Structure and Activity of Schistosomicidal Agents of the Pyrimidine Type

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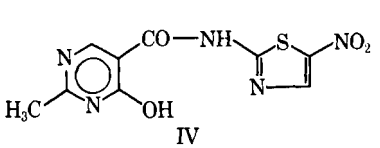
Abstract □ To profit from the concept of metabolite antagonism in the area of schistosomiasis chemotherapy, new compounds containing combinations of both substituted pyrimidine (closely related to the pyrimidine moiety present in thiamine) and 5-nitrothiazole or 2-imidazolidinethione moieties were synthesized.

Keyphrases □ Schistosomicidal agents, potential—synthesis and biological activity of pyrimidine moiety with 5-nitrothiazole or 2-imidazolidinethione □ Pyrimidines, substituted—synthesized as potential schistosomicidal agents, biological activity

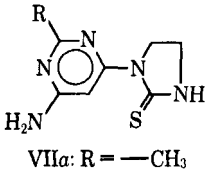
In a previous report (1) the schistosomicidal activity of a new compound, 2-*N*-(2′-methyl-4′-amino-5′-pyrimidoyl)amino-5-nitrothiazole (I), was described. The rational approach for the introduction of this compound was based upon its close relation to thiamine in an attempt to profit from the concept of metabolic antagonism or antimetabolites in the area of schistosomiasis chemotherapy. In another communication[1], the effect of the potent schistosomicidal agent, 1-(5-nitro-2-thiazolyl)-2-imidazolidinone[2] (II) (2), on pyruvate metabolism was reported.

The purpose of this work was to introduce new compounds, including structural combinations between differently substituted pyrimidines (closely related to that included in thiamine) and 5-nitrothiazole or 2-imidazolidinethione moieties. The latter is the thione analog of imidazolidinone, structurally represented as the schistosomicidal agent II. The combination between these systems was suggested to be through an amide linkage because this may be biologically hydrolyzed to free both parts. Thus, the molecule may act as a whole or as separate components in the presence of one another. Also, attachment was thought to be through direct binding between both systems, similar to that represented in II between 5-nitrothiazole and 2-imidazolidinone.

The desired pyrimidine moiety, 2-methyl-4-hydroxy-5-carbethoxypyrimidine (III) (3), was prepared through condensation of acetamidine hydrochloride and ethyl ethoxymethylenemalonate (4). Condensation of III with 2-amino-5-nitrothiazole gave 2-*N*-(2′-methyl-4′-hydroxy-5′-pyrimidoyl)amino-5-nitrothiazole (IV). Furthermore, 2-methyl-4-amino-6-hydroxypyrimidine (V) (5) was obtained through condensation of acetamidine hydrochloride and ethyl cyanoacetate. Treatment of V with phosphorus oxychloride gave the 6-chloro derivative (VI) (6). Condensation of VI with 2-imidazolidinethione in an acidic medium gave 1-*N*-(2′-methyl-4′-amino-6′-pyrimidyl)-2-imidazolidinethione (VII) in a fairly good yield.



IV



VII*a*: R = —CH_3
VII*b*: R = —SCH_3

Another substituted pyrimidine moiety of interest was 2-methylmercapto-4-amino-6-chloropyrimidine (VIII) (7). This was obtained through the action of phosphorus oxychloride upon the 6-hydroxy analog (IX) (8). Reaction of VIII with 2-imidazolidinethione gave 1-*N*-(2′-methylmercapto-4′-amino-6′-pyrimidyl)-2-imidazolidinethione (VII*b*).

Replacement of the 2-methylmercapto group from the pyrimidine system with different amines could be easily effected if the nitroso group was present at the 5-position (9). For this, 2-methylmercapto-4-amino-5-nitroso-6-hydroxypyrimidine (X) (8) was prepared. The 2-methylmercapto group in X was replaced with 2-aminothiazole to give 2-*N*-(4′-amino-5′-nitroso-6′-hydroxy-2′-pyrimidyl)aminothiazole (XI*a*). Similarly, 3-(4′-amino-5′-nitroso-6′-hydroxy-2′-pyrimidyl)-2-imidazolidinethione (XI*b*) was prepared through the reaction of X with 2-imidazolidinethione. Tentative structural assignments to Compounds VII*a*, VII*b*, and XI*b*, as drawn from analytical data and IR analyses, suggest the *N*-aryl formation within these products.

Furthermore, 2-(β-diethylaminoethylamino)-4,6-diamino-5-nitrosopyrimidine (XI*c*) was prepared through the reaction of β-diethylaminoethylamine with 2-methylmercapto-4,6-diamino-5-nitrosopyrimidine (XII) (10). The diamino side chain was reported to be necessary for the carcinostatic and schistosomicidal activities in agents of the thioxanthone type (11).

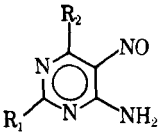
EXPERIMENTAL[3]

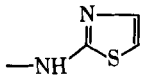
2-*N*-(2′-Methyl-4′-hydroxy-5′-pyrimidoyl)amino-5-nitrothiazole (IV)—A mixture of III (1.82 g., 0.01 mole) and 2-amino-5-nitrothiazole (1.45 g., 0.01 mole) in 20 ml. of absolute ethanol was refluxed for 6–8 hr. The mixture was cooled, and yellowish needles of IV precipitated. These were filtered off and recrystallized from methanol as long dark-yellow needles; yield 1.95 g. (70%); m.p. 202°; IR: 3120 (—OH, chelated), 1720 (=C=O), and 1380 cm.$^{-1}$ (thiazole —NO_2 group).

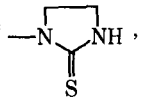
Anal.—Calc. for $C_9H_7N_5O_4S$: C, 38.44; H, 2.40; N, 24.91; S, 11.38. Found: C, 38.65; H, 2.63; N, 24.99; S, 11.02.

[1] I. Nabih, M. El-Hawarry, and H. Zoorob, to be published.
[2] Coded as Niridazole.

[3] All melting points were taken in open glass capillaries using a Gallenkamp melting-point apparatus and are uncorrected. IR spectra were determined on a Carl Zeiss Jena model UR 10 infracord spectrophotometer using mineral oil. Microanalyses were performed by the Microanalytical Laboratory, National Research Centre, Cairo, and Spang Microanalytical Laboratory, Ann Arbor, Mich.



XI*a*: R_1 =  , R_2 = —OH

XI*b*: R_1 =  , R_2 = —OH

XI*c*: R_1 = —HN—CH_2—CH_2—$N(C_2H_5)_2$, R_2 = —NH_2

1-*N*-(2′-Methyl-4′-amino-6′-pyrimidyl)-2-imidazolidinethione (VII*a*)—A mixture of VI (6) (1.4 g., 0.01 mole) and 2-imidazolidinethione (1 g., 0.01 mole) in 7 ml. of water and 0.8 ml. of hydrochloric acid (37%) was heated to boiling on a hot plate for 3 hr. Upon cooling, a crystalline precipitate formed; upon basification with 5% ammonium hydroxide solution, the precipitate turned to a white crystalline mass. This was filtered off and recrystallized from methanol to give a white fibrous material of VII*a*; yield 1.5 g. (75%); m.p. 214°; IR: 3390 (—NH) and 1170 cm.$^{-1}$ (=C=S).

Anal.—Calc. for $C_8H_{11}N_5S$: C, 45.93; H, 5.26; N, 33.49. Found: C, 45.97; H, 5.36; N, 33.80.

1-*N*-(2′-Methylmercapto-4′-amino-6′-pyrimidyl)-2-imidazolidinethione (VII*b*)—A mixture of VIII (7) (0.7 g., 0.004 mole) and 2-imidazolidinethione (0.4 g., 0.004 mole) in glacial acetic acid (5 ml.) and concentrated hydrochloric acid (1 drop) was heated on a steam bath for 1.5 hr. The mixture was refrigerated, made basic with 5% ammonium hydroxide solution, and kept in a deep freeze overnight. The formed precipitate was filtered off and recrystallized from methanol to give white crystals of VII*b*; yield 0.65 g. (68%); m.p. 224°; IR: 3310 (—NH) and 1170 cm.$^{-1}$ (=C=S).

Anal.—Calc. for $C_8H_{11}N_5S_2$: C, 39.83; H, 4.56; N, 29.04; S, 26.55. Found: C, 39.57; H, 4.90; N, 29.28; S, 26.30.

2-*N*-(4′-Amino-5′-nitroso-6′-hydroxy-2′-pyrimidyl)-2-aminothiazole (XI*a*)—A mixture of X (8) (1.86 g., 0.01 mole) and 2-aminothiazole (1 g., 0.01 mole) in 35 ml. of water was refluxed until the odor of methylmercaptan could not be detected (about 24–30 hr.). The content of the flask was then filtered off while hot. The filtrate was acidified just to pH 5 with glacial acetic acid and then was cooled. The formed precipitate was isolated. Recrystallization from water gave a light-brown precipitate of XI*a*; yield 1.3 g. (55%); m.p. >300°; IR: 3620 (—OH, free), 3320 (—NH), and 1620 cm.$^{-1}$ (the pyrimidine nitroso group).

Anal.—Calc. for $C_7H_6N_6O_2S$: C, 35.92; H, 2.52; N, 35.29; S 13.44. Found: C, 34.88; H, 3.01; N, 34.98; S, 13.02.

3-(4′-Amino-5′-nitroso-6′-hydroxy-2′-pyrimidyl)-2-imidazolidinethione (XI*b*)—A mixture of X (8) (1.86 g., 0.01 mole) and 2-imidazolidinethione (1.02 g., 0.01 mole) in 30 ml. of water was refluxed until no odor of methylmercaptan could be detected (about 24–36 hr.). Then the procedure was continued as described for Compound XI*a* to give XI*b*. Recrystallization from water yielded 1.12 g. (50%); m.p. >300°; IR: 3610 (—OH), 3300 (—NH), 1170 (=C=S), and 1650 cm.$^{-1}$ (—NO).

Anal.—Calc. for $C_7H_8N_6OS \cdot H_2O$: C, 32.56; H, 3.87; N, 32.55. Found: C, 32.48; H, 3.84; N, 32.49.

2-(β-Diethylaminoethylamino)-4,6-diamino-5-nitrosopyrimidine (XI*c*)—A mixture of XII (10) (1 g., 0.006 mole) and β-diethylaminoethylamine (2.6 ml., 0.02 mole) in 20 ml. of water was refluxed for 20 min. The content in the flask was then cooled until a red crystalline precipitate formed. This precipitate was filtered off, washed with cold water, and recrystallized from water to give XI*c*; yield 0.92 g. (60%); m.p. 165°; IR: 3200 (—NH) and 1650 cm.$^{-1}$ (—NO).

Anal.—Calc. for $C_{10}H_{19}N_7O$: C, 47.43; H, 7.51; N, 38.74. Found: C, 47.56; H, 7.62; N, 38.78.

BIOLOGICAL TESTING

Compounds IV, XI*b*, and XI*c*, in water solutions, were submitted to biological screening. Groups of mice (six each), with an average weight of 29–31 g., were infected with *Schistosoma mansoni*. All mice were showing viable ova in their stools.

Group A was kept as the control group. Group B received oral doses of 75 mg. Compound XI*c*/kg. body weight. Oral administration[4] of the compound continued for 10 consecutive days. Two other groups (C and D) were similarly given Compounds IV and XI*b*, respectively, at the same dosage levels as Group B for the same period. After 1 week from the end of treatment, ova excretion in each group was examined once weekly for 6 weeks. Only Group B showed a gradual decrease in the rate, and final cessation, of ova excretion. Examinations continued for 4 weeks. No ova could be detected in the mice stools of Group B. In Groups C and D, given Compounds IV and XI*b*, respectively, no remarkable decrease in the rate of ova excretion was observed as compared with the control group (A).

These biological findings showed that Compound XI*c* demonstrated schistosomicidal activity in mice, while Compounds IV and XI*b* failed to exhibit such activity. For the purpose of screening, Compounds IV, XI*b*, and XI*c* were selected as representatives of structural combinations between the pyrimidine moiety and other systems.

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[4] Administrations were through stomach-feeding polyethylene tubes.