Potential Antiarrhythmic Agents III:
4-Amino-N-[2-(substituted amino)ethyl]-2,6-dimethylbenzamides

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Abstract ☐ 4-Amino-N-(2-morpholinoethyl)-2,6-dimethylbenz-amide, 4-amino-N-(2-piperidinoethyl)-2,6-dimethylbenzamide, 4-amino-N-[2-(4-phenyl-1-piperazino)ethyl]-2,6-dimethylbenzamide, and 4-amino-N-[2-(3-azabicyclo[3.2.2]nonano)ethyl]-2,6-dimethyl-benzamide were synthesized as potential antiarrhythmic agents. These compounds were found to be capable of prolonging the effective refractory period of the isolated rabbit atria. They also demonstrated prophylactic activity against ouabain. In addition, 4-amino-N-(2-piperidinoethyl)-2,6-dimethylbenzamide was able to terminate ouabain-induced arrhythmia, and 4-amino-N-(2-mor-pholinoethyl)-2,6-dimethylbenzamide was active in antagonizing aconitine-evoked arrhythmia. The four compounds, especially 4-amino-N-[2-(3-azabicyclo[3.2.2]nonano)ethyl]-2,6-dimethylbenza-mide, also showed some degree of local anesthetic activity in the corneal reflex test in rabbits. The pKa and LD_{50} values of the compounds were determined.

Keyphrases ☐ 4-Amino-N-[2-(substituted amino)ethyl]-2,6-di-methylbenzamides —synthesized as potential antiarrhythmic agents ☐ Antiarrhythmic agents, potential—synthesis of 4-amino-N-[2-(substituted amino)ethyl]-2,6-dimethylbenzamides

The observed effectiveness of 4-amino-N-(2-diethyl-aminoethyl)-2,6-dimethylbenzamide (Ia) in preventing ouabain-induced arrhythmias (1) prompted the investigation of other 4-amino-N-[2-(substituted amino)-ethyl]-2,6-dimethylbenzamides (Ib, Ic, Id, and Ie). These analogs differed from the parent Ia in that the terminal tertiary nitrogen atom was included in a heterocyclic base. The rationale for modifying the structure in this manner was based on the results of a study by Thyrum et al. (2). These workers reported that inclusion of the basic tertiary nitrogen atom in the procaine amide molecule as part of a heterocyclic ring (e.g., piperidine) produced compounds with considerable antiarrhythmic activity.

The synthesis of the title compounds was achieved by allowing N-(4-nitro-2,6-dimethylbenzoyl)ethylenimine (II) to react with the corresponding heterocyclic bases

Ia: R = —N(CH₂CH₃)₂

Ib: R = —N⟨ ⟩O

Ic: R = —N⟨ ⟩

Id: R = —N⟨ ⟩N—⟨○⟩

Ie: R = —N⟨ ⟩

II
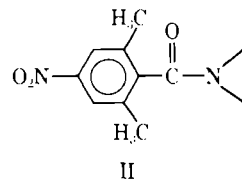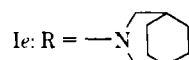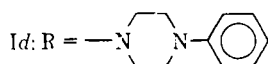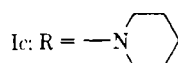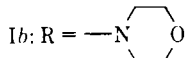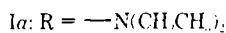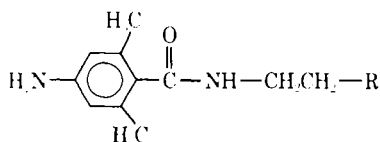
in acetone, followed by catalytic reduction of the 4-nitro-N-[2-(substituted amino)ethyl]-2,6-dimethylbenz-amides. Compound II was obtained by the condensation of 4-nitro-2,6-dimethylbenzoyl chloride and ethyl-enimine.

EXPERIMENTAL[1]

Chemical Synthesis **4-Nitro-2,6-dimethylbenzoyl Chloride** —This acid chloride was prepared by the reaction of 4-nitro-2,6-dimethyl-benzoic acid with thionyl chloride. The acid was obtained by converting 4-nitro-2,6-dimethylaniline to 4-nitro-2,6-dimethylbenzoni-trile via the Sandmeyer reaction, followed by acidic hydrolysis of the nitrile.

N-(4-Nitro-2,6-dimethylbenzoyl)ethylenimine (II)— The compound was synthesized by the reaction of 4-nitro-2,6-dimethylbenzoyl chloride with ethylenimine via the Schotten-Baumann reaction. The yield was 97%, m.p. 128–130.5°, after recrystallization from a mixture of acetone and n-hexane.

Anal.— Calc. for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72. Found: C, 60.36; H, 5.54; N, 12.86.

4-Nitro-N-[2-(substituted amino)ethyl]-2,6-dimethylbenzamides — The method described by Thyrum and Day (3) was employed to prepare these compounds. A mixture of N-(4-nitro-2,6-dimethyl-benzoyl)ethylenimine (0.018 mole) and a heterocyclic base (0.018 mole) in acetone was refluxed for 20 min. The product was isolated after removal of the solvent and recrystallized from a mixture of acetone and n-hexane. The physical data and yields of the four 4-nitro-N-[2-(substituted amino)ethyl]-2,6-dimethylbenzamides are listed in Table I.

4-Amino-N-[2-(substituted amino)ethyl]-2,6-dimethylbenzamides (Ib–Ie) – A mixture of a 4-nitro-N-[2-(substituted amino)ethyl]-2,6-dimethylbenzamide (0.01 mole), platinum oxide (200 mg.), and absolute ethanol (200 ml.) was hydrogenated in a Parr apparatus at room temperature and 60 p.s.i. After the calculated quantity of hydrogen gas had been consumed (about 2 hr.) the catalyst was filtered and the solvent was removed. The reduction product thus obtained was recrystallized from benzene. The physical data and yields of the 4-amino-N-[2-(substituted amino)ethyl]-2,6-dimethyl-benzamides are listed in Table II.

Determination of pKa Values –The method of the determination was previously described (1). It consisted of dissolving the base in a stoichiometric quantity of 0.1 N HCl and then adding a known amount of 0.1 N NaOH to the solution. After the pH of the solution was determined, the pKa value of the compounds was calculated, using the formula pKa = pH − log [B]/[BH⁺].

Pharmacological Testing—The compounds prepared in this study were tested for activity against arrhythmias induced by ouabain and by aconitine in anesthetized cats. Other studies, such as acute toxicity in mice, local anesthetic activity as determined by the cor-
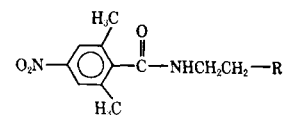
Table I—4-Nitro-N-[2-(substituted amino)ethyl]-2,6-dimethylbenzamides

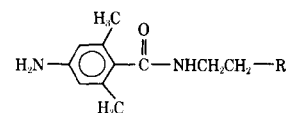| R	Yield, %	Melting Point	Formula	Analysis, % Calc.	Found	C=O Frequency, cm.$^{-1}$
—N◯O	97	133.5–135.5°	$C_{15}H_{21}N_3O_4$	C 58.62 H 6.89 N 13.67	58.81 6.73 13.57	1620
—N◯O · HCl	89	221–223° dec.	$C_{15}H_{22}ClN_3O_4$	C 52.40 H 6.45 N 12.22	52.40 6.36 12.01	1660
—N◯	91	145.4–147°	$C_{16}H_{23}N_3O_3$	C 62.93 H 7.59 N 13.76	63.26 7.43 13.82	1635
—N◯N—◯	97	132.5–133.5°	$C_{21}H_{26}N_4O_3$	C 65.95 H 6.85 N 14.65	66.24 6.76 14.57	1635
—N◯N—◯ · HCl	90	245–247° dec.	$C_{21}H_{27}ClN_4O_3$	C 60.21 H 6.50 N 13.37	59.92 6.17 13.13	1650
—N◯	87	170–171.5°	$C_{19}H_{27}N_3O_3$	C 66.06 H 7.88 N 12.16	66.03 7.87 12.34	1630

Table II—4-Amino-N-[2-(substituted amino)ethyl]-2,6-dimethylbenzamides

Compound	R	Yield, %	Melting Point	Formula	Analysis, % Calc.	Found	C=O Frequency, cm.$^{-1}$
I*b*	—N◯O	98	180–181.5°	$C_{15}H_{23}N_3O_2$	C 64.96 H 8.36 N 15.15	64.96 8.02 14.94	1625
I*c*	—N◯	56	103–104°	$C_{16}H_{25}N_3O$	C 69.78 H 9.15 N 15.26	70.06 8.79 15.11	1625
I*d*	—N◯N—◯	91	137–139°	$C_{21}H_{28}N_4O$	C 71.56 H 8.01 N 15.90	71.81 7.85 15.66	1635
I*e*	—N◯	95	131–133°	$C_{19}H_{29}N_3O$	C 72.34 H 9.27 N 13.32	72.45 9.53 13.32	1620

neal reflex test in rabbits, and effect on the maximum stimulation rates of isolated rabbit atria, were also carried out. The results of the pharmacological testing are summarized in Tables III–VII.

RESULTS AND DISCUSSION

On the basis of the results of the biological testing, some general comments can be made regarding the effect of the inclusion of a heterocyclic ring in I*a* on the various pharmacological properties of the compound.

Substitution of the diethylamino group in the parent I*a* with piperidino, N-phenylpiperazino, or 3-azabicyclo[3.2.2]nonano greatly increased the acute toxicity of the compound, as shown in Table III. However, substitution with morpholine had an opposite effect. Compound I*b* was at least three times less toxic than I*a*.

As indicated by the results in Table III, inclusion of the terminal tertiary amino group in I*a* as part of the morpholine or N-phenyl-piperazine ring caused a large drop in the local anesthetic activity.

Table III—Local Anesthetic Activity[a], Ionization Constant (Water, 25°), and Median Lethal Dose[b] (LD$_{50}$) of Compounds

Compound	Number of Tests[c]	Average Percent Anesthesia[d]	pKa	LD$_{50}$, mg./kg. i.p.
I*b*	4	16.3	5.96	746
I*c*	4	33.8	8.27	14
I*d*	5	19.5	6.16	74
I*e*	4	85.6	8.57	19
I*a*	3	54.1[e]	8.68[e]	209[e]
Procaine hydrochloride	16	39.1	8.86[e]	—
Saline (control)	6	0	—	—

[a] As determined by the corneal reflex test in rabbits. [b] Calculated by the method of Litchfield and Wilcoxon (4) and based on 1 day's observations on mice. [c] Referred to local anesthetic determinations only. [d] Number of actual failures of corneal reflex divided by the possible maximum number of failures. [e] Values taken from *Reference 1*.

The piperidine ring also reduced the activity of Ia but to a lesser extent. On the other hand, Ie, which has its tertiary nitrogen atom included in the heterocyclic base 3-azabicyclo[3.2.2]nonane, was a much more active local anesthetic agent than Ia and procaine. The pKa values of the active Ia, Ic, and Ie were similar to that of procaine.

Results in Table IV show that the effect of Ia on the effective refractory period of isolated rabbit atria was greatly reduced when the diethylamino moiety of the molecule was replaced by morpholino or piperidino. However, N-phenylpiperazine and 3-azabicyclo[3.2.2]nonane replacements enhanced the activity of Ia in prolonging the effective refractory period. In addition, it was found that the 3-azabicyclo[3.2.2]nonane derivative (Ie) had a strong depressant action on the force and rate of contraction of isolated

rabbit atria. Therefore, Ie was tested in this series of experiments at low concentrations. The high activity of Ie in prolonging the effective refractory period appeared to be related to its strong local anesthetic action. A local anesthetic agent could probably prolong the effective refractory period by raising the threshold for excitation.

Prior to the testing for antiarrhythmic activity in cats, the maximum tolerated doses of the compounds were determined, using the procedures previously described (1). According to the results in Table V, the maximum tolerated doses of Ia–Id were not significantly different from each other. It would appear, therefore, that the substitution of the diethylamino group in Ia with morpholino, piperidino, or N-phenylpiperazino had no serious effect on the cardiotoxicity of Ia. However, substitution with a 3-azabicyclo-[3.2.2]nonane ring increased the cardiotoxicity of Ia severalfold. The extreme toxic action of Ie on the heart seemed to be in agreement with its strong depressant effect on the isolated rabbit atria.

Inclusion of the four heterocyclic rings studied in this work did not significantly alter the effectiveness of Ia in preventing ouabain-induced arrhythmias. As is evident in Table V, a higher dose of ouabain was required to evoke ectopic rhythm in cats that were pretreated with Ib, Ic, Id, or Ie. On a weight basis, Ie provided the best protection against ouabain-induced arrhythmias.

According to the results in Table VI, of the four compounds tested only Ic exhibited ability to convert ouabain-evoked arrhythmias to normal sinus rhythm in cats. Although Ie was not able to provide complete protection, it did partially restore the normal QRS complex and T segment to the ECG. No significant activity was detected for Ib and Id. In view of the fact that Ia was totally devoid of activity in terminating arrhythmias evoked by ouabain (1), modifying the structure by substitution of the diethylamino group with a piperidine or 3-azabicyclo[3.2.2]nonane ring appeared to have a favorable effect on the activity.

Results in Table VII show that Ib was effective in antagonizing the action of aconitine. The ectopic rhythm was converted to sinus rhythm by Ib in three of the five cats tested. Complete antagonism was not accomplished in the other two cats. However, in one of these two animals, normal sinus rhythm did not return even after the aconitine-saturated cotton pledget was removed and subsequent injection of propranolol (5 mg./kg.) was made. Another compound that indicated some degree of activity was Ie, which was able to improve the ECG in two of the three cats. Therefore, it would seem

Table IV—Effects of Compounds on Maximum Stimulation Rates (MSR) of Isolated Rabbit Atria

Compound	Concentration, mcg./ml.	Number of Tests	Average Percent Depression, MSR ± SE
Ib	10	5	5.7 ± 1.8
	20	5	9.9 ± 0.9
	30	5	18.6 ± 2.5
Ic	1	5	5.5 ± 1.4
	5	5	8.2 ± 2.3
	10	5	9.8 ± 1.4
Id	10	7	27.1 ± 9.0
	20	7	41.7 ± 4.4
Ie	1	9	16.4 ± 2.4
	3	5	23.7 ± 4.4
Ia	10	5	17.1 ± 1.3[a]
	20	5	30.7 ± 1.4[a]
	30	5	36.3 ± 2.2[a]
Procaine amide hydrochloride	10	5	13.2 ± 2.2
	20	5	16.7 ± 3.6
	30	5	21.0 ± 2.9

[a] Values taken from *Reference 1*.

Table V—Arrhythmic and Lethal Doses of Ouabain[a] in Cats Pretreated with Compounds

Compound	Dose of Compound[b], mg./kg.	Number of Cats	Average Dose to Ectopic Rhythm, mg./kg. ± SE	p[c]	Average Lethal Dose, mg./kg. ± SE	p[c]
Control	—	3	104 ± 6	—	158 ± 10	—
Ib	10	3	139 ± 8	<0.01	191 ± 20	<0.05
Ic	8	3	148 ± 6	<0.01	211 ± 8	<0.01
Id	10	2	130 ± 13	<0.05	193 ± 13	<0.05
Ie	1	3	157 ± 12	<0.01	220 ± 12	<0.01
Ia	8.5	3	129 ± 11[d]	<0.05	215 ± 20[d]	<0.01
Procaine amide hydrochloride	18.5	3	143 ± 15	<0.01	228 ± 1	<0.01

[a] Ouabain administered by intravenous infusion at a rate of 5 mcg./kg./min. [b] Maximum tolerated doses of compounds. [c] p values compared with control. [d] Values taken from *Reference 1*.

Table VI—Effects of Compounds on Ouabain-Induced Arrhythmias Given by the Geometric Increment Procedures

Compound	Number of Complete Antagonism[a]	Number of Improvement[a]	Number of No Improvement[a]	Cumulated Dose, mg./kg.
Ib	1/3	0/3	2/3	10 (2.5, 2.5, 5)
Ic	3/3	—	—	8 (1, 1, 2, 4)
Id	—	1/2	1/2	10 (2.5, 2.5, 5)
Ie	—	2/2	—	1 (0.5, 0.5)

[a] Number of complete antagonism, improvement, and no improvement/number of experiments. Improvement is defined as partial restore of the normal QRS complex and T segment to the ECG.

Table VII—Effects of Compounds on Aconitine-Induced Arrhythmias Given by the Titration Procedures

Compound	Number of Complete Antagonism[a]	Number of Improvement[a]	Number of No Improvement[a]	Cumulated Dose, mg./kg.
Ib	3/5	1/5	1/5[b]	10
Ic	1/3	1/3	1/3	8
Id	—	1/3	2/3	10
Ie	—	2/3	1/3[b]	1

[a] Number of complete antagonism, improvement, and no improvement/number of experiments. Improvement is defined as partial restore of the normal QRS complex and T segment to the ECG. [b] Sinus rhythm did not return after removal of the cotton pledget saturated with aconitine nitrate and the subsequent injection of propranolol at a dose of 5 mg./kg.

that in comparison with Ia, Ib and Ie showed more potential in abolishing aconitine-induced arrhythmias, since Ia was found to be completely inactive in this respect (1).

The carbonyl absorption bands of the 4-nitro-N-[2-(substituted amino)ethyl]-2,6-dimethylbenzamides and their amino analogs appeared in the IR spectra in the lower 1620–1635-cm.$^{-1}$ region (Tables I and II), which was somewhat lower than expected for a secondary amide. It was suspected that the shift in frequency was due to the existence in the compounds of an intramolecular hydrogen bonding between the terminal tertiary nitrogen and the amide hydrogen atom. This postulation appeared to gain support from the fact that in the IR spectra of the hydrochloride salt of 4-nitro-N-(2-morpholinoethyl)-2,6-dimethylbenzamide and of 4-nitro-N-[2-(4-phenyl-1-piperazino)ethyl]-2,6-dimethylbenzamide, the carbonyl absorption bands occurred in the vicinity of 1660 cm.$^{-1}$, a region normally assigned for secondary amide carbonyl absorption. Due to the hygroscopic nature of the hydrochloride salts of Ib–Ie, it was not possible to prepare these compounds for IR studies.

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Effect of Environmental Conditions and Polymer Ratio on Water Vapor Transmission through Free Plasticized Cellulose Films

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Abstract ☐ Experiments were conducted to evaluate the effects of changes in the polymer ratio and environmental conditions on the water vapor transmission properties of plasticized films containing combinations of hydroxypropyl methylcellulose and ethylcellulose. Rates of water vapor transmission were calculated from a formula based on Fick's first law of diffusion. Inverse relationships were observed between the rate of water vapor transmission and film thickness for all films studied. In these plasticized systems, the polymer ratio of hydroxypropyl methylcellulose to ethylcellulose produced essentially no difference in the water vapor transmission properties from one film composition to another. Films subjected to a water vapor environment at both film surfaces were more permeable to water vapor than films subjected to a water vapor environment at only one surface. In the thickness range studied, films subjected to 40 and 50° conditions had lower rates of water vapor transmission than those studied at 30°. The findings of this study demonstrated the presence of another mechanism of vapor transmission, in addition to diffusion, that is apparently related to the hydrophilic character of the film.

Keyphrases ☐ Cellulose films, plasticized—effects of environment and hydroxypropyl methylcellulose–ethylcellulose ratio on water vapor transmission ☐ Transmission (water vapor) through plasticized cellulose films—effects of environment and hydroxypropyl methylcellulose–ethylcellulose ratio ☐ Films, plasticized cellulose —effects of environment and hydroxypropyl methylcellulose–ethylcellulose ratio on water vapor transmission ☐ Permeation of water vapor through plasticized cellulose films—effects of environment and hydroxypropyl methylcellulose–ethylcellulose ratio

All polymer membranes possess the ability to transmit liquids, gases, and vapors, a property termed permeation. This property is an important parameter in determining the potential or actual usefulness of polymeric materials in many pharmaceutical applications. In recent years the pharmaceutical industry has increasingly utilized synthetic, polymeric, film-forming materials as specialized coatings for drug particles and dosage forms, as packaging materials, as topical films and bandage components, as dialysis membranes and filters, and for many other purposes. With the rapid increase in the use of polymers for pharmaceutical purposes, a corresponding growth of scientific studies concerning the properties of these materials has become increasingly important.

The rate of permeation through films or membranes is highly dependent upon the barrier's nature. Polymeric, film-forming materials with low moisture permeability are said to possess five main characteristics (1): (a) a saturated or nearly saturated carbon chain, (b) a minimum of chain branching, (c) a high degree of lateral symmetry, (d) a fair degree of longitudinal symmetry,