2.3 g. (0.025 mole) of thiosemicarbazide in 35 ml. of water; the mixture was boiled for 5 min. and filtered hot, to give 7.7 g. of very pale yellow crystals which were recrystallized from boiling, glacial acetic acid (25 vols.); m.p. above 245° (slight browning at 190°, dark brown at 220°). Its infrared spectrum differed from those of the two parent compounds. The spectrum of compound II showed bands that were absent from the spectrum of thiosemicarbazide: at 3356, 1664, 1245, 1179, 973, 953, 877, 834, 824, and 732 cm.$^{-1}$. Compound II showed bands that were absent from the spectra of both compound V and thiosemicarbazide: at 1600, 1555, 1497, 1412, 1346, 1087, 926, 860, and 759 cm.$^{-1}$. The spectrum of thiosemicarbazide showed bands at 1311, 1156, and 995 cm.$^{-1}$ that were absent from the spectrum of compound II.

Anal. Calcd. for $C_{10}H_{10}Cl_2N_4OS$: C, 39.35; H, 3.30; Cl, 23.24; N, 18.36; S, 10.50. Found: C, 39.44; H, 3.20; Cl, 23.21; N, 18.41; S, 10.54.

Antiviral Activity.[7]—In tests for antivaccinial activity, Swiss mice (weighing approximately 15 g. each) were inoculated intranasally with one LD_{50} of a mouse lung-adapted strain of International Health Division neutrotropic vaccinia virus.[8] Groups of treated and control mice were compared as to life span, survival rate, and infectivity score (a weighted score on lung consolidation). For each drug, half of the daily dose (one third of the single-dose intraperitoneal LD_{50}) was given intraperitoneally to each mouse (a) in the morning and (b) in the late afternoon, for 5 days, beginning 30 to 60 min. after virus inoculation; the daily dose of each drug was 167 mg./kg. The results are given in Table I.

Both compounds were inactive in mice infected intranasally with (a) feline pneumonitis virus or (b) influenza A (PR 8) virus; and in mice infected by intracerebral inoculation of (a) Type II (Lansing) poliomyelitis virus or (b) Western equine encephalomyelitis virus.

TABLE I

EFFECTS OF THIOSEMICARBAZONE TREATMENT ON VACCINIAL INFECTION IN MICE

Compound	Dead/Total	Survivors, %	Infectivity score[a]	Survival index[b]
I	2/20	90	1.6	>1.96
II	1/19	95	0.5	>2.06
—(Control)	16/20	20	4.3	

[a] Infectivity score. Averages of all mice scored as follows: death with complete lung consolidation, 5; alive at 10–14 days with 4/4, 3/4, 1/2, and 1/4 lung consolidation, 4, 3, 2, and 1, respectively. [b] Survival index = (average survival time, in days, of treated mice)/(average survival time, in days, of control mice). Treated mice were sacrificed on the 14th post-infection, 9th post-treatment day for infectivity scoring, and, for purposes of calculating the survival index, were considered to have died on the sacrifice day.

(7) These tests were kindly performed by Dr. Frank M. Schabel, Jr., Head of the Chemotherapy Division, Southern Research Institute, Birmingham, Alabama.

(8) The procedures used have been described: F. M. Schabel, Jr., W. R. Laster, Jr., R. W. Brockman, and H. E. Skipper, *Proc. Soc. Exptl. Biol. Med.*, **83**, 1 (1953); F. M. Schabel, Jr., and H. E. Skipper, *Cancer Research*, Suppl. No. **3**, 52 (1955).

Communications to the Editor

[4-(Aminoalkylamino)-1-naphthylazo]heterocyclic Compounds, a Novel Class of Schistosomicides

EDWARD F. ELSLAGER, DAVID B. CAPPS, LESLIE M. WERBEL, DONALD F. WORTH, JACK E. MEISENHELDER, HAIG NAJARIAN, AND PAUL E. THOMPSON

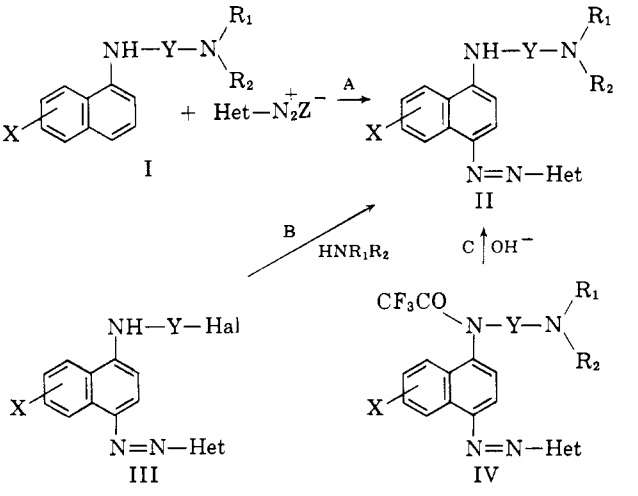
Research Laboratories, Parke, Davis and Company, Ann Arbor, Michigan

Received December 12, 1962

We wish to report the synthesis and biological evaluation of a novel class of schistosomicides, namely, [4-(aminoalkylamino)-1-naphthylazo]heterocyclic compounds of structure II, where R_1 and R_2 represent alkyl groups, Y an alkylene radical, X a hydrogen or halogen atom or a lower alkoxy or alkyl group, and Het a heterocyclic radical.

A majority of the compounds was synthesized by coupling a diazotized heterocyclic amine with the appropriate 1-(aminoalkyl)naphthylamine (I) (route A). These naphthylamine intermediates were prepared by: (1) alkylation of a 1-naphthylamine[1] or an alkaline metal salt thereof with a dialkylaminoalkyl halide; (2) hydrogenation of a Schiff base resulting from the condensation of 1-naphthylamine with a dialkylamino aldehyde or ketone; (3) condensation of 1-naphthol with an alkylaminoalkylamine in the presence of sodium bisulfite or sodium hydrosulfite; (4) amination of an ω-haloalkyl-1-naphthylamine with an aliphatic amine.

Alternatively, compounds of structure II were prepared by allowing a N-(ω-haloalkyl)-4-(heterocyclicazo)-1-naphthylamine (III) to react with the appropriate amine (route B) or by alkaline hydrolysis of the corresponding N-(aminoalkyl)-2,2,2-trifluoro-N-[4-(heterocyclicazo)-1-naphthyl]acetamides IV (route C). Analyses for all intermediates and products were satisfactory.



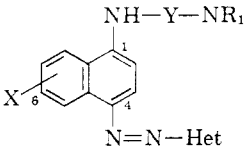
The [4-(dialkylaminoalkylamino)-1-naphthylazo]-heterocycles (II) were evaluated in albino mice infected with a Puerto Rican strain of *Schistosoma mansoni*.[2] Antischistosome activity is widespread within this series. Compounds II a through o (Table I), which are representative of the more promising members of the series, effected a 97–100% reduction of live worms at

(1) M. A. Stahmann and A. C. Cope, *J. Am. Chem. Soc.*, **68**, 2494 (1946).

(2) For a description of test methods, see P. E. Thompson, J. E. Meisenhelder, and H. Najarian, *Am. J. Trop. Med. Hyg.*, **11**, 31 (1962).

TABLE I

4-[(DIALKYLAMINOALKYLAMINO)-1-NAPHTHYLAZO]HETEROCYCLIC COMPOUNDS



II	X	—Y—NR1R2	Het	Route	M.p., °C. (uncorr.)
a	H	$CH_2CHCH_3CH_2N(CH_3)_2$	2-Pyridyl-1-oxide	A	185–186 dec.
b	H	$CH_2CHOHCH_2N(C_2H_5)_2 \cdot 3HCl$	3-Pyridyl	A	154–157
c	H	$(CH_2)_2N(C_2H_5)_2$	2-Butoxy-5-pyridyl	A	92–94
d	H	$(CH_2)_2N(C_2H_5)_2$	3-Quinolyl	A	124–125
e	H	$(CH_2)_2N(C_2H_5)_2 \cdot 2HCl$	8-Quinolyl	A	156–158
f	H	$(CH_2)_2N(C_2H_5)_2$	8-Hydroxy-5-quinolyl	C	158–162
g	H	$(CH_2)_2N(C_2H_5)_2$	2,4-Dihydroxy-5-pyrimidyl	A	210–211
h	H	$(CH_2)_2N(C_2H_5)_2$	2,4,6-Trihydroxy-5-pyrimidyl	C	209–213 dec.
i	H	$(CH_2)_2N(CH_2)_5$	2-Thiazolyl	A	135–137
j	H	$CHCH_3CH_2N(CH_3)_2$	5-Benzotriazolyl	A	197–199
k	H	$(CH_2)_2N(C_2H_5)_2$	2,1,3-Benzothiadiazol-5-yl	A	126–128
l	H	$(CH_2)_2N(CH_2)_4$	1,2-Benzisothiazol-5-yl	A	161–165
m	H	$(CH_2)_2N(C_2H_5)_2$	3,4-Dihydro-3-oxo-2H-1,4-benzothiazin-6-yl	A	213–214
n	6-OCH_3	$(CH_2)_2N(C_2H_5)_2 \cdot 2HCl$	4-Antipyrinyl	A	200–202
o	H	$(CH_2)_2N(CH_2)_5$	3-Dibenzofuranyl	B	164–165

doses ranging from 85 to 750 mg./kg./day when administered orally in the diet for 14 days or by gavage for 10 days. We found several of them to be distinctly more promising in mice than lucanthone hydrochloride,[2,3] the tris(*p*-aminophenyl)carbonium salts,[2,4] 4,4′-(heptamethylenedioxy)dianiline dihydrochloride,[5,6] N-[5-(*p*-aminophenoxy)pentyl]phthalimide,[7] or 3-[4-(3-chloro-*p*-tolyl)-1-piperazinylcarbonyl]acrylic acid.[8]

5-[4-(2-Diethylaminoethylamino)-1-naphthylazo]-uracil (IIg) was selected for expanded studies. When administered in the diet to mice infected with the Puerto Rican strain of *S. mansoni* at doses ranging from 167 to 489 mg./kg./day for 14 days, it caused an 82–100% reduction in live worms and was tolerated well. The drug also killed very high proportions of the worms and was tolerated well in gavage doses ranging from 50 to 328 mg./kg. given twice daily for 10 days. Treatment of mice infected with the Liberian strain of *S. mansoni* by the drug-diet method produced similar results. Although the drug was tolerated and active when given intraperitoneally in doses of 100 to 400 mg./kg. daily for 10 days, it was less active than when given orally; much unabsorbed drug remained in the peritoneal cavity. The drug exhibited partial but not highly encouraging prophylactic activity. In mice, IIg killed schistosomes (Puerto Rican strain) more rapidly than lucanthone hydrochloride[2,3] or the tris(*p*-aminophenyl)carbonium salts.[2,4] On a weight basis, the compound was considerably more active against the Puerto Rican strain in golden hamsters than in mice.

In rhesus monkeys infected with the Puerto Rican strain, doses of 12.5 mg./kg./day for 10 days suppressed egg production but were not curative. Doses of 25 through 200 mg./kg./day for 10 or 15 days usually effected a cure and were tolerated well except for transient weight loss and mucoid diarrhea toward the end of the treatment period at daily doses of 100 mg./kg. and above. In all species studied, the azouracil compound exhibits a steep dose-response curve. Consequently, large differences in efficacy are likely to be associated with small differences in dose at low drug levels.

Preclinical toxicity studies in experimental animals[9] and observations on the tolerance of the drug by normal human volunteers[10] indicated that IIg might cause gastrointestinal side effects, but otherwise should be safe for trial in the treatment of human schistosomiasis. The efficacy and tolerance of the drug were subsequently studied in 132 people infected with *S. mansoni* and *S. haematobium* in various areas of Brazil, Puerto Rico, Liberia, Uganda and Nyasaland.[10] Each investigator reported the drug to be moderately effective in suppressing egg production but rarely curative. Effective doses produced a high incidence of gastrointestinal side effects: nausea, vomiting, diarrhea, and abdominal cramping.

More than 500 naphthylazo compounds have been synthesized and tested in these laboratories against *S. mansoni* in mice. Results to date indicate that a 4-amino-1-naphthylazo moiety is essential for significant antischistosome activity. Among compounds of structure II, activity is abolished or drastically reduced when R_1 and/or R_2 represent hydrogen, when the secondary amine at position 1 is alkylated, or when a carbonyl group is substituted for the methylene group adjacent to the terminal aliphatic amine. Representative 4-azo-1-(dialkylaminoalkyl)aniline derivatives[11] were synthesized and tested, but none of these exhibited antischistosome activity.

Laboratory investigations with the 4-[(dialkylaminoalkylamino)-1-naphthylazo]heterocyclic compounds are continuing and we are actively engaged in extending this work to other series. More detailed publications

(3) W. Kikuth and R. Gönnert, *Ann. Trop. Med. Parasitol.*, **42**, 256 (1948).

(4) E. F. Elslager, F. W. Short, D. F. Worth, J. E. Meisenhelder, H. Najarian, and P. E. Thompson, *Nature*, **190**, 628 (1961).

(5) C. G. Raison and O. D. Standen, *Brit. J. Pharmacol.*, **10**, 191 (1955).

(6) R. F. Collins, M. Davis, N. D. Edge and J. Hill, *ibid.*, **13**, 238 (1958).

(7) R. F. Collins, M. Davis, N. D. Edge, J. Hill, H. W. Reading, and E. R. Turnbull, *ibid.*, **14**, 467 (1959).

(8) G. Lämmler, *Z. Tropenmed. u. Parasitol.*, **9**, 294 (1958).

(9) K. Weston, unpublished data in the files of Parke, Davis and Company, Ann Arbor, Michigan.

(10) K. O. Courtney, unpublished data in the files of Parke, Davis and Company, Ann Arbor, Michigan.

(11) F. Mietzsch and J. Klarer, U. S. Patent 2,022,921, December 3, 1935.

concerning the chemical and biological aspects of these studies will be forthcoming.

Acknowledgments.—The authors are indebted to A. C. Bratton, Jr., and L. M. Long for advice and encouragement. Appreciation is also extended for the respective technical contributions of A. Bayles, C. E. Childs, Z. Gavrilis, D. H. Kurtz, P. McClay, B. Olszewski, F. W. Short, and J. M. Vandenbelt.

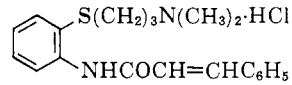
2′-(3-Dimethylaminopropylthio)cinnamanilide and Related Compounds: A New Class of Potent and Relatively Specific Serotonin Inhibitors

JOHN KRAPCHO, BERNARD RUBIN, ANNE M. DRUNGIS, ERVIN R. SPITZMILLER, CHESTER F. TURK, JUNE WILLIAMS, BRADFORD N. CRAVER, AND JOSEF FRIED

The Squibb Institute for Medical Research, New Brunswick, New Jersey

Received January 8, 1963

We wish to report the discovery of a new class of serotonin inhibitors show ng activity of a high order. Characteristic of these compounds is I



I

which *in vitro* at concentrations ranging from 0.0005 to 0.03 mcg./ml. inhibited the spasmogenic effect of 0.2 mcg./ml. of serotonin on the excised rat uterus. This corresponds to 157 times the antiserotonin activity of BAS[1] with a potency range of 60 to 407 times BAS at $p = 0.05$. The specificity of action of I was evident from the findings that concentrations as high as 2 mcg./ml. failed to inhibit the contractile response to acetylcholine (0.4 mcg./ml.) of the rat uterus and that concentrations of 0.5 to 8 mcg./ml. were necessary to inhibit histamine (2 mcg./ml.)-induced contractions of excised guinea pig ileum.

In vivo antiserotonin activity of I has been demonstrated in dogs and mice. Intravenous doses of about 150 mcg./kg. of I in pentobarbitalized-flaxedilized dogs produced 50% inhibition of the bronchoconstrictor effect of *i.v.* serotonin (20 mcg./kg.). About 4 mg./kg. *i.v.* of BAS was required for a similar degree of inhibition. Pretreatment of mice with oral doses of 32–128 mg./kg. of I partially to completely inhibited spontaneous head-twitch caused by 500 mg./kg. i.p. of *dl*-5-hydroxytryptophan. In contrast, BAS failed to inhibit this type of head-twitch after oral doses as high as 310 mg./kg.

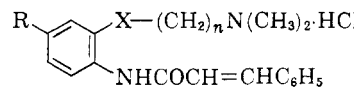
Compounds related to I have been synthesized (Table I) and studied on the excised rat uterus in order to define the structure–activity relationships in this series. The introduction of a chlorine atom into the *ortho* (II) or *para* (III) position of the cinnamoyl group gave compounds having essentially the same activity as I; replacement of the cinnamoyl moiety, however, by dihydrocinnamoyl (IV), benzoyl (V), and propionyl (VI) yielded compounds having activity comparable to BAS. Substitution of a diethylamino (VII) for the dimethylamino group in I scarcely altered activity. Oxidation of I to the sulfone (VIII) led to a compound of considerably lower activity. The *para* isomer of I, 4′-(3-dimethylaminopropylthio) cinnamanilide (IX), which no longer possesses the *ortho* relationship of the non-basic nitrogen and the basic substituent, exhibited little or no antiserotonin activity at 2 mcg./ml.

TABLE I

Compound	M.p., °C. (cor.) hydrochloride salt	Compound	M.p., °C. (cor.) oxalate salt
I	146–148	IV	124–126
II	144–145	V	152–153
III	148–150	VI	131–133
VII	179–181	VIII	189–191
IX	238–240		

Other structural variants of I (Table II) are represented by the general formula II.

TABLE II



II

Cpd.	R	X	*n*	M.p., °C (cor.)
X	H	O	4	165–167
XI	H	O	3	179–181
XII	CH_3O	O	3	223–225
XIII	H	O	2	212–214
XIV	H	CH_2	1	189–191

The oxygen analog of I (XI) had antiserotonin activity comparable to that exhibited by I. It is of interest that the introduction of a methoxyl group into this compound (XII) led to considerable deactivation. Decreasing the length of the side chain by one carbon atom (XIII) resulted in minor reduction of activity, whereas an increase by one carbon atom (X) yielded a considerably less active compound. Moderate activity was shown by a compound (XIV) which contained only methylene groups in the side chain. The *meta* isomer of IV, 3′-(3-dimethylaminopropoxy)cinnamanilide maleate (XV), m.p. 136–138° corr., showed little or no antiserotonin activity at 2 mcg./ml.

The following examples are illustrative of the synthetic procedures used. Reaction of 2-aminobenzenethiol with 3-dimethylaminopropyl chloride in the presence of sodium methoxide or sodamide gave 2-(3-dimethylaminopropylthio)aniline. This intermediate exhibited no significant antiserotonin activity. Acylation with cinnamoyl chloride gave I. The alkylation of *o*-nitrophenol with 3-dimethylaminopropyl chloride yielded 2-(3-dimethylaminopropoxy)nitrobenzene. Catalytic reduction of this compound followed by reaction of the resulting amine with cinnamoyl chloride gave XI. In a similar manner, 2-(2-dimethylaminoethyl)nitrobenzene (from *o*-nitrophenethyl bromide and dimethylamine) was converted to XIV.

Further laboratory investigations of I and related compounds are in progress. Papers describing chemical and biological aspects in more detail will be forthcoming.

(1) BAS is 1-benzyl-2-methyl-3-(2-aminoethyl)-5-methoxyindole hydrochloride. The antiserotonin activity of this compound was reported by E. N. Shaw and D. W. Woolley, *J. Pharm. Expll. Therap.*, **116**, 164 (1956).