Potential Antidiabetic Agents. Pyrazolo[3,4-*b*]pyridines

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A series of 4-alkoxypyrazolo[3,4-*b*]pyridine-5-carboxylic acid esters (III) has been synthesized *via* the appropriate 4-hydroxy- (I) or 4-chloropyrazolo[3,4-*b*]pyridine-5-carboxylic acid esters (II). Since some of them displayed hypoglycemic activity, a series of derivatives of 1-cyclohexyl-3-[*p*-(aminoalkyl)phenylsulfonyl]urea (VIII) was synthesized by acylating *p*-(aminoalkyl)benzenesulfonamides with various pyrazolo[3,4-*b*]pyridinecarboxylic acids (VI) followed by reaction with cyclohexyl isocyanate. 1-Cyclohexyl-3-[*p*-[2-[(1-ethyl-4-isopentoxy-3-methyl-1*H*-pyrazolo-[3,4-*b*]pyridin-5-yl)formamido]ethyl]phenylsulfonyl]urea (**32**, glicaramide) was found to have a pronounced hypoglycemic effect that was superior to that of glidazamide or tolbutamide and about equal to that of glibenclamide.

In the development of a synthetic program directed toward the preparation of various fused-ring derivatives of 5-aminopyrazoles, a series of 4-substituted pyrazolo[3,4-*b*]-pyridine-5-carboxylic acids was synthesized. Compounds of this type have been reported[1,2] to be potent inhibitors of phosphodiesterase (PDE) isolated from several sources. This enzyme controls the degradation of adenosine 3′,5′-monophosphate (cAMP), a mediator of effects of various hormones. Recent reports have shown that cAMP might be involved in the stimulation by various drugs of insulin release.[3-7] For instance, methylxanthines, which are inhibitors of PDE, also modulate the release of insulin in response to glucose,[3,6-8] and pyrazolopyridines with a hydrazino or keto group have been found to stimulate insulin release *in vitro*.[9] To obtain information on structure-activity relationships in the pyrazolo[3,4-*b*]pyridine family, we extended synthetic work on 4-substituted alkoxy compounds and tested them for their PDE-inhibiting activity *in vitro* and their hypoglycemic activities *in vivo*. Furthermore, we prepared and investigated derivatives possessing a sulfonylurea moiety in position 5 of the molecule, since several compounds with that side chain are known to be antidiabetic agents.

Chemistry. Variously substituted 4-hydroxypyrazolo[3,4-*b*]pyridine-5-carboxylic acid esters (I), whose synthesis has recently been described by us,[10] were used as starting materials. In addition, 6-substituted derivatives were prepared by condensing acylmalonic acid diesters with 5-aminopyrazoles in polyphosphorous acid. According to Scheme I, the desired 4-alkoxypyrazolo[3,4-*b*]pyridine-5-carboxylic acid esters (III) were obtained either by reaction of the 4-chloro compounds II with sodium alcoholates (procedure A) or by treatment of the 4-hydroxy derivatives I with alkylating agents (procedure B); subsequent saponification of the esters (II–V) provided the acids of formula VI (procedure C).

When methyl iodide was used as an alkylating agent, the 7-methyl-substituted 4,7-dihydropyrazolo[3,4-*b*]pyridine (IV) was also obtained (procedure G). Catalytic reduction of the chloro derivative II furnished the 4-unsubstituted pyrazolopyridine V.

The synthesis of pyrazolo[3,4-*b*]pyridin-5-ylformamidoalkylphenylsulfonylureas of the general formulas VIII was carried out according to Scheme II, utilizing the following procedures.

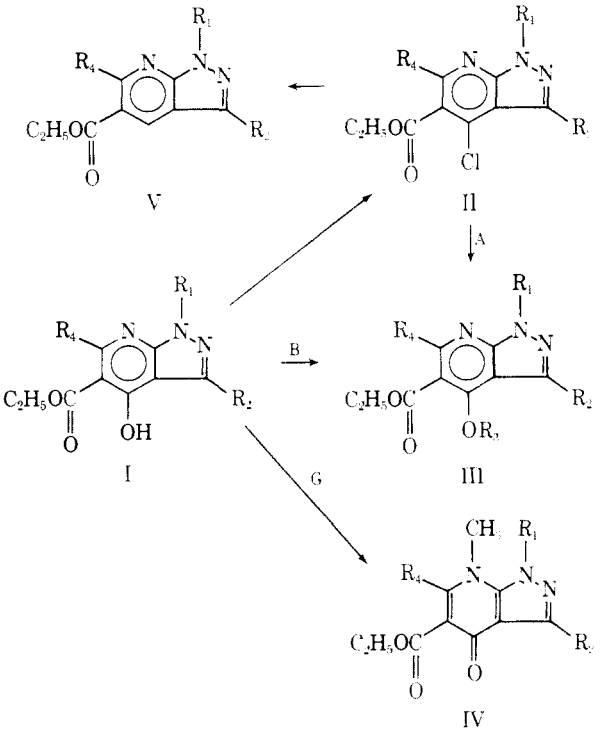
Reaction of the 4-chloropyrazolo[3,4-*b*]pyridine-5-carboxylic acid chlorides, accessible from compounds of formula VI, with *p*-(aminoalkyl)benzenesulfonamide provided the sulfonamides VII, that, when treated with cyclohexyl isocyanate, yielded the sulfonylureas VIII. Replacement of the chlorine atom at position 4 of the molecule made the preparation of variously 4-substituted sulfonylureas possible (procedure D).

When the starting materials were 4-alkoxypyrazolo[3,4-*b*]pyridine-5-carboxylic acid chlorides related to formula VI, treatment with *p*-(aminoalkyl)benzenesulfonamide yielded compounds VII that were, in turn, converted directly to the final sulfonylureas (procedure E).

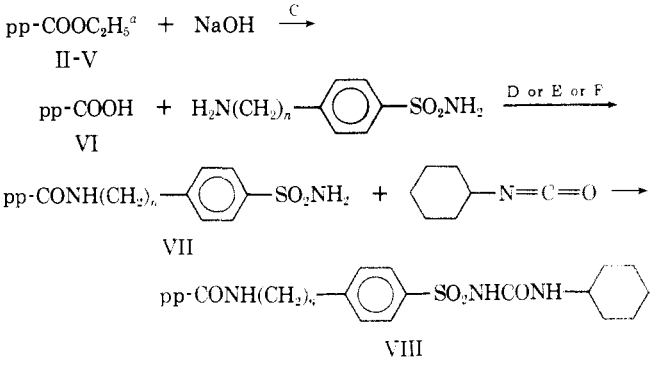
Conversion of pyrazolo[3,4-*b*]pyridine-5-carboxylic acids of formula VI, carrying alkoxy, oxo, or hydrogen in position 4 of the molecule, to the corresponding sulfonamides VII was achieved *via* the nonisolated mixed anhydrides. Treatment with cyclohexyl isocyanate provided the sulfonylureas as well (procedure F).

Biological Activity. The compounds were tested *in vitro* for inhibition of PDE and *in vivo* for hypoglycemic activity. PDE was isolated from cat heart and rat brain

Scheme I



Scheme II



[a] pp-$COOC_2H_5$ represents abbreviations for the pyrazolopyridines II–V.

according to Brooker and coworkers.[11] Its activity was assayed by a modification of their method, with labeled cAMP as substrate. The index I_{50} expresses the concentration of compound that caused 50% inhibition of the enzyme activity.

The effect on blood sugar concentration was studied in Sprague–Dawley rats of both sexes, 150–190 g, fasted overnight and during the experiment, but given access to water. Water-soluble compounds, mainly sodium salts, were administered as aqueous solutions, whereas insoluble ones (esters) were given as suspensions with 1% carboxymethylcellulose, in a volume 10 ml/kg. The animals received the compounds intraperitoneally or orally in three graded doses, usually 1, 10, and 100 mg/kg, and, when active, also at 0.1 or 0.01 mg/kg. The controls were given corresponding volumes of the vehicle. Blood samples (0.1 ml) were obtained from the sublingual vein before and 1, 3, 5, and 24 hr after dosing. The blood was transferred immediately to 1 ml of double-distilled water containing 0.1 mg of heparin, sodium salt. The glucose concentration was determined with an Auto-Analyzer by our modification of the Hoffmann ferricyanide reduction method.[12]

The following parameters were calculated: (a) minimum effective dose (MED)—the smallest dose that produces a statistically significant decrease ($p < 0.05$, Student's t test) in glucose concentration, as compared with the control group, provided that the starting levels are not significantly different; (b) maximum reduction of blood-glucose concentration—the lowest observed value, expressed as a percentage of the control group, taking into account the difference in the starting values; (c) duration of activity (in hours)—the time in which the blood-glucose concentration after any dose was significantly lower than that in the control group.

Some compounds were retested in rats that had been made diabetic by a slow intravenous injection of streptozotocin (Upjohn, Kalamazoo, Mich.) dissolved in citrate buffer, pH 5.0, 50 mg/kg. They were used 6–7 days after the injection if their blood-sugar concentration was greater than 300 mg %.

1. **Nonsulfonylurea Pyrazolo[3,4-*b*]pyridine Derivatives.** The results are summarized in Table I which indicates that most compounds are good or very good inhibitors of PDE isolated from rat brain. This activity is associated with an alkyl radical in position 1 and an ester group in position 5. A substitution in position 1 by benzyl (**21** *vs.* **2**, **22** *vs.* **7**) decreased PDE-inhibiting activity substantially and free acids were devoid of any activity (**17** *vs.* **2**, **18** *vs.* **6**). When R_4 was an arylalkyl radical (**13**, **14**), the compounds proved to be the most potent inhibitors of rat brain PDE in the series. In almost all cases, this enzyme was more susceptible to inhibition by pyrazolopyridines than was PDE isolated from cat heart. There is a tendency in the series of alkyl derivatives for brain PDE-inhibiting activity to decrease as the size of the substituent increases (**2** > **3** = **7** > **8** > **9**).

Only one compound displayed slight, yet significant, hypoglycemic properties in normal rats. In streptozotocin-diabetic rats, at least five compounds lowered the concentration of blood sugar significantly, the maximum decrease being 22% of the appropriate control group (compound **8**). It is obvious that in these animals, hypoglycemia could not have resulted from the release of insulin.

2. **Sulfonylurea Derivatives.** Table II summarizes biochemical and pharmacological data. Most of the compounds retained the ability of nonsulfonylurea derivatives to inhibit PDE of both origins, but the inhibition was much less marked in the case of rat brain PDE. Direct comparison can be made between the activities of compounds **27**, **29**, and **31–33** of this table and those of the "parent" derivatives, **2**, **3**, and **7–9**, respectively, of Table I. Although the inhibition of cat heart PDE was not changed substantially, greater concentrations of the sulfonylurea derivatives were required to cause 50% inhibition of rat brain PDE than had been true of the "parent" derivatives. In about half the cases, rat brain PDE was less susceptible than cat heart PDE to inhibition by the compounds.

Hypoglycemic activity improved remarkably when the sulfonylurea side chain was introduced into the molecule. Almost all compounds decreased the blood-glucose concentration after their ip or po administration. An alkoxy group in position 4 seems to be an important prerequisite for pharmacologic activity, but compounds with a hydrogen atom (**34**) or an oxo group (**36**) in position 4 were similarly effective. However, substitution by a chlorine atom (**35**) or a butylamino rest (**37**) led to a complete loss of activity.

In terms of a minimum effective dose, the best activity after intraperitoneal administration of a compound was associated with a bulky branched alkoxy group: *i*-C_6H_{13} (**33**) > *i*-C_5H_{11} (**32**) = *i*-C_4H_9 (**31**) > *n*-C_4H_9 (**30**) = *i*-C_3H_7 (**29**) > CH_3 (**26**). The optimal substituent for peroral effectiveness seems to be one of the size of the isopentyl group. Whereas the maximum effect was not influenced by changes in size of the substituent, the duration of this effect was directly related to the size of the substituent.

Alteration of the aliphatic side chain connecting the formamido group with the para position of the phenylsulfonylurea moiety markedly influenced the hypoglycemic activity, in that shortening of the chain led to a sharp decrease in effectiveness: $n = 0$ (**46**) < $n = 1$ (**45**) < $n = 2$ (**27**); Table III. The literature data indicate that a further lengthening of the chain does not increase the biological activity.[13]

Replacement of ethyl by higher alkyl radicals in position 1 of the molecule had no significant influence of the degree of hypoglycemic activity (compounds **38**, **40** *vs.* **26**, **27**) nor did branching of the side chain (compounds **39** *vs.* **38**).

In summary, in this series of phenylsulfonylurea derivatives, maximal activity was observed when a branched alkoxy group with four to seven carbon atoms was present in the 4 position of the pyrazolopyridyl group and a two-carbon chain was present between the amide nitrogen and the benzene ring.

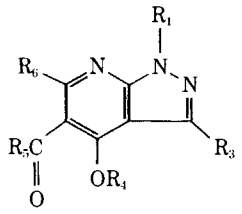
Sulfonylurea derivatives are not usually hypoglycemic in animals with pancreatic lesions. However, at least three compounds of the pyrazolopyridine series decreased the blood sugar concentration in rats made diabetic by injection of streptozotocin (Table III). The minimum effective dose was 10 or 100 mg/kg ip. Although an effect on the remaining β cells of these animals cannot be excluded, we feel that the compounds may possess an extrapancreatic site of action and cause hypoglycemia, as in the case of compound **8**, Table I.

Experimental Section

The procedures given below are representative for the variously substituted compounds described in Table I. The melting points, determined in a Lindstroem apparatus, are not corrected, and the yields are generally for unrecrystallized products. The identification of compounds **1–52** is supported by spectral and analytical data. Analyses (C, H, Cl, N, S) for compounds reported in this paper were within ±0.4% of the theoretical values. Except for 6-substituted pyrazolo[3,4-*b*]pyridine-5-carboxylic acids, the synthesis of which has been included in this section, all the other derivatives of this pyrazolopyridine family used as starting materials were prepared by the procedure given by Höhn and coworkers.[10]

Ethyl 3,6-Dimethyl-1-ethyl-4-hydroxy-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate (I). Acetomalonic acid diethyl ester[14]

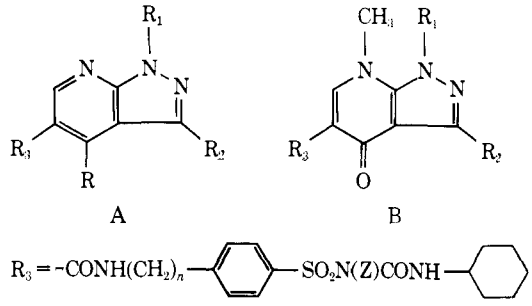
Table I. Alkoxypyrazolopyridines III



No.	R_1	R_3	R_4	R_5	R_6	Procedure	Yield, %	Mp, °C	Bp, °C (mm)	Recrystn solvent[a]	Empirical formula	PDE activity,[b] I_{50} Cat heart	PDE activity,[b] I_{50} Rat brain	Hypoglycemic activity[c] Fasted rat	Hypoglycemic activity[c] Diabetic rat
1	CH_3	H	CH_3CH_2	OC_2H_5	H	A, B	91	128–129		L	$C_{12}H_{15}N_3O_3$		8.0–62	0	
2	CH_3CH_2	CH_3	CH_3CH_2	$OC_2H_5 \cdot HCl$	H	A	60	98 dec		Ea	$C_{14}H_{20}ClN_3O_3$	30	2.2	H	
3	CH_3CH_2	CH_3	$(CH_3)_2CH$	$OC_2H_5 \cdot HCl$	H	A	70	108–109		Ea	$C_{15}H_{22}ClN_3O_3$	45	6.8	0	
4	CH_3CH_2	H	$CH_2{=}CHCH_2$	OC_2H_5	H	B	71	85–87		P	$C_{14}H_{17}N_3O_3$	24–80	3.2	H	
5	CH_3CH_2	CH_3	$CH_2C{\equiv}CH$	OC_2H_5	H	B	50	112		L	$C_{15}H_{17}N_3O_3$	32	11	0	
6	CH_3CH_2	H	$CH_3CH_2CH_2CH_2$	OC_2H_5	H	B	85	70–71		L	$C_{15}H_{21}N_3O_3$	3.4–25	1.7 5.6	0	0
7	CH_3CH_2	CH_3	$(CH_3)_2CHCH_2$	OC_2H_5	H	B	65		150–152 (0.3)		$C_{16}H_{23}N_3O_3$	18	5.2	0	+
8	CH_3CH_2	CH_3	$(CH_3)_2CHCH_2CH_2$	OC_2H_5	H	B	95		158–160 (0.3)		$C_{17}H_{25}N_3O_3$	20-60	9.0	++	+
9	CH_3CH_2	CH_3	$(CH_3)_2CHCH_2CH_2CH_2$	OC_2H_5	H	B	88		177–178 (0.6)		$C_{18}H_{27}N_3O_3$	33	12	H	++
10	CH_3CH_2	CH_3	$CH_3CH_2CH(CH_2CH_3)CH_2$	OC_2H_5	H	B	85		160–161 (0.3)		$C_{18}H_{27}N_3O_3$	32	4.0	H	+
11	CH_3CH_2	CH_3	$(CH_3)_2CHCH_2CH_2$	$OCH_2CH_2CH_3 \cdot HCl$	H	B	95	62–64			$C_{18}H_{28}ClN_3O_3$	90	13	H	0
12	CH_3CH_2	CH_3	CH_3CH_2	$OCH_2CH_2CH(CH_3)_2$	H	A	92		190–191 (0.9)		$C_{17}H_{25}N_3O_3$			H	
13	CH_3CH_2	H	$C_6H_5CH_2$	OC_2H_5	H	B	91	128		L	$C_{18}H_{19}N_3O_3$	>100	1.6	0	
14	CH_3CH_2	CH_3	$C_6H_5CH_2CH_2$	OC_2H_5	H	B	88		209–210 (0.6)		$C_{20}H_{23}N_3O_3$	10.5	1.4	H	0
15	CH_3CH_2	CH_3	CH_3	OH	H	B/C	94	189–190		Et	$C_{11}H_{13}N_3O_3$	>100	>100		
16	CH_3CH_2	H	CH_3CH_2	OH	H	A/C	93	198–199		Et	$C_{11}H_{13}N_3O_3$	86	>100	0	
17	CH_3CH_2	CH_3	CH_3CH_2	OH	H	A/C	92	201–202		Et	$C_{12}H_{15}N_3O_3$	>100	>100	0	
18	CH_3CH_2	H	$CH_3CH_2CH_2CH_2$	OH	H	B/C	62	148		B	$C_{13}H_{17}N_3O_3$	>100	>100	0	
19	CH_3CH_2	CH_3	$(CH_3)_2CHCH_2CH_2$	OH	H	B/C	98	137–138		C	$C_{15}H_{21}N_3O_3$	40	>100	0	0
20	$(CH_3)_2CH$	H	CH_3CH_2	OC_2H_5	H	B	84	104–105		L	$C_{14}H_{19}N_3O_3$	13	5.0	0	
21	$C_6H_5CH_2$	CH_3	CH_3CH_2	OC_2H_5	H	B	79	77–78		H	$C_{19}H_{21}N_3O_3$	47	90	0	
22	$C_6H_5CH_2$	H	$(CH_3)_2CHCH_2$	OC_2H_5	H	B	86	50–52		H	$C_{20}H_{23}N_3O_3$	>100	36	0	
23	$C_6H_5CH_2$	H	$(CH_3)_2CHCH_2$	$OCH_2CH(CH_3)_2$	H	A	71	58–59		H	$C_{22}H_{27}N_3O_3$	>100	120	0	
24	$C_6H_5CH_2$	H	$(CH_3)_2CHCH_2CH_2$	$OCH_2CH_2CH(CH_3)_2$	H	A	85	65–67		H	$C_{24}H_{31}N_3O_3$	>100	>100	0	++
25	CH_3CH_2	CH_3	$(CH_3)_2CHCH_2CH_2$	OC_2H_5	CH_3	B	92		147–149 (0.2)		$C_{18}H_{27}N_3O_3$	80	27	H	

[a]The abbreviations have following meaning: B, benzene; C, cyclohexane; Ea, EtOAc; Et, EtOH; H, hexane; L, ligroine (90–100°); P, PrOH. [b]Values in μM; concentration of drug producing 50% inhibition of PDE. [c]The symbols have following meaning: 0, no activity; H, hyperglycemia at 10–100 mg/kg; +, significant hypoglycemia at 100 mg/kg; ++, significant hypoglycemia at 10 mg/kg.

Table II. Sulfonylureas VIII



No.	Structure	R_1	R_2	R	*n*	Z	Procedure[a]	Mp, °C (uncor)	Recrystn solvent[b]	Formula[c]
26	A	CH_3CH_2	CH_3	OCH_3	2	Na	D	210 dec	MeOH*	$C_{26}H_{33}N_6NaO_5S$
27	A	CH_3CH_2	CH_3	OCH_2CH_3	2	Na	E	246–248 dec	EtOH*	$C_{27}H_{35}N_6NaO_5S$
28	A	CH_3CH_2	CH_3	$OCH_2CH{=}CH_2$	2	H	D	165–167 dec	A	$C_{28}H_{36}N_6O_5S$
29	A	CH_3CH_2	CH_3	$OCH(CH_3)_2$	2	Na	D	216–218 dec	PrOH*	$C_{28}H_{37}N_6NaO_5S\cdot0.5H_2O$
30	A	CH_3CH_2	CH_3	$OCH_2CH_2CH_2CH_3$	2	H	D	184–185	A	$C_{29}H_{40}N_6O_5S$
31	A	CH_3CH_2	CH_3	$OCH_2CH(CH_3)_2$	2	Na	D	224–225 dec	A*	$C_{29}H_{39}N_6NaO_5S\cdot H_2O$
32	A	CH_3CH_2	CH_3	$OCH_2CH_2CH(CH_3)_2$	2	H	D	166–167 dec	A	$C_{30}H_{42}N_6O_5S$
33	A	CH_3CH_2	CH_3	$OCH_2CH(CH_2CH_3)_2$	2	H	D	180–181	MeOH	$C_{31}H_{44}N_6O_5S\cdot0.5H_2O$
34	A	CH_3CH_2	CH_3	H	2	Na	F	295	A*	$C_{25}H_{31}N_6NaO_4S\cdot0.5H_2O$
35	A	CH_3CH_2	CH_3	Cl	2	H	D	218	D	$C_{25}H_{31}ClN_6O_4S$
36	B	CH_3CH_2	CH_3		2	Na	F	246–249 dec	MeOH*	$C_{26}H_{33}N_6NaO_5S\cdot0.5H_2O$
37	A	CH_3CH_2	CH_3	$NHCH_2CH_2CH_2CH_3$	2	H	D	105–110 dec	MeOH	$C_{29}H_{41}N_7O_4S$
38	A	$CH_3CH_2CH_2$	CH_3	OCH_2CH_3	2	Na	D	242–244 dec	A*	$C_{28}H_{37}N_6NaO_5S$
39	A	$(CH_3)_2CH$	CH_3	OCH_2CH_3	2	Na	E	252–254 dec	EtOH	$C_{28}H_{37}N_6NaO_5S$
40	A	$CH_3CH_2CH_2CH_2$	CH_3	OCH_2CH_3	2	H	D	187–188	A	$C_{29}H_{40}N_6O_5S$
41	A	$(CH_3)_2CH$	CH_3	OCH_3	2	Na	D	249–251 dec	A*	$C_{27}H_{35}N_6NaO_5S\cdot2H_2O$
42	A	$CH_3CH_2CH_2CH_2$	CH_3	OCH_3	2	Na	D	195–197 dec	MeOH–Et_2O	$C_{28}H_{37}N_6NaO_5S\cdot H_2O$
43	A	CH_3CH_2	H	OCH_3	2	Na	E	245 dec	D*	$C_{25}H_{31}N_6NaO_5S\cdot2H_2O$
44	A	CH_3CH_2	H	OCH_2CH_3	2	Na	E	199–201	EtOH	$C_{26}H_{33}N_6NaO_5S$
45	A	CH_3CH_2	CH_3	OCH_2CH_3	1	H	E	208–209	MeOH	$C_{26}H_{34}N_6O_5S$
46	A	CH_3CH_2	CH_3	OCH_2CH_3	0	H	E	225–226	MeOH	$C_{25}H_{32}N_6O_5S$

[a]For procedures, refer to Experimental Section. [b]The abbreviations have the following meaning: A, acetone; D, dioxane; *, recrystallized as acid. [c]All compounds were analyzed correctly for C, H, N, Na, and S.

(60 g, 0.3 mol) was added dropwise to a stirred mixture of 5-amino-1-ethyl-3-methylpyrazole[15] (37 g, 0.3 mol) and 125 g of PPA, heated to 130°. This reaction temperature was maintained for 45 min. After the mixture had cooled to room temperature, 350 ml of H_2O was added with stirring; 18 g of precipitated crystals (mp 74–76°) was collected by filtration. The filtrate was extracted four times with 150 ml of $CHCl_3$. Evaporation of the $CHCl_3$ extract gave a second crop of crystals (27 g, mp 70–73°) when the residue was recrystallized from hexane; the total yield was 45 g (57%). A purified sample melted at 79–81° (hexane).

Ethyl **4-Chloro-3,6-dimethyl-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate (II).** A mixture of ethyl 3,6-dimethyl-1-ethyl-4-hydroxy-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate (10.5 g, 0.04 mol) and 75 ml of $POCl_3$ was refluxed for 4 hr. The excess $POCl_3$ was removed *in vacuo* and the residue was treated with H_2O. The oily compound was extracted with Et_2O, washed with an aqueous Na_2CO_3 solution (10%), and washed again with H_2O. Evaporation of the dried (Na_2SO_4) ethereal extract provided 8.6 g (77%) of the oily chloro compound that was used in the alkylating step without further purification.

Ethyl **3,6-Dimethyl-1-ethyl-4-isopentoxy-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate (III, 25). Procedure A.** Ethyl 4-chloro-3,6-dimethyl-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate (18.4 g, 0.07 mol) was added to a solution of Na (1.61 g, 0.07 mol) in 200 ml of 2-pentanol. The reaction mixture was stirred and heated at 50° for 3 hr, then the precipitated NaCl was filtered off, and the filtrate was evaporated under reduced pressure. The remaining oil (21.7 g, 93%) was dissolved in Et_2O. The ethereal solution was washed with H_2O, dried (Na_2SO_4), and evaporated. The residue was distilled *in vacuo* to give the product, bp 147–149° (0.2 mm).

Ethyl **1-Ethyl-4-isopentoxy-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate (III, 8). Procedure B.** Ethyl 1-ethyl-4-hydroxy-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate (41.6 g, 0.167 mol) and 110 g of well-pulverized K_2CO_3 in 480 ml of DMF were stirred at 60° (bath temperature) for 2 hr. In the next hour 2-pentyl bromide (61 g, 0.4 mol) was added dropwise to the mixture. During this time and for the following 13 hr, the whole mixture was stirred and kept at 60°. After cooling and removing the inorganic material by filtration, the filtrate was evaporated *in vacuo* and the remaining oil was dissolved in Et_2O. The ethereal solution was washed with H_2O, dried (Na_2SO_4), and evaporated once more. Distillation of the oil (51.46 g, 96.5%) gave 46 g of the product, bp 158–160° (0.3 mm).

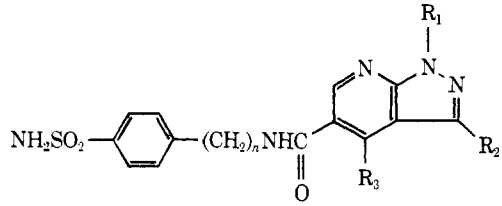
1-Ethyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic Acid (VI) and Ethyl Ester (V). 4-Chloro-1-ethyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic acid ethyl ester (53 g, 0.2 mol) dissolved in absolute EtOH (500 ml) and triethylamine (20 g) was mixed with Pd/charcoal (1.5 g, 10%) and hydrogenated for 4 hr at room temperature. The reaction mixture was filtered and evaporated *in vacuo*. The residue when treated with H_2O, filtered, and dried yielded 42.5 g (91%) of the product, mp 53–56°. Recrystallization from hexane raised the melting point to 55–57°. Saponification according to procedure C gave the acid, mp 200–202°. Recrystallization from EtOH raised the melting point to 201–203°.

Table III. Hypoglycemic and PDE-Inhibiting Activities

	Fasted normal rat																
	Ip administration					Po administration					Diabetic rat, ip administration					PDE inhibition,[c] I_{50}, μM	
			Maximum response				Maximum response					Maximum response					
Compd no.	n[a]	MED,[b] mg/kg	Dose, mg/kg	% decrease	Duration, hr	n[a]	MED,[b] mg/kg	Dose, mg/kg	% decrease	Duration, hr	n[a]	MED,[b] mg/kg	Dose, mg/kg	% decrease	Duration, hr	Rat brain	Cat heart
26	12	10	100	33	>5 <24											36	34
27	18	1	10	40	5	10	10	100	36	>5 <24	10	>100				48	29
28	10	1	100	37	>5 <24	6	>1 <10	100	53	>5 <24						52	27
29	14	>1 <10	100	42	24	17	>1 <10	100	48	>5 <24						>100	27
30	13	>1 <10	100	46	24	14	10	100	31	24						27	15
31	20	>0.1 <1	100	40	24	16	>1 <10	100	32	24	6	100	100	31	>5 <24	12	28
32	18	1	10	36	>5 <24	16	1	10	42	>5 <24	12	10	100	30	>24	22	15
33	12	0.1	10	46	>5 <24	6	10	100	34	>5 <24						35	16
34	10	>1 <10	100	49	>5 <24	12	10	100	46	>5 <24	12	>100				34	29
35	4	>100														34	58
36	18	>1 <10	100	54	>5 <24	6	>10									>100	
37	6	>100														14	14
38	16	>1 <10	10	37	3						12	100	100	17	>3 <5	88	44
39	14	>1 <10	100	36	>5 <24	10	10	100	28	>5 <24						54	35
40	8	1	100	43	24											50	48
41	8	10	100	27	>5 <24						6	>100				100	14
42	8	>10 <100	100	34	>3 <5	4	10	100	32	1						>100	18
43	8	>1 <10	100	36	>5 <24											48	47
44	16	10	10	31	1	4	>10									25	17
45	11	>10 <100	100	39	>3 <5											48	33
46	11	>100														28	26
T[d]	12	10	100	37	>5 <24	12	30	100	36	>5 <24	12	100	300	18	>3 <5	>100	>100
HB[e]	22	0.01	10	42	>5 <24	12	1	10	36	>24	6	100	100	23	>5	>100	52
HZ[f]	18	10	100	45	>5 <24	18	10	100	39	>5 <24	18	100	100	15	>3 <5		

[a]Number of animals. [b]Minimum effective dose (see the text for definition). [c]Concentration causing 50% inhibition of the enzyme. [d]Tolbutamide or 3-(*p*-tolyl-4-sulfonyl)-1-butylurea. [e]Glibenclamide or *N*-4-[2-(5-chloro-2-methoxybenzamido)ethyl]phenylsulfonyl-*N*[1]-cyclohexylurea. [f]Glidazamide or 1-(indan-5-sulfonyl)-3-(1-hexahydroazepinyl)-urea.

Table IV. Sulfonamides VII



No.	R_1	R_2	R_3	n	Procedure[a]	Mp, °C	Recrystn solvent[b]	Formula[c]
47	CH_3CH_2	CH_3	OCH_2CH_3	2	E	243–245	EtOH	$C_{20}H_{25}N_5O_4S$
48	CH_3CH_2	CH_3	H	2	F	224–226	EtOH	$C_{18}H_{21}N_5O_3S \cdot 0.75H_2O$
49	$(CH_3)_2CH$	CH_3	OCH_2CH_3	2	E	255–257	AcOH	$C_{21}H_{27}N_5O_4S$
50	CH_3CH_2	H	OCH_3	2	E	252–254	AcOH	$C_{18}H_{21}N_5O_4S$
51	CH_3CH_2	CH_3	OCH_2CH_3	1	E	234–236	AcOH	$C_{19}H_{23}N_5O_4S$
52	CH_3CH_2	CH_3	OCH_2CH_3	0	E	243–245	EtOH	$C_{18}H_{21}N_5O_4S$

[a–c] See footnotes in Table II.

1-Ethyl-4-isopentoxy-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic Acid (VI, 19). Procedure C. A suspension of 8 (20 g, 0.06 mol) in 300 ml of aqueous 1 *N* NaOH and 180 ml of EtOH was stirred for 33 hr at room temperature. After removal of EtOH, the clear solution was acidified with aqueous 6 *N* HCl to yield 16.5 g (95%) of compound that, after recrystallization from cyclohexane, melted at 137–138°.

4-Chloro-1-ethyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic Acid Chloride. 1-Ethyl-4-hydroxy-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic acid (22 g, 0.1 mol) and 75 ml of $SOCl_2$ were refluxed for 4 hr. The clear $SOCl_2$ solution was evaporated to dryness *in vacuo*. The residue, weighing 24 g (93%), contained the crude acid chloride, which could be used without further purification for the next reaction step. A sample recrystallized from cyclohexane melted at 68–70°.

4-[β-(4-Chloro-1-ethyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-formamido)ethyl]benzenesulfonamide (VII). Procedure D. *p*-(β-Aminoethyl)benzenesulfonamide[16] (13 g, 0.077 mol) was added to a solution of 4-chloro-1-ethyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic acid chloride (9 g, 0.053 mol) in 100 ml of anhydrous pyridine. After the reaction mixture had been stirred for 3 hr at room temperature, the precipitated *p*-(β-aminoethyl)-benzenesulfonamide hydrochloride was filtered off under suction and the filtrate was evaporated to dryness *in vacuo*. The residual product was treated with H_2O, filtered under suction, and recrystallized from a mixture of EtOH and dioxane: yield 10 g (68%); mp 258–260°.

1-Cyclohexyl-3-[[*p*-[2-[(4-chloro-1-ethyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-5-yl)formamido]ethyl]phenyl]sulfonyl]urea (VIII, 35). 4-[β-(Chloro-1-ethyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-formamido)ethyl]benzenesulfonamide (16.8 g, 0.04 mol) and well-pulverized K_2CO_3 (11.2 g, 0.08 mol) were suspended in 300 ml of acetone. The mixture was refluxed for 1 hr and then cyclohexyl isocyanate (6 g, 0.44 mol) was added rapidly, 1 drop at a time. The whole mixture was refluxed for 4 hr, with stirring. After cooling, the precipitate was filtered off under suction, washed with acetone, and, in turn, dissolved in 450 ml of hot H_2O. Undissolved matter was separated by filtration, and the filtrate was acidified with dilute HCl. The sulfonylurea, recrystallized from dioxane, had mp 218°, yield 14 g (68%).

1-Cyclohexyl-3-[[*p*-[2-[(1-ethyl-4-isopentoxy-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-5-yl)formamido]ethyl]phenyl]sulfonyl]urea (VIII, 32). **35** (5.5 g, 0.01 mol) was added to a solution of Na (0.5 g, 0.022 mol) in 75 ml of isoamyl alcohol. This mixture was kept at room temperature for 6 hr and then the precipitate was filtered, washed with isoamyl alcohol and Et_2O, and treated with 50 ml of H_2O. The undissolved matter was separated by filtration, and the filtrate was acidified with dilute HCl, to give the sulfonylurea. After being dried in the desiccator and recrystallized from acetone, the product melted at 166–167°, yield 5.0 g (84%).

4-[β-(4-Ethoxy-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-formamido)ethyl]benzenesulfonamide (VII). Procedure E. *p*-(β-Aminoethyl)benzenesulfonamide (29.4 g, 0.14 mol) was added to a suspension of 4-ethoxy-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic acid chloride (17.5 g, 0.07 mol) in 200 ml of anhydrous pyridine. The reaction mixture was stirred for 2 hr at room temperature. The precipitate, consisting of 4-[β-(4-ethoxy-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-formamido)ethyl]benzenesulfonamide and *p*-(β-aminoethyl)benzenesulfonamide hydrochloride, was filtered off under suction and washed with pyridine and Et_2O. In order to remove the hydrochloride, the precipitate was washed with H_2O and then dried: yield 10 g; mp 220–222°. After evaporation of the mother liquor and treatment of the residue with H_2O, an additional 10 g was obtained; the total yield was 20 g (69%). The compound was recrystallized from dioxane: mp 221–222°.

1-Cyclohexyl-3-[[*p*-[2-[(4-ethoxy-1-ethyl-1*H*-pyrazolo[3,4-*b*]-pyridin-5-yl)formamido]ethyl]phenyl]sulfonyl]urea (VIII, 44). 4-[β-(4-Ethoxy-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-formamido)-ethyl]benzenesulfonamide (4.2 g, 0.01 mol) and well-pulverized K_2CO_3 (2.8 g, 0.02 mol) were suspended in 75 ml of acetone. The mixture was refluxed for 1 hr and then cyclohexyl isocyanate (1.3 g, 0.01 mol) was added rapidly, 1 drop at a time. The whole mixture was refluxed for 4 hr, with stirring. After cooling, the precipitate was filtered off under suction, washed with acetone, and, in turn, dissolved in 150 ml of hot H_2O. Undissolved matter was separated by filtration, and the filtrate was acidified with dilute HCl. The sulfonylurea was recrystallized from a mixture of MeOH and dioxane: mp 213–214°; yield 3.7 g (68%).

For the preparation of the Na salt, 44 was treated with an equimolar amount of EtONa in EtOH. At room temperature, the Na salt precipitated in the form of white crystals, mp 199–201°.

4-[β-(4-Ethoxy-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-formamido)ethyl]benzenesulfonamide (VII). Procedure F. A solution of 4-ethoxy-1-ethyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic acid (23.5 g, 0.1 mol) in 300 ml of $CHCl_3$ and 20 ml of triethylamine was cooled to 0°. At this temperature, isobutyl chloroformate (19 g, 0.14 mol) was added dropwise. The whole mixture was stirred for 2 hr, during which time the temperature might increase to 15°. A suspension consisting of *p*-(β-aminoethyl)benzenesulfonamide (20 g, 0.1 mol) in 200 ml of $CHCl_3$ and 20 ml of triethylamine was added to the mixture. The mixture was stirred at room temperature for 4 hr and then the precipitate was filtered off under suction and washed with $CHCl_3$. With the crop isolated from the mother liquor, 26.5 g of the product, mp 213–214°, was obtained; it was converted to 1-cyclohexyl-3-[[*p*-[2-[4-ethoxy-1-ethyl-1*H*-(pyrazolo[3,4-*b*]pyridin-5-yl)formamido]ethyl]phenyl]sulfonyl]-urea by procedure E.

Ethyl 1-Ethyl-4,7-dihydro-3,7-dimethyl-4-oxo-1*H*-pyrazolo-[3,4-*b*]pyridine-5-carboxylate (IV). Procedure G. A mixture containing ethyl 1-ethyl-4-hydroxy-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate (49.8 g, 0.2 mol), well-pulverized K_2CO_3 (71 g), DMF (290 ml), and CH_3I (71 g, 0.5 mol) was stirred for 14 hr at 50°. Then the excess of K_2CO_3 was removed by filtration. On standing overnight, the K salt of ethyl 1-ethyl-4-hydroxy-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate-7-methiodide crystallized with a certain amount of KI and K_2CO_3. To obtain the 4-oxo compound, the K salt was dissolved in 75 ml of H_2O. After a short time, the ethyl 1-ethyl-4,7-dihydro-3,7-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate began to crystallize. Evaporation of the DMF mother liquor and treatment of the oily residue with Et_2O provided an additional amount of the desired compound. The total yield amounted to 25 g (47.5%). The compound was recrystallized from EtOH, mp 192–193°. The ethereal solution contained the isomeric ethyl 1-ethyl-4-methoxy-3-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate. After removing the Et_2O, 27.5 g (52%) of the 4-methoxy compound was obtained.

1-Ethyl-4,7-dihydro-3,7-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic Acid (VI). Ethyl 1-ethyl-4,7-dihydro-3,7-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate (26.3 g, 0.1 mol) was saponified with 130 ml of aqueous NaOH (15%)

while being stirred at room temperature. The clear solution was allowed to stand overnight. After acidification with HCl (18%), the precipitated acid was filtered off under suction, washed with H_2O, and dried at 100° to yield 23.2 g (99%), mp 254°. A sample recrystallized from acetonitrile melted at 256°.

4-[2-(4,7-Dihydro-3,7-dimethyl-1-ethyl-4-oxo-1*H*-pyrazolo-[3,4-*b*]pyridine-5-formamido)ethyl]benzenesulfonamide (VII). A solution of 4,7-dihydro-3,7-dimethyl-1-ethyl-4-oxo-1*H*-pyrazolo-[3,4-*b*]pyridine-5-carboxylic acid (14 g, 0.06 mol) in 300 ml of $CHCl_3$ and 12 ml of triethylamine was cooled to 0°. At this temperature, isobutyl chloroformate (11.5 g, 0.085 mol) dissolved in 60 ml of $CHCl_3$ was added dropwise and the whole mixture was stirred at 0–5° for 20 min. A solution of *p*-(β-aminoethyl)benzenesulfonamide (12 g, 0.06 mol) in 120 ml of $CHCl_3$ and 12 ml of triethylamine was then added to the mixture. After being stirred at room temperature for 2 hr, the precipitate was filtered off under suction and washed with $CHCl_3$ to give 24.7 g (98.8%), mp 236–238°. A sample recrystallized from glacial AcOH melted at 238–240°.

1-Cyclohexyl-3-[[*p*-[2-[(4,7-dihydro-3,7-dimethyl-1-ethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridin-5-yl)formamido]ethyl]phenyl]-sulfonyl]urea (VIII, 36). 4-[2-(4,7-Dihydro-3,7-dimethyl-1-ethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-formamido)ethyl]benzenesulfonamide (6.3 g, 0.015 mol) was added to a solution of K (0.65 g, 0.0165 mol) in 50 ml of absolute MeOH, and the mixture was stirred at room temperature for 45 min. The MeOH was evaporated *in vacuo*, and 75 ml of acetone and cyclohexyl isocyanate (2.1 g, 0.0165 mol) were added to the K salt. The whole mixture was stirred for 2 hr at 65–70° (bath temperature) and then the acetone was decanted from the oily residue. The latter was dissolved in 125 ml of H_2O, with stirring, and the aqueous solution was filtered and then acidified with HCl (18%), and the precipitated product was filtered off, washed with H_2O, and dried in the desiccator to yield 6.5 g (80%). Shortly after the crude product was dissolved in acetone, the sulfonylurea crystallized, mp 190–192°; when recrystallized from MeOH, it melted at 195–196°. For the preparation of the Na salt, the sulfonylurea was treated with an equimolar amount of EtONa in EtOH. At room temperature the precipitated Na salt was filtered off under suction and washed with EtOH and Et_2O, mp 246–249° dec.

In Table IV are listed the melting points of those pyrazolo[3,4-*b*]pyridin-5-ylformamidoalkylbenzenesulfonamides (VII) that have not been described in the Experimental Section.

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Pyrazolodiazepines. 1,3- (and 2,3-) Dialkyl-4,6-dihydro-8-arylpyrazolo[4,3-*e*][1,4]diazepin-5-ones as Antianxiety Agents

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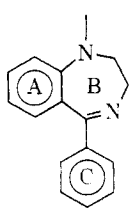
A series of 1,3- (and 2,3-) dialkyl-4,6-dihydro-8-arylpyrazolo[4,3-*e*][1,4]diazepin-5-ones was synthesized and evaluated for psychotropic activity. Intermediates are new dialkylnitropyrazolyl aryl ketones VIII and IX prepared from dialkylnitropyrazolecarboxylic acids. Many of these pyrazolodiazepines exhibit high CNS activity in animals. One compound, 1-ethyl-4,6-dihydro-3-methyl-8-phenylpyrazolo[4,3-*e*][1,4]diazepin-5(1*H*)-one (98), is about as potent as diazepam as an antianxiety agent with less sedative properties and is being studied in the clinic (CI-683).

In extensive research efforts, thousands of benzodiazepines and related compounds have been synthesized and studied, but the vast majority of these efforts have been directed to changes in ring B and/or C. At the time this work was started in 1967, the only published change from the fused benzo ring A, aside from nuclear substitution, was the synthesis of 5-aryl-1,3-dihydro-2*H*-pyrido-1,4-diazepin-2-ones.[1] These appeared to be electronically analogous to the requirement of an electronegative substituent at position 7 to obtain high drug potency. Our work was directed toward the incorporation of other hetero systems in place of the fused ring A of I. The first compounds to be synthesized were 8-arylpyrazolo[4,3-*e*][1,4]diazepin-5-ones[2] II and are the subject of this paper. An isomeric pyrazole series, the 4-arylpyrazolo[3,4-*e*][1,4]diazepin-7-



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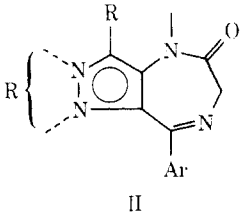

II

ones,[3] was developed simultaneously and is to be the subject of a future communication.†

Chemistry. Potential intermediates to II have been

†A third series, the thienodiazepinones, was prepared and studied concurrently in these laboratories. A recent paper[4] and also prior reports[5] describe some of these thienodiazepinones.