4-arylazo-3,5-dimethyl-(2*H*)-1,2,6-thiadiazine 1,1-dioxides prepd in a similar way are listed in Table I.

4-Phenylazo-3,5-diphenyl-(2*H*)-1,2,6-thiadiazine 1,1-Dioxide. Sulfamide (0.48 g) when reacted with 1.64 g of 1,3-diphenyl-1,2,3-propanetrione-2-phenylhydrazone as in method A, gave pale yellow plates (55%), mp 268° (EtOH). *Anal.* ($C_{21}H_{16}N_4O_2S$) C, H, N, S. Properties of the other 4-arylazo-3,5-diphenyl-(2*H*)-1,2,6-thiadiazine 1,1-dioxides prepd are given in Table I.

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Potential Antitumor Agent Dicyclohexylammonium 2-{4-[*N*,*N*-Bis(2-chloroethyl)amino]phenoxy}-2-methylpropionate

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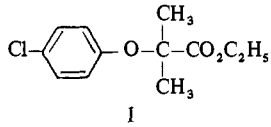
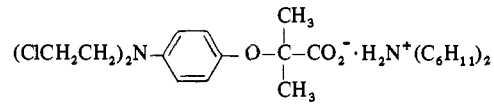
The antihyperlipidemic agent ethyl 2-(*p*-chlorophenoxy)-2-methylpropionate (1) (clofibrate)[1] has been applied as a drug to reduce serum lipids. After absorption from the gastrointestinal tract clofibrate undergoes rapid hydrolysis by serum enzymes to the free acid which is strongly bound to plasma proteins. Animal studies indicate that clofibrate remains almost exclusively in the blood. Distribution of the free acid is limited to the plasma and extracellular fluids. After administration of effective doses, no trace was found in muscle, fat, heart, spleen, cerebrospinal fluid, or bile. With larger doses transient amounts were detected in the liver.[1-4]



I

In man, absorption from the gastrointestinal tract is uniform. Serum levels are linearly proportional to dosage, from 3 to 24 hr after administration of effective doses. Furthermore, clofibrate is cleared from the plasma in an average half-life time of 12 hr.

It was surmised that a cytotoxic compound, selective to malignant cells, of similar pharmacological properties as described above, could serve as a potential chemotherapeutic agent against neoplastic diseases of blood. Thus, the N mustard II was synthesized which differs from I, mainly, in that Cl on the Ph ring is replaced by a bis(chloroethyl)amino group. The preparation of an amine salt of the free acid rather than the ester was decided upon in order to favor slow absorption from the gastrointestinal tract. These modifications in structure, it was anticipated, would not significantly alter the ability of the molecule to bind to plasma proteins. Hence, a distribution limited predominantly to blood and extracellular fluids might be expected.



Dicyclohexylammonium 2-{4-[*N*,*N*-bis(2-chloroethyl)-amino]phenoxy}-2-methylpropionate (II) was prepared in a 6-step procedure as outlined in Scheme I.

Scheme I

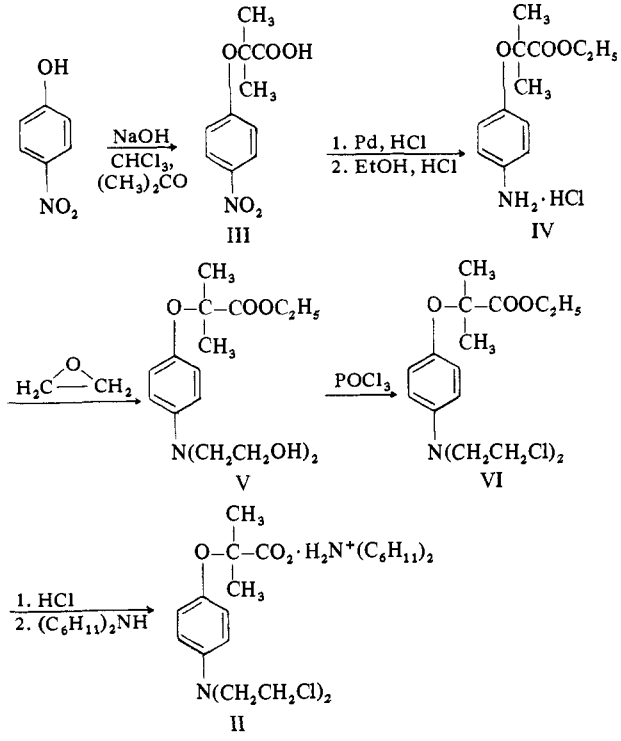


Table I. Antitumor Activity of Dicyclohexylammonium 2-{4-[*N*,*N*-Bis(2-chloroethyl)amino[phenoxy}-2-methylpropionate against Lymphoid Leukemia L-1210 in Mice

Dose,[a] mg/kg	Survivors	Animal wt diff[b] ($T - C$), g	Survival days Test	Survival days Control	T/C,[c] %
400	0/6	−0.4	0	9.4	
300	0/6	−0.4	0	9.6	
150	4/6	−4.3	7.5	9.6	
75	6/6	−3	13.7	9.6	142
50	6/6	−1.8	12.2	9.6	127
33	6/6	−1	10.8	9.6	112
22	6/6	−1.1	10.5	9.6	109
75[d]	6/6	−3.6	8.7	9.3	93
50[d]	6/6	−4.5	8.8	9.3	94
33[d]	6/6	−3.8	9.0	9.3	96
22[d]	6/6	−3.7	13.7	9.3	147

[a] Ip route. [b] Average wt change of test group minus control group. [c] Ratio of survival time of test to control animals. [d] Dose was repeated for 9 consecutive days.

The activity against L-1210 lymphoid leukemia is presented in Table I.

Experimental Section

2-(4-Nitrophenoxy)-2-methylpropionic Acid (III).[5] $CHCl_3$ (100 g, 0.83 mole) was gradually added to a mixt of *p*-nitrophenol

(54 g, 0.39 mole), anhyd Me_2CO (400 g, 6.9 moles), and NaOH (100 g, 2.5 moles) at such a rate (*ca.* 1 hr) to maintain gentle reflux. The reaction mixt was then refluxed for an addl 4 hr and concd under reduced pressure. After addn of H_2O (600 ml) the mixt was passed through a filter while hot, the filtrate cooled, acidified (HCl), and extd ($CHCl_3$). The ext was purified by treatment with decolorizing C. After evapn of the solvent the residue was taken up in 2 *N* NaOH, the soln treated with activated C and, subsequently, acidified with dil HCl. On cooling a solid formed that was filtered off, washed (cold H_2O), dried, and recrystd from CCl_4 to give 36 g (40%) of product, mp 123–124°. *Anal.* ($C_{10}H_{11}NO_5$) C, H, N, O.

Ethyl 2-(4-Aminophenoxy)-2-methylpropionate HCl Salt (IV). A mixt of 2-(4-nitrophenoxy)-2-methylpropionic acid (14 g, 0.062 mole), 10% Pd/C (1 g), and 10 ml of concd HCl in 100 ml of EtOH was shaken under H_2 until the uptake of gas ceased (*ca.* 3 hr). After filtration the solvent was evapd under reduced pressure. The wet residue was dissolved in EtOH–PhH. After evapn of the azeotropic mixt, 14.3 g (99%) of cryst 2-(4-aminophenoxy)-2-methylpropionic acid·HCl was obtained. This product (14.3 g, 0.062 mole), without further purification, was dissolved in 200 ml of abs EtOH. Dry HCl was passed through the soln which was then heated under reflux for 1 hr, concd to 25 ml, and cooled. Addn of 200 ml of Et_2O caused pptn of a white cryst material which was collected on a filter, washed (Et_2O), and dried. Crude material (13.4 g, 83%) (mp 159–163°) was obtd. A sample was recrystd from MeCN, mp 163–165°. *Anal.* ($C_{12}H_{18}ClNO_3$) C, H, Cl, N, O.

Ethyl 2-{4-[*N*,*N*-Bis(2-hydroxyethyl)amino]phenoxy}-2-methylpropionate (V). To the crude ester IV (11.4 g, 0.044 mole) in 100 ml of H_2O was added 5 g of $NaHCO_3$, 80 ml of glac AcOH, and 10 ml of EtOH. The soln was cooled to −5°, 30 ml of liq ethylene oxide added, and the mixt allowed to stand at room temp for 4 days. The mixt was then neutralized with $NaHCO_3$ and extd (CH_2Cl_2, 3 × 125 ml). The combined exts were washed (H_2O, 4 × 125 ml) to remove glycol polymers, and dried ($MgSO_4$). The soln was evapd under reduced pressure to yield 11.1 g of an oily material. The oil was dissolved in warm Et_2O and treated with activated C. After evapn of the solvent under reduced pressure 11 g (80%) of a light tan oily product was obtd. The HCl salt had mp 113–115°. *Anal.* ($C_{16}H_{26}ClNO_5$) C, H, Cl.

Ethyl 2-{4-[*N*,*N*-Bis(2-chloroethyl)amino]phenoxy}-2-methylpropionate (VI). To V (8.7 g, 0.028 mole) was added 65 ml of $POCl_3$ and the mixt heated on a steam bath for 0.5 hr. The soln was poured on 800 ml of crushed ice while stirring, neutralized to pH 5 with NaOAc, and extd (CH_2Cl_2, 3 × 200 ml). The combined exts were washed (H_2O, 2 × 200 ml), dried ($MgSO_4$), and treated with activated C. The solvent was evapd under reduced pressure. The remaining oily material was dissolved in CH_2Cl_2–PhMe (30:200 ml) and the soln evapd to dryness at 50° under reduced pressure. A brown oily product (9.8 g, 98%) was obtd which was used without further purification in the subsequent reaction.

Dicyclohexylammonium 2-{4-[*N*,*N*-Bis(2-chloroethyl)amino]-phenoxy}-2-methylpropionate (II). VI (8.6 g, 0.024 mole) was heated on a steam bath in 150 ml of concd HCl for 0.5 hr, cooled, neutralized with NaOAc to pH 5, and extd with CH_2Cl_2 (3 × 200 ml). The combined exts were washed (H_2O, 2 × 200) and dried ($MgSO_4$). The solvent was then evapd under reduced pressure. The remaining oily material was dissolved in PhMe and the soln evapd to dryness under reduced pressure. A viscous oily product (4.2 g, 55%) was obtd. *Anal.* ($C_{14}H_{19}Cl_2NO_3$), C, H, N, O.

The dicyclohexylamine salt was made by add of dicyclohexylamine in Et_2O and subsequent pptn with petr ether (bp 30–60°), mp 139–141°. The recrystd salt (Et_2O–petr ether) melted at 141–143°. *Anal.* ($C_{26}H_{42}Cl_2N_2O_3$) C, H, Cl.

Antitumor Test. The tests against lymphoid leukemia L-1210 were carried out by CCNSC, National Cancer Institute, according to test procedures described in ref 6.

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Structure–Activity Studies on Sulfamyl Diuretics

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An examination of the structure–activity relationship of thiazide and hydrothiazide diuretics led to the conclusion that apart from other factors a substituent in neighboring position to the sulfonamide group must be present and that compounds having Cl, Br, NO_2, and CF_3 groups in this position are highly active.[1] In generalizations about structural features that lead to diuretic activity in both the aromatic disulfonamides and the thiazides and their related structures this substituent was designated the "activating group."[2] It was suggested that the outstanding activating groups are halogen or a "pseudohalogen" as CF_3. Even the non-thiazide-type high-ceiling diuretic 4-chloro-*N*-(2-furylmethyl)-5-sulfamylanthranilic acid[3,4] (furosemide) could be reduced to the empirical rules.[2] The recently described diuretic activity of 3-*n*-butylamino-4-chloro-5-sulfamylbenzoic acid[5] seemed to provide further support for the predicted structural requirements. On the other hand recent reports[6,7] disclosed that certain 4-substituted 3-amino-5-sulfamylbenzoic and 5-sulfamylanthranilic acid derivatives are greatly superior in potency to the corresponding 4-Cl compounds. Both similarities and differences in the influence of the different 4 substituents on the diuretic activity of these 2 series were seen. In the 4-RNH-3-amino-5-sulfamylbenzoic acid series, highly potent compounds were found while among the anthranilic acid derivatives the 4-RNH group afforded less active agents. C_6H_5O and C_6H_5S in the 4 position, however, provided increased activity in both series.

These facts prompted us to synthesize analogs of selected thiazide-type diuretics[4,8] of different structures bearing C_6H_5O or C_6H_5S groups instead of Cl or CF_3 ortho to the sulfonamide group. Furthermore, we investigated the corresponding analogs of 4-chloro-5-sulfamylsalicylic acid[9] (**21**), which had been shown in a preclinical study,‡ to be a high-ceiling diuretic although about 0.1 as potent as furosemide.

Chemistry. As analogs to chlorothiazide (**1**) and flumethiazide, hydrochlorothiazide and hydroflumethiazide, and bendroflumethiazide, 6-phenylthio-7-sulfamyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide (**2**), 6-phenylthio-7-sulfamyl-2,3-dihydro-4*H*-1,2,4-benzothiadiazine 1,1-dioxide (**4**), and the corresponding 3-benzyl compound **5** were synthesized according to Scheme I. The preparation of the disulfamylaniline **3** by halogen exchange in 3-chloro-4,6-disulfamylaniline failed in our hands. However, ring closure to chlorothiazide (**1**) increased the reactivity of the Cl to such a degree as to allow the exchange reaction with the thiophenoxide ion to form **2**. For purification crude **2** is conveniently hydrolyzed to **3**. Ring-closure reactions to **2**, **4**, and **5** were performed by established methods.[4]

‡Personal communication from Dr. K. H. Olesen, Medical Department B, Rigshospital, University of Copenhagen, Medical School.