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Chemistry and Hypoglycemic Activity of N-[[(Dialkylamino)alkoxy]phenyl]benzamidines[1]

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A series of N-[[(dialkylamino)alkoxy]phenyl]benzamidines was synthesized and evaluated for hypoglycemic activity in the glucose-primed rat. Structure–activity relationships indicated that N′-phenyl-N-[4-[2-(diisopropylamino)-ethoxy]phenyl]benzamidine dihydrobromide (7), N′-(4-chlorophenyl)-N-[4-[2-(diisopropylamino)ethoxy]phenyl]-benzamidine dihydrochloride (31), and N′-phenyl-N-[4-[(diisopropylamino)propoxy]phenyl]benzamidine dihydrobromide (11) are some of the more interesting compounds. A comparison of these hypoglycemic agents with classical standards (tolazamide, phenformin, and buformin) in several experimental models showed that the benzamidines seem to combine in one molecule some of the biological activities of the β-cytotrophic sulfonylureas and some of the activities of the biguanides.

A number of synthetic amidines have been reported to have biological activity. Fastier has presented an SAR study of some amidines that revealed a variety of activities, e.g., muscle relaxation, antifungal, and lowering of blood pressure and blood glucose.[3] However, the toxicity of these compounds had precluded extensive use. We now describe a group of benzamidines with hypoglycemic activity associated with a relatively wide margin of safety.

Chemistry. Two synthetic routes were used to prepare the benzamidines described in this paper and are shown in Scheme I.

Method A. The benzamidines were prepared by allowing the substituted benzimidoyl chlorides to react with [(dialkylamino)alkoxy]anilines. The resulting benzamidine hydrochlorides were converted to the free base by treatment with ammonium hydroxide. The base was then converted to the desired addition salts.

The intermediate benzimidoyl chlorides were prepared from the corresponding N-substituted benzamides by treatment with $SOCl_2$ or PCl_5 according to published procedures.[4] When possible, the benzimidoyl chlorides were purified by distillation under vacuum, but some were utilized in a crude state since distillation caused elimination of hydrogen chloride with the resultant formation of the nitrile.[5] The [(dialkylamino)alkoxy]anilines were prepared by the catalytic reduction of [(dialkylamino)-alkoxy]nitrobenzenes obtained by the reaction of the sodium salt of a nitrophenol with a β-(dialkylamino)alkyl chloride.[6]

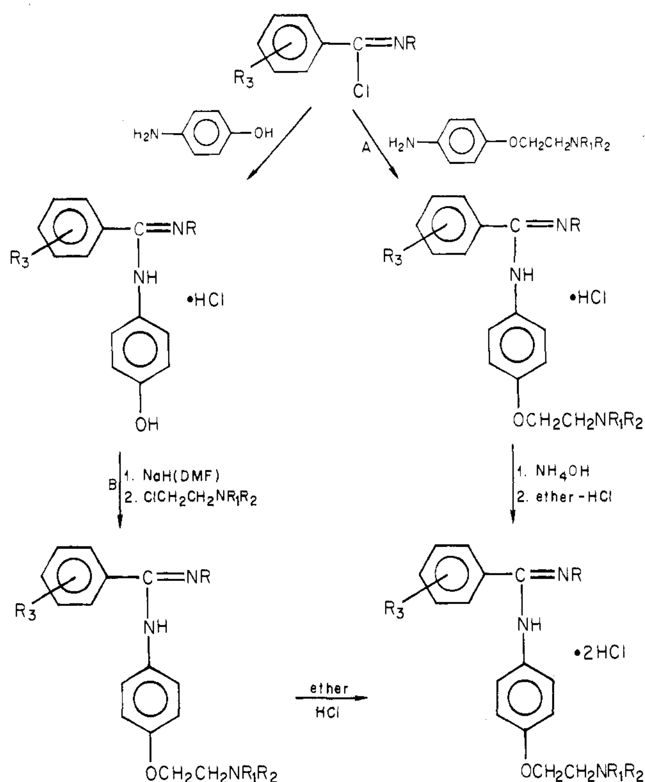
Method B. The benzamidines were also obtained by the reaction of hydroxyphenylbenzamidine hydrochloride with a β-(dialkylamino)alkyl chloride in the presence of NaH in DMF. The intermediate hydroxyphenylbenzamidines were synthesized by reacting the corresponding substituted benzimidoyl chloride with an aminophenol in acetone.

Results and Discussion

The hypoglycemic activity of the N-[[(dialkylamino)-alkoxy]phenyl]benzamidines are listed in Table I. Results are expressed as the mean percent difference in blood glucose concentration between treated and control groups. Of the 39 compounds screened in the glucose-primed rat (Table I) at a dose level of 100 mg of free base/kg of body weight, nine compounds (**7**, **8**, **11**, **24–26**, **30**, **31**, and **39**) showed a reduction of 30% or more in blood glucose levels 2 h after drug administration.

Some trends were observed when we used N′-phenyl-N-[4-[2-(diisopropylamino)ethoxy]phenyl]benzamidine hydrobromide (7) as the standard for comparison of all the other benzamidines. When the phenyl group of 7 at R was substituted with methyl to yield **1**, with ethyl to yield **2**, with isobutyl to yield **3**, with cyclohexyl to yield **4**, with allyl to yield **5**, or with benzyl to yield **6**, the hypoglycemic activity of the resultant compound was lower (**2–5**) or absent (**6**), or the compound produced hyperglycemia as in the case of **1**. Similarly, when the diisopropylamino side chain of 7 at NR_1R_2 was substituted with dimethylamino to yield **12**, with diethylamino to yield **13**, with dibutylamino to yield **14**, with methylphenylamino to yield **16**, with methylbenzylamino to yield **17**, with dibenzylamino to yield **18**, with morpholino to yield **19**, with pyrrolidino to yield **20**, or with piperidino to yield **23**, the hypoglycemic activity of the resultant compound was reduced (**13–17**)

Scheme I



(1) This paper has been presented in part. See "Abstracts of Papers", 175th National Meeting of the American Chemical Society, Anaheim, CA, Mar 7, 1979, American Chemical Society, Washington, DC, 1979, Abstr MEDI 18.

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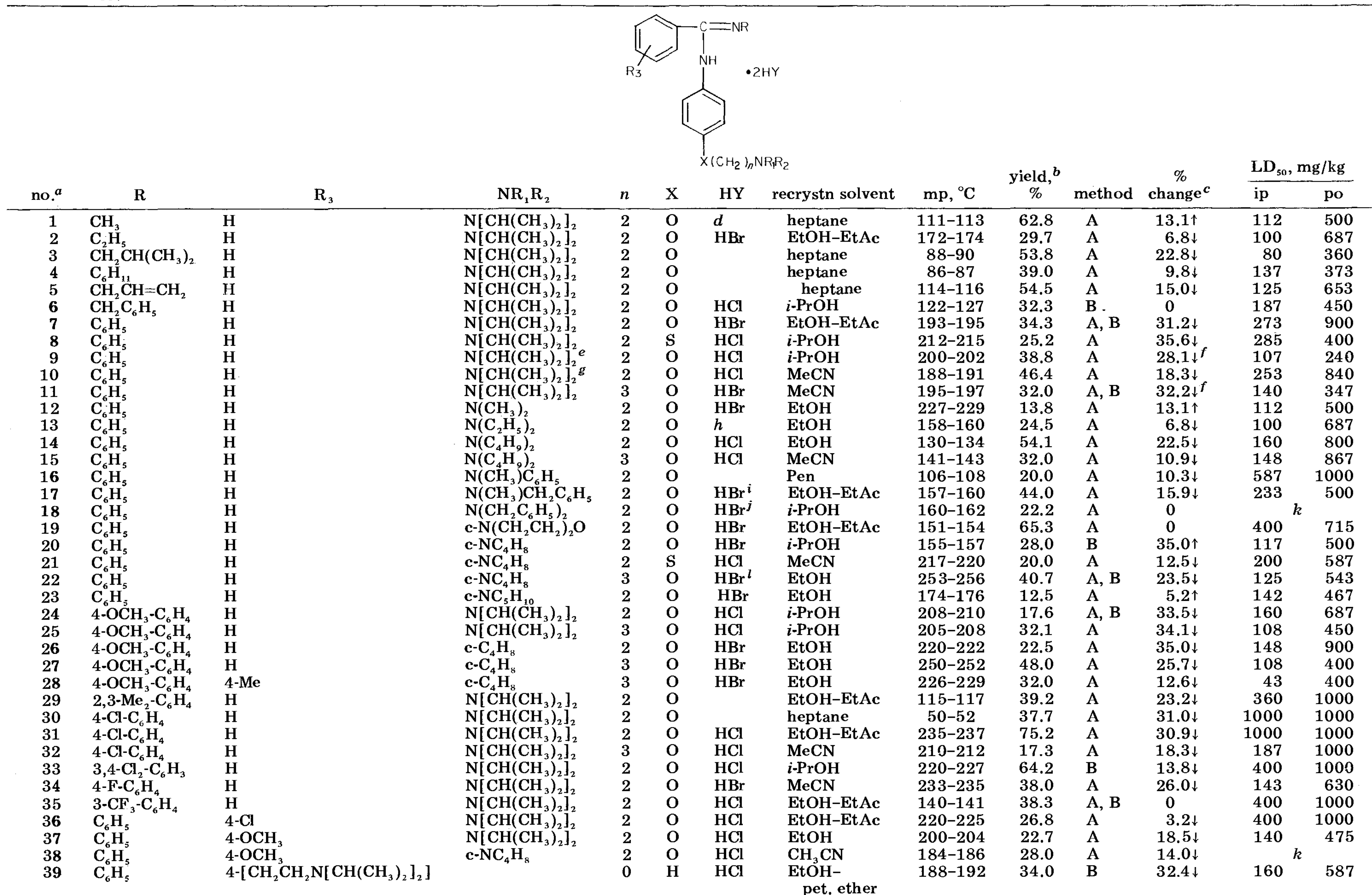
Table I. *N*-[[(Dialkylamino)alkoxy]phenyl]benzamidines



no.[a]	R	R_3	NR_1R_2	*n*	X	HY	recrystn solvent	mp, °C	yield,[b] %	method	% change[c]	LD_{50}, mg/kg ip	LD_{50}, mg/kg po
1	CH_3	H	$N[CH(CH_3)_2]_2$	2	O	*d*	heptane	111-113	62.8	A	13.1↑	112	500
2	C_2H_5	H	$N[CH(CH_3)_2]_2$	2	O	HBr	EtOH-EtAc	172-174	29.7	A	6.8↓	100	687
3	$CH_2CH(CH_3)_2$	H	$N[CH(CH_3)_2]_2$	2	O		heptane	88-90	53.8	A	22.8↓	80	360
4	C_6H_{11}	H	$N[CH(CH_3)_2]_2$	2	O		heptane	86-87	39.0	A	9.8↓	137	373
5	$CH_2CH{=}CH_2$	H	$N[CH(CH_3)_2]_2$	2	O		heptane	114-116	54.5	A	15.0↓	125	653
6	$CH_2C_6H_5$	H	$N[CH(CH_3)_2]_2$	2	O	HCl	*i*-PrOH	122-127	32.3	B	0	187	450
7	C_6H_5	H	$N[CH(CH_3)_2]_2$	2	O	HBr	EtOH-EtAc	193-195	34.3	A, B	31.2↓	273	900
8	C_6H_5	H	$N[CH(CH_3)_2]_2$	2	S	HCl	*i*-PrOH	212-215	25.2	A	35.6↓	285	400
9	C_6H_5	H	$N[CH(CH_3)_2]_2$[e]	2	O	HCl	*i*-PrOH	200-202	38.8	A	28.1↓[f]	107	240
10	C_6H_5	H	$N[CH(CH_3)_2]_2$[g]	2	O	HCl	MeCN	188-191	46.4	A	18.3↓	253	840
11	C_6H_5	H	$N[CH(CH_3)_2]_2$	3	O	HBr	MeCN	195-197	32.0	A, B	32.2↓[f]	140	347
12	C_6H_5	H	$N(CH_3)_2$	2	O	HBr	EtOH	227-229	13.8	A	13.1↑	112	500
13	C_6H_5	H	$N(C_2H_5)_2$	2	O	*h*	EtOH	158-160	24.5	A	6.8↓	100	687
14	C_6H_5	H	$N(C_4H_9)_2$	2	O	HCl	EtOH	130-134	54.1	A	22.5↓	160	800
15	C_6H_5	H	$N(C_4H_9)_2$	3	O	HCl	MeCN	141-143	32.0	A	10.9↓	148	867
16	C_6H_5	H	$N(CH_3)C_6H_5$	2	O		Pen	106-108	20.0	A	10.3↓	587	1000
17	C_6H_5	H	$N(CH_3)CH_2C_6H_5$	2	O	HBr[i]	EtOH-EtAc	157-160	44.0	A	15.9↓	233	500
18	C_6H_5	H	$N(CH_2C_6H_5)_2$	2	O	HBr[j]	*i*-PrOH	160-162	22.2	A	0	*k*	
19	C_6H_5	H	$c\text{-}N(CH_2CH_2)_2O$	2	O	HBr	EtOH-EtAc	151-154	65.3	A	0	400	715
20	C_6H_5	H	$c\text{-}NC_4H_8$	2	O	HBr	*i*-PrOH	155-157	28.0	B	35.0↑	117	500
21	C_6H_5	H	$c\text{-}NC_4H_8$	2	S	HCl	MeCN	217-220	20.0	A	12.5↓	200	587
22	C_6H_5	H	$c\text{-}NC_4H_8$	3	O	HBr[l]	EtOH	253-256	40.7	A, B	23.5↓	125	543
23	C_6H_5	H	$c\text{-}NC_5H_{10}$	2	O	HBr	EtOH	174-176	12.5	A	5.2↑	142	467
24	$4\text{-}OCH_3\text{-}C_6H_4$	H	$N[CH(CH_3)_2]_2$	2	O	HCl	*i*-PrOH	208-210	17.6	A, B	33.5↓	160	687
25	$4\text{-}OCH_3\text{-}C_6H_4$	H	$N[CH(CH_3)_2]_2$	3	O	HCl	*i*-PrOH	205-208	32.1	A	34.1↓	108	450
26	$4\text{-}OCH_3\text{-}C_6H_4$	H	$c\text{-}C_4H_8$	2	O	HBr	EtOH	220-222	22.5	A	35.0↓	148	900
27	$4\text{-}OCH_3\text{-}C_6H_4$	H	$c\text{-}C_4H_8$	3	O	HBr	EtOH	250-252	48.0	A	25.7↓	108	400
28	$4\text{-}OCH_3\text{-}C_6H_4$	4-Me	$c\text{-}C_4H_8$	3	O	HBr	EtOH	226-229	32.0	A	12.6↓	43	400
29	$2,3\text{-}Me_2\text{-}C_6H_4$	H	$N[CH(CH_3)_2]_2$	2	O		EtOH-EtAc	115-117	39.2	A	23.2↓	360	1000
30	$4\text{-}Cl\text{-}C_6H_4$	H	$N[CH(CH_3)_2]_2$	2	O		heptane	50-52	37.7	A	31.0↓	1000	1000
31	$4\text{-}Cl\text{-}C_6H_4$	H	$N[CH(CH_3)_2]_2$	2	O	HCl	EtOH-EtAc	235-237	75.2	A	30.9↓	1000	1000
32	$4\text{-}Cl\text{-}C_6H_4$	H	$N[CH(CH_3)_2]_2$	3	O	HCl	MeCN	210-212	17.3	A	18.3↓	187	1000
33	$3,4\text{-}Cl_2\text{-}C_6H_3$	H	$N[CH(CH_3)_2]_2$	2	O	HCl	*i*-PrOH	220-227	64.2	B	13.8↓	400	1000
34	$4\text{-}F\text{-}C_6H_4$	H	$N[CH(CH_3)_2]_2$	2	O	HBr	MeCN	233-235	38.0	A	26.0↓	143	630
35	$3\text{-}CF_3\text{-}C_6H_4$	H	$N[CH(CH_3)_2]_2$	2	O	HCl	EtOH-EtAc	140-141	38.3	A, B	0	400	1000
36	C_6H_5	4-Cl	$N[CH(CH_3)_2]_2$	2	O	HCl	EtOH-EtAc	220-225	26.8	A	3.2↓	400	1000
37	C_6H_5	$4\text{-}OCH_3$	$N[CH(CH_3)_2]_2$	2	O	HCl	EtOH	200-204	22.7	A	18.5↓	140	475
38	C_6H_5	$4\text{-}OCH_3$	$c\text{-}NC_4H_8$	2	O	HCl	CH_3CN	184-186	28.0	A	14.0↓	*k*	
39	C_6H_5	$4\text{-}[CH_2CH_2N[CH(CH_3)_2]_2]$		0	H	HCl	EtOH-pet. ether	188-192	34.0	B	32.4↓	160	587

[a] Except for compound 18, elemental analyses were within ±0.4% of theoretical values. [b] No attempt made to maximize yields. [c] Blood sugar lowering in the glucose-primed rat at a dose of 100 mg/kg (all doses expressed as free base). Arrow indicates direction of glycemic change. [d] Where no salt is given, the melting point is that of the free base. [e] The aminoalkoxy group is meta to the amino group. [f] Blood sugar lowering in the glucose-primed rat at a dose of 50 mg of base/kg. [g] The aminoalkoxy group is ortho to the amino group. [h] Oxalate. [i] Monohydrate salt. [j] C: calcd, 62.41; found, 61.78. [k] LD_{50} not determined. [l] Hemihydrate salt.

or abolished (**18** and **19**), or the compound produced hyperglycemia (**12**, **20**, and **23**). Replacement of oxygen by sulfur in the side chain of **7** did not modify the hypoglycemic activity of the oxygen derivative (cf. **7** and **8**), although the sulfur compound (**8**) was more toxic when given orally than the oxygen derivative.

In the case of replacement of oxygen by sulfur in the side chain of **20** to yield **21**, the hyperglycemic activity of **20** was changed to a slight hypoglycemic action, and compound **21** was slightly less toxic. Substituents were also placed on the phenyl group of **7** at R in place of hydrogen. The 4-methoxyphenyl compound (**24**) was equipotent with **7** but was slightly less toxic. The 2,3-dimethylphenyl compound (**29**) was less potent as a hypoglycemic agent and slightly less toxic. Similarly, the potency of the 4-chlorophenyl compound (**31**) decreased as the 4-chlorophenyl position was replaced with 3,4-dichloro to yield **33**, with 4-fluoro to yield **34**, or with 3-trifluoromethyl to yield **35**. Lengthening of the methylene chain from $n = 2$ to $n = 3$ between the phenyl ring and NR_1R_2 producted variable results. The activity was unchanged (cf. **7** and **11** with **24** and **25**), reversed (cf. **20** hyperglycemia with **25** hypoglycemia), or decreased (cf. **14** with **15**, **26** with **27**, and **31** with **32**).

Moving the diisopropylaminoethoxy chain from the para position, as in **7**, to the meta position, as in **9**, enhanced the hypoglycemic activity, although the acute toxicity of **9** was greater than that of **7**; on the other hand, when the chain was moved to the ortho position, as in **10**, the hypoglycemic activity was reduced. Moving the entire side chain from the *N*-phenyl ring, as in **7**, to the benzimidoylphenyl ring, as in **39**, did not alter the hypoglycemic activity, although **39** was more toxic than **7**.

Based on the initial screen (glucose-primed rat) and the acute toxicity data, three compounds (**7**, **11**, and **31**) were selected for further hypoglycemic evaluation. These compounds were tested orally in the normal, fasted guinea pig, alloxanized rat, the glucose-primed adrenalectomized rat, and the normal, fasted monkey. As a rule, the sulfonylureas (tolbutamide and tolazamide) are active in the glucose-primed rat, while the biguanides (phenformin and buformin) are active in the normal, fasted guinea pig. The hypoglycemic activity of **7**, **11**, and **31** was compared to phenformin, buformin, and tolazamide in the above models, and the results are summarized in Table II. The data reveal that in the primary hypoglycemic screen (glucose-primed rat) the order of potency was as follows: tolazamide > **7** = **11** = **31**; phenformin and buformin were inactive. In the normal, fasted guinea pig model, **11** was the most active of the three benzamidines; however, they were less potent than phenformin, buformin, or tolazamide. The order of potency in the alloxanized rat was as follows: phenformin > **7** = **11** ≥ **31** > buformin; tolazamide was inactive. In the glucose-primed, adrenalectomized rat, the order of potency was as follows: tolazamide > **7** = **11** = **31** ≥ buformin; in the normal, fasted monkey, the order of hypoglycemic activity was as follows: phenformin ≥ tolazamide > buformin ≥ **7** = **11** > **31**.

At the present time there are two clinically useful classes of oral hypoglycemic compounds, viz., the sulfonylureas and the biguanides.[7] The sulfonylureas (e.g., tolazamide) produce hypoglycemia by stimulating the release of insulin from the pancreas, as well as by potentiating the action of insulin.[7] Tolazamide lowered blood glucose in all of the

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Table II. Comparison of *N*-[[(Dialkylamino)alkoxy]phenyl]benzamidines in Standard Hypoglycemic Assays

	glucose-primed rat		normal, fasted guinea pig		alloxanized rat		adrenalectomized rat		normal, fasted monkey		mouse LD_{50},[c] mg/kg	
compd	oral dose,[c] mg/kg	% redn in blood glucose[a,d]	oral dose,[c] mg/kg	% redn in blood glucose[b]	oral dose,[c] mg/kg	% redn in blood glucose[b]	oral dose,[c] mg/kg	% redn in blood glucose[a,d]	oral dose,[c] mg/kg	% redn in blood glucose[b]	ip	po
7	50	12.9	100	10.6	100	16.5	25	33.4	25	0	273	900
	100	31.2			150	37.3			50	31.9		
	150	32.0							60	45.0		
									70	23.5		
11	10	10.9	100	20.0	100	15.0	25	30.6	25	0	140	347
	25	21.2			200	21.5			50	26.5		
	50	32.2							60	32.3		
	100	33.5							70	39.0		
									100	75.0		
31	25	18.8	100	14.0	100	7.4	25	31.4	150	35.0	1000	1000
	50	23.5			200	28.8						
	100	30.9										
tolazamide	10	28.1	100	33.5	100	0	10	75.8	20	11.0	1000	1000
	25	50.0	125	26.7			20	85.6	40	26.7		
	250	42.9	150	30.4								
	500	51.1	160	25.0								
phenformin	100	0	25	46.0	100	62.0			10	50.0	160	900
buformin	100	0	30	50.0	300	21.9	350	42.2	70	50.0	140	500

[a] Hypoglycemia measured 2 h after drug administration. [b] Hypoglycemia measured 5 h after drug administration. [c] Milligrams of free base per kilogram body weight. [d] $p < 0.05$ by two-tailed Student's t test.

models except in the alloxanized, diabetic rat, since alloxan produces diabetes by destroying the insulin-producing β cells of the pancrease. In contrast, the biguanides (e.g., buformin and phentormine) do not stimulate the release of insulin from the pancreas, although small quantities of insulin do enhance their hypoglycemic action.[7] In addition, the biguanides potentiate the action of insulin to produce hypoglycemia. The present data with buformin and phenformin are consistent with this mechanism; i.e., they produced hypoglycemia in all of the models except in the glucose-primed rat. The benzamidines, on the other hand, had hypoglycemic activity in all of the models, including the alloxanized, diabetic rat and the glucose-primed rat. Thus, the benzamidines seem to combine in one molecule some of the biological activities of the sulfonylureas and some of the activities of the biguanides.

Experimental Section

Melting points were determined with a Fisher-Johns apparatus and are uncorrected. Elemental analyses were determined by Midwest Microlab, Ltd., Indianapolis, IN, and were within ±0.4% of the theoretical values. No attempt was made to maximize yields.

***N*′-(4-Chlorophenyl)-*N*-[4-[2-(diisopropylamino)ethoxy]-phenyl]benzamidine Dihydrochloride (31). Method A.** To a solution of 125 g (0.05 mol) of *N*-(4-chlorophenyl)benzimidoyl chloride in 100 mL of dry CH_3CN was added 11.8 g (0.05 mol) of 4-[(diisopropylamino)ethoxy]aniline dissolved in 100 mL of dry CH_3CN. The reaction mixture was heated to reflux and maintained at this temperature for a period of 8 h. The CH_3CN was removed by distillation, and the crude product (19.1 g) was made basic with NH_4OH. Crystallization of the organic material from 250 mL of *n*-heptane yielded 9.5 g (42.3%) of the benzamidine, mp 50–52 °C. Hydrogen chloride was bubbled through an ethereal solution of the benzamidine, and the dihydrochloride salt was recrystallized from EtOH–EtAc (1:1) to yield 8 g of salt: 75.2% recovery; mp 235–237 °C.

***N*′-Phenyl-*N*-(4-hydroxyphenyl)benzamidine Hydrochloride.** To a suspension of 4-aminophenol (270 g, 2.5 mol) in 2.7 L of Me_2CO was added, with stirring, 539 g (2.5 mol) of *N*-phenylbenzimidoyl chloride dissolved in 250 mL of Me_2CO. After the addition was complete, the reaction mixture was refluxed for a period of 30 min. After the mixture was cooled to 15 °C, the product was collected by filtration. Recrystallization from MeOH–H_2O (1:1) yielded 353.4 g (49%) of product, mp 289–291 °C.

***N*′-Phenyl-*N*-[4-(2-pyrrolidinylethoxy)phenyl]benzamidine Dihydrochloride (20). Method B.** To a suspension of NaH (99.7 g, 2.07 mol) in 470 mL of dry, distilled DMF was added 353.4 g (1.09 mol) of *N*′-phenyl-*N*-(4-hydroxyphenyl)-benzamidine hydrochloride over a period of 1 h under nitrogen. The reaction temperature was maintained between 60 and 70 °C by means of an ice bath. When the addition was complete, the temperature of the reaction mixture was elevated to 100–110 °C, and a solution of 2-pyrrolidinylethyl chloride (186.2 g, 1.43 mol) in 310 mL of toluene was added dropwise over a period of 8 h and then cooled. The NaCl and excess NaH were filtered off, and the solvents were removed from the filtrate. The residue was extracted with Et_2O (3 × 500 mL), and the Et_2O was washed with H_2O and dried ($MgSO_4$). The HCl gas was bubbled into the dry ethereal solution to form the benzamidine dihydrochloride. Recrystallization from 2-propanol–acetone (1:1) yielded 320 g (64%) of product, mp 155–157 °C.

Pharmacological Methods. Glucose-Primed, Fasted Rat. The primary screen employed to test these compounds and to establish the SAR was the method described by Dulin[8] using glucose-primed, fasted rats (Table I). Groups of six male Sprague–Dawley rats, weighing 140–150 g, were fasted for 16 h. Each animal then received 100 mg of glucose subcutaneously in a volume of 0.5 mL of isotonic NaCl solution. An aqueous carboxymethylcellulose suspension of the test compound (0.5 mL) was administered orally immediately thereafter. Untreated control animals received the vehicle (0.5 mL). Tolazamide [1-(hexahydro-1*H*-azepin-1-yl)-3-(4-tolylsulfonyl)urea], phenformin (1-phenethylbiguanide), and buformin (1-butylbiguanide) were orally administered to additional groups of rats as positive controls. After 2 h the rats were lightly anesthetized with ether, and blood was withdrawn by cardiac puncture. Blood glucose concentrations were determined by the Autoanalyzer modification[9] of the method described by Hoffman.[10] The initial screening data in Table I are expressed as mean percent difference in blood glucose concentration between treated and control groups, while the data in Table II are expressed as percent reduction in blood glucose as compared to control groups.

Normal, Fasted Guinea Pig. Male and female Hartley strain guinea pigs (450–500 g) were deprived of food, but not water, for a period of 18 h. They were separated into groups of four by sex. Blood samples were obtained by cardiac puncture. Immediately after taking the zero time or control blood sample, the compound was administered to the animals. Blood samples were drawn 5 h after the drugs were given. Results (found in Table II) were expressed as percent reduction in blood glucose as compared to pretreatment blood glucose values.

Alloxanized, Diabetic Rat.[11] Male rats of the Sprague–Dawley strain (180–220 g) were made diabetic by an intraabdominal injection of 225 mg/kg of alloxan monohydrate. Three days later, the urine of each rat was tested for the presence of glucose using Clinistix reagent strips. Those showing glucose in the urine were segregated into groups of six rats each. After obtaining control blood samples from the tail vein of all rats, the test compounds were administered orally. Blood samples were again obtained for glucose assay 5 h after compound administration. Results are expressed as percent reduction in blood glucose as compared to pretreatment blood glucose values (see Table II).

Normal, Fasted Monkey.[12] Male and female rhesus monkeys (2–3 kg) were deprived of food, but not water, for a period of 18 h. Pretreatment blood samples were obtained from the popliteal vein. Compounds were given orally via a stomach tube. Blood samples were again taken 5 h after drug administration. Results are expressed as percent reduction in blood glucose as compared to pretreatment blood glucose values (see Table II).

Glucose-Primed, Adrenalectomized Rat.[8] Hypoglycemic activity was studied in male Sprague–Dawley rats, adrenalectomized 7 days prior to testing. At the end of 7 days they were deprived of food for 16 h and then injected subcutaneously with 100 mg of glucose per rat. Immediately thereafter, the treatment groups received the test drug orally, and the control group was administered an equal volume of saline. Blood samples were withdrawn after 2 h by cardiac puncture. Results are expressed as percent reduction in blood glucose as compared to pretreatment blood glucose values (see Table II).

Acute Toxicity Determinations. Male and female Carworth Farms CF-1 strain mice weighing 16–25 g were used. Four mice per dose level were used in this procedure. LD_{50}'s (the dose that produces lethality in 50% of mice dosed) for all drugs were determined following oral and intraperitoneal routes of administration. Drugs were dissolved in distilled water and suspended in 0.5% carboxymethylcellulose or 0.5% gelatin mixtures. Drugs were also solubilized by pH adjustment with 1 N HCl or 1 N NaOH if the drugs were not affected by such treatment. Dosages were logarithmically spaced, and the mice were observed for 7 days after drug administration. The determination of the LD_{50}'s was based on the method of Bliss.[13]

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