New Schistosomicidal and Carcinostatic Agents of the Thiaxanthone Type (I)

I. NABIH and A. ZAYED

Abstract □ A new class of compounds, structurally related to axanthones and including the naphthalene system in its reduced tetrahydro form (tetralin), was synthesized and examined for activity in the areas of schistosomiasis and cancer. While 4-(β-diethylaminoethylamino)-1,2-cyclohexenothiaxanthone hydrochloride showed pronounced activity in both areas, its isomer, 2-(β-diethylaminoethylamino)-3,4-cyclohexenothiaxanthone hydrochloride, was completely inactive in schistosomiasis but still active against biologically inoculated tumors.

Keyphrases □ Thiaxanthone derivatives—synthesis as potential schistosomicidal and carcinostatic agents □ 4(or 2)-(β-Diethylaminoethylamino)-1,2(or 3,4)-cyclohexenothiaxanthone hydrochloride—synthesis as potential schistosomicidal and carcinostatic agents

Thiaxanthone derivatives are considered the first nonmetallic organic compounds found to be biologically active for the oral treatment of schistosomiasis (1).

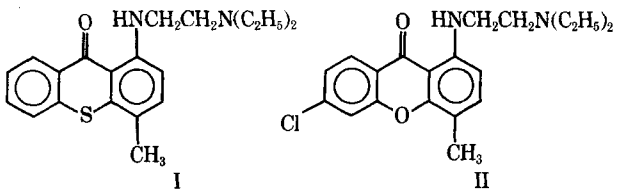
DISCUSSION

The most interesting representatives in this group relative to structure–activity relationships are 1-(β-diethylaminoethylamino)-4-methylthiaxanthone[1] (I) and its oxygen isostere, 1-(β-diethylaminoethylamino)-4-methyl-6-chloroxanthone[2] (II).

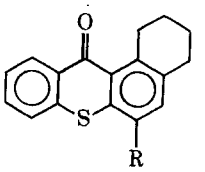
Studies showed that the structural feature necessary for the biological activity within this group is a dialkylaminoalkylamino side chain *para* to a methyl on the aromatic ring (2).

This class of compounds has received increasing interest, since they exert strong carcinostatic activity (3, 4). The most promising member in this group, I, is rather poorly tolerated by the human and produces toxic effects during treatment.

These considerations suggested the synthesis of new compounds of the thiaxanthone type, containing a methylene group in a cyclic six-membered, saturated ring system *para* to an amino side chain, thus incorporating the naphthalene system in its reduced tetrahydro form. This structure type was suggested because tetrahydronaphthalene tends to fix molecular oxygen under mild conditions to form the peroxide (5, 6). It also was biologically demonstrated that molecular oxygen is directly incorporated in the naphthalene metabolism through an oxygenation process (7, 8). Such oxygen carriers may affect glycolysis in the parasite, which seeks its main source of energy for living and reproduction through this metabolic process. Some involved enzymes, such as phosphoglyceraldehyde dehydrogenase and phosphofructokinase, contain sulfhydryl groups essential to their operation. The oxidation of these groups through the presence of oxygen may result in decreased enzymatic activity and, hence, diminished glycolysis.



[1] Lucanthone or Miracil D, Farbenfabriken Bayer AG, Leverkusen, West Germany.
[2] Miracil C, Farbenfabriken Bayer AG, Leverkusen, West Germany.



III: R = —Cl
IV: R = —$NHCOCH_3$
V: R = —NH_2
VI: R = —$NHCH_2CH_2N(C_2H_5)_2$

Another reason for including tetrahydronaphthalene in these structures is that they may biologically undergo oxidation or dehydrogenation to quinoids of the naphthoquinone type, and it is already established that naphthoquinones can highly reduce the rate of glycolysis in schistosomes (9).

Upon condensation of thiosalicylic acid with 6-chlorotetralin in the presence of concentrated sulfuric acid, 4-chloro-1,2-cyclohexenothiaxanthone (III) was obtained.

6-Chlorotetralin was prepared, as well as its 5-chloro isomer, through direct chlorination of tetralin in the presence of iodine as a catalyst (10). The separation of each was effected through sulfonation, by which only 5-chlorotetralin was sulfonated.

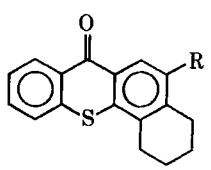
Replacement of the 4-chloro atom in III by the diethylaminoethylamino side chain failed to occur under different experimental conditions. An alternative route was adopted where 6-acetaminotetralin (11, 12) was the component in the condensation reaction with thiosalicylic acid. This tetralin derivative was prepared through nitration of tetralin, and a mixture of 5- and 6-nitrotetralins was formed (13). The latter was isolated through fractional distillation, reduced by iron and hydrochloric acid (14), and acetylated to give 6-acetaminotetralin. Its condensation with thiosalicylic acid in the presence of concentrated sulfuric acid gave 4-acetamino-1,2-cyclohexenothiaxanthone (IV), which, upon hydrolysis, gave 4-amino-1,2-cyclohexenothiaxanthone (V).

Diazotization of V and treatment with cuprous chloride gave a chloro product identical to III.

Treatment of the amino compound, V, with 2-diethylaminoethyl chloride gave 4-(β-diethylaminoethylamino)-1,2-cyclohexenothiaxanthone (VI).

Similarly, 5-acetaminotetralin was prepared through acetylation of the corresponding amine. Its condensation with thiosalicylic acid in the presence of concentrated sulfuric acid gave 2-acetamino-3,4-cyclohexenothiaxanthone (VII), which, upon hydrolysis, yielded 2-amino-3,4-cyclohexenothiaxanthone (VIII). Upon heating VIII with 2-diethylaminoethyl chloride, 2-(β-diethylaminoethylamino)-3,4-cyclohexenothiaxanthone (IX) was obtained.

Diazotization of VIII and treatment with cuprous chloride gave 2-chloro-3,4-cyclohexenothiaxanthone (X).



VII: R = —$NHCOCH_3$
VIII: R = —NH_2
IX: R = —$NHCH_2CH_2N(C_2H_5)_2$
X: R = —Cl

Biological Screening—*Schistosomiasis*—A group of mice infected with *Schistosoma mansoni* and showing viable ova in their stools were given oral doses of 60 mg./kg. of the hydrochloride of VI for 12 consecutive days.

Another infected group was similarly treated with the hydrochloride of IX at the same dosage level for the same period.

A third infected group was given no treatment and served as the control.

One week after treatment, collected stools from the groups were examined for ova excretion; the examination was continued for 6 weeks at 1-week intervals. The animals were sacrified 8 weeks thereafter, and the liver and mesenteric venules were examined for the presence of dead and living worms.

In the first group, treated with the hydrochloride of VI, there was a gradual decrease in ova excretion until none was found. Only dead worms were found in the liver.

In the second group, treated with the hydrochloride of IX, no depression in ova count was observed. Living worms were distributed in the liver and mesenteric venules.

In the third group, the control, no remarkable change in the ova count was observed. Living worms were found in the liver and in mesenteric venules.

From these findings, it could be concluded that VI is biologically active in experimental schistosomiasis, while its isomer (IX) is devoid of such activity.

Tumor Experiments—Two groups of mice, inoculated with 10^6 tumor cells of Ehrlich ascites carcinoma, were given 80 mg./kg. i.p. of both IV and VII hydrochlorides in aqueous solutions for 5 consecutive days.

Cell counts and weight observations after inoculation showed that both compounds possessed an inhibitory effect on tumor growth (Fig. 1).

Detailed data, methods, and techniques used for biological screening will be fully described in a separate report.

EXPERIMENTAL[3]

6-Chlorotetralin—This compound was prepared as described by Schroeter (10).

4-Chloro-1,2-cyclohexenothiaxanthone (III)—A mixture of 20 g. (0.13 mole) of thiosalicylic acid, 21.6 g. (0.13 mole) of 6-chlorotetralin, and 30 ml. of concentrated sulfuric acid was shaken at room temperature for 25 hr.; it was then cooled and poured over ice water. The product formed was collected and thoroughly washed with an ammonia solution and then water. After drying, it weighed 27 g. (70%). Upon recrystallization from acetone, it melted at 100–101°.

Anal.—Calc. for $C_{17}H_{13}ClOS$: C, 67.88, H, 4.36; S, 10.66. Found: C, 68.25; H, 4.58; S, 10.39.

4-Acetamino-1,2-cyclohexenothiaxanthone (IV)—To a mixture of 4 g. (0.026 mole) of thiosalicylic acid and 75 ml. of concentrated sulfuric acid was added portionwise, with shaking, 5 g. (0.026 mole) of 6-acetaminotetralin. Shaking continued at room temperature for 20 hr. Then the mixture was cooled and poured over ice water. The precipitate formed was collected and washed in succession with water, aqueous ammonium hydroxide, and water. After drying, the product weighed 6.7 g. (82%). Upon recrystallization from benzene, it melted at 230–231°.

Anal.—Calc. for $C_{19}H_{17}NO_2S$: C, 70.56; H, 5.3; S, 9.89. Found: C, 70.00; H, 5.49; S, 9.90.

4-Amino-1,2-cyclohexenothiaxanthone (V)—To a solution of 5 g. of II in 100 ml. of ethanol was added 30 ml. of hydrochloric acid (37%). This was refluxed for 5 hr. and then cooled. The precipitate formed was isolated and boiled with NaOH (50%) for 10 min. The product was filtered and washed with water to give 4.2 g. of III after drying. Upon recrystallization from ethanol, it melted at 185–186°.

Anal.—Calc. for $C_{17}H_{15}NOS$: C, 72.56; H, 5.37; N, 4.94; S, 11.39. Found: C, 72.53; H, 5.66; N, 4.79; S, 11.41.

[3] Melting points are uncorrected and were taken in open capillary tubes, using a Gallenkamp melting-point apparatus. Microanalyses were performed by the Microanalytical Laboratory, National Research Centre, Cairo, U.A.R., and the Spang Microanalytical Laboratory, Ann Arbor, Mich. IR spectra were determined on a U.R. 10 Carl Zeiss-Jena IR, using KBr pellets.

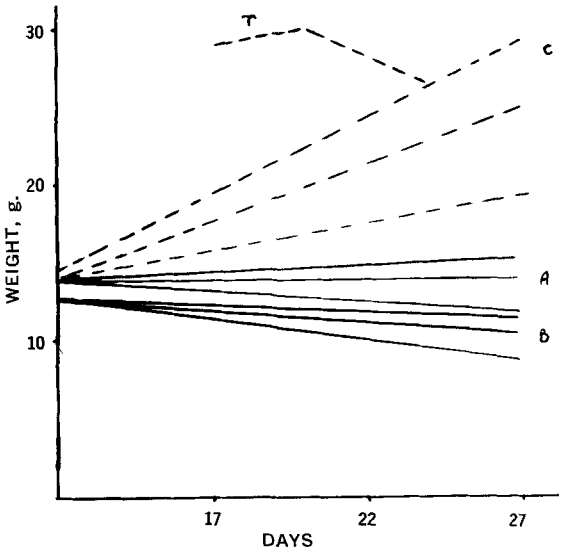


Figure 1—*Effect of treatment with Compound VI hydrochloride (A) and IX hydrochloride (B) on growth of mice tumors of the Ehrlich ascites carcinoma type. Each mouse was implanted with 10^6 tumor cells. C = control. T illustrates effect of treatment by A on a control mouse after 20 days of inoculation.*

4 - (β - Diethylaminoethylamino) - 1,2 - cyclohexenothiaxanthone (VI)—A mixture of 1 g. of III and 0.5 ml. of 2-diethylaminoethyl chloride was heated under reflux at 170° for 3 hr.; after cooling, the product was boiled with NaOH (50%) for 10 min. After decantation of the alkali, the residue was recrystallized from ethanol to give 1.2 g. (90%) of IV, m.p. 95–96°. IR: 1630 (C=O), 3310 (—NH—), 680 (—S—), and 3017 $cm.^{-1}$ (cyclohexyl).

Anal.—Calc. for $C_{23}H_{28}N_2OS$: C, 72.59; H, 7.41; N, 7.36; S, 8.43. Found: C, 72.44; H, 7.27; N, 7.34; S, 8.51.

The hydrochloride was prepared by dissolving VI in dry ether and passing a stream of dry HCl through the solution. The yellow product was filtered, washed with dry ether, and dried to give a hydrochloride, m.p. 150–155°.

4-Chloro-1,2-cyclohexenothiaxanthone (III)—To a cold mixture of 40 ml. of hydrochloric acid (37%), 10 ml. of acetic acid, and 2 g. of V was added a solution of 1 g. of sodium nitrite in 50 ml. water. This mixture was poured over a solution of 1 g. of cuprous chloride in 50 ml. of hydrochloric acid (37%). The product was filtered to give 1.3 g. After recrystallization from acetone, it melted at 100–101° (identical to that obtained through the direct condensation of 6-chlorotetralin with thiosalicylic acid, with no depression in a mixed melting-point determination).

2-Acetamino-3,4-cyclohexenothiaxanthone (VII)—A mixture of 4 g. (0.026 mole) of thiosalicylic acid, 5 g. (0.026 mole) of 5-acetaminotetralin, and 80 ml. of concentrated sulfuric acid was shaken at room temperature for 30 hr., stirred at 60° for 2 hr., and then poured after cooling over ice water. The precipitate formed was filtered and washed with water, ammonia solution, and water. After drying, it gave 6 g. (70%) of VII. Upon recrystallization from benzene, it melted at 298–300°.

Anal.—Calc. for $C_{19}H_{17}NO_2S$: C, 70.56; H, 5.3; N, 4.32, S, 9.89. Found: C, 70.57; H, 5.16; N, 4.05; S, 9.80.

2-Amino-3,4-cyclohexenothiaxanthone (VIII)—A mixture of 5 g. of VII, 125 ml. of absolute ethanol, and 30 ml. of hydrochloric acid (37%) was refluxed for 10 hr. and then cooled. The hydrochloride formed was filtered and dried; it melted at 285–287°. This was boiled with 50% sodium hydroxide for about 15 min. and then filtered, washed with water, and dried to give 4.2 g. (96%) of the free amine VIII. It was recrystallized from ethanol as yellow needles, m.p. 239–240°.

Anal.—Calc. for $C_{17}H_{15}NOS$: C, 72.56, H, 5.37; N, 4.94; S, 11.39. Found: C, 72.86; H, 5.64; N, 5.23; S, 11.52.

2 - (β - Diethylaminoethylamino) - 3,4 - cyclohexenothiaxanthone (IX)—A mixture of 1 g. of VIII and 0.5 ml. of 2-diethylaminoethyl chloride was heated under reflux at 180° for 7 hr. The mixture was boiled with a few milliliters of 50% sodium hydroxide and then

washed with water. Recrystallization of the residue from dilute ethanol gave 1.3 g. (94%) of IX, m.p. 114–115°. IR: 1620 cm.$^{-1}$ (C=O), 3340 cm.$^{-1}$ (—NH—), 700 cm.$^{-1}$ (—S—), and 3017 cm.$^{-1}$ (cyclohexyl).

Anal.—Calc. for $C_{23}H_{28}N_2OS$: C, 72.59; H, 7.41; N, 7.36; S, 8.43. Found: C, 72.53; H, 7.25; N, 7.28; S, 8.55.

The hydrochloride was similarly prepared as described for IV, m.p. 169–170°.

2-Chloro-3,4-cyclohexenothiaxanthone (X)—Diazotization of VIII and treatment of the diazonium solution with cuprous chloride, as described for III, gave X. After recrystallization from ethanol, it melted at 186–187°.

Anal.—Calc. for $C_{17}H_{13}ClOS$: C, 67.88; H, 4.36; S, 10.66. Found: C, 68.41; H, 4.67; S, 10.73.

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Preparation and Activity of β-Substituted Acetylcholine Iodides

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Abstract □ The enantiometers of (−)- and (+)-acetyl β-ethylcholine iodide were prepared following the resolution of 1-dimethylamino-2-butanol from (+)-tartaric acid and (+)-bromocamphorsulfonic acid for the (−)- and (+)-isomers, respectively. The absolute configuration of the (−)-enantiomer was determined by the synthesis of (−)-acetyl β-ethylcholine iodide from R(−)-2-hydroxybutyric acid. The optically active acetyl β-phenylcholine iodides were prepared from the optically active mandelic acids. These enantiomers show that the R(−)-isomers have greater affinity for the acetylcholinesterase receptor and that the S(+)-isomers are more potent agonists on guinea pig ileum.

Keyphrases □ Acetylcholine iodides, β-substituted—preparation, activity □ Acetyl β-ethylcholine iodide, enantiomers—preparation, absolute configuration, activity

It was shown previously (1, 2) that acetylcholinesterase and the smooth muscle of guinea pig ileum each reacts best with only one of the enantiomers of acetyl β-methylcholine. Cocolas *et al.* (3) also pointed out that the enantiomers of acetyl β-methylcholine likely assume a conformation in which the interface between them and the corresponding susceptible receptor area, during interaction, is the same as that for acetylcholine; that is, the facet of the choline fragment facing the receptor is the side that resembles acetylcholine.

We sought to extend the study of stereochemical requirements at the acetylcholinesterase and the smooth muscle receptor in guinea pig ileum by examining the activity of β-ethyl and β-phenyl acetylcholines. Some investigators (1, 4, 5) assumed that S(+)-acetyl β-methylcholine has better affinity for acetylcholinesterase than the R(−)-enantiomer. It might be expected that acetylcholinesterase would produce a greater disparity between the action of the enantiomers of β-ethyl- and β-phenyl-substituted acetylcholines, since the increase in bulk would produce a more remote possibility of similar accommodation of the enantiomeric pairs. An examination of the enzymatic activity of each optical isomer of β-ethyl and β-phenyl acetylcholine iodide was undertaken to test the hypothesis of enantiomeric stereoselectivity of the acetylcholinesterase receptor area. The potency of these enantiomeric pairs on guinea pig ileum was also investigated as a measure of their biologic activity on the muscarinic receptor site.

EXPERIMENTAL[1]

1-Dimethylamino-2-butanol—To a solution of 50 g. 1-nitro-2-butanol (6) in 50 ml. 90% formic acid and 125 ml. formalin (40%)

[1] Melting points were taken on a Mel-Temp apparatus and are uncorrected. Specific rotations were taken on a Cary 60 spectropolarimeter. The IR spectra were run on a Perkin-Elmer model 257 spectrophotometer. Microanalyses were carried out by M-H-W Laboratories, Garden City, Mich.