traction (7) and equilibration (8) reflexes were not changed, and pentylenetetrazol- or electroshock-induced convulsive effects (9, 10) were not antagonized. No anxiolytic activity was observed until 30 mg/kg was administered (11). No central anticholinergic action was noted at 100 mg/kg by the classical antipilocarpine test in rats (14).

Prochlorperazine-induced catalepsy (13) was inhibited at the ED_{50} value of 30 mg/kg ($p \leq 0.05$, Student t test). The amphetaminic group toxicity effect was potentiated at 50 mg/kg (13). Thus, the results reported here suggest that I may be a significant and selective antidepressant compound.

Structural Determinations—The crystal data were: space group P2/C; a = 7.109(3), b = 12.766(3), and c = 16.110(4) Å; β = 97.52(5); V = 1395.2 Å^3; Z = 4; D_c = 1.33; and R = 0.052.

The distances between the center of the phenyl ring and N-6 and N-8 atoms are 3.73 and 5.30 Å, respectively (Fig. 1). The interatomic distance[3] between N-6 and N-8 is 2.40 Å.

PMR Spectrographic Results—The shifts (expressed in δ parts per million using deuterated dimethyl sulfoxide as the solvent) (Fig. 2) corresponding to the methylene protons (c and d) of I were designed unambiguously and compared to shifts reported previously for other compounds (II and III) (1).

The chemical shifts of protons c and d unequivocally indicate that the anisotropic cone of the phenyl ring is closed to proton d (deshielding effect) and that, in solution, I exhibits a privileged conformation in accordance with that observed in the solid state.

DISCUSSION

A number of binding sites at the receptor are known to be important for α-adrenergic activity: the catechol moiety, the β-hydroxyl group, and the ammonium function (Fig. 3). The extended Hückel theory (16) and the perturbative configuration interaction using localized orbitals (17, 18) calculations have permitted postulation of the distance between the center of the aryl ring and the oxygen atom (3.6–3.7 Å), the center of the ring and the nitrogen atom (5.1–5.2 Å), and the nitrogen–oxygen distance (2.8–2.9 Å) as the main characteristics of the molecule binding the α-adrenergic receptor sites.

The spatial configuration of I fits this proposed model.

It can be concluded that I displays structural features making it suitable for binding the presynaptic receptor sites. Nevertheless, I cannot activate them; the bulky group attached to the nitrogen atom accounts for this inhibition. As such, when it partially occupies the site, it hinders the feedback inhibition of norepinephrine, increasing its release in the synaptic cleft. This effect could account for its pharmacological properties and provides information on the activity of certain antidepressant drugs.

[3] Full details concerning the crystallographic data (interatomic distances, angles, and coordinates) are available (15).

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Effect of Sodium Oleate on Salicylic Acid Binding to Human Serum Albumin

PETER A. SCHWARTZ, DOUGLAS S. GREENE, and C. T. RHODES ˣ

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Abstract □ The pharmacological activity of salicylates is related to the nonprotein-bound fraction of drug in the plasma. Free fatty acids have been shown to displace bound drug and to increase the serum levels of salicylates. Continuous ultrafiltration was used to measure unbound salicylic acid at 37°. A nonlinear analysis of the ultrafiltration data using whole number values for the number of binding sites indicates that sodium oleate displaces the salicylic acid competitively at both binding sites. Increased concentrations of fatty acids due to disease or the infusion of fatty acid emulsions perhaps may produce toxic levels of salicylic acid.

Keyphrases □ Sodium oleate—effect on binding of salicylic acid to human serum albumin □ Salicylic acid—binding to human serum albumin, effect of sodium oleate □ Protein binding—effect of sodium oleate on binding of salicylic acid to human serum albumin

Since the pharmacological activity of many drugs is related to the non-protein-bound fraction of the drug in plasma (1–3), any decrease in the fraction of protein-bound drug may be expected to result in an increased pharmacological effect. Free fatty acids bind rapidly to albumin at more than one site (4–6), have a higher affinity for albumin than do most drugs (7, 8), and increase the concentration of unbound drug *in vivo* by displacement of

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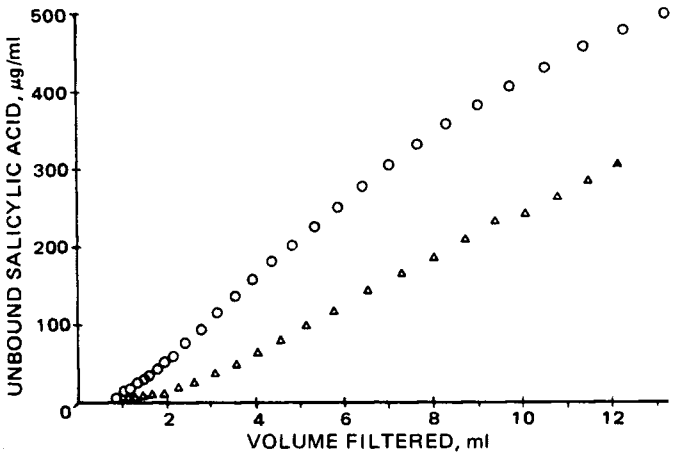

Figure 1—*Relationship between unbound salicylic acid and ultrafiltration volume for salicylic acid alone (△) and salicylic acid with 2.0 mEq of sodium oleate (○).*

bound drug (9, 10). Since salicylates can be relatively highly protein bound at certain concentrations (11–14), drug displacement due to elevated free fatty acid concentrations may have significant clinical implications (15).

Palmitic, oleic, and linoleic acids are the major free fatty acids present in serum (16, 17). Elevated concentrations of free fatty acids, *i.e.*, above the normal range of 0.3–0.8 mEq/liter, have been reported in renal disease (18), diabetes (19), hyperthyroidism (20), stress and exercise (21–23), myocardial infarction (24), and infection (25). Because free fatty acid increases are common in clinical practice and may result in significant drug displacement, the changes in salicylic acid binding in the presence of varying free fatty acid concentrations were investigated *in vitro* using human serum albumin at 37°.

EXPERIMENTAL

Human serum albumin[1] was prepared in pH 7.4 phosphate buffer at a physiological concentration of 4 g/100 ml. Salicylic acid[2] solutions were prepared in pH 7.4 phosphate buffer at concentrations between 700 and 800 μg/ml. Sodium oleate[3] was added to the human serum albumin to yield concentrations of 0.5, 1.0, 1.5, and 2.0 mEq/liter.

The bound fraction of drug was determined using continuous ultrafiltration (26). The salicylic acid solution was placed in the reservoir above the 25-mm ultrafiltration cell, equipped with a membrane having a 10,000 Dalton molecular weight cutoff[4]. The cell then was filled with either human serum albumin or a human serum albumin–sodium oleate mixture. The contents of the cell and reservoir were kept at 37° in a water bath.

The ultrafiltrate was collected under nitrogen (<20 psi) in tared, glass-stoppered test tubes at a flow rate of ~10 ml/hr. After an initial collection of 1 ml (to allow for the void volume), samples were collected and the collected filtrate volumes were determined by reweighing the tubes and converting weights to volumes, assuming a solution density of one (filtrate volumes varied between 0.1 and 0.7 ml).

Salicylate concentrations were determined spectrophotometrically at the absorption maximum of 295 nm from suitably diluted filtrate samples. A regression line of absorbance as a function of salicylate concentration was linear up to 40 μg/ml ($r = 0.999$, $n = 8$). A control study performed without protein showed no evidence of drug binding by the membrane or other parts of the cell.

The method of Greene and Nightingale (27) was used to calculate the number of binding sites and the affinity constants for each site. The raw data, *i.e.*, the filtrate concentration, C_f, as a nonlinear function of the volume filtered, V, were fitted using a computer[5] and the NONLIN program (28):

$$\frac{dC_f}{dV} = \frac{(C_r - C_f)/V_c}{1 + \frac{n_1K_1P}{(1 + K_1C_f)^2} + \frac{n_2K_2P}{(1 + K_2C_f)^2}} \quad \text{(Eq. 1)}$$

where C_r, V_c, and P are the reservoir concentration, ultrafiltration cell volume, and moles of protein, respectively.

All calculations were corrected by subtraction of the void volume from the total volume filtered (26). The void volume, V', was calculated using the NONLIN program to fit the following equation iteratively (27):

$$C_f = C_r - C_re^{\left(\frac{-V+V'}{V_c}\right)} \quad \text{(Eq. 2)}$$

Initial estimates of binding constants were obtained from the computer program MAP (28), which calculates the map of the sum of squares surface. All fits were obtained for a two-site model either with four parameters, *i.e.*, n_1, K_1, n_2, and K_2, or with two parameters, *i.e.*, K_1 and K_2, with whole numbers assigned to n_1 and n_2.

RESULTS AND DISCUSSION

The salicylic acid concentration in the ultrafiltrate is a nonlinear function of the volume filtered (Fig. 1). From these data, a Scatchard plot (Fig. 2) can be constructed using a mass balance analysis of the cell (29). Examination of the intercept on the r axis in Fig. 2 for salicylic acid alone and in the presence of 2.0 mEq of sodium oleate/liter suggests at least four salicylate binding sites on human serum albumin distributed between

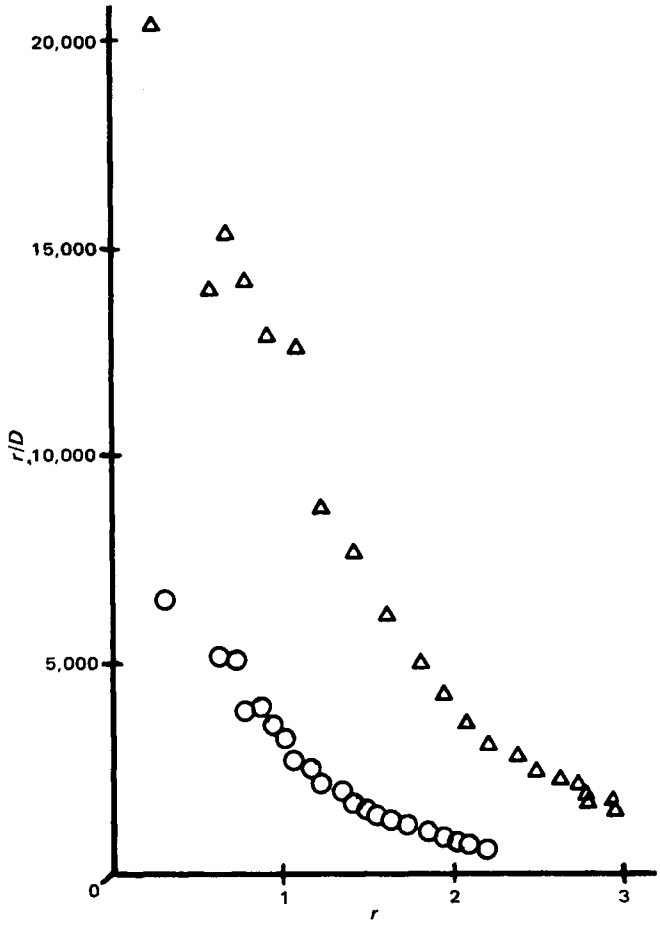

Figure 2—*Scatchard plot of salicylic acid binding. Key: △, salicylic acid alone; and ○, salicylic acid with 2.0 mEq of sodium oleate; r is the moles of salicylic acid bound per mole of human serum albumin and D equals moles of unbound salicylic acid.*

[1] Fraction V powder, Calbiochem.
[2] A-277, Fisher.
[3] Pfaltz and Bauer.
[4] Millipore, PTSC.
[5] Itel AS-5 model 3.

Table I—Scatchard Parameters for Salicylic Acid Binding to 4% Human Serum Albumin at 37°

Model	n_1	K_1, liters/mole	n_2	K_2, liters/mole
Unconstrained	1.33 (0.22)[a]	18,750 (10,600)	2.48 (0.21)	840 (310)
Constrained	2	7440 (2460)	2	430 (48)

[a] The standard deviation is given in parentheses.

two classes of sites. The data are quite clear for the systems that did not contain sodium oleate but are not unequivocal for the systems containing sodium oleate.

Table I lists the Scatchard parameters of a two-class binding model that were obtained by fitting the filtrate data as a function of volume to Eq. 1 using the NONLIN program. The estimates for these parameters are in reasonable agreement with those of other investigations at 37° (30, 31).

The NONLIN program can be modified to constrain the number of binding sites to whole numbers (32). The most likely combination of whole numbers of a two-class binding model, *i.e.*, n_1 and n_2, appears to be one of the following constrained systems: 1, 3; 1, 4; 1, 5; 2, 2; 2, 3; and 2, 4. The best-fit constrained model for the two binding sites (n_1 and n_2) using the criteria of smaller sums of squares was 2, 2. This result differs from a similar analysis of a Scatchard plot of salicylic acid binding to 0.3% human serum albumin at 25°, in which 1, 4 was reported for n_1 and n_2, respectively (32). The 2, 2 model also appeared to describe adequately the salicylic acid binding in the presence of 0.5–2.0 mEq of sodium oleate/liter.

Figure 3 demonstrates that the apparent dissociation constants for both classes of binding sites decreased apparently linearly with increasing oleate concentration. Decreased affinity for binding without a change in the number of binding sites also was shown for diazepam in the presence of oleic acid (10).

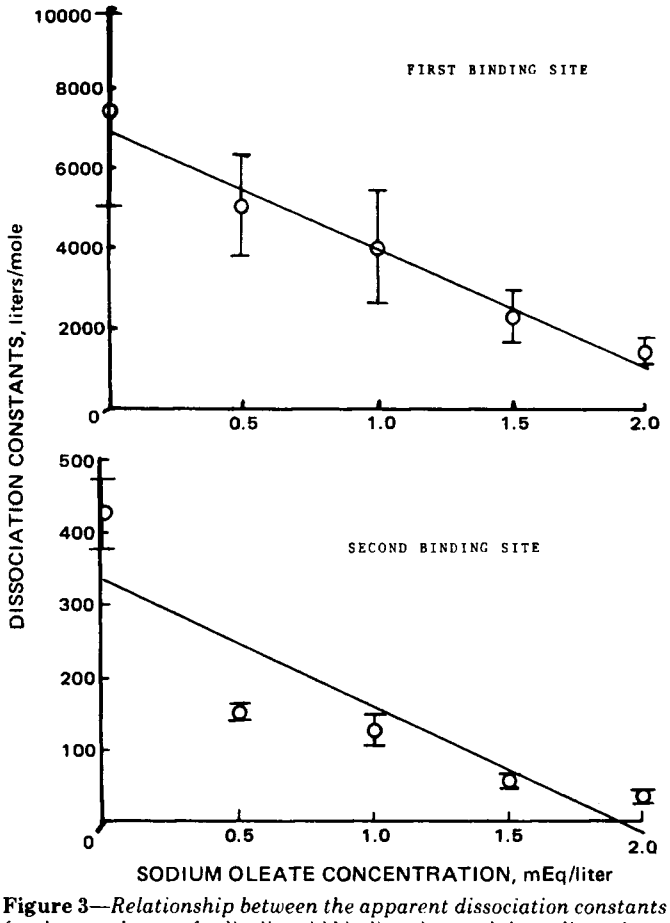

Figure 3—*Relationship between the apparent dissociation constants for the two classes of salicylic acid binding sites and the sodium oleate concentration.*

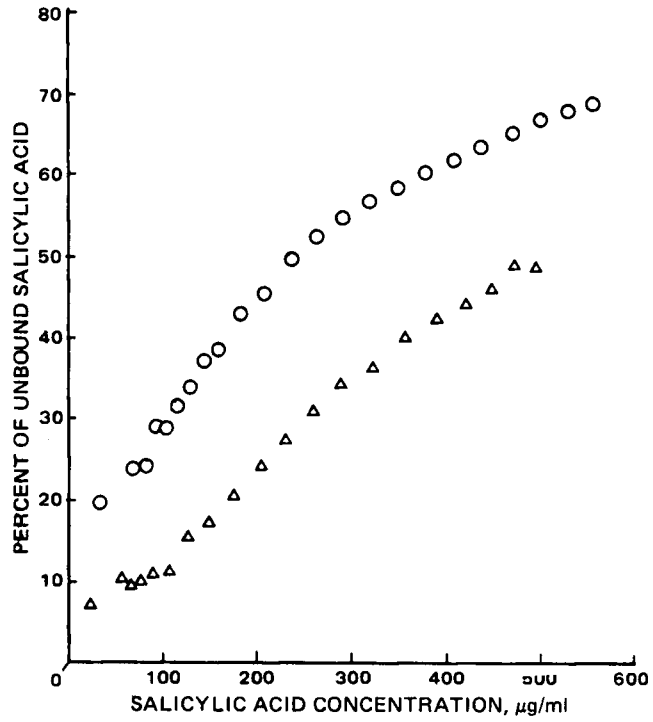

Figure 4—*Relationship between the percent of unbound salicylic acid and the total drug concentration. Key: △, salicylic acid alone; and ○, salicylic acid with 2.0 mEq of sodium oleate.*

Oleic acid appears to alter the binding affinity for salicylic acid to human serum albumin without affecting the number of binding sites. Similar competitive effects of free fatty acids on the binding of salicylic acid and other drugs have been attributed to allosteric conformational changes of protein molecules (32).

The unbound percent of salicylic acid as a function of total drug concentration calculated from the mass balance of cell contents is shown in Fig. 4. A twofold increase in the percent unbound salicylic acid, *i.e.*, from ~20 to 40%, occurred at oleic acid levels of 2.0 mEq/liter, as compared to normal free fatty acid levels of ~0.5 mEq/liter.

This investigation was purely *in vitro* in nature, and there always is an uncertainty in any attempt to extrapolate *in vitro* data to *in vivo* clinical situations. However, it is known that the toxic effects of salicylic acid are related to its unbound concentration, the dose-related volume of distribution, and the clinical status of the patient, *e.g.*, acidosis (15). Since salicylic acid binding can be reduced considerably by elevation of the free fatty acid level, any substantial increase in free fatty acids due to disease or the use of fatty acid emulsion infusions may perhaps be potentially clinically relevant. Since salicylic acid has a quite high therapeutic ratio, the effect may not be hazardous; however, it would be desirable to study the effects of the free fatty acid concentration on several strongly bound drugs with low therapeutic indexes.

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Novel Potential Anticancer Agents Derived from Benzimidazole

EL-SEBAII A. IBRAHIM, A.-MOHSEN M. E. OMAR ˣ, and M. A. KHALIL

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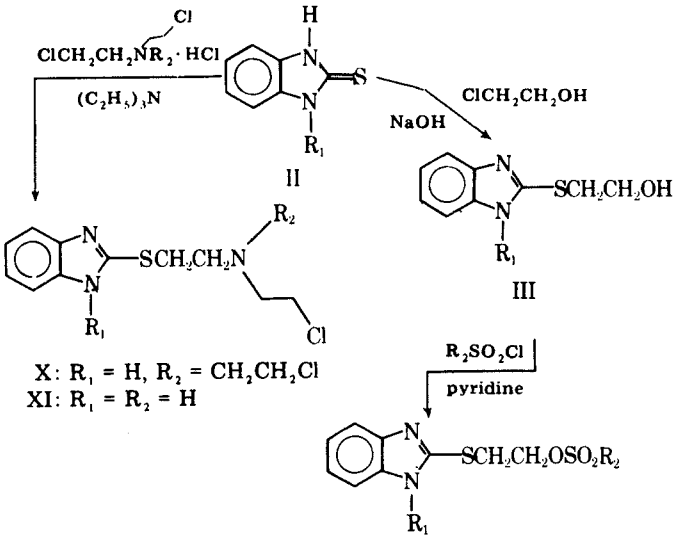
Abstract □ Two novel series of benzimidazole derivatives bearing structural modifications of certain drugs were prepared for evaluation for potential anticancer activity. The first series was a group of alkylating agents, and the second series was a variety of 4-substituted-1-thioacetyl-3-thiosemicarbazides. The tests of some representative products for antileukemic activity against P-388 lymphocytic leukemia indicated no significant effects.

Keyphrases □ Antineoplastic agents, potential—benzimidazole sulfonic esters and nitrogen mustards, synthesis and evaluation for antileukemic activity □ Benzimidazole derivatives—sulfonic esters and nitrogen mustards, synthesis and evaluation for anticancer activity

Among the benzimidazole alkylating agents whose syntheses have been described (1–5), only 2-[bis(2-chloroethyl)aminoethyl]benzimidazole (benzimidazole mustard NSC 23891, I) has shown pronounced anticancer activity (6, 7). Interest in the effect of structural modification on the anticancer activity of such compounds prompted the synthesis of benzimidazole sulfonic esters (IV–IX) and nitrogen mustards (X and XI) (Scheme I), in which the thioethyl chain replaced the methylenic group in I. In addition, 4-substituted-1-(2-mercaptoacetylbenzimidazole)-3-thiosemicarbazides (XIV–XX) (Scheme II) were prepared to compare their anticancer properties with those reported for some heterocyclic α-formylthiosemicarbazone derivatives (8–11).



Scheme I

RESULTS AND DISCUSSION

Chemistry—The sulfonic esters (IV–IX) were prepared starting with *S*-alkylation of benzimidazole-2-thione or the 1-substituted derivatives (II) with ethylene chlorohydrin in the presence of potassium hydroxide, followed by treatment of the resulting alcohols (III) with methanesulfonyl chloride or *p*-toluenesulfonyl chloride and pyridine (12) (Scheme I). When the 1-position in the alcohols (III) was vacant, the reactions proceeded with the production of the disulfonated products (VIII and IX) (Table I). The bi- and monofunctional nitrogen mustards (X and XI) were synthesized by reacting benzimidazole-2-thione with a mixture of tris(2-chloroethyl)amine hydrochloride or bis(2-chloroethyl)amine hydrochloride and triethylamine in absolute ethanol.

Treatment of benzimidazole-2-thione (II) with ethyl bromoacetate gave

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