New Schistosomicidal and Carcinostatic Agents of the Thiaxanthone Type III

I. NABIH[▲] and A. ZAYED

Abstract □ Compounds structurally related to cyclohexenothiaxanthone were synthesized. In biological testing, a representative compound, 1-(β-diethylaminoethylamino)-3,4-cyclohexenothiaxanthone, showed pronounced schistosomicidal and antitumor activity.

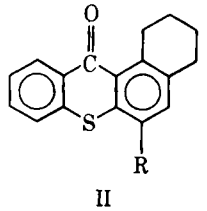
Keyphrases □ Thiaxanthone derivatives—synthesized and screened as potential schistosomicidal and carcinostatic agents □ 1-(β-Diethylaminoethylamino)-3,4-cyclohexenothiaxanthone—synthesized and screened as potential schistosomicidal and carcinostatic agent □ Schistosomicidal agents, potential—synthesis and screening of cyclohexenothiaxanthone analogs □ Carcinostatic agents, potential—synthesis and screening of cyclohexenothiaxanthone analogs

The thiaxanthone derivatives are considered to be the first nonmetallo-organic compounds of biological activity in the chemotherapy of schistosomiasis (1). They have received increasing interest since the discovery of their carcinostatic activity (2, 3). The most promising member in this group is 1-(β-diethylaminoethylamino)-4-methylthiaxanthone (I), but this agent is poorly tolerated by patients and usually induces severe unfavorable side effects during treatment (4). Because of these considerations, efforts were made to synthesize and develop new related structures suitable for application in both schistosomiasis and cancer.

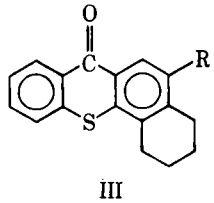
DISCUSSION

In a previous article[1] (5), the total syntheses and biological activity of two new agents related to cyclohexenothiaxanthone, namely, 1,2-cyclohexeno-4-(β-diethylaminoethylamino)thiaxanthone (II) and 2-(diethylaminoethylamino)-3,4-cyclohexenothiaxanthone (III), were described. The rationale for including the reduced tetralin system in these structures was also discussed.

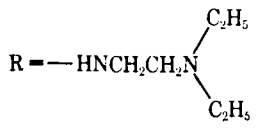
The approach to the synthesis of both II and III began with sulfuric acid condensation of thiosalicylic acid with the corresponding chlorotetralin compound. However, attempts to introduce the desired diamino side chain by replacing the chlorines in both condensation products failed. The chlorine atoms in both products, 2-chloro-3,4-cyclohexenothiaxanthone and its isomer 4-chloro-1,2-cyclohexenothiaxanthone, were resistant to any further substitution. This could be explained by the absence of the activating effect of the carbonyl groups on the lability of both these chlorine atoms. Such an effect suggested preferential condensation of the diamino side chain with 1-chloro-4-methylthiaxanthone, as with the synthesis of I (1).



II



III



Alternatively, both II and III were obtained through the condensation of the corresponding acetaminotetralin with thiosalicylic acid in the presence of sulfuric acid, followed by subsequent hydrolysis and condensation of the free amine with the desired side chain. Generally, in this type of condensation of thiosalicylic acid with aromatic components by sulfuric acid, the sulfide function forms first. The configuration of the condensation products depends upon the nature of these components (6). In the case of 5-chlorotetralin or 5-acetaminotetralin, the sulfide linkage initially formed at the 8-position, since this is activated due to the presence of the chlorine located *para* and the cyclic methylene group located *ortho*. In electrophilic substitution, *e.g.*, sulfonation, 5-chloro-8-sulfonic acid is the exclusive product (7). With 6-chlorotetralin and 6-acetaminotetralin, the 7-position is preferred for sulfide linkage formation.

In this work, the synthesis of another isomer of II and III, namely, 1-(β-diethylaminoethylamino)-3,4-cyclohexehothiaxanthone (IV), was of interest. This compound represents a direct structural analogy to I, where the cyclic methylene stands for the 4-methyl group located *para* to the diamino side chain. The synthesis of IV by any described approach using sulfuric acid condensation with chlorotetralin or acetaminotetralin was not successful, so other routes were tried. Diazotized 5-amino-7-nitrotetralin (8) reacted with thiosalicylic acid to give the intermediate, 2',3'-cyclohexeno-5'-nitrodiphenylsulfide-2-carboxylic acid (V). Reactions of the diazotized amines with thiophenols led to the formation of the sulfide linkage (9). Reduction of V gave the free amine VI which, upon cyclization with polyphosphoric acid, gave 1-amino-3,4-cyclohexenothiaxanthone (VII). Condensation of VII with β-chlorodiethylaminoethylamine gave the desired Compound IV.

The acidic intermediate V could also be obtained in better yield through the reaction of 5-iodo-7-nitrotetralin (10) with thiosalicylic acid in the presence of sodium carbonate. Furthermore, this route of synthesis was applied as an alternative for the preparation of II. For this purpose, diazotized 6-amino-7-nitrotetralin (11) reacted with thiosalicylic acid to build the acid intermediate, 2'-nitro-4',5'-cyclohexenodiphenylsulfide-2-carboxylic acid (VIII). Similarly, this was obtained through the reaction of 6-iodo-7-nitrotetralin (12) with thiosalicylic acid in the presence of sodium carbonate. Upon cyclization of VIII, 1,2-cyclohexeno-4-nitrothiaxanthone (IX) was obtained. This compound was reduced by hydrochloric acid and iron powder to give the free amine, 1,2-cyclohexeno-4-aminothiaxanthone (X). This product was identical to that obtained from the previously described synthesis of II (5).

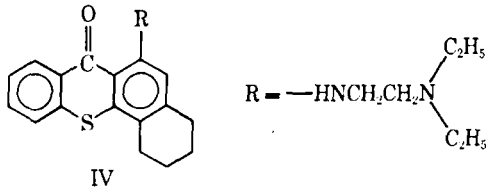
Compound III also was prepared by this route. Diazotized 5-amino-8-nitrotetralin (13) was reacted with thiosalicylic acid; the product, 2',3'-cyclohexeno-4'-nitrodiphenylsulfide-2-carboxylic acid (XI), was reduced to give 2',3'-cyclohexeno-4'-aminodiphenylsulfide-2-carboxylic acid (XII). Compound XII, upon cyclization with polyphosphoric acid, gave the amine, 2-amino-3,4-cyclohexenothiaxanthone (XIII). This product was identical to that obtained from the previously described synthesis of III (5).

BIOLOGICAL TESTING

The biological data for Compounds II and III in schistosomiasis and cancer were reported previously (5). In this work the isomer IV was submitted for testing as follows.

Schistosomiasis—Two groups of six mice, weighing 20–24 g. each, were infected with cercaria of *Schistosoma mansoni* obtained from a number of snails to ensure that both sexes were in the infecting cercaria. Ten weeks after infection, both groups showed viable eggs in their stools. The six members of one group were given

[1] Part I of this work. Part II will be published in *J. Chem. UAR.*



IV

daily oral doses (in water solution) of 80 mg./kg. body weight of IV as the hydrochloride, through a polyethylene feeding tube, for 12 consecutive days. The six mice in the other group were controls. After 1 week from the end of treatment, regular examination for ova excretion (every other day) was carried out for 3 weeks. In the treated group, a gradual decrease in the ova count was observed and ova excretion ceased by the end of the observation period (3 weeks). In the control group, ova excretion continued without remarkable change. Upon sacrificing the animals in both groups, living worms were found distributed in the liver and mesenteric venules. In the treated group, dead worms were present in the liver.

Experimental Tumors—*ELD (Ehrlich Ascites Carcinoma)*—Two groups of four mice each (ABYXdba strain), weighing 20–25 g., were inoculated intraperitoneally with 10^6 living tumor cells of the ELD type. In one group, Compound IV (as the hydrochloride in water solution) was injected intraperitoneally daily at doses of 80 mg./kg. body weight on the 3rd day after inoculation and for 6 consecutive days. The other group was kept as the control. Observation and weight control continued for 6 weeks. After 10 days, the control group showed clear tumor development with a remarkable increase in weight. In the treated group, no signs of tumor development were observed and there was no appreciable change in weight. Upon sacrificing the animals in both groups, living tumor cells were found in the controls; in the treated group, no tumor cells were found.

TA_3 (Mammary Carcinoma)—Two groups of four mice, weighing 16–18 g. each, were inoculated with 10^6 cells of the TA_3 type. On the 3rd day after inoculation and for 6 consecutive days, one group of four mice was treated intraperitoneally with 80 mg./kg. of Compound IV (as the hydrochloride in water solution). The other group was left untreated as a control. After 10 days, the control animals showed development of the transplanted tumor accompanied by an increase in weight (20–22 g.). In the treated mice, no remarkable change in weight was observed. After sacrificing the animals in both groups, no tumor cells were found in treated animals; in the controls, living tumor cells were present.

Regression of well-established experimental tumors by purely chemical means is seldom seen (14). Thus, testing the compound's effect on established tumors was of interest.

Treatment of established ascites tumors of the ELD type (14–18 days postinoculation with 2×10^6 living tumor cells) by daily doses of 80 mg./kg. for 4 consecutive days showed marked regression in the tumor sizes and cell counts, *e.g.*, the number counted before treatment was 900×10^6 cells/ml., and after treatment the number found was 26×10^6 cells/ml. After sacrificing the treated animals, masses of dead cells were found on the wall of the abdominal cavity.

Established mammary tumors, TA_3 type (8–10 days postinoculation with 10^6 living tumor cells) were treated with 80 mg./kg. of the compound for 4 days. Marked regression in tumor size and cell count was observed. For example, a mouse bearing a tumor cell count of 171×10^6/ml. showed a posttreatment cell count of 15×10^6/ml. The treated cells showed vacuolization and dispersion. This was contrary to control, nontreated cells which usually aggregate in colonized groups. This observed intracellular dispersion could be attributed to the electrostatic interaction between the polarized thiaxanthone molecule [due to electron delocalization (15), measured dipole moment for the compound 5.8 Debye units] and the tumor cells which carry high electronegative charges (16). This property provides a clue for the selective attack on tumor cells.

Preliminary studies in human leukemia (myeloblastic and monocytic types) *in vitro* showed pronounced effect of the compound toward the leucocytes.

The observed antitumor activity of the compound could be rationalized on the basis that the thiaxanthone system serves as a target-seeking device or carrier to bring the tetralin part of the molecule to the tumor cells, since tetralin tends to fix molecular oxygen in an oxygenation process (17). This would affect the glycolytic rate in the cell through oxidation of the sulfhydryl (—SH) enzymes engaged in this metabolic process. If cell division depends on the presence of small quantities of sulfhydryl, then the division could be inhibited by keeping the sulfhydryl in an oxidized condition by the presence of such oxygen carriers. Furthermore, the tetrahydronaphthalene system could undergo biological transformation to a naphthoquinone-type structure which depresses cell division. Also, the thiaxanthone system could act through complexing with DNA strands in the tumor cells, thus inhibiting DNA-dependent RNA synthesis. Biochemical studies are underway to determine the possibility of complex formation with this compound.

EXPERIMENTAL[2]

2′,3′-Cyclohexeno-5′-nitrodiphenylsulfide-2-carboxylic Acid (V)—*Method A*—To a mixture of 5-amino-7-nitrotetralin (10 g.) and sulfuric acid (40 ml., 50%) at 0° was added dropwise a solution of sodium nitrite (5 g. in 30 ml. water). The diazonium solution was then poured into a stirred solution of thiosalicylic acid (7 g. in 100 ml. 35% NaOH), heated on a steam bath under stirring for 3 hr., and cooled, and the supernate was then decanted. The residue was boiled with water and filtered while hot, and the filtrate was acidified with acetic acid. The separated product was purified by redissolving it in warm ammonium hydroxide solution and filtering. The filtrate was acidified with acetic acid and filtered. The product was dried to give 11 g. of V (69%), and it was recrystallized from benzene–alcohol, m.p. 210–212°.

Anal.—Calc. for $C_{17}H_{15}NO_4S$: C, 62.07; H, 4.59; N, 4.25; S, 9.73. Found: C, 62.18; H, 4.63; N, 4.42; S, 10.12.

Method B—A mixture of 5-iodo-7-nitrotetralin (2.4 g.), thiosalicylic acid (1.2 g.), sodium carbonate (1 g.), amyl alcohol (15 ml.), and 0.2 g. copper powder was refluxed for 20 hr. Five milliliters of sodium hydroxide solution was then added and the alcohol was steam distilled. The residue was boiled with excess water (25 ml.) and filtered, and the filtrate was acidified after cooling with dilute hydrochloric acid. The formed product weighed 1.90 g. (74%) after drying. Upon recrystallization from benzene–alcohol, it had a melting point of 210–212°, with no depression in the melting point when mixed with the product obtained by Method A.

2′,3′-Cyclohexeno-5′-aminodiphenylsulfide-2-carboxylic Acid (VI)—The nitro Compound V (3 g.) was stirred on a water bath with acetic acid (30 ml.), and then iron powder (3 g.) was added portionwise. After addition of half of the iron, 10 ml. acetic acid was added. Heating was continued for 2 hr. The mixture was filtered while hot, and the residue was washed with additional hot 50% acetic acid. The combined filtrate was poured into ice water. The formed precipitate was collected, washed with water, and dried to give 2.10 g. (74%) of VI. It was recrystallized from ethanol, m.p. 214–216°.

Anal.—Calc. for $C_{17}H_{17}NO_2S$: C, 68.27; H, 5.73; N, 4.68. Found: C, 68.08; H, 5.69; N, 4.65.

1-Amino-3,4-cyclohexenothiaxanthone (VII)—Sixteen grams of phosphoric acid was heated at 190°; then 2 g. of VI was added portionwise, and heating at this temperature was continued for 2 hr. Then the mixture was cooled and poured into ice water. The formed precipitate was collected, washed with hot ammonium hydroxide solution and then water, and dried. It gave 1.7 g. (90%) of VII. Upon recrystallization from benzene, it melted at 198–200°.

Anal.—Calc. for $C_{17}H_{15}NOS$: C, 72.56; H, 5.37; N, 5.37. Found: C, 72.78; H, 5.43; N, 5.46.

1-(β-Diethylaminoethylamino)-3,4-cylohexenothiaxanthone (IV)—A mixture of VII (2 g.) and β-chlorodiethylaminoethylamine (1.5 ml.) was heated at 180° for 4 hr. It was boiled with 25 ml. water for 0.5 hr., and then 4 ml. of concentrated hydrochloric acid was added; it was heated and filtered while hot. The filtrate was basified with sodium hydroxide solution; the formed precipitate was collected, washed with water, and then dried. It gave 2.26 g. of V (82%). Upon recrystallization from ethanol, it melted at 94–96°; IR: 1630 (C=O), 3340 (—NH—), 3017 (cyclohexyl), and 680 (—S—) $cm.^{-1}$.

Anal.—Calc. for $C_{23}H_{28}N_2OS$: C, 72.29; H, 7.41; N, 7.36; S, 8.43. Found: C, 72.34; H, 7.48; N, 7.39; S, 8.51.

The hydrochloride was prepared by dissolving the base in dry ether and allowing a stream of hydrogen chloride gas to pass through the solution, whereby it separated as a deep-orange product, m.p. 202–204°. It was highly hygroscopic and soluble in water.

[2] All melting points were taken in open capillaries using a Gallenkamp melting-point apparatus and are uncorrected. Microanalyses were performed at the National Research Center (microanalytical unit) and Spang Microanalytical Laboratory, Ann Arbor, Mich.

2′-Nitro-4′,5′-cyclohexenodiphenylsulfide-2-carboxylic Acid (VIII)—*Method A*—A mixture of 6-amino-7-nitrotetralin (6 g.) and concentrated hydrochloric acid (20 ml.) in 10 ml. of water was stirred at 0°, and then a solution of sodium nitrite (2.5 g. in 10 ml. water) was added. The diazonium solution was added portionwise to a stirred solution of thiosalicylic acid (3.5 g. in 50 ml. 20% NaOH solution) and then heated on a steam bath for 3 hr. After cooling, the supernatant layer was decanted and the residue was boiled with water. This was filtered while hot, and the filtrate was acidified after cooling with acetic acid. The product was redissolved in hot ammonium hydroxide solution, filtered, and then acidified with acetic acid. The collected product was dried to give 5.83 g. of VIII (64%). Upon recrystallization from benzene–alcohol, it melted at 162–164°.

Anal.—Calc. for $C_{17}H_{15}NO_4S$: C, 62.07; H, 4.59; N, 4.25; S, 9.73. Found: C, 62.32; H, 4.68; N, 4.37; S, 9.92.

Method B—A mixture of 6-iodo-7-nitrotetralin (3 g.), thiosalicylic acid (1.5 g.), amyl alcohol (30 ml.), sodium carbonate (1.3 g.), and 0.3 g. of copper powder was refluxed for 17 hr., and then 6 ml. of 50% NaOH was added. The alcohol was removed through steam distillation. The residue was boiled with 50 ml. water and filtered, and the filtrate was acidified with dilute hydrochloric acid. The formed product was collected, washed with water, and dried to give 2.4 g. of VIII. Upon recrystallization from benzene–alcohol, it melted at 162–164°, with no depression in melting point when mixed with the product prepared by Method A.

1,2-Cyclohexeno-4-nitrothiaxanthone (IX)—A mixture of VIII (2 g.) and phosphorus oxychloride (10 ml.) was refluxed for 1 hr. Then the excess of the oxychloride was removed under reduced pressure, and the residue was refluxed with 1 *N* HCl (50 ml.) for 30 min. The formed precipitate was filtered, washed with hot ammonia solution and then with water, and dried to give 1.5 g. of IX (78%). Upon recrystallization from benzene, it melted at 167–169°.

Anal.—Calc. for $C_{17}H_{13}NO_3S$: C, 65.58; H, 4.20; S, 10.29. Found: C, 65.67; H, 4.26; S, 10.38.

1,2-Cyclohexeno-4-aminothiaxanthone (X)—Compound IX (1 g.) and concentrated hydrochloric acid (5 ml.) in 20 ml. of ethyl alcohol were refluxed on a steam bath, and 1 g. of iron powder was added portionwise. Reflux continued for 4 hr. Then the mixture was cooled and the formed product was filtered. Sodium hydroxide solution (50%) was added to the filtrate to give 0.6 g. of X. It was recrystallized from benzene, m.p. 182–183°. The compound was identical to that previously prepared (5) as an intermediate in the synthesis of II.

2′,3′-Cyclohexeno-4′-nitrophenylsulfide-2-carboxylic Acid (XI)—A mixture of 5-amino-8-nitrotetralin (3 g.) and 50% sulfuric acid (20 ml.) was stirred at 0°. A solution of sodium nitrite (1 g.) in 15 ml. of water was added dropwise. Then the diazonium solution was added gradually to a solution of 1.6 g. of thiosalicylic acid in 50 ml. of 50% NaOH and heated on a water bath. After 2 hr., the mixture was cooled and the supernate was decanted. The residue was boiled with water and filtered, and the filtrate was acidified with acetic acid. The obtained product was dissolved in dilute ammonia and filtered. The filtrate was acidified, and the product was dried to give 2.8 g. of XI. Upon recrystallization from benzene–alcohol, it melted at 220°.

Anal.—Calc. for $C_{17}H_{15}NO_4S$: C, 62.07; H, 4.59; N, 4.25. Found: C, 61.78; H, 4.82; N, 4.51.

2′,3′-Cyclohexeno-4′-aminodiphenylsulfide-2-carboxylic Acid (XII)—A solution of IX (2 g.) in 25 ml. of acetic acid was stirred on a steam bath, and 2 g. of iron powder was added portionwise. Then 20 ml. of water was added. Heating was continued for 3 hr. The hot mixture was filtered and washed with dilute acetic acid. The filtrate was cooled and poured into ice water; the formed product was collected, washed with water, and dried to give 1.2 g. of XII. After recrystallization from benzene, it melted at 217–218°; IR: 3010 (cyclohexyl) and 690 (—S—) $cm.^{-1}$.

Anal.—Calc. for $C_{17}H_{17}NO_2S$: C, 68.34; H, 5.73; N, 4.69. Found: C, 68.42; H, 5.78; N, 4.76.

2-Amino-3,4-cyclohexenothiaxanthone (XIII)—To 10 g. of polyphosphoric acid heated at 170° was added 1 g. of XII; then heating was continued for 1 hr. After cooling, the mixture was poured into ice water. An excess of ammonia solution was added, and the solution was filtered. The formed product was washed with hot water and dried to give 0.70 g. of XIII, m.p. 235–237° [lit. (5) m.p. 239–240°].

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▲To whom inquiries should be directed. Present address: Karolinska Institutet, Medicinska Nobelinstitutet, Biokemiska avdelningen, Laboratorium för Enzymforskning, Solnavägen 1, S-104 01 Stockholm 60, Sweden.