TABLE II

No.	Toxicity LD_{50}, mg/kg per os (mice)	Gastric antisecretory activity ED_{50}, mg/kg Shay rat[b]	Anticholinergic activity pA_2[c]
1	570[d] (545–595)	1.42	$>10^{-4}$ —
2	618 (573–668)	2.30	$3.5 \cdot 10^{-5}$
3	673 (576–787)	5.10	$>10^{-4}$
4	628 (576–686)	4.05	$>10^{-4}$
5	>1.000	1.90	$1.25 \cdot 10^{-4}$
6	470 (398–554)	2.64	$>10^{-4}$
7	>1.000	11.50	$>10^{-4}$
8	>1.000	3.70	$>10^{-4}$
9	575 (532–623)	3.25	$>10^{-4}$
10	70 (58.6–83.6)	NA (20)	
11	>1.000	NA (100)	
12	>1.000	16.2	$>10^{-4}$
13	830 (686–1004)	4.28	$>10^{-4}$
14	924 (775–1101)	4.32	$>10^{-4}$
15	>1.000	7.90	$>10^{-4}$
16	>1.000	1.50	$>10^{-4}$
17	>1.000	7.82	$>10^{-4}$
18	>1.000	NA (100)	
19	>1.000	50	$>10^{-4}$
20	>1.000	82	
21	653 (568–751)	NA (50)	$>10^{-4}$
22	>1.000	4.60	$>10^{-4}$
23	>1.000	NA (100)	$>10^{-4}$

Reference products	LD_{50}[a]	ED_{50} Shay-rat	pA_2[c]
Atropine sulfate[e]	207 ip (182–227)	0.462 ip	$1.6 \cdot 10^{-8}$
PPT·HCl[f]	592 po (526–667)	5.40[b]	$>10^{-4}$
Thioacetamide[g]	≃220 ip	35.0 ip	

[a] Acute toxicity was determined orally in male albino mice CD strain. LD_{50} calcd by C. S. Weil's method [*Biometrics*, **8**, 249 (1962)]. Numbers in parentheses are fiduciary limits of LD_{50}. [b] Compounds are given by intraduodenal route. NA = inactive compound. Numbers in parentheses are the maximum dose assayed. [c] Anticholinergic activity was established on perfused guinea pig ileum by pA_2 technique [E. Bulbring, A. Grema, and O. R. Saxby, *Brit. J. Pharmacol.*, **13**, 440 (1958)]. Drugs were dissolved in 0.9% saline. [d] Van Tamelen and Baran (footnote *a*, Table I) have LD_{50} mice as 750 mg/kg sc. [e] Atropine sulfate from FLUKA AG, Buchs (Switzerland). [f] 2-Phenyl-2-(2-pyridyl)thioacetamide·HCl synthesized in our laboratories. [g] Thioacetamide from Schuchardt, München (Germany).

this position, were inactive (**18**) or showed only mild activity (**19**).

Experimental Section

2-(2-Pyridyl)butanothioamide (5a).—2-(2-Pyridyl)butanonitrile (8.2 g, 0.056 mole) was dissolved into a mixture of Et_3N (5.6 g, 0.056 mole) and pyridine (8 g). The soln was satd with dry H_2S at room temp and treated in a sealed tube to 100° and maintained for 15 hr. After cooling, the mixture was poured into H_2O (100 ml). The suspension was extracted with $CHCl_3$, the extracts were washed (H_2O), dried (Na_2SO_4), and concd under vacuum. The solid residue was recrystd from C_6H_6 to give 6 g (60%), mp 108–109°. *Anal.* ($C_9H_{12}NS$) N, S.

The hydrochloride was prepared by adding etheral 4 *N* HCl (6 ml) to **3a** (4 g) in EtOH (150 ml). The solvents were removed under vacuum and the residue was recrystd from 90 ml of EtOAc–EtOH (50:40); yield 3.9 g (80%), mp 180–181° dec. *Anal.* ($C_9H_{12}NS \cdot HCl$) N, S.

Gastric Antisecretory Activity in the Rat.—Gastric antisecretory activity was evaluated in the 4 hr polyrus-ligated rat, using the technique of Shay.[5] The compds were suspended in 20% gum syrup, and were administered intraduodenally immediately after pyloric ligation to groups of 6 male Sprague Dawley/CD rats weighing 221 ± 1.77 g.† Free acid output was calcd for each rat and expressed as μequiv/4 hr per 100 g of body weight. Data of the whole test series have been pooled for the control group (185 rats),‡ and the mean value of each group receiving drugs was compared to the mean value of this control group using the Student's "*t*" test. Per cent inhibition was calcd in comparison with the control values representing 100%, and plotted on semilogarithmic paper *vs.* mg/kg of dose. ED_{50} was read from the graph. Three to five doses were used for each compound.

After completion of this manuscript, some pharmacological results on 2-pyridyl thioacetamide were described by J. Borsy, *et al.*, at the 4th World Congress of Gastroenterology in Copenhagen. These results are in full agreement with ours.

(5) H. G. Shay, S. A. Komarow, S. S. Felds, D. Meranze, M. Gruenstein, and H. Siplet, *Gastroenterology*, **5**, 43 (1945).

† *m* ± standard error of the mean.

‡ Control values for these experiments with 185 rats were: vol: 3.09 ± 0.06 ml/4 hr per 100 g. Free acid concn: 85.8 ± 1.2 mequiv/l. Free acid output: 271.8 ± 7.9 μequiv/4 hr per 100 g. Total acid concn: 111.0 ± 1.0 mequiv/l. Total acid output: 348 ± 8.6 μequiv/4 hr per 100 g.

Antimicrobial Compounds. 1. Synthesis and Antimicrobial Activity of Some Alkylidene, Cycloalkylidene, and Arylidene Derivatives of 3-Hydrazinopyridazine

M. LIKAR, B. DRINOVEC,

Institute of Microbiology, University of Ljubljana, Ljubljana, Yugoslavia

M. JAPELJ,* A. POLLAK, A. POVŠE, AND P. JERMAN

Research and Development Institute, Krka, Pharmaceutical and Chemical Works, Novo Mesto, Yugoslavia

Received June 1, 1970

Various sulfonamidopyridazines[1,2] have been reported to have antibacterial activity with a low toxicity. In our experiments we have found that 3-thenoylamino-6-chloropyridazine[3] exhibits good antimicrobial activity. A number of hydrazinopyridazines[4] and a few alkylidenehydrazinopyridazines[5] have been reported to

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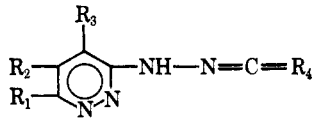
(5) (a) D. Libermann, *Bull. Soc. Chim. Fr.*, 1430 (1954); (b) British Patent 856,409; *Chem. Abstr.*, **55**, 13459 (1961); (c) British Patent 788,502; *Chem. Abstr.*, **55**, 11967 (1961); (d) E. Bellasio, F. Parravicini, and E. Testa, *Farmaco Ed. Sci.*, **24**, 919 (1969).

TABLE I

ALKYLIDENE, CYCLOALKYLIDENE, AND ARYLIDENE DERIVATIVES OF 3-HYDRAZINOPYRIDAZINE



No.	R_1	R_2	R_3	R_4	Mp, °C	Recrystn[a] solvent	% yield[b]	Formula[c]
1	Cl	H	H	$=C(Ph)CH_3$	175–177	A	49.0	$C_{12}H_{11}ClN_4$
2	Cl	H	H	$=C(CH_2COOC_2H_5)CH_3$	122–125	B	55.0	$C_{10}H_{13}ClN_4O_2$
3	Cl	CH_3	H	$=C(CH_2COOC_2H_5)CH_3$	207–208	B	49.8	$C_{11}H_{15}ClN_4O_2$
4	Cl	CH_3	H	$=C(CH_2CH(CH_3)_2)CH_3$	104–107	A	60.0	$C_{11}H_{17}ClN_4$
5	Cl	CH_3	H	$=C(CH_3)_2$	142–144	C	62.2	$C_8H_{11}ClN_4$
6	Cl	H	H	$=C(CH_3)_2$	156–159	D	50.0	$C_7H_9ClN_4$
7	Cl	H	H	$=C(CH_2CH(CH_3)_2)CH_3$	128–129	A	61.6	$C_{10}H_{15}ClN_4$
8	Cl	CH_3	H	$=C(Ph)CH_3$	205–207	A	53.6	$C_{13}H_{13}ClN_4$
9	Cl	H	H	$=C_6H_{10}$	158–160	A	68.0	$C_{10}H_{13}ClN_4$
10	Cl	CH_3	H	$=C_6H_{10}$	160–162	A	55.7	$C_{11}H_{15}ClN_4$
11	Cl	CH_3	CH_3	$=C_6H_{10}$	113–116	D	65.0	$C_{12}H_{17}ClN_4$
12	Cl	CH_3	CH_3	$=C(Ph)CH_3$	120–122	A	61.0	$C_{14}H_{15}ClN_4$
13	Cl	CH_3	CH_3	$=C(CH_3)_2$	102–104	B	56.0	$C_9H_{13}ClN_4$
14	Cl	H	CH_3	$=C_6H_{10}$	150–153	A	48.0	$C_{11}H_{15}ClN_4$
15	Cl	CH_3	H	$=C(C_2H_5)CH_3$	109–112	E	57.0	$C_9H_{13}ClN_4$
16	Cl	H	H	$=C(C_2H_5)CH_3$	99–103	E	48.0	$C_8H_{11}ClN_4$
17	Cl	CH_3	CH_3	$=C(C_2H_5)CH_3$	187–189	A	35.0	$C_{10}H_{15}ClN_4$

[a] A, 100% EtOH; B, 100% MeOH; C, 67% MeOH; D, 67% EtOH; E, *n*-hexane. [b] Yields are given for the recrystallized products. [c] Compounds were analyzed for C, H, N. Results were within 0.4% of calculated values.

have good hypotensive properties, but no mention was made of their antimicrobial activities. Recently we have prepared a number of new alkylidene, cycloalkylidene, and arylidene derivatives of substituted 3-hydrazinopyridazines (Table I) in order to examine their antiviral, antibacterial, and antifungal activities.

Results of Microbiological Assays.—New alkylidene, arylidene, and cycloalkylidene derivatives of 3-hydrazinopyridazine have been tested for their antimicrobial effects using standard techniques as described previously.[6] Representative strains of pathogenic and saprophytic bacteria, fungi, and viruses have been used as test organisms and the positive results are presented Table II.

TABLE II

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF THE TEST COMPOUNDS[a]

Microorganism	1	2	5	6	8	11	12	13	14	15	16
Diplococcus pneumoniae		+									
Hemophylus influenzae		+									
Streptococcus viridans									+	+	
Salmonella paratyphi B							+				
Mycobacterium photochromogenes				+							
M. scotochromoges					+						
M. nonphotochromogenenes				+	+						
M. rapid growers				+							
Nocardia asteroides		+	+	+		+	+				
Cryptococcus neoformans		+	+	+	+		+				
Trychophyton rubrum	+	+		+		+		+	+	+	
T. schoenleini					+	+		+	+		
T. interdigitale						+			+		+
T. mentagrophites	+			+		+		+	+		

[a] + means a total inhibition of microbial growth.

None of the compounds were active against *Shigella flexneri, Staphylococcus aureus, Streptococcus pyogenes, Neisseria catarrhalis, Listeria monocytogenes, Pasteurella pseudotuberculosis, Corynebacterium diphtheriae, Bacillus anthracis, Salmonella typhi, Klebsiella, Pseudomonas aeruginosa, Streptococcus hemolyticus, Candida albicans, Microsporum canis,* and *Aspergillus fumigatus.*

Compounds **6**, **10**, **11**, **12**, **13**, **14**, **15**, and **16** markedly reduced the titer of influenza virus A_2 grown in allantoic cavities of embryonated eggs. Compounds **1**, **2**, and **11** were also active against influenza virus A_2. Compounds **3** at 0.0026 *M* and **5** at 0.0054 *M* protected 30% of mice infected with 100 LD_{50} dose of Semliki forest virus (arbovirus group A). None of the compounds showed an inhibitory effect on the growth of herpesvirus and poliovirus type 1 grown in human embryonic kidney cell cultures.

Experimental Section[7]

Chemistry.—Syntheses of the compounds listed in Table I were carried out by a general procedure, using the acid-catalyzed reaction[8] of the substituted 3-hydrazinopyridazines with aliphatic ketones, aliphatic-aromatic ketones, cyclopentanone, and cyclohexanone. Intermediary 3-hydrazino-6-chloropyridazine,[9] 3-hydrazino-4-methyl-6-chloropyridazine, 3-hydrazino-5-methyl-6-chloropyridazine,[10] and 3-hydrazino-4,5-dimethyl-6-chloropyridazine[11] were obtained according to the procedures described earlier.

Preparation of Compounds 1–15.—The appropriate ketone (0.013 mole), the corresponding 3-hydrazinopyridazine (0.01 mole), H_2SO_4 (0.0005 mole), and aliphatic alcohol (MeOH or EtOH) were heated under reflux for 2 hr, cooled, and filtered. The crude products were purified by recrystallization (see Table I).

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(7) All melting points were determined on a Bötius Mikroheiztisch apparatus and are uncorrected. All compounds exhibited the expected ir spectra.

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