disubstituted hydrazones, only one derivative (**24**) was active.

Compound **9**, was most active *in vitro* but inactive when tested topically in the guinea pig according to the method of Arnold, *et al.*[3]

Experimental Section[4]

Preparation of *para*-Substituted Phenylglyoxal N,N-Disubstituted Hydrazones. Method A.—A mixture of 0.01 mole of α-ketoaldehyde, 0.011 mole of N,N-disubstituted hydrazine hydrochloride, and 0.011 mole of NaOAc in 10 ml of AcOH was stirred for 2 hr at 20–25°. Then a 20% aqueous Na_2CO_3 was added to alkalinity. Some products precipitated as solids, other as thick oils which solidified on standing. The solids were collected and crystallized. Compounds **7, 9,** and **34** did not solidify and were extracted into Et_2O. After drying, the solvent was evaporated and the residue was crystallized.

Method B.—The N,N-disubstituted hydrazine hydrochloride (0.01 mole) and 0.01 mole of NaOAc were added at 20° to a solution of 0.01 mole of α-ketoaldehyde in 20 ml of EtOH, and the mixture was stirred for 24 hr at 20°. When the products crystallized, they were collected, washed (H_2O), and recrystallized. If no crystallization took place, H_2O was added to cloudiness, and the solution was filtered with charcoal and cooled. The separate crystals were collected and recrystallized.

Method C.—The N,N-disubstituted hydrazine hydrochloride (0.01 mole) and 0.01 mole of NaOAc were added at 20° to a solution of 0.01 mole of α-ketoaldehyde in 20 ml of EtOH, and the mixture was stirred for 24 hr at 20°. The mixture was filtered and the solvent was evaporated to dryness *in vacuo* at 20°. Compounds **24, 27,** and **32** were crystallized, while **40** and **41** were dissolved in Et_2O and the extracts were washed with aqueous NaOAc. After drying (Na_2SO_4), the solvents were evaporated and the residues were crystallized.

Method D.—A solution of 0.01 mole of α-ketoaldehyde and 0.01 mole of N,N-disubstituted hydrazine in 20 ml of EtOH was stirred at 25° for 4 hr. After cooling the crystals were collected and recrystallized.

Pharmacological Methods.—For all tests NMRI albino mice (18–20 g) and Wistar albino rats (200–250 g) were used. For *T. mentagrophytes* infection, Pirbright guinea pigs (250–300 g) were used.

Acute Toxicity.—LD_{50} values were determined in mice intraperitoneally, and the mortality over 48 hr was recorded. The animals were also observed for behavior and objective symptoms according to the Irwin scheme.[5]

Other Tests.—All compounds were screened also for their antispasmodic activity *in vitro* following the methods described by Setnikar and Tirone[6] and for their coronary vasodilator activity on the isolated rabbit heart following the method of Setnikar, *et al.*[7]

Antimicrobial and antifungal activity, antiviral activity, anticonvulsant activity, and antiinflammatory activity were determined according to the methods previously described.[8]

Acknowledgments.—We wish to thank Miss B. Olgiati for microanalytical data and Miss M. J. Magistretti for pharmacological screening of these compounds.

(3) H. Arnold, L. Degen, J. Potel, and R. Rebling, *Arzneimittel-Forsch.*, **14**, 68 (1964).

(4) Melting points are uncorrected and were determined on a Kofler micro hot stage. Where analyses are indicated only by symbols of elements analytical results obtained for those elements were within ±0.4% of the theoretical values.

(5) This scheme was discussed informally by S. Irwin at a Gordon Research Conference, New London, N. H., 1959.

(6) I. Setnikar and P. Tirone, *Arzneimittel-Forsch.*, **16**, 1146 (1966).

(7) I. Setnikar, W. Murmann, and M. T. Ravasi, *Arch. Intern. Pharmacodyn.*, **131**, 187 (1961).

(8) E. Massarani, D. Nardi, L. Degen, and M. J. Magistretti, *J. Med. Chem.*, **9**, 617 (1966).

Synthesis and Antimicrobial Activity of Some Thenoyl Amides

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Antitrichomonal activity of 2-(2-thenoylamino)-5-nitrothiazole[2] has often been ascribed to the nitrothiazole moiety[3a] in accord with the same activity of related heterocyclic compounds,[3b] especially 5-nitroimidazoles.[4] A variety of derivatives of thiophene-2-carboxylic acid have also been found to have some antimicrobial effects.[5] We have now synthesized a number of amides of thiophene-2-carboxylic acid with various aromatic and heteroaromatic amines (Table I) in order to examine their biological properties.

Results of Microbiological Assays.—Thenoyl amides have been tested for their antimicrobial effectiveness according to standard techniques as described elsewhere.[6] A number of pathogenic and saprophytic bacteria, fungi, and viruses were used as test organism and the results are presented in Table II.

None of the compounds were active against *Shigella flexneri, Haemophilus influenzae, Staphylococcus aureus, Streptococcus viridans, Streptococcus pyogenes, Listeria monocytogenes, Pasteurella pseudotuberculosis, Corynebacterium diphtheriae, Bacillus anthracis, Diplococcus pneumoniae, Salmonella typhi, Microsporum canis,* and *Trichophyton interdigitale.*

Some of the compounds (**1, 4, 13**) markedly reduced the titers of influenza A_2 virus grown in allantoic fluids of embryonated eggs. The most active substances were **1** and **13** which reduced the hemagglutinins of influenza A_2 virus in allantoic fluids 20-fold. Compound **12** at 0.00406 *M* protected all the mice infected with $100LD_{50}$ dose of pseudorabies virus. A number of compounds protected 30–60% of mice infected with $100LD_{50}$ dose of Semliki forest virus (arbovirus group A), *i.e.*, **3, 4, 8,** and **11.**

(1) Correspondence should be addressed to this author.

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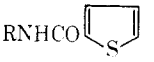
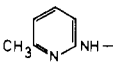
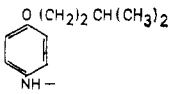
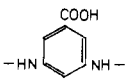
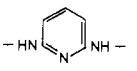
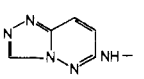
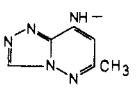
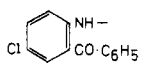
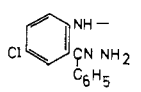
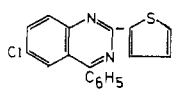
TABLE I
PHYSICAL PROPERTIES OF



No.	RNH	Recrystn[b] solvent	% yield[c]	Mp, °C	Formula	Analyses
1		A	34	275–280 dec	$C_9H_{12}NO_3S$	C, H, S; N[d]
2		A	47	260–265 dec	$C_9H_{12}NO_3S$	N, S
3		B	48	158–160	$C_9H_7N_3OS$	C, H, N, S
4		B	37	112–114	$C_{11}H_{10}N_2OS$	C, H, N, S
5		C	58	270–275 dec	$C_9H_6ClN_3OS$	C, H, N, S
6		C	43	143–144	$C_{14}H_{18}NO_2S$	H, N, S; C[e]
7		C	41	306–310 dec	$C_{17}H_{12}N_2O_4S$	C, H, N, S
8		D	35	188–189	$C_{15}H_{11}N_3O_2S$	C, H, N, S
9		E	40	224–225	$C_{10}H_7N_5OS$	C, H, N, S
10		C	46	227–229	$C_{11}H_9N_5OS$	C, H, N, S
11		E	82, 5	159–161	$C_{18}H_{12}ClNO_2S$	H, N, S; C[f]
12		F	58	198–199	$C_{18}H_{14}ClN_3OS$	C, H, N, S
13[a]		F	61, 5	238–240	$C_{18}H_{11}ClN_2S$	C, H, N, S

[a] Complete formula is given. [b] A = DMF–H_2O (1:2), B = Me_2CO, C = EtOH, D = C_6H_6, E = dioxane, F = $CHCl_3$. [c] Yields are given for the recrystallized products. [d] N: calcd, 5.66; found, 6.24. [e] C: calcd, 66.40; found, 66.89. [f] C: calcd, 63.24; found, 62.69.

Experimental Section[7]

Chemistry.—Syntheses of the compounds listed in Table I were carried out by the general procedures which are illustrated below. The amines for the preparation of **1–4, 7,** and **8** were commercial reagent grade chemicals. Intermediary amines for the preparation **5, 6, 9,** and **10** were obtained according to the procedures earlier described.[8–11] 2-Amino-5-chlorobenzophenone,

(7) All melting points were determined on a Bötius Mikroheiztisch apparatus and are uncorrected. Elemental analyses were performed by microanalytical laboratory, Department of Organic Chemistry, Faculty of Chemistry, Ljubljana. Where analyses are indicated only by symbols, the elements were within ±0.4% of the theoretical values. All compounds exhibited ir spectra as expected.

(8) J. Durey, K. Meier, and K. Eichenberger, *Helv. Chim. Acta*, **37**, 121 (1954).

(9) G. O. Gutenkunst and H. L. Gray, *J. Amer. Chem. Soc.*, **44**, 1741 (1922).

(10) N. Takahayashi, *J. Pharm. Soc. Japan*, **76**, 765 (1956).

(11) E. A. Steck and R. P. Brundage, *J. Amer. Chem. Soc.*, **81**, 6289 (1959).

TABLE II
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF THE COMPOUNDS TESTED[a]

Microorganism	1	3	4	5	6	8	9	11	12	13
Neisseria catharrhalis						+				
Salmonella paratyphi B									+	
Klebsiella sp.	+									
Pseudomonas aeruginosa										+
Candida albicans					+	+				
Cryptococcus neoformans										+
Nocardia asteroides								+		
Aspergillus fumigatus	+									
Trichophyton rubrum	+	+	+	+			+			
Trichophyton schoenleini	+			+						
Trichophyton mentagrophytes										+
Histoplasma capsulatum	+									

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

required for the preparation of **11**, was obtained employing the procedure described for analogous compound.[12]

Preparation of Compounds 1–11.—Thenoyl chloride (3.66 g, 0.024 mole) was added dropwise during 0.5 hr to a well-stirred and ice-cooled solution of 0.02 mole of the particular amine in 20 ml of pyridine. To complete the reaction in the case of **1**, **2**, **5**, **6**, and **10**, the reaction mixture was stirred for 2 hr at room temperature. To prepare **3**, **4**, **7–9**, and 11 the mixture was refluxed for 6 hr. After cooling overnight, **9** and **10** separated. Crude products were collected on a filter, washed with dilute HCl (*ca.* 3%), and recrystallized. Other products crystallized on pouring the reaction mixture on ice and were filtered off, washed with dilute HCl, and recrystallized.

2-(2-Thenoylamino)-5-chlorobenzophenone Hydrazone (**12**).—Compound **10** (6.7 g, 0.025 mole) and 0.81 g (0.025 mole) of hydrazine in 15 ml of EtOH were placed in a sealed tube and heated for 5 hr at 150°. The reaction mixture was cooled and poured on ice. The crude product which separated was filtered off, dried (6.3 g, 70%), and recrystallized as indicated in Table I.

2-(2-Thenyl)-4-phenyl-6-chloroquinazoline (**13**).—Compound **10** (2 g, 0.0076 mole) in 13 ml of 6.5% NH_3 solution in EtOH was sealed in a glass tube and heated for 5 hr at 140°. On cooling the crude product which separated was collected on a filter, washed (EtOH), and recrystallized.

(12) J. F. J. Dippy and V. Mass, *J. Chem. Soc.*, 2205 (1952).

Synthesis of 5,8-Quinazolinedione

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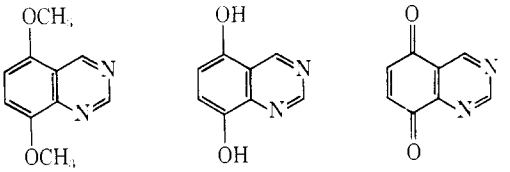
Quinones containing heterocyclic rings appear to be interesting from various points of view. Of a large number of 4,7-indolequinone derivatives related to mytomicin antibiotics,[1] several had interesting antibacterial activities. Various 5,8-quinolinediones were studied in relation to their antibacterial and cytostatic activities.[2] Recently also 5,8-isoquinolinedione[3] and some 5,8-quinoxalinedione derivatives have been prepared and studied. We were therefore led to investigate the closely related 5,8-quinazolinedione.

We recently prepared 5,8-dimethoxyquinazoline (**1**) and some derivatives,[5] for instance, 5-methoxy-8-hydroxyquinazoline. This compound was found to have antibacterial properties analogous to those of 8-hydroxyquinoline.



1 **2** **3**

The complete demethylation of **1** was achieved by heating the substance with $AlCl_3$ at 180°. 5,8-Quinazolinedione (**3**) was prepared by $K_2Cr_2O_7$ oxidation. It was stable under normal conditions of storage and displayed typical quinonic behavior to KI–H_2SO_4, diphenylbenzidine–H_2SO_4,[6a] and ethyl cyanoacetate–alcohol NH_3[6b] test reagents. Mixed with 5,8-dihydroxyquinazoline it easily formed the quinhydrone derivative.

Biological Results[7]—5,8-Dihydroxyquinazoline (**2**) was tested on three strains of *Staphylococcus aureus* (I 67, Pd 2, Ba 61) and on *Streptococcus pyogenes* (N.T.C.C.S.T.A.), both in the absence and in the presence of equal molar amounts of Fe^{3+}. 5,8-Quinazolinedione (**3**) was tested on the same strains of *S. aureus* and of *S. pyogenes* and on *Escherichia coli* (Pd 3), *Salmonella typhi* (murium), and *Candida albicans*. Neither **2** nor **3** exhibited antibacterial activity below a concentration of 100 μg/ml.

5,8-Dihydroxyquinazoline (**2**) has a structure analogous to that of 8-hydroxyquinoline; the antibacterial activity of this substance is related to complex formation with various transition metal ions.[8] The ineffective antimicrobial activity of **2**, which does form stable metal ion complexes, may be attributable to its low partition coefficient in oleyl alcohol–H_2O (0.33). This factor in many cases can be correlated with the antibacterial activity of 8-hydroxyquinoline derivatives.[9a,b]

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(7) The tests for antimicrobial activity were carried out by Dr. P. Benetti in the Institute of Health of the University of Ferrara, and those for antitumor activity by Dr. F. Baccichetti in this institute.

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