[CONTRIBUTION FROM THE SCIENTIFIC DEPARTMENT, ISRAELI MINISTRY OF DEFENCE]

NEW SUBSTANCES OF POSSIBLE CHEMOTHERAPEUTICAL VALUE. I

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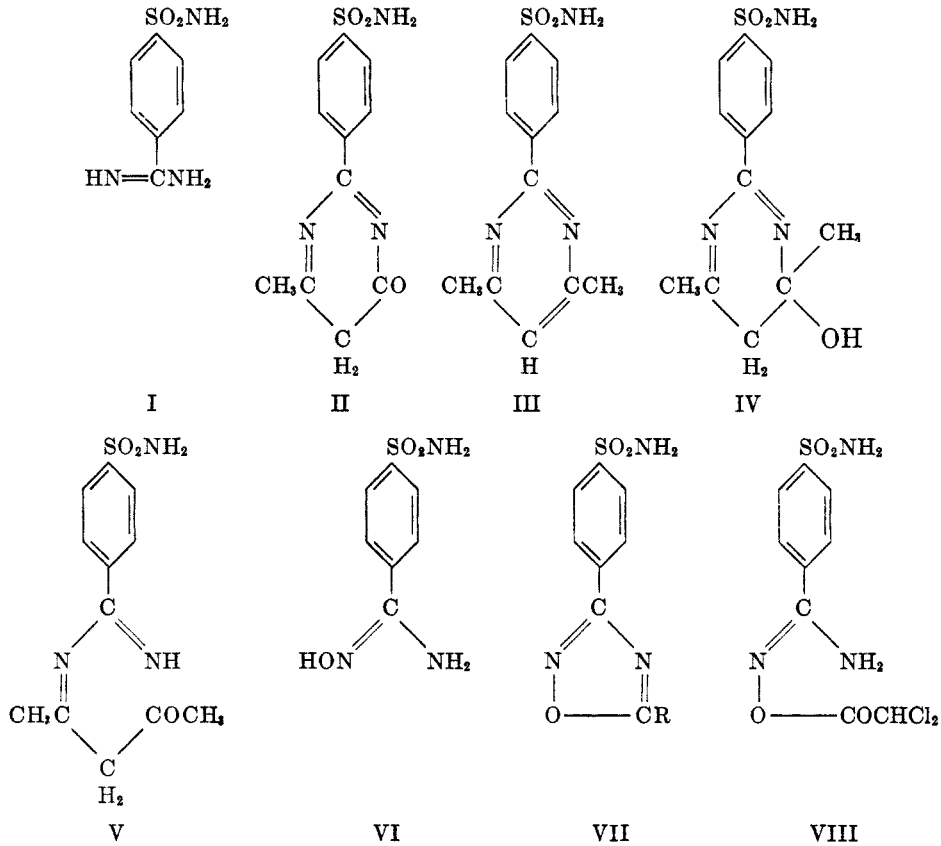
The aim of the present investigation was to explore the potentialities of two structural principles discovered recently in chemotherapeutically active substances: nitrogenous derivatives of *p*-sulfonamidobenzoic acid and nitro-aromatic nuclei. Andrewes and co-workers (1) have reported that *p*-sulfonamidobenzamidoxime and *p*-sulfonamidobenzamidine hydrochlorides are active against typhus, and the discovery of the antibiotic chloramphenicol [*threo*-3-(*p*-nitrophenyl)-2-dichloroacetylamido-1,3-propanediol] emphasized the pro-therapeutic properties of the nitro-group which had become known from observations on nitrofuran derivatives and similar compounds (2–4) and on the German "Nitroakridin 3582" (5). The latter was found active against typhus (6), rickettsial (7), and virus infections (8), whilst it had no effect on *Leptospira* (9). Also the recent investigation of 1,1-di-(4′-nitrophenyl)-2,2,2-trichloroethane for the treatment of spotted fever (10) is recalled in this connection.

(I). A number of substances were synthesized which contained *the p-sulfonamidobenzamidine* (I) structure incorporated in heterocyclic ring systems. With ethyl acetoacetate, 2-(*p*-sulfonamidophenyl)-4-methyl-6-keto-5, 6-dihydropyrimidine (II) was obtained (Compare Hu and Chao, 11). The infrared spectrum (measured on a suspension in liquid paraffin) showed the presence of a carbonyl group by the 1686 cm^{-1} band, although the absorption in the double bond region was generally not well defined. The carbonyl group is not enolized, as the absence of the absorption in the OH-region proves (12). The condensation with acetylacetone gave a product which contained one molecule of water more than expected for 2-(*p*-sulfonamidophenyl)-4,6-dimethylpyrimidine (III). The infrared investigation of the product (suspension in liquid paraffin) showed bands at 3250, 1671, and 1593 $cm.^{-1}$. The absence of the hydroxyl absorption proves that the cyclic formula (IV) does not apply and that, evidently, the intermediate (V) had remained uncyclized. The absorption at 3250 $cm.^{-1}$ can be ascribed to the C=NH grouping [3380 cm^{-1}, according to Colthup (13), possibly modified by hydrogen bonding with the carbonyl], while the band at 1671 cm^{-1} can be explained as superposition of the C=C, C=N and C=O absorptions, and that at 1593 cm^{-1} as the phenyl band.

(II). *p-Sulfonamidobenzamidoxime* (VI) gave with boiling chloroacetyl chloride and dichloroacetyl chloride, 5-chloromethyl- and 5-dichloromethyl-3-(*p*-sulfonamidophenyl)-1,2,4,-oxadiazole (VII a and b), respectively. Condensation of VI with dichloroacetic acid gave a product different from (VIIb); according to the analysis, it was a dichloroacetyl derivative of the starting material, probably VIII. This is in keeping with the observation of Tiemann and Krueger (14) that benzamidoxime reacts at room temperature with benzoyl or acetyl chloride to give O-acyl derivatives, which cyclize, upon heating, to the corresponding oxadiazoles.



I II III IV

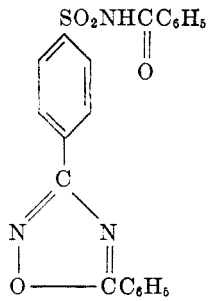
V VI VII VIII

a. R = CH_2Cl
b. R = $CHCl_2$
c. R = C_6H_5
d. R = CH_3

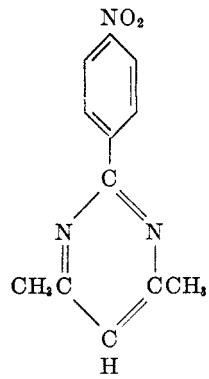
In boiling glacial acetic acid, benzoyl chloride condenses with VI to 5-phenyl-3-(*p*-sulfonamidophenyl)-1,2,4-oxadiazole (VIIc); at 180°, an additional benzoyl group enters the molecule. Formula IX is supported by the determination of active hydrogen and by the appearance of the NH— absorption in the infrared spectrum (3380 cm^{-1}). Analogously, boiling acetic anhydride leads to a compound which according to the analysis is the N-acetyl derivative of 5-methyl-3-(*p*-sulfonamidophenyl)-1,2,4-oxadiazole (VIId). An abnormal product was isolated when VI was heated with glacial acetic acid at 150°; it was crystalline, but very difficultly soluble and high-melting (309°), and contained only two (not three) nitrogen atoms per sulfur atom in the molecule. Its structure has not been elucidated, but it was shown that it is not identical with the "infusible" crystalline product which has been obtained upon prolonged heating of *p*-sulfonamidobenzoic acid (15–17) and which according to Hu and Chao (11) is *p*-sulfobenzamidine.

(III). Analogously, *p-nitrobenzamidine and p-nitrobenzamidoxime* were stud-

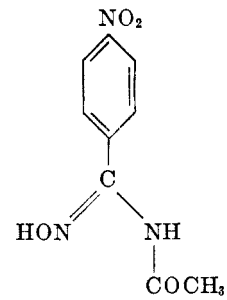
ied; the former reacted with acetylacetone to give 2-(*p*-nitrophenyl)-4,6-dimethylpyrimidine (X). The latter gave with glacial acetic acid an acetyl derivative (XI), while acetic anhydride and benzoyl chloride gave 5-methyl- and 5-phenyl-3-(*p*-nitrophenyl)-1,2,4-oxadiazole (XII, R = CH_3, C_6H^5), respectively. Also chloroacetyl chloride reacted analogously.



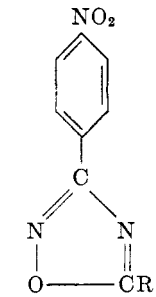
IX



X



XI



XII

For the acetyl derivative, the formula XI (N-acetyl) has been proven by the infrared spectrum which showed only one strong band at 1650 cm^{-1} which is to be interpreted as a superposition of the C=N absorption [acetoxime: 1647 cm^{-1} (18)] and the monosubstituted amide absorption (1680 cm^{-1}). The isomeric O-acetyl compound would show, apart from the C=N frequency, the ester absorption at about 1750 cm^{-1}.

The chemotherapeutic properties of the substances prepared in this investigation are now being explored; they will be reported elsewhere.

Acknowledgement. The infrared spectra have been determined by Dr. S. Pinchas, Optics Department, Weizmann Institute of Science, Rehovoth.

EXPERIMENTAL

p-Cyanobenzenesulfonamide was best prepared according to Iris *et al.* (19); however, the purification of the crude product was preferably effected by recrystallization from water in presence of charcoal. Yield, 90%; m.p. 169°.

Anal. Calc'd for $C_7H_6N_2O_2S$: C, 46.2; H, 3.3.

Found: C, 46.2; H, 3.4.

p-Sulfonamidobenzamidine (I) was obtained from the foregoing substance according to Andrewes *et al.* (1). Yield, 60 g. of crude product from 60 g. of the nitrile. This was purified by precipitation of the solution in hydrochloric acid with ammonia, and recrystallization from water. M.p. 225° [compare also for derivatives, (19a)].

2-(p-Sulfonamidophenyl)-4-methyl-6-keto-5, 6-dihydropyrimidine (II). To a mixture of equimolecular quantities of *p*-sulfonamidobenzamidine hydrochloride (2 g.) and ethyl acetoacetate (1.12 g.), 10% sodium hydroxide solution was added until the alkaline reaction persisted. The mixture was heated on the water-bath for one hour. Filtration and recrystallization from alcohol gave crystals of m.p. 265–266°. Yield, 1 g. (43.5%).

Anal. Calc'd for $C_{11}H_{11}N_3O_3S$: S, 12.1. Found: S, 11.9.

Condensation product (V) *of p-sulfonamidobenzamidine* (I) *and acetylacetone.* When a

solution of 5 g. of *p*-sulfonamidobenzamidine (I) in a small quantity of concentrated hydrochloric acid was mixed with 2.5 cc. of acetylacetone and 7.5 cc. of pyridine and kept at normal temperature for several days, needles crystallized. These were filtered, washed with dilute hydrochloric acid and water, and recrystallized from alcohol. M.p. 195°; Yield, 2.5 g. (40%).

Anal. Calc'd for $C_{12}H_{15}N_3O_3S$: C, 51.2; H, 5.3; N, 14.9.
Found: C, 51.0; H, 5.4; N, 14.6, 14.6, 14.8.

p-Sulfonamidobenzamidoxime (VI). *p-Cyanobenzenesulfonamide* (14.5 g.) was refluxed for 2 hours with a methanolic solution (20) of hydroxylamine (5.28 g.), and the resulting solution was concentrated to a small volume. Cooling gave 13.5 g. (80%) of the desired product, which was recrystallized from water. Yield, 12 g.

Anal. Calc'd for $C_7H_9N_3O_3S$: C, 39.1; H, 4.2; S, 14.9.
Found: C, 39.4; H, 4.7; S, 15.0, 14.6.

The *hydrochloride*, obtained from VI and aqueous hydrochloric acid by heating until dissolution and subsequent cooling, crystallized from isopropyl alcohol as a hemihydrate of m.p. 149–150° (dec.); the anhydrous product (prepared by heating at 100° *in vacuo*) has m.p. 198°.

Anal. Calc'd for $C_7H_{10}ClN_3O_3S$: Cl, 14.1. Found: Cl, 13.9.

The *hydrobromide*, prepared analogously, had m.p. 198°.

Anal. Calc'd for $C_7H_{10}BrN_3O_3S$: Br, 27.0. Found: Br, 27.1.

5-Chloromethyl-3-(p-sulfonamidophenyl)-1,2,4-oxadiazole (VIIa). The mixture of 2 g. of VI and 3 cc. of chloroacetyl chloride was refluxed for 30 minutes; removal of the excess acid chloride and treatment with sodium carbonate solution and water gave 2.5 g. of the product (yield, quantitative) which melted at 145° after recrystallization from alcohol.

Anal. Calc'd for $C_9H_8ClN_3O_3S$: C, 39.5; H, 2.9; N, 15.4; S, 11.7; Cl, 12.8.
Found: C, 39.8; H, 2.8; N, 15.0; S, 11.4; Cl, 12.7

5-Dichloromethyl-3-(p-sulfonamidophenyl)-1,2,4-oxadiazole (VIIb). The above procedure, applied to 2 g. of VI and 3 cc. of dichloroacetyl chloride, gave the condensation product in 80% yield. From aqueous alcohol, it had m.p. 130°.

Anal. Calc'd for $C_9H_7Cl_2N_3O_3S$: C, 35.2; H, 2.3; N, 13.7; S, 10.4; Cl, 22.8.
Found: C, 35.0; H, 2.2; N, 14.0; S, 10.7; Cl, 23.1.

O-Dichloroacetyl-p-sulfonamidobenzamidoxime (VIII). The mixture of 2.5 g. of VI and 3 g. of dichloroacetic acid was refluxed for 15 minutes with 5 cc. of glacial acetic acid. Cooling and addition of ether gave 2.8 g. (75%) of the condensation product (VIII). Recrystallization from glacial acetic acid gave m.p. 210°.

Anal. Calc'd for $C_9H_9Cl_2N_3O_4S$: C, 33.2; H, 2.8; N, 12.9; S, 9.8; Cl, 21.8.
Found: C, 33.0; H, 3.0; N, 12.6; S, 10.4; Cl, 21.8.

3-(p-Sulfonamidophenyl-5-phenyl-1,2,4-oxadiazole (VIIc). A mixture of 1 g. of VI, 1.2 g. of benzoyl chloride, and 3 cc. of glacial acetic acid was heated at 160° (bath temperature) for 30 minutes. Upon cooling, the reaction product (1 g., 71%) crystallized; it was filtered and recrystallized from benzene or alcohol, m.p. 241°.

Anal. Calc'd for $C_{14}H_{11}N_3O_3S$: C, 56.0; H, 3.7; N, 14.0; S, 10.7; active H, 0.67.
Found: C, 55.7; H, 3.5; N, 14.0; S, 10.9; active H, 0.68.

N^4-Benzoyl derivative (IX) *of* VIIc. A mixture of 2.5 g. of VI and 6 g. of benzoyl chloride was heated at 180° for 20 minutes. The crystals, which separated on cooling, were filtered (3.5 g., 74%) and recrystallized from benzene, m.p. 216°.

Anal. Calc'd for $C_{21}H_{15}N_3O_4S$: C, 62.5; H, 3.7; N, 10.4; S, 7.9; active H, 0.25.
Found: C, 62.1; H, 3.7; N, 10.3; S, 7.9; active H, 0.36.

N^4-Acetyl derivative of VIId. When 1 g. of VI was refluxed for one hour with 10 g. of acetic acid and 2 g. of acetic anhydride and the resulting solution cooled, a product crystallized which after recrystallization from alcohol had m.p. 206–207°. Yield, 1 g.

Anal. Calc'd for $C_{11}H_{11}N_3O_4S$: C, 47.0; H, 3.9; N, 15.0; S, 11.4.
Found: C, 47.2; H, 4.0; N, 14.7; S, 11.2.

Reaction of VI *with acetic acid.* When VI was heated at 150° for 30 minutes with an excess

of glacial acetic acid, very difficultly soluble colorless crystals were formed, which melted at 309°.

Anal. Found: C, 44.2; H, 3.0; N, 14.7; S, 16.9.

p-Nitrobenzamidine was prepared from *p*-nitrobenzonitrile (21, 22) *via* the imino-ether, in the same manner as the *p*-sulfonamido-compound. Purification by dissolution in conc'd hydrochloric acid and precipitation with ammonia. M.p. 194°; yield, 10.8 g. (60%) from 15 g. of the nitrile.

Anal. Calc'd for $C_7H_7N_3O_2$: N, 25.5. Found: N, 25.5, 25.3.

2-(p-Nitrophenyl)-4,6-dimethylpyrimidine (X). The condensation of *p*-nitrobenzamidine (2.48 g.) with acetylacetone (1.5 g.) was carried out in the manner described above. Instead of pyridine, a solution of potassium carbonate (4 times the theoretical quantity) could be used as condensing agent. The precipitate was filtered, washed with a little alcohol and ether, and recrystallized from dilute alcohol. M.p. 167°; yield, 1.4 g. (40%).

Anal. Calc'd for $C_{12}H_{11}N_3O_2$: C, 63.0; H, 4.8.
Found: C, 63.4; H, 4.7.

p-Nitrobenzamidoxime. p-Nitrobenzonitrile (5 g.) was refluxed for one hour with twice the theoretical quantity (40 cc.) of a 2 *N* hydroxylamine solution in methanol (20). The solid reaction product which separated upon cooling, was filtered and recrystallized from alcohol or acetone. M.p. 180°, [literature: 150°; 169° (23)]; yield, 4 g. (66%).

Anal. Calc'd for $C_7H_7N_3O_3$: C, 46.4; H, 3.9; N, 23.2.
Found: C, 46.4; H, 3.8; N, 23.2.

Acetyl derivative (XI), from 1 g. of the base and 5 cc. of boiling glacial acetic acid (15 minutes). Upon addition of water, the product separated (1.1 g.; 90%). It was recrystallized from glacial acetic acid, m.p. 226°.

Anal. Calc'd for $C_9H_9N_3O_4$: N, 18.8. Found: N, 18.8; 18.8.

3-(p-Nitrophenyl)-5-methyl-1,2,4-oxadiazole (XII, R = CH_3). When 1 g. of nitrobenzamidoxime and 5 cc. of acetic anhydride were refluxed for 30 minutes, the condensation product crystallized upon cooling in well-defined crystals of m.p. 140°. After recrystallization from anhydrous alcohol, yield, 1 g. (80%).

Anal. Calc'd for $C_9H_7N_3O_3$: C, 52.7; H, 3.4; N, 20.5.
Found: C, 52.5; H, 3.2; N, 20.9.

3-(p-Nitrophenyl)-5-chloromethyl-1,2,4-oxadiazole (XII, R = CH_2Cl). The mixture of 1 g. of nitrobenzamidoxime and 1 cc. of chloroacetyl chloride was heated at 120° for 30 minutes, and cooled. The crystalline reaction product was washed with aqueous sodium carbonate solution and water, and recrystallized from dilute alcohol. M.p. 88°, yield, 1.2 g. (90%).

Anal. Calc'd for $C_9H_6ClN_3O_3$: C, 45.2; H, 2.5; N, 17.6.
Found: C, 45.6; H, 2.3; N, 17.9.

3-(p-Nitrophenyl)-5-phenyl-1,2,4-oxadiazole (XII, R = C_6H_5). The mixture of 1 g. of *p*-nitrobenzamidoxime and 5 g. of benzoyl chloride was heated for 30 minutes on the water-bath. Upon cooling and with the addition of ether, crystals separated which were recrystallized from benzene; m.p. 195°. Yield, 90%.

Anal. Calc'd for $C_{14}H_9N_3O_3$: C, 62.9; H, 3.4; N, 15.8.
Found: C, 62.8; H, 3.2; N, 16.2.

SUMMARY

1. The condensation of *p*-sulfonamidobenzamidine with ethyl acetoacetate and acetylacetone has been studied.

2. The reaction of *p*-sulfonamidobenzamidoxime with benzoyl chloride, chloroacetyl chloride, dichloroacetyl chloride, acetic anhydride, acetic acid, and dichloroacetic acid has been investigated.

3. *p*-Nitrobenzamidine has been condensed with acetylacetone, and *p*-nitro-

benzamidoxime with acetic anhydride, chloroacetyl chloride, and benzoyl chloride, respectively, to heterocyclic compounds.

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