} M [^{3}H]estradiol in the absence and presence of increasing amounts (10^{-9}–10^{-5} M) of the cyclic tamoxifen analogue (**5**–**8**) or unlabeled estradiol (control). Unbound compounds were then removed by dextran-coated charcoal, and the amounts of estrogen receptor bound [^{3}H]estradiol were measured. The relative concentrations of estradiol and cyclic tamoxifen analogues required to achieve 50% inhibition of [^{3}H]estradiol binding is the RBA; i.e., RBA = ([I_{50}] of estradiol/[I_{50}] of test compound) × 100. This procedure gives values of the same order of magnitude with cytosol from rat immature uterus, human breast tumors, or MCF-7 cells.

An MCF-7 whole cell assay was additionally carried out on the hydroxylated compounds **6** and **8**. MCF-7 cells were incubated at 37 °C for 50 min with 10^{-9} M [^{3}H]estradiol in the absence or presence of increasing amounts (10^{-10}–10^{-5} M) of **6**, **8**, or unlabeled estradiol (control). Bound compounds were then extracted with ethanol, and the amounts of estrogen receptor bound [^{3}H]estradiol were measured. The RBA values were calculated as for the cytosol assay.

Effect of the Cyclic Hydroxytamoxifen Analogues 6 and 8 on MCF-7 Cell Growth.[23] MCF-7 cells were plated at a density of 5000 cells/mL in 96-multiwell dishes. After 24 h of culture, compounds **6** and **8** were added to the culture dishes according to the protocol described previously. Estradiol was also added to evaluate its extent of antagonism of growth inhibition of compounds **6** and **8**. Final concentrations were as follows: estradiol, 10^{-8} M; compounds **6** and **8**, 10^{-8}, 10^{-7}, and 10^{-6} M. After 5 days of culture, the monolayer was fixed with 90% ethanol and colored with hematoxylin. The intensity of the coloration giving a measure of the number of cells was determined with a multiscan spectrophotometer at 540 nm (Flow Laboratories Inc).

Determination of the Effect of Compounds on Rat Uterine Growth. Immature female Sprague–Dawley rats were injected subcutaneously daily with solutions of compounds (Table II) in 0.1 mL of peanut oil for 3 days and then sacrificed on day 4. Uteri were removed, excess liquid was removed by blotting, and the uteri were weighed.

Acknowledgment. This investigation was supported by grants from the Cancer Research Campaign and Medical Research Council (to R.M.), from the Caisse Generale d'Epargne et de Retraite de Belgique (to G.L.), and from the National Institute of Health (Grant R01-CA 32713), Bethesda, MD (to V.C.J.).

Registry No. **6**, 113748-77-9; **7**, 113748-78-0; **8**, 113748-79-1; **9**, 6500-65-8; **10**, 113748-80-4; **11**, 113748-81-5; **12**, 113748-82-6; **13**, 109610-82-4; **14**, 113748-83-7; **15**, 113748-84-8; **16**, 113748-85-9; **17**, 113748-86-0; **17** (phenol), 113748-88-2; PhLi, 591-51-5; $Me_2NCH_2CH_2Cl$·HCl, 4584-46-7; $ClCH_2CH_2Cl$, 107-06-2; PhMgBr, 100-58-3; Me_2NH, 124-40-3; 4-bromophenyl 2,3,5,6-tetrafluoro-4-(trifluoromethyl)phenyl ether, 97631-87-3; 3-methoxy-9-phenyl-6,7-dihydro-5*H*-benzocycloheptene, 113748-87-1.

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Electrophysiologic and Antiarrhythmic Activities of 4-Amino-*N*-[2-(diethylamino)ethyl]-3,5-dimethylbenzamide, a Sterically Hindered Procainamide Analogue

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Procainamide is a widely used antiarrhythmic that is fraught with therapeutic limitations such as a short half-life, production of autoimmune antibodies and a lupus-like syndrome, and complex pharmacokinetics. We synthesized the congeners of procainamide possessing one or two methyl substituents ortho to the 4-amino moiety (compounds **4** and **5**, respectively), in order to sterically encumber the 4-amino substituent and prevent or diminish the rate of metabolic N-acetylation. Moreover, we anticipated that this structural alteration might eliminate the autoimmune toxicities associated with procainamide. Like procainamide, the two methylated analogues significantly reduced the rate of rise and amplitude of the action potential when studied in isolated canine Purkinje fibers. Whereas procainamide caused no significant change in action potential duration (APD), both methylated congeners significantly reduced APD at 70% and 95% repolarization. Moreover, the dimethylated congener was significantly more efficacious than procainamide in reducing ERP (effective refractory period) and increasing the ERP/APD_{70}. The ability of these compounds to block ouabain-induced arrhythmias was studied in anesthetized dogs. Addition of two methyl groups ortho to the amine produced an increase in potency: The conversion doses for procainamide and the monomethyl and dimethyl congeners were 19.0, 18.3, and 14.3 mg/kg, respectively, following iv administration. After iv administration to rats, procainamide was extensively metabolized to *N*-acetylprocainamide and displayed a half-life of 0.4 h. In contrast, dimethylprocainamide was *not* metabolized by N-acetylation, had a half-life of 1.4 h, and provided greater peak plasma concentrations. Thus, addition of methyl substituents ortho to the 4-amino group of procainamide alters the electrophysiological characteristics of the compound, increases its potency against ouabain-induced arrhythmias in vivo, increases its plasma half-life, and prevents N-acetylation.

Procainamide (**1**; Chart I) is a widely used antiarrhythmic drug that is sometimes effective in the management of ventricular premature depolarizations, life-threatening paroxysmal ventricular tachycardia, atrial

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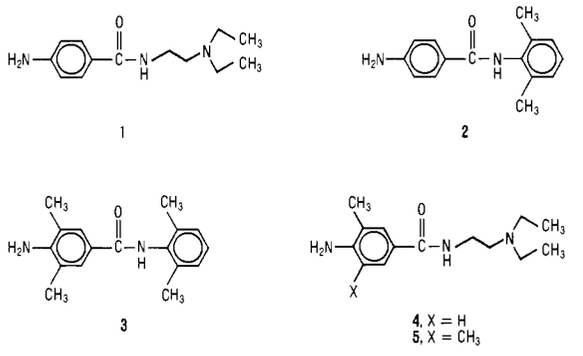
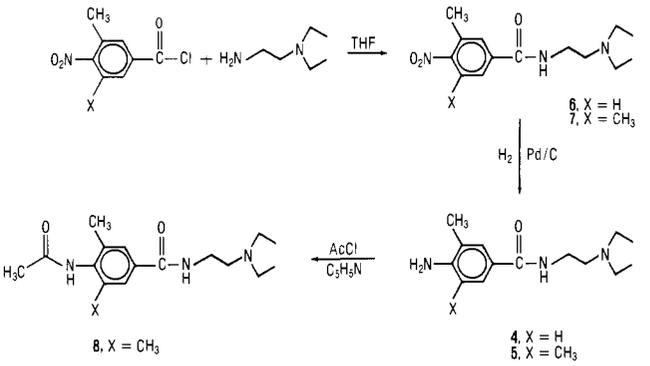
Chart I



flutter, and atrial fibrillation.[1] Mechanistically, the compound decreases action potential upstroke velocity and either does not affect or slightly prolongs total action potential duration; thus, the drug serves as a prototypical class 1A antiarrhythmic drug according to the Vaughan-Williams electrophysiological classification scheme.[2]

Despite its widespread use, procainamide is fraught with therapeutic limitations.[1,3] The compound has a short half-life in humans, which mandates dosing every 3–4 h in order to maintain effective plasma concentrations. A more serious concern, however, are the debilitating side effects produced by procainamide. Nearly all patients receiving chronic procainamide therapy develop autoimmune antibodies; approximately 30% of all patients display an overt clinical disease resembling idiopathic systemic lupus erythematosus and must discontinue therapy.[4,5] Although the molecular basis for this lupus-like syndrome has not been firmly established, cytochrome P-450 derived metabolites of procainamide, the *N*-hydroxy and nitroso analogues, have been implicated in the genesis of the immunologically mediated side effects of procainamide.[6] Finally, procainamide has complex pharmacokinetics. It is rapidly and efficiently metabolized to form *N*-acetylprocainamide (NAPA), an electrophysiologically active compound.[7–9] Since there is considerable interpatient variability in the quantity of NAPA produced[7,8] and since NAPA has important electrophysiological differences relative to procainamide,[10] the facile metabolism of procainamide complicates its therapeutic use.[11–13]

Scheme I



We recently reported our efforts to alter the metabolism of the benzamide anticonvulsant **2** [4-amino-*N*-(2,6-dimethylphenyl)benzamide, LY201116] by structural modification.[14] This compound is extensively metabolized by N-acetylation, and to preclude or diminish the rate of N-acetylation, we prepared 4-amino-*N*-(2,6-dimethylphenyl)-3,5-dimethylbenzamide (**3**). Studies in rodents revealed that this compound was not metabolized by N-acetylation and provided exceptionally high and long-lived plasma concentrations of parent drug.[14] We anticipated that the corresponding structural modification of procainamide might retain the desirable antiarrhythmic features of procainamide while providing the following advantages: (1) the two flanking methyl groups would sterically encumber the 4-amino substituent to prevent cytochrome P-450 induced N-hydroxylation and its toxicological sequelae; (2) the same steric hindrance would preclude metabolic N-acetylation to form NAPA, an active but electrophysiologically distinct metabolite; and (3) because of this altered metabolism, the half-life of procainamide might be prolonged. We have synthesized the monomethyl and dimethyl analogues of procainamide (Chart I, compounds **4** and **5**, respectively), and herein we report their antiarrhythmic activities and electrophysiological characteristics compared to procainamide. Moreover, we have determined that the metabolism of the dimethyl analogue (LY264729) is markedly different from that of procainamide.

Results and Discussion

Chemistry. The procainamide congeners were prepared by well-known synthetic procedures (Scheme I). The acid chlorides of the appropriately methylated 4-nitrobenzoic acids were formed by use of thionyl chloride and then reacted with *N,N*-diethylethylenediamine. The resulting nitro precursors were subjected to catalytic hydrogenation to afford the final products possessing one or two methyl substituents ortho to the amino group (compounds **4** and **5**, respectively). Despite the fact that the structure–activity relationships (SAR) of procainamide have been extensively studied, these congeners have not been prepared previously. Finally, **8**, the *N*-acetyl derivative of dimethylprocainamide, was prepared as a GC standard for metabolism studies.

General Electrophysiologic Properties. The electrophysiological properties of procainamide and its analogues were examined in isolated canine Purkinje fibers. The concentration–response relationship for procainamide on upstroke velocity (V_{max}) was studied previously, and the concentration that reduced V_{max} by 50% was (7.3 ±

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Table I. General Electrophysiological Properties of Procainamide and Methylated Congeners in Canine Purkinje Fibers[a]

drug	V_{max}, V/s	amplitude, mV	APD_{70}, ms	APD_{95}, ms	resting potential, mV
control	501 ± 40	127 ± 3	285 ± 19	347 ± 17	−93 ± 1
procainamide	345 ± 29	119 ± 3	284 ± 7	361 ± 11	−91 ± 2
percent change	−31 ± 3[b]	−7 ± 1[b]	1 ± 5	5 ± 4	−2 ± 3
control	462 ± 30	129 ± 5	292 ± 16	363 ± 6	−89 ± 2
4	282 ± 20	114 ± 3	244 ± 11	336 ± 6	−85 ± 5
percent change	−39 ± 3[b]	−12 ± 2[b]	−16 ± 5[b,c]	−7 ± 0.2[b,c]	−4 ± 6
control	632 ± 34	131 ± 1	363 ± 23	426 ± 22	−92 ± 3
5	387 ± 31	117 ± 5	227 ± 11	353 ± 9	−81 ± 8
percent change	−38 ± 5[b]	−11 ± 3[b]	−37 ± 3[b-d]	−17 ± 3[b-d]	−12 ± 7

[a] All measurements were made at the time of maximum drug effect, i.e., >30 min. Values are mean ± SEM of four or five experiments. Action potential durations were measured at 70% repolarization (APD_{70}) and 95% repolarization (APD_{95}). Each cell served as its own control. Drugs were tested at 3×10^{-4} M. V_{max} = maximal upstroke velocity. [c] $P < 0.05$ compared to procainamide. [d] $P < 0.05$ compared to monomethylated congener **4**.

0.82) $\times 10^{-4}$ M.[15] Consequently, all studies with procainamide and its congeners in this report were conducted at concentrations of 3×10^{-4} M so potential potency differences among compounds would be detectable. Effects of procainamide and the methylated congeners on V_{max} and action potential duration (APD) were rapid in onset and had attained steady-state values within approximately 30 min; the results are displayed in Table I.

Procainamide produced a 31% decrease in V_{max} and a 7% decrease in amplitude. No significant effects were observed on resting potential or on action potential duration measured at 70% repolarization (APD_{70}) or 95% repolarization (APD_{95}). At the same concentration, the two methylated analogues also significantly reduced the rate of rise and amplitude of the action potential. The magnitude of these effects were greater than those observed with procainamide, but the differences were not statistically significant. All three compounds tended to increase conduction time, although the increase achieved statistical significance only in the procainamide group. Surprisingly, there were some significant electrophysiological differences among compounds with respect to action potential duration (APD). Whereas procainamide caused no significant change in APD, both **4** and **5** significantly reduced APD at both stages of repolarization. For example, at 70% repolarization, **4** and **5** decreased APD by 16% and 37%, respectively. Effects on APD increased progressively as each methyl moiety was added to procainamide, and the magnitude of the differences between **4** and **5** was significant (Fischer's LSD procedure). With both methylated congeners, the magnitude of the effect on APD was greater at 70% than at 95% of full repolarization, resulting in a triangulation of the action potential plateau phase.

The effects of these compounds on the effective refractory period (ERP) and the refractory period to action potential duration ratios (ERP/APD) were also examined, and the results are displayed in Table II. Only **5** produced a significant reduction (−18 ± 6%) in ERP relative to control values. For each group of control tissues, the ERP/APD_{70} ratio was near unity, suggesting that the effective refractory period was best approximated by the APD_{70}. Both procainamide and its dimethyl congener **5** increased ERP/APD_{70}, but the increase was greater with **5**. Thus, refractory period increases out of proportion to APD, suggesting a reduced availability of sodium current during repolarization. The ratio of the V_{max} of a premature action potential elicited immediately upon completion of repolarization was compared to the V_{max} of last-paced action potential. This ratio serves as an index of the rate of recovery from sodium-channel inactivation. Both methylated analogues tended to decrease this ratio (Table II) compared to procainamide, suggesting a drug-induced delay in sodium-channel recovery as is seen with potent class 1 agents.[16]

Table II. Effects of Procainamide and Methylated Congeners on Effective Refractory Period (ERP) and Refractory Period to Action Potential Duration Ratios (ERP/APD)[a]

drug	ERP, ms	ERP/APD_{70}	Na^+ inactivation[b]
control	308 ± 26	1.05 ± 0.01	1.00 ± 0.02
procainamide	321 ± 11	1.10 ± 0.01	0.96 ± 0.02
percent change	6 ± 6	5 ± 0.6[c]	−3 ± 2
control	303 ± 27	1.05 ± 0.03	0.96 ± 0.01
4	283 ± 18	1.19 ± 0.09	0.88 ± 0.02
percent change	−6 ± 3	12 ± 6	−9 ± 2[c]
control	351 ± 32	1.02 ± 0.02	0.99 ± 0.01
5	282 ± 5	1.29 ± 0.05	0.91 ± 0.02
percent change	−18 ± 6[c,d]	27 ± 7[c,d]	−7 ± 3

[a] All measurements were made at the time of maximum drug effect, i.e., >30 min. Values are mean ± SEM of four or five experiments. Action potential durations were measured at 70% repolarization (APD_{70}) and 95% repolarization (APD_{95}). Each cell served as its own control. Drugs were tested at 3×10^{-4} M. [b] The V_{max} of a premature action potential elicited immediately upon complete repolarization divided by the V_{max} of the paced action potential. [c] $P < 0.05$ compared with corresponding control values. [d] $P < 0.05$ compared to procainamide.

The effects of methyl groups on APD and ERP could result from an alteration of molecular lipophilicity in a global sense, steric encumbrance in the microenvironment of the amino group, an altered basicity of the amino group, or a combination of these factors; it is difficult, if not impossible, to dissect these factors experimentally. Previous investigators have studied effects of certain physicochemical properties such as molecular weight and lipophilicity on sodium-channel inactivation by procainamide analogues.[17,18] For example, Ehring and Hondeghem found that lower molecular weight procainamide congeners blocked a greater fraction of the sodium channels per activation than did larger analogues. Moreover, they determined that there was a positive correlation between lipophilicity and the rate of recovery from sodium-channel inactivation.[17] Thus, our current data, in conjunction with prior SAR reports, suggest that electrophysiological changes can be induced by relatively subtle alterations in molecular structure and physicochemical characteristics of procainamide analogues.

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Table III. Effects of Procainamide and Methylated Congeners on Ouabain-Induced Arrhythmias in Pentobarbital-Anesthetized Dogs[a]

compound	ouabain dose, μg/kg	conversion dose, mg/kg	n
procainamide	68.0 ± 1.2	19.0 ± 3.0	5
4	65.0 ± 2.2	18.3 ± 2.0	5
5	69.0 ± 5.6	14.3 ± 3.4[b]	5

[a] Ouabain was administered iv until sustained arrhythmias were present. Drugs were administered by iv infusion until conversion to sinus rhythm was obtained. Values are mean ± SEM. For complete details, consult the Experimental Section. [b] $P < 0.05$ compared to the procainamide group.

In Vivo Antiarrhythmic Effects. Because of these unanticipated in vitro electrophysiological differences among the methylated congeners and the parent structure, procainamide, we investigated the in vivo antiarrhythmic effects of these three compounds. Ouabain was infused through the cephalic vein of pentobarbital-anesthetized dogs until sustained arrhythmias were produced, and the ability of the test compounds to abolish these arrhythmias was examined; the results are displayed in Table III. Approximately 65–69 μg/kg of ouabain produced sustained arrhythmias in all three groups of animals. A 19.0 mg/kg iv dose of procainamide caused a reversion to sinus rhythm. This value compares favorably with literature results.[19] Addition of two methyl groups ortho to the amine produced an increase in potency. The conversion doses for **4** and **5** were 18.3 and 14.3 mg/kg, respectively, and although these potency increases were slight, the difference between **5** and procainamide was statistically significant. Although the molecular basis for this increase in potency would be difficult to establish in this whole-animal model, it is not related to differences in acetylation of the 4-amino group since dogs do not N-acetylate aromatic amines, including procainamide.[7,20] Either the previously discussed methyl-induced electrophysiological differences or the possible methyl-mediated increases in plasma half-lifes may be responsible for the increases in potency.[21]

Metabolism Studies in Rats. Since dogs do not N-acetylate aromatic amines, the metabolism of dimethylprocainamide was studied in rats. A 25 mg/kg dose of either procainamide or dimethylprocainamide was administered iv to rats, and at various times plasma samples were obtained by cardiac puncture; concentrations of parent compounds and *N*-acetyl metabolites were determined by GC. The results are summarized in Figure 1. Rats dosed with procainamide had parent drug plasma concentrations of 9.98 ± 1.3 μg/mL 5 min after drug administration. These plasma concentrations declined rapidly, and at 2 h only 0.36 ± 0.06 μg/mL was detected. The plasma half-life of parent drug was 0.4 h. In these same animals the *N*-acetyl metabolite was present at the first observation point (5 min), and plasma concentrations peaked at 2.29 ± 0.04 μg/mL 30 min after dosing. The 2.0-h plasma half-life of *N*-acetylprocainamide was longer than that of procainamide, and after 2 h, plasma concentrations of the metabolite exceeded those of parent drug. Other investigators have previously documented the rapid and extensive metabolism of procainamide to *N*-acetylprocainamide in the rat.[22,23]

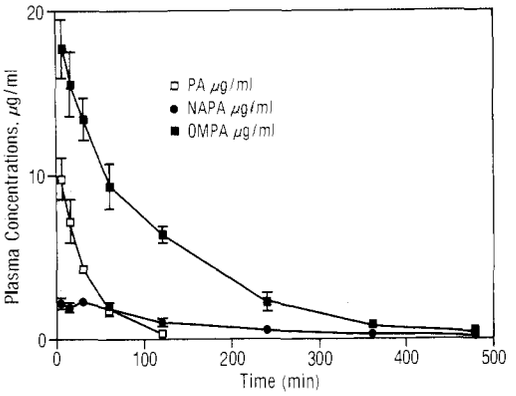

Figure 1. Plasma concentrations of procainamide (PA), dimethylprocainamide (**5**) (DMPA), and *N*-acetylprocainamide (NAPA). Groups of three rats were administered either PA or DMPA (25 mg/kg, iv), and at the indicated times plasma samples were collected and drug concentrations determined by GC as described in the Experimental Section. Each point is the mean ± SEM of experimental values, and symbols without error bars indicate the error fell within the area of the symbol. Whereas considerable NAPA was detected following administration of PA, no *N*-acetyl metabolite of DMPA was observed.

After administration of dimethylprocainamide, its peak plasma concentration was considerably greater than observed with procainamide. The plasma concentration 5 min after dosing was 17.7 ± 1.8 μg/mL, and 4 h after dosing the plasma concentration was still 2.3 ± 0.5 μg/mL; the plasma half-life of dimethylprocainamide was 1.4 h, a 3.5-fold increase relative to procainamide. No metabolically derived *N*-acetyldimethylprocainamide could be detected in these studies. Thus, the two methyl groups flanking the 4-amino substituent in dimethylprocainamide preclude metabolic N-acetylation and prolong the plasma half-life of parent drug.

Conclusions

This work has demonstrated that methyl substituents may be used to flank the 4-amino substituent of procainamide. The electrophysiological effects of procainamide on V_{max} and amplitude of the action potential were maintained by this structural modification, but in addition, important electrophysiological differences were introduced in that the two methylated procainamide congeners reduced APD at all phases of repolarization. Moreover, dimethylprocainamide was significantly more efficacious than procainamide in reducing ERP and increasing the ERP/APD_{70}. Both methylated analogues effectively antagonized ouabain-induced arrhythmias in pentobarbital dogs, and dimethylprocainamide was significantly more potent than procainamide. In marked contrast to procainamide, dimethylprocainamide was not metabolized by N-acetylation after iv administration to rats and had a 3.5-fold greater half-life. Thus, addition of methyl substituents ortho to the 4-amino group of procainamide alters the electrophysiological characteristics of the drug, increases its potency against ouabain-induced arrhythmias in vivo, increases its plasma half-life, and prevents metabolic N-acetylation. Unfortunately, whether these methylated congeners produce less autoimmune-mediated toxicity cannot be determined without clinical studies

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because of the dearth of relevant animal models for procainamide-induced lupus.[24]

Experimental Section

Methods. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are not corrected. Proton magnetic resonance (^{1}H NMR) spectra were obtained with a General Electric QE-300 spectrometer. Chemical shifts are reported in ppm downfield from a tetramethylsilane internal standard (δ scale). ^{1}H NMR data are presented in the form: (solvent in which spectra were taken), δ value of signal (peak multiplicity, integrated number of protons, and assignment). Mass spectra were recorded from a Varian MAT CH-5 spectrometer at the ionization voltage expressed in parentheses. Only peaks of high relative intensity or of diagnostic importance are presented in the form: m/e (intensity relative to base peak). Microanalytical data were provided by the Physical Chemistry Department of Lilly Research Laboratories; only symbols of elements analyzed are given, and they were within 0.4% of theoretical values unless indicated otherwise.

Except where noted, a standard procedure was used for product isolation. This involved quenching by addition to water, filtration or exhaustive extraction with a solvent (washing of extract with aqueous solutions, on occasion), drying over an anhydrous salt, and evaporation of solvent under reduced pressure. Particular solvents, aqueous washes (if needed), and drying agents are mentioned in parentheses after the phrase "product isolation."

***N*-[2-(Dimethylamino)ethyl]-3,5-dimethyl-4-nitrobenzamide Ethanedioate** (1:1) (**7**). A mixture of 3,5-dimethyl-4-nitrobenzoic acid[25] (10 g, 51.3 mmol) and thionyl chloride (160 mL) was refluxed for 1 h. Excess thionyl chloride was removed under reduced pressure, and toluene was added to the residue. Toluene was then removed under reduced pressure. The acid chloride was dissolved in THF and added dropwise to a solution of *N*,*N*-diethylethylenediamine (14.4 mL, 102.6 mmol) in THF. The reaction was stirred at room temperature overnight, and then solvent was removed in vacuo. Product isolation (ethyl acetate, 10% sodium bicarbonate, water, brine, Na_2SO_4) provided 11.5 g of a nearly homogeneous, low-melting solid. The oxalate salt was generated and recrystallized from ethyl acetate/methanol to provide 11.7 g (59%) of **7** with mp 178–179 °C. Anal. ($C_{15}H_{23}N_3O_3 \cdot C_2H_2O_4$) C, H, N.

4-Amino-*N*-[2-(diethylamino)ethyl]-3,5-dimethylbenzamide Dihydrochloride (**5**). A mixture of the free base of *N*-[2-(dimethylamino)ethyl]-3,5-dimethyl-4-nitrobenzamide (10 g, 34.1 mmol) and 8.9 g of 5% Pd/C in 181 mL of THF was stirred under 60 psi of hydrogen until the theoretical amount had been consumed. The reaction was filtered through Celite. Removal of solvent under reduced pressure and recrystallization of the dihydrochloride salt from ethanol provided 9.0 g (78%) of **5** as white crystals with mp 240–242 °C. Anal. ($C_{15}H_{25}N_3O \cdot 2HCl$) C, H, N.

***N*-[2-(Diethylamino)ethyl]-3-methyl-4-nitrobenzamide Ethanedioate** (1:1) (**6**). This material was prepared from 3-methyl-4-nitrobenzoic acid following the procedure outlined for the preparation of **7**. The melting point of the oxalate salt was 178–180 °C. Anal. ($C_{14}H_{21}N_3O_3 \cdot C_2H_2O_4$) C, H, N.

4-Amino-*N*-[2-(diethylamino)ethyl]-3-methylbenzamide Dihydrochloride (**4**). This material was prepared from **6** via the procedure outlined for the preparation of **5**. The melting point of the dihydrochloride salt was 223–226 °C. Anal. ($C_{14}H_{23}N_3O \cdot 2HCl$) C, H, N.

***N*-[2-(Diethylamino)ethyl]-4-(acetylamino)-3,5-dimethylbenzamide** (**8**). Pyridine (2.7 mL, 33.3 mmol) was rapidly added to a suspension of **5** (3.7 g, 11 mmol) in approximately 25 mL of DMF at room temperature. Acetyl chloride (805 μL, 11.3 mmol) was added dropwise, and then the reaction was stirred overnight. Product isolation (brine, 5 N sodium hydroxide, ethyl acetate, brine, Na_2SO_4) and recrystallization from THF/hexane provided 2.3 g (70%) of **8** as white crystals with mp 161–162 °C. Anal. ($C_{17}H_{27}N_3O_2$) C, H, N.

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Pharmacological Methods. In Vitro Electrophysiology. Adult mongrel dogs weighing between 15 and 25 kg were anesthetized by iv administration of 30 mg/kg sodium pentobarbital, and their hearts were removed. Free-running false tendons, the distal portions of the right or left bundle branches, and ventricular papillary muscles were quickly dissected. Tissues were pinned to the bottom of a wax-lined bath and continuously superfused (6 mL/min) with modified Tyrode's solution having a pH of 7.4 and aerated with 95% O_2-5% CO_2; the temperature was maintained at 36 ± 0.5 °C. The solution contained the following ions (mequiv/L): Na^+, 156.7; K^+, 4.0; Mg^{2+}, 1.0; Ca^{2+}, 4.0; Cl^-, 145.9; $H_2PO_4^-$, 1.8; HCO_3^-, 18.0; and glucose (5.5 mM). Tissues were stimulated through bipolar Teflon-coated stainless-steel electrodes at a basic cycle length of 1000 ms with square wave pulses of 0.5-ms duration at 1.5 times the threshold voltage (usually 4–6 V). Transmembrane potentials were recorded with glass microelectrodes filled with 3 M KCl (5–20 MΩ, DC resistance), connected to a high-impedance unity gain electrometer (W-P Instruments, Model 725, 2×10^{10} Ω). Tyrode's solution was superfused over the tissues for at least 0.5 h while the action potential was monitored to insure stability. Electronic differentiation (type 740 operational amplifier, field effect transistor input) was used to obtain the maximum rate of rise of the upstroke of the action potential (V_{max}). The output of the amplifier was linear for upstroke velocities from 10 to 1200 V/s. Signals were displayed on a storage oscilloscope (Tektronix Model 564) and recorded photographically.

Action potential amplitude, duration, and refractory period were measured by an on-line computer (DEC, PDP-11/45, coupled to an analog/digital converter; sampling frequency was 50 μs for 10 ms and then 1 ms for the next 800 ms). Values of APD were obtained at 70% (APD_{70}) and 95% (APD_{95}) of full repolarization. Cellular refractory period was determined by initiating premature stimuli (S_2, twice the intensity of S_1 impulses) various times after the basic driving stimuli (S_1) during the absolute refractory period. The effective refractory period (ERP) was defined as the longest S_1, S_2 interval in which S_2 failed to initiate a second action potential. Conduction time was also measured by computer and was defined as the time from the stimulus artifact to action potential upstroke. The recovery from sodium-channel inactivation was measured by applying a premature stimulus (S_2) immediately after full repolarization of the action potential and was expressed as a ratio of the rate of rise of the premature action potential to that of the paced action potential.

When the action potential was stable, either procainamide, **4**, or **5** was added to the superfusion medium to a final concentration of 3×10^{-4} M. All variables were monitored until the action potential was stable again (~30 min). Five tissues from different dogs were exposed to each drug. The data were analyzed by one-way analysis of variance, and differences among the drug groups were tested by Fisher's LSD procedure ($P < 0.05$).

In Vivo Inhibition of Ouabain-Induced Arrhythmias. Male beagle dogs weighing 12–15 kg were anesthetized with 35 mg/kg of sodium pentobarbital (iv) followed by an additional 5 mg/kg 15 min later. A Harvard dog ventilator provided ventilation through a cuffed endotracheal tube (18 strokes/min, 20 mL/kg per stroke), and a heating pad was used to maintain body temperature at 37–38 °C. Lead(II) electrocardiogram was monitored by use of subdermal needle electrodes and recorded on a Beckman Dynograph recorder. A 23-gauge butterfly infusion needle was placed in the cephalic vein for administering ouabain and test compounds.

Ouabain was administered at a dose of 50 μg/kg followed at 15-min intervals by additional doses of 10 μg/kg until an arrhythmia was produced. If less than 50% abnormal beats were present 15 min after the ouabain dose or the arrhythmia did not persist for 30 min, an additional 5 μg/kg of ouabain was administered. Following 30 min of continuous arrhythmia, test drugs were infused at 200 μg/kg per min in water with an infusion rate of 0.388 mL/min. If arrhythmic beats were present after 10 min of infusion (total dose, 2 mg/kg), the infusion rate was increased to 500 μg/kg per min (0.97 mL/min) until the material in the infusion syringe was depleted. Once conversion to 100% sinus beats was observed, the infusion was continued until twice the dose required for conversion or the entire contents of the infusion syringe had been administered. The EKG was then monitored

for 2 h or until the arrhythmia returned, and the duration of conversion was noted. The data were analyzed by paired t test, and $p < 0.05$ was considered significant.

Benzamide Antiarrhythmic Plasma Assay. Plasma concentrations of the benzamide antiarrhythmics were quantitated by a modification of the GC procedure of Yamaji and co-workers.[26] The assay was linear from 1 to 10 μg/mL, and concentrations as low as 50 ng/mL in plasma could be detected. Compounds were dissolved in water and administered to Fischer 344 rats via the jugular vein, and blood samples were obtained by cardiac puncture. To 100 μL of plasma were added 500 ng of internal standard and 1 mL of 2 N sodium hydroxide. This mixture was applied to a Chem Elut column (Analytichem Int., Harbor City, CA), and after 15 min the column was eluted with 10 mL of dichloromethane. The eluate was taken to dryness under nitrogen and reconstituted with 50 μL of ethyl acetate, and 2 μL was injected into the GC system. Procainamide was used as the internal standard for the analysis of plasma concentrations of **5** and **8**, and **5** was used as the internal standard for analysis of plasma concentrations of procainamide and *N*-acetylprocainamide. A Hewlett-Packard Model 5890 gas chromatograph, equipped with a nitrogen–phosphorus detector, a Model 3393A reporting integrator, and a Model 7673A autosampler, was used for analysis of plasma concentrations of the benzamides. The capillary column used was an 30-m, 0.32 mm column with a 0.25 μm DB-5 coating (J. W. Scientific, Folsom, CA). The injector and detector temperatures were 300 °C, and the column temperature was 235 °C. Flow rate of carrier helium was 2.7 mL/min. Under these conditions, retention times were as follows: procainamide, 3.45 min; *N*-acetylprocainamide, 7.8 min; dimethylprocainamide, 5.11 min; and *N*-acetyldimethylprocainamide, 8.98 min.

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Registry No. **1**, 51-06-9; 1 (*N*-acetyl), 32795-44-1; **4**, 112740-76-8; **4**·2HCl, 112740-75-7; **5**, 112740-74-6; **5**-2HCl, 112740-73-5; **6**, 112740-71-3; **6**·oxalate, 112740-70-2; **7**, 112740-69-9; **7**-oxalate, 112740-72-4; **8**, 112740-77-9; $H_2N(CH_2)_2N(CH_2CH_3)_2$, 100-36-7; 3,5-dimethyl-4-nitrobenzoic acid, 3095-38-3; 3,5-dimethyl-4-nitrobenzoic acid chloride, 3558-73-4; 3-methyl-4-nitrobenzoic acid, 3113-71-1.

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Synthesis and Antitumor Activity of Quaternary Ellipticine Glycosides, a Series of Novel and Highly Active Antitumor Agents

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A series of ellipticine glycosides [2-*N*-glycosyl quaternary pyridinium salts of three ellipticines: ellipticine (**1**), 9-methoxyellipticine (**2**), and 9-hydroxyellipticine (**4**)] were stereoselectively synthesized in good yields by an improved condensation reaction between ellipticines [**1**, **2**, and 9-acetoxyellipticine (**3**)] and protected (peracylated and perbenzylated) glycosyl halides with cadmium carbonate, followed by deprotection. These glycosides were preliminarily evaluated for their antitumor activity in the L1210 leukemia system. Twenty-six (53%) of the 49 glycosides tested were curative, and five [9-hydroxyellipticine L-arabinopyranoside (**41b**), D-lyxofuranoside (**43a**), L-lyxopyranoside (**44b**), D-xylofuranoside (**49a**), and L-rhamnopyranoside (**56**)] were selected for extended evaluation on the basis of their high levels of activity. The structure–activity relationships are discussed. The selected glycosides showed remarkable activity in six different murine tumor systems with excellent therapeutic ratios; their efficacy surpassed that of doxorubicin against three of these systems. On the basis of these results and ease of formulation, the two glycosides **41b** (SUN4599) and **49a** (SUN5073) were selected for further preclinical evaluation and possible clinical development.

Ellipticine (**1**, 5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole) and 9-methoxyellipticine (**2**) are alkaloids isolated from various plants of the Apocynaceae family.[1-4] These alkaloids and some of their derivatives exhibit antitumor properties in tests with experimental animal tumors.[5-8] Most ellipticine derivatives are insoluble in water because of their hydrophobic structures and this has led to considerable difficulty in developing formulations for clinical use. To date, of these compounds, 9-methoxyellipticine lactate[9] and 9-hydroxy-2-methylellipticinium acetate (celiptium, **5**),[10,11] water-soluble derivatives, have been found to be effective against human myeloblastic leukemia

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