in the form of the drug must be taking place at the higher concentrations. Evidence in the literature indicates that a variety of surface-active compounds may undergo association (13), and when such association takes place the monomer concentration can no longer be equated to stoichiometric concentrations. Certain β-lactam antibiotics have shown surface-active properties (14).

Figure 4 shows a plot of log concentration *versus* surface tension for the sodium salts of nafcillin and ampicillin. The apparent critical micelle concentration (CMC) for sodium nafcillin (the break in the plot) is in the range of 20–30 mg/ml, the same point where saturation of the transport was observed. Sodium ampicillin, on the other hand, has a CMC above the concentrations used in the transport studies.

In summary, these studies show that the β-lactam antibiotics (both penicillins and cephalosporins) are not transported across the everted rat gut by any specialized transport mechanism. This was true for both penicillinase-sensitive and penicillinase-resistant compounds in this series, as well as for compounds (amphoteric) resembling amino acids. In addition, the results obtained with the model compound, glucose, demonstrate the utility of the everted rat gut for this type of investigation.

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## ACKNOWLEDGMENTS AND ADDRESSES


Received February 27, 1974, from the *Exploratory Pharmacy Section, Pharmacy Research and Development Division, Wyeth Laboratories, Inc., Radnor, PA 19087*

Accepted for publication June 11, 1974.

The authors thank Dr. James Boylan of Eli Lilly and Co. for kindly supplying cephalexin monohydrate and cephaloglycin dihydrate, and Paul Osimo for technical assistance.



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# New Carcinostatic Agent with Possible Selective Activity on Tumor Cells


**I. NABIH [x] and A. ZAYED**



**Abstract** □ The nitrogen mustard analog of the antitumor agent 1,2-cyclohexeno-4-(β-diethylaminoethylamino)thiaxanthone was synthesized. Its biological activity in experimental tumors *in vivo* and on human leukemia *in vitro* is described.



**Keyphrases** □ Nitrogen mustard analog of 1,2-cyclohexeno-4-(β-diethylaminoethylamino)thiaxanthone—synthesis and screening for carcinostatic and antitumor activities □ 1,2-Cyclohexeno-4-(β-diethylaminoethylamino)thiaxanthone, nitrogen mustard analog—synthesized and screened for carcinostatic and antitumor activities □ Antitumor activity—synthesis and screening of nitrogen mustard analog of 1,2-cyclohexeno-4-(β-diethylaminoethylamino)-thiaxanthone


Previous articles (1, 2) reported the antitumor activity of a new group of compounds related to the cyclohexenothiaxanthones. These are mainly represented by the three isomers 1,2-cyclohexeno-4-(β-diethylaminoethylamino)thiaxanthone (I), 2-(β-diethylaminoethylamino)-3,4-cyclohexenothiaxanthone (II), and 1-(β-diethylaminoethylamino)-3,4-cyclohexenothiaxanthone (III).

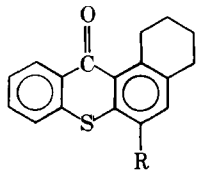
## DISCUSSION

*Structurally*, the cyclohexenothiaxanthone system incorporates the thiaxanthone skeleton along with that of tetrahydronaphthalene. The rational for this combination is that the thiaxanthone tends to polarization due to resonance (3), thus indicating separation of charges and high dipole moments. This physical property would permit a selective attack on the tumor cells, avoiding the normal ones, at the appropriate concentrations, since the cellular membranes of the former carry considerably higher negative charges than their normal homologs (4, 5). Studies indicate that the thiaxanthone moiety in these compounds could be used as a carrier or a target-seeking device for the selective attack on tumor cells through electrostatic attraction.

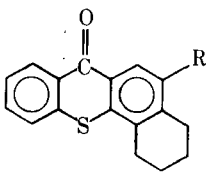
The other structural part of the molecule, the tetralin, tends to attach molecular oxygen in an oxygenation process (6). Such oxygen carriers could affect the glycolysis in the cell by oxidation of the essential SH-groups of some enzymes necessary for cell growth and replication. In addition, the tetralins could undergo biological transformation to naphthoquinone-type structures, which are known to depress cell division.

In experimental tumors of the Ehrlich ascites carcinoma type, mammary carcinoma, Maloney virus lymphoma, and mouse ascites leukemia, the three isomers showed pronounced antitumor effects (1, 2). *In vitro* experiments on cells of these tumor types, as well as on leucocytes from several forms of human leukemia, showed that the compounds possessed varying but definite damaging effects on malignant cells.

The present work illustrates the synthesis of an alkylating agent with properties similar to those of nitrogen mustards, where the cyclohexenothiaxanthone structure may retain its antitumor effect and also serve as a carrier for the alkylating group. This is of importance in relation to the transport and chemical reactivity of this group. This approach had received considerable interest con-



I



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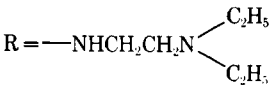
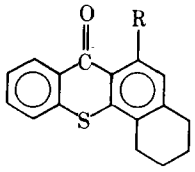
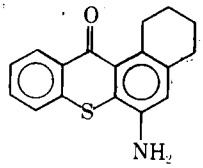
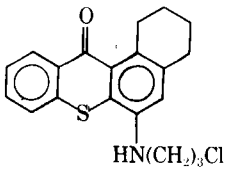




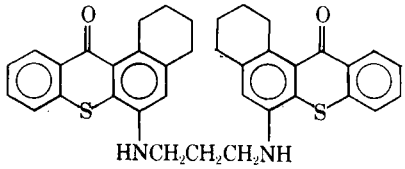
III



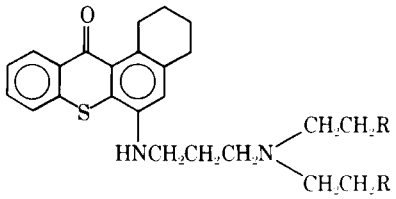
IV



V



VI



VII: R = OH
VIII: R = Cl

cerning the so-called antimalarial mustards as possible chemotherapeutic agents in the management of neoplastic diseases (7). In chloroquine and quinacrine, which possess side chains that terminate in tertiary diethylamino groups, both terminal hydrogen atoms on each group were substituted with chlorine to give the nitrogen mustard analogs. In experimental and clinical trials, both analogs showed pronounced antitumor activity (8).

This finding initiated the synthesis of the nitrogen mustard analog of the compound, I. It was synthesized with three carbon atoms between the two nitrogens of the side chain. Compounds with increased number of carbon atoms between the nitrogens of the side chain of the antimalarials nitrogen mustard analogs showed increased antitumor activity and decreased host toxicity (9).

Reaction of 1,2-cyclohexeno-4-aminothiaxanthone (IV) (1) with 1-bromo-3-chloropropane in the presence of sodium acetate gave a mixture of 1,2-cyclohexeno-4-(β-chloropropylamino)thiaxanthone (V) and 4,4′-bis(1,2-cyclohexenothiaxanthone)-1,3-diaminopropane (VI). When this reaction was carried out in boiling *n*-butanol, V was the predominant product. Condensation of V with diethanolamine in the presence of sodium acetate gave 4-bis(2′-hydroxyethyl)aminopropylamino-1,2-cyclohexenothiaxanthone (VII). This compound, upon treatment with phosphorus oxychloride or thionyl chloride, gave the desired product, 4-bis(2′-chloroethyl)aminopropylamino-1,2-cyclohexenothiaxanthone (VIII).

## EXPERIMENTAL

**Biological**—The product VIII is slightly soluble in water but is freely soluble in 70% ethanol. It was used as a colloidal suspension (through dissolution in ethanol and then dilution with water up to 8%).

In *in vivo* experiments, tumors of the Ehrlich ascites carcinoma, mammary carcinoma, and Maloney virus lymphoma cell lines were used for tests in mice, along with the appropriate controls. Treatment began on the 3rd day postinoculation of $10^6$ cells of Ehrlich ascites carcinoma and $10^4$ cells of mammary carcinoma and Maloney virus lymphoma types in the proper mice per host strains. (For Ehrlich ascites carcinoma type, CBA mice strains were used; for mammary carcinoma and Maloney virus lymphoma types, AxSn mice strains were used.) Doses were 60 mg/kg body weight daily for 5 consecutive days. In the treated mice, tumors did not develop throughout the follow-up 6-week postinoculation observation. In controls, mice that had been similarly inoculated but not treated developed tumors.

In the *in vitro* experiments, human leucocytes from acute myeloblast leukemia were used as substrates for the test compound. The leucocytes were collected and suspended in a balanced salt solution. Normal leucocytes collected from normal blood were used in the same manner as controls to test for the selectivity of the compound.

The compound to be tested was added as a colloidal suspension in 8% ethanol to correspond to two different concentrations of 0.2 and 0.5 mg/ml of the total medium. The normal leucocytes used as a control were also suspended in a balanced salt solution diluted with 8% ethanol and similarly treated at both concentration levels.

Examinations were made of dead and living leucocytes 24 hr after the addition of the material at both concentrations to the normal leucocytes and to the malignant ones. For differentiation between living and dead cells, trypan blue staining solution was used in aliquots taken from the treated leucocytes. This test showed that all malignant leucocytes were dead at both concentrations. Only a few normal cells were dead (10–15%) at both concentrations.

These results indicate that a certain selectivity exists for the uptake of the compound by the malignant leucocytes, which is not seen with the normal ones.

**Chemical**[1]—*2-Cyclohexeno-4-(β-chloropropylamino)thiaxanthone (V) and 4,4-Bis(1,2-cyclohexenothiaxanthone)-1,3-diaminopropane (VI)*—A mixture of IV (1) (3 g, 0.01 mole), 1-bromo-3-chloropropane (1.8 g, 0.01 mole), and sodium acetate (1.5 g, 0.01 mole) was heated at 150° for 3 hr. The gummy product was washed with sodium carbonate solution and water and extracted with ethyl acetate. The ethyl acetate was distilled and the residue was recrystallized twice from ethanol to give 1.4 g (37%) of V, mp 130–132°.

*Anal.*—Calc. for $C_{20}H_{20}ClNOS$: C, 67.12; H, 5.63; Cl, 9.90; N, 3.91. Found: C, 66.97; H, 5.56; Cl, 10.02; N, 3.83.

The ethyl acetate-insoluble residue fraction was recrystallized from benzene to give 1.2 g (19%) of VI, mp 220–222°.

*Anal.*—Calc. for $C_{37}H_{34}N_2O_2S_2$: C, 73.72; H, 5.69; N, 4.65. Found: C, 73.68; H, 5.65; N, 4.59.

The reaction was repeated under the following conditions. The same mixture of reactants was heated under reflux in *n*-butanol (30 ml) for 10 hr. The butanol was removed by steam distillation and the residue was recrystallized from ethanol to give 2.9 g (75%) of V.

*4-Bis(2′-hydroxyethyl)aminopropylamino-1,2-cyclohexenothiaxanthone (VII)*—A mixture of V (3.3 g, 0.0092 mole), diethanolamine (1 ml), and sodium acetate (2 g) was heated at 150° for 5 hr. The reaction product was washed with sodium carbonate solution and water and recrystallized from ethanol to give 3 g (84%) of VII, mp 151–153°.

*Anal.*—Calc. for $C_{24}H_{30}N_2O_3S$: C, 67.57; H, 7.07; N, 6.58. Found: C, 67.48; H, 6.98; N, 6.56.

[1] Melting points were taken in open capillary tubes by the use of a sulfuric acid bath and Gallenkamp melting-point apparatus and are uncorrected. Microanalyses were performed by the Microanalytical Laboratory, National Research Center, Cairo, U.A.R. IR spectra were determined on a UR 10 Zeiss-Jena IR, using KBr pellets.

*4-Bis(2'-chloroethyl)aminopropylamino-1,2-cyclohexenothiaxanthone (VIII)*—Compound VII (2 g, 0.0047 mole) was refluxed with phosphorus oxychloride (6 ml) for 3 hr, and excess oxychloride was removed under reduced pressure. The product was washed with sodium carbonate solution and water and recrystallized from benzene–petroleum ether to give 1.8 g (84%) of VIII, mp 160–162°.

*Anal.*—Calc. for $C_{24}H_{28}Cl_2N_2OS$: C, 62.26; H, 6.10; Cl, 15.32; N, 6.05. Found: C, 62.28; H, 6.17; Cl, 14.98; N, 5.97.

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## ACKNOWLEDGMENTS AND ADDRESSES


Received September 21, 1973, from the *National Research Center, Dokki, Cairo, U.A.R.*

Accepted for publication June 11, 1974.



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# *COMMUNICATIONS*

## Evaluation of Mathematical Models for Diffusion from Semisolids


**Keyphrases** □ Diffusion, semisolids—evaluation of mathematical models □ Semisolids—evaluation of mathematical models for diffusion of drugs from semisolid systems


*To the Editor:*

Recently, Ayres and Laskar (1) reported an evaluation of models used to study the release of drugs from semisolid systems. Their analysis encompassed literature data (2–7) obtained on diverse experimental systems containing suspended or dissolved drugs in the form of gels, ointments, and emulsions. We find ourselves critical of both the approach and methods used in this analysis.

To establish the framework for our objections, it is necessary to present again the fundamental equations governing release of drugs from such systems. For semisolid systems initially containing uniformly dissolved drug in a homogeneous base, the amount of drug released in one dimension is given by Eq. 1 (8):

$$Q = hC_0\left[1 - \frac{8}{\pi^2}\sum_{m=0}^{\infty}\frac{1}{(2m+1)^2}\exp\left(-\frac{D(2m+1)^2\pi^2t}{4h^2}\right)\right] \quad \text{(Eq. 1)}$$

where:

$Q$ = amount of drug released per unit area of application
$h$ = thickness of vehicle layer
$C_0$ = initial concentration of drug in the vehicle
$D$ = diffusion constant of drug in the vehicle
$t$ = time after application
$m$ = integer, with values from 0 to $\infty$

Equation 1, which is derived from Fick's second law of diffusion, is a valid expression for release from one side of a layer of vehicle containing drug in solution as long as the following assumptions are met (8):

1. There is a single drug in true solution and initially uniformly distributed throughout the vehicle.
2. The composition of the vehicle remains fixed during the diffusion process, *i.e.*, components other than the drug do not leave or enter the vehicle phase.
3. The diffusion constant of the drug is independent of time and position in the vehicle.
4. The drug reaching the receptor side of the vehicle layer is cleared rapidly.

For most practical applications of Eq. 1, a simplified form (Eq. 2) may be used up to about 30% drug release (8):

$$Q = 2C_0\left(\frac{Dt}{\pi}\right)^{1/2} \quad \text{(Eq. 2)}$$

Higuchi (9) derived an equation (Eq. 3) for studying the rate of drug release from ointment bases containing drugs in suspension. This equation relates the amount of drug released to time and other variables of the system:

$$Q = (2C_0 - C_s)\sqrt{Dt\left/1 + \frac{2(C_0 - C_s)}{C_s}\right.} \quad \text{(Eq. 3)}$$

where $C_s$ = solubility of drug as units per centimeter$^3$ in the external phase of the ointment, and $D$ = diffusion constant of drug in the external phase of the ointment.

The operative boundary conditions for the use of Eq. 3 are as follows: *(a)* the suspended drug is in a