solved in water, basified with potassium carbonate, and extracted with benzene. The free base was obtained by chromatography on basic alumina. Elution with benzene and methylene chloride gave 0.85 g. (8%) of VII, which was converted to the hydrochloride salt and recrystallized from *n*-butanol to give an analytical sample decomposing at 213–215°. NMR($CDCl_3$ + D_2O), p.p.m.: 8.15 (s, 1, ArH), 6.85 [d, 1, (J_o = 9 Hz.), ArH], 6.68 (s, 2, ArH), 6.67 [d, 1, (J_m = 5 Hz.), ArH], 6.55 [q, 1, (J_o = 9 Hz., J_m = 5 Hz.), ArH), 3.90 (s, 6, OCH_3), 3.88 (s, 3, OCH_3), 3.85 (s, 3, OCH_3), 3.70 (s, 3, OCH_3), 3.9–3.7 (broad, 2, CH_2), 3.5–2.7 (m, 4, CH_2), 2.94 (s, 3, NCH_3). M^+ *m/e* 475,477.

Anal.—Calc. for $C_{28}H_{32}ClNO_6$: C, 65.43; H, 6.28; N, 2.73. Found: C, 65.19; H, 6.20; N, 2.96.

REFERENCES

(1) N. M. Mollov, K. B. Duchevska, K. Silyanovska, and S. Stoichev, *C. R. Acad. Bulg. Sci.*, **21**, 605(1968); through *Chem. Abstr.*, **69**, 58087z(1968).

(2) S. M. Kupchan, K. K. Chakravarti, and N. Yokoyama, *J. Pharm. Sci.*, **52**, 985(1963); M. Tomita, H. Furukawa, S.-T. Lu, and S. M. Kupchan, *Tetrahedron Lett.*, **1965**, 4309.

(3) M. P. Cava, K. Bessho, B. Douglas, S. Markey, and J. A. Weisbaen, *ibid.*, **1966**, 4279.

(4) S. M. Kupchan and A. J. Liepa, *Chem. Commun.*, **1971**, 599.

(5) R. I. Metzler and J. Doczi, *J. Amer. Chem. Soc.*, **72**, 4986 (1950).

(6) W. M. Woley and N. Meadow, *J. Chem. Soc.*, **1953**, 1067.

(7) *Cancer Chemother. Rep.*, **25**, 3(1962).

ACKNOWLEDGMENTS AND ADDRESSES

Received January 24, 1972, from the *Drug Development Branch, Drug Research and Development, Chemotherapy, National Cancer Institute, National Institutes of Health, Bethesda, MD 20014*

Accepted for publication April 19, 1972.

The authors thank Dr. Tadashi Hirata of the Drug Development Branch for helpful discussions.

Kenneth D. Paull gratefully acknowledges the National Institutes of Health Postdoctoral Research Fellowship CA42650.

▲ To whom inquiries should be directed.

Synthesis of Pyrazine Derivatives as Potential Hypoglycemic Agents

V. AMBROGI, K. BLOCH, S. DATURI, W. LOGEMANN▲, and M. A. PARENTI

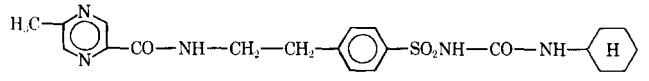
Abstract □ A series of new pyrazine derivatives was synthesized and screened for hypoglycemic activity. Some of these compounds showed weak activity at high dosage levels. Pyrazinoyl-4-ethylthiosemicarbazide was active both in mice and rats.

Keyphrases □ Pyrazine derivatives—synthesized and screened as potential hypoglycemic agents □ Hypoglycemic agents, potential—synthesis and pharmacological screening of pyrazine derivatives □ Pyrazinoyl-4-ethylthiosemicarbazide—synthesis, tested as potential hypoglycemic agent

Previous reports from this laboratory on hypoglycemic drugs showed that certain substituted pyrazine phenylsulfonylureas possess high antidiabetic activity at very low dosage levels. The most favorable compound of this series was *N*-{4-[β-(5-methylpyrazine-2-carboxamido)ethyl]benzenesulfonyl}-*N'*-cyclohexylurea or glipizide (I) (1–4).

Contrary to the other known phenyl-substituted sulfonylureas, the pyrazineamide moiety of this molecule shows a certain hypoglycemic activity (5). Based on this observation, the authors synthesized a series of pyrazine derivatives without the sulfonyl moiety in the molecule for screening for hypoglycemic activity.

Among other derivatives, compounds obtained by introduction of the pyrazine moiety into compounds not belonging to the sulfonylurea class were studied,

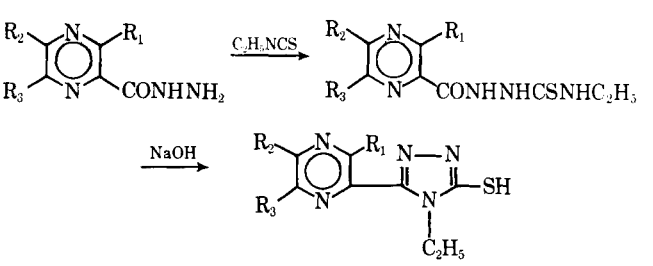


I

whose antidiabetic activity was known from the literature. It was hoped that this activity could be increased. Thus, the authors synthesized 5-pyrazinyl-4-alkyl-4*H*-1,2,4-triazole-3-thiols. The corresponding 5-phenyl derivatives, according to Mhasalkar *et al.* (6), showed a hypoglycemic activity comparable to tolbutamide. In this paper the synthesis of some corresponding pyrazine oxadiazoles and thiadiazoles is also described.

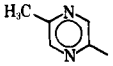
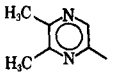
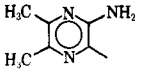
3-Mercapto-5-(2-pyrazinyl)-4-ethyl-4*H*-1,2,4-triazoles were synthesized from the corresponding thiosemicarbazides by cyclization with sodium hydroxide, according to Girard (7), to obtain the 3-mercapto-1,2,4-triazoles from acyl thiosemicarbazides. The requisite thiosemicarbazides were obtained by reacting acid hydrazides and ethyl isothiocyanate as shown in Scheme I.

2-Mercapto-5-(2-pyrazinyl)-1,3,4-thiadiazoles were obtained from potassium salts of the corresponding 3-pyrazinoyldithiocarbazic acid by cyclization with concentrated sulfuric acid, according to Young and Wood (8). Potassium salts were obtained by treating the requisite hydrazides with carbon disulfide and potassium



Scheme I

Table I—1-Pyrazinoyl-4-ethylthiosemicarbazides R—CONHNHCSNHC$_2$H$_5$

Sample Number	R	Yield, %	Melting Point	Formula	Analysis, % Calc.	Found	Hypoglycemic Activity at 50 mg./kg. Mice[a]	Rats[b]
1		75.6	206°	$C_8H_{11}N_5OS$	N 31.12 S 14.20	31.04 14.0	Accepted	18%
2		79.5	198°	$C_9H_{13}N_5OS$	N 29.3 S 12.64	29.17 12.81	Rejected	—
3		77.8	195°	$C_{10}H_{15}N_5OS$	N 27.65 S 12.62	27.36 12.81	Rejected	—
4		61.8	189°	$C_8H_{12}N_6OS$	N 35 S 13.3	34.89 13.52	Accepted	13%
5		51	232°	$C_{10}H_{16}N_6OS$	N 31.3 S 11.92	31.20 11.78	Accepted	—

[a] Accepted = hypoglycemic activity > than that of tolbutamide at the same dosage. [b] Maximal hypoglycemic activity as percent decrease in comparison to controls. Tolbutamide activity = 38%.

hydroxide in alcohol at 35–40° as described by Hoggarth (9) (Scheme II).

2-(2-Pyrazinyl)-5-mercapto-1,3,4-oxadiazoles were obtained, as described by Young and Wood (8) for similar compounds, by refluxing the appropriate hydrazides, carbon disulfide, and alcoholic potassium hydroxide without isolating the intermediate potassium salts, until most of the hydrogen sulfide evolved (Scheme III).

2-Mercapto-5-pyrazin-2′-yl-1,3,4-oxadiazole was prepared, as described by Wilder Smith and Frommel (10), by reacting pyrazinoic acid hydrazide with thiophosgene.

EXPERIMENTAL

Chemical—*5-Methylpyrazinoylhydrazide*—A mixture of 5-methylpyrazinamide (23 g., 0.168 mole) and hydrazine (160 ml. of 98%) was refluxed for 1 hr. on a steam bath. The excess of hydrazine was removed, and the residue was crystallized from ethanol, yielding 20.7 g., 81%, m.p. 128–130°.

Anal.—Calc. for $C_6H_8N_4O$: C, 47.35; H, 5.3; N, 36.85. Found: C, 47.1; H, 5.4; N, 36.31.

1-(5′-Methyl)pyrazinoyl-4-ethylthiosemicarbazide—5-Methylpyrazinoylhydrazide (4.56 g., 0.03 mole) and ethyl isothiocyanate (2.87 ml., 0.033 mole) were dissolved in dioxane (45 ml.) and refluxed for 4½ hr. The mixture was then cooled and filtered; the solid was washed with water and crystallized from ethanol, yielding 5.7 g., 79.5%, m.p. 198°.

Similarly, a number of 1-pyrazinoyl-4-ethylthiosemicarbazides were prepared (Table I).

3-Mercapto-5-(5′-methyl)pyrazin-2′-yl-4-ethyl-4H-1,2,4-triazole—To 1-(5′-methyl)pyrazinoyl-4-ethylthiosemicarbazide (4.8 g., 0.02 mole) was added 2 *N* NaOH (60 ml.), and the mixture refluxed for 2 hr. It was cooled and acidified with hydrochloric acid. The precipitate was filtered, washed with water, and crystallized, yielding 3.3 g., 74.5%, m.p. 238°.

All the new triazoles (Table II) were prepared similarly.

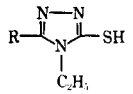
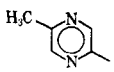
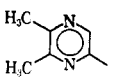
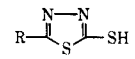


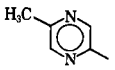
Table II—3-Mercapto-5-(2-pyrazinyl)-4-ethyl-4*H*-1,2,4-triazoles

Sample Number	R	Yield, %	Melting Point	Formula	Analysis, % Calc.	Found	Hypoglycemic Activity at 50 mg./kg. Mice[a]	Rats[b]
1		76.2	200°	$C_8H_9N_5S$	C 46.4 H 4.35 N 33.8 S 15.45	46.1 4.28 33.75 15.73	Accepted	11%
2		74.5	234°	$C_9H_{11}N_5S$	C 48.85 H 5.02 N 31.65 S 14.48	48.83 5.06 31.8 14.6	Rejected	—
3		83.2	188°	$C_{10}H_{13}N_5S$	C 51.0 H 5.54 N 29.8 S 13.62	50.74 5.59 29.42 13.55	Rejected	—
4		95	292°	$C_8H_{10}N_6S$	C 43.2 H 4.54 N 37.5 S 14.4	43.0 4.59 37.8 14.27	Rejected	—

[a] Accepted = hypoglycemic activity > than that of tolbutamide at the same dosage. [b] Maximal hypoglycemic activity as percent decrease in comparison to controls. Tolbutamide activity = 38%.

Table III—2-Mercapto-5-(2-pyrazinyl)-1,3,4-thiadiazoles



Sample Number	R	Yield, %	Melting Point	Formula	Analysis, % Calc.	Analysis, % Found	Hypoglycemic Activity at 50 mg./kg. Mice[a]	Hypoglycemic Activity at 50 mg./kg. Rats[b]
1		32	215°	$C_6H_4N_4S_2$	C 36.75 H 2.05 N 28.55 S 32.6	36.5 2.04 28 32.4	Accepted	—
2		41.5	>280°	$C_7H_6N_4S_2$	C 40 H 2.88 N 26.65 S 3.04	39.8 2.8 26.41 30.6	Rejected	—

[a] Accepted = hypoglycemic activity > than that of tolbutamide at the same dosage. [b] Maximal hypoglycemic activity as percent decrease in comparison to controls. Tolbutamide activity = 38%.

Potassium 3-(5'-Methyl)pyrazinoyldithiocarbazate—5-Methylpyrazinoylhydrazide (5.1 g., 0.0335 mole), carbon disulfide (3 ml., 0.05 mole), and potassium hydroxide 85% (3.31 g., 0.05 mole) in ethanol (200 ml.) were stirred together for 3 hr. at 35° and then for 18 hr. at room temperature. The mixture was cooled and filtered, yielding 5 g. of pale-yellow needles (56.1%), m.p. 270° dec.

Anal.—Calc. for $C_7H_7KN_4OS_2$: C, 31.50; H, 2.63; S, 24. Found: C, 31.8; H, 2.2; S, 24.1.

Potassium 3-pyrazinoyldithiosemicarbazate was prepared similarly, yielding 91%, m.p. 280° dec.

Anal.—Calc. for $C_6H_5KN_4OS_2$: C, 25.75; H, 2.0; S, 25.4. Found: C, 27.8; H, 2.1; S, 25.1.

2-Mercapto-5-(5'-methyl)pyrazin-2'-yl-1,3,4-thiadiazole—Potassium 3-(5'-methyl)pyrazinoyldithiocarbazate (4.5 g., 0.0169 mole) was dissolved in concentrated sulfuric acid (180 ml.) at room temperature. When solution was complete, the reaction mixture was poured into ice, cooling externally. The resultant precipitate was filtered and recrystallized from ethanol, yielding 1.55 g., 41.5%, m.p. > 280°.

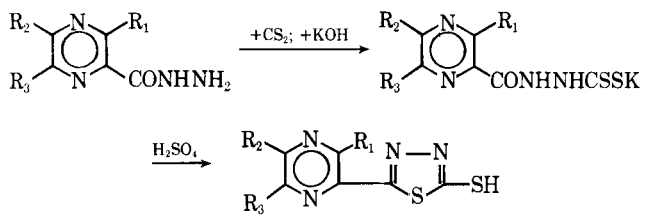
Compound 1 of Table III was prepared similarly.

2-Mercapto-5-(5'-methyl)pyrazin-2'-yl-1,3,4-oxadiazole—A mixture of 5-methylpyrazinoylhydrazide (3.03 g., 0.02 mole), carbon disulfide (1.2 ml., 0.02 mole), and potassium hydroxide 85% (1.32 g., 0.02 mole) in ethanol (85 ml.) was heated and refluxed for 3 hr. After removal of the solvent, the residue was dissolved in water and acidified with dilute hydrochloric acid. The precipitate was filtered and crystallized from ethanol, yielding 1.3 g., 33.4%, m.p. 232–234°.

Compound 3 of Table IV was prepared similarly, while Compound 1 was synthesized by reacting pyrazinoic acid hydrazide with thiophosgene (10).

Biological—Screening to determine hypoglycemic activity of the compounds under investigation was carried out as follows. Each compound was first tested in mice at the dose of 50 mg./kg.; then the compounds that were found as active as tolbutamide ("accepted" in the table) were further tested in rats at the dose of 50 mg./kg., corresponding to the dose at which tolbutamide displays a high hypoglycemic activity in rats (decrease of 38% in respect to controls).

Outbred male CF_1 SPF mice (20–24 g.), fasted for 3 hr. before the experiment, and outbred CFE SPF rats (130–160 g.), fasted for 10 hr. before the experiment, were used. The animals were from the authors' own colony.



Scheme II

The compounds to be tested were administered by gavage, suspended in 0.5% methylcellulose[1]. Doses were contained in a volume of 0.1 ml./10 g. of animal weight for mice and 0.2 ml./100 g. for rats.

Blood samples for glucose assay were collected at 60 and 180 min. after administration of the compounds to the mice and 30, 60, 90, and 180 min. after administration of the compounds to the rats. For each time, groups of six animals were used.

In both animal species (mice and rats), blood samples were obtained by decapitation. Blood glucose was assayed by the colorimetric method with *o*-toluidine described by Dubowski (11)[2].

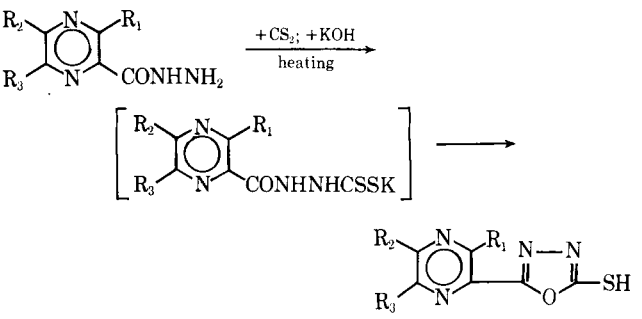
In Tables I–IV, activities in the rat are reported as maximum percent decrease of blood glucose as compared to controls independently of the time of administration; inactivity of the product is indicated by a short line.

RESULTS AND DISCUSSION

None of the compounds in Tables I–IV possess outstanding hypoglycemic activity comparable with tolbutamide or with the newer sulfonylureas. However, some of the synthesized compounds showed weak activity in mice (Table I, Compounds 1, 4, and 5). Of the bicyclic compounds, 2-mercapto-5-pyrazin-2'-yl-4-ethyl-4*H*-1,2,4-triazole (Table II, Compound 1) and 2-mercapto-5-pyrazin-2'-yl-1,3,4-thiadiazole (Table III, Compound 1) were active in mice.

Pyrazinoyl-4-ethylthiosemicarbazide (Table I, Compound 1) was also active in the rat. Among the other compounds, only 1-(3'-amino)pyrazinoyl-4-ethylthiosemicarbazide (Table I, Compound 4) and 2-mercapto-5-pyrazin-2'-yl-4-ethyl-4*H*-1,2,4-triazole (Table II, Compound 1) showed very slight activity in rats. All of the other compounds (listed in the tables) not mentioned above were inactive at the dosage tested.

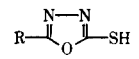
These results were surprisingly different from those reported by Mhasalkar *et al.* (6). Therefore, we tested two of the most active

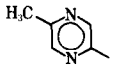
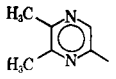


Scheme III

[1] Methocel.
[2] "Clinical Kits," Carlo Erba.

Table IV—2-(2-Pyrazinyl)-5-mercapto-1,3,4-oxadiazoles



Sample Number	R	Yield, %	Melting Point	Formula	Analysis, % Calc.	Analysis, % Found	Hypoglycemic Activity at 50 mg./kg. Mice[a]	Hypoglycemic Activity at 50 mg./kg. Rats[b]
1		30.5	213°	$C_6H_4N_4OS$	C 40 H 2.24 N 31.1 S 17.78	39.8 2.4 31 17.6	Rejected	—
2		33.4	230°	$C_7H_6N_4OS$	C 43.3 H 3.1 N 28.85 S 16.51	43.38 3.15 28.6 16.41	Rejected	—
3		43	198°	$C_8H_8N_4OS$	C 46.12 H 3.87 N 26.93 S 15.36	46 3.72 26.1 15.43	Rejected	—

[a] Accepted = hypoglycemic activity > then that of tolbutamide at the same dosage. [b] Maximal hypoglycemic activity as percent decrease in comparison to controls. Tolbutamide activity = 38%.

compounds synthesized by the above-mentioned authors but, under our conditions, found no hypoglycemic activity.

REFERENCES

(1) V. Ambrogi, K. Bloch, S. Daturi, P. Griggi, W. Logemann, M. A. Parenti, T. Rabini, and R. Tommasini, *Arzneim.-Forsch.*, **21**, 200(1971).

(2) V. Ambrogi, K. Bloch, S. Daturi, P. Griggi, W. Logemann, V. Mandelli, M. A. Parenti, T. Rabini, M. M. Usardi, and R. Tommasini, *ibid.*, **21**, 208(1971).

(3) S. Marigo, G. del Nero, P. P. Bini, and G. Sacchetti, *ibid.*, **21**, 215(1971).

(4) F. Pedrazzi, A. Pisani Casetti, S. Losi, F. Bommartini, D. Artini, and A. Emanueli, *ibid.*, **21**, 220(1971).

(5) F. Uchimaru, S. Okada, A. Kosasayma, and T. Konno, *Chem. Pharm. Bull.*, **19**, 1337(1971), and unpublished data of the present authors.

(6) M. Y. Mhasalkar, M. H. Shah, S. T. Nikam, K. G. Anantanarayanan, and G. V. Deliwala, *J. Med. Chem.*, **13**, 672(1970); *ibid.*, **14**, 260(1971).

(7) M. Girard, *Compt. Rend.*, **225**, 458(1947).

(8) R. W. Young and K. H. Wood, *J. Amer. Chem. Soc.*, **77**, 400(1955).

(9) E. Hoggarth, *J. Chem. Soc.*, **1952**, 4811.

(10) A. E. Wilder Smith and E. Frommel, *Arzneim.-Forsch.*, **12**, 485(1962).

(11) K. M. Dubowski, *Clin. Chem.*, **8**, 215(1962).

ACKNOWLEDGMENTS AND ADDRESSES

Received January 13, 1972, from the *Antidiabetic Department and the Chemistry Department, Carlo Erba Research Institute, Milan Italy.*

Accepted for publication April 12, 1972.

The authors gratefully acknowledge the careful technical assistance of Mr. L. Bertone, Mr. A. Calabrese, Mr. C. Confalonieri, and Mr. G. Longo and thank Dr. E. Pella and his staff for the microanalyses.

▲ To whom inquiries should be directed.

Synthesis and Antimicrobial Activity of Thiocarbohydrazide-1,5-dicarboxylic Acid Diesters

I. LALEZARI▲, N. REZVANI, and F. MALEKZADEH

Abstract □ Eight thiocarbohydrazide-1,5-dicarboxylic acid diesters were prepared and found to be active against some pathogenic microorganisms.

Keyphrases □ Thiocarbohydrazide-1,5-dicarboxylic acid diesters—synthesis, evaluation of antimicrobial activity □ Antimicrobial activity—eight thiocarbohydrazide-1,5-dicarboxylic acid diesters

Recently the synthesis and antiviral and antibacterial activities of *N*-thiadiazolylcarbamic acid esters (1, 2) were reported. In the present work, a series of thiocarbohydrazide-1,5-dicarboxylic acid diesters was syn-