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RESEARCH PAPER

Profiling of Drugs for Membrane Activity Using Liposomes as an In Vitro Model System

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ABSTRACT

The increasing size of chemical libraries being analyzed by high-throughput screening results in a growing number of active compounds that need to be assessed before moving forward in the drug development process. As a consequence, more rapid and highly sensitive strategies are required to accelerate the process of drug discovery without increasing the cost. Due to the fact that significant numbers of compounds from combinatorial libraries are hydrophobic in nature, approaches are needed to evaluate the potential for these compounds to interfere with the functions of biological membranes. The liposome system was used to detect agents that act as follows: (i) ionophores able to induce specific ion permeability, e.g., valinomycin for K^+ and protonophoric uncouplers for H^+; (ii) ion antiporters which exchange H^+ for other ions, e.g., nigericin; (iii) agents that form low specificity ion channels in the membrane, e.g., gramicidin; and (iv) detergents and other membrane-disrupting agents. We propose using this liposome assay during the drug development process to identify compounds that have membrane activity and, as a consequence, produce a biological effect by altering the physico-chemical properties of the cell membrane rather than interacting with a protein target. Screening of a representative set of biologically-active compounds (198) indicated that the majority of systemic antimicrobial drugs, but not topical drugs, lack membrane activity in this model system.

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Key Words: *Drug testing; Fluorescence; Hydrophobicity; Liposomes; LogP; Membrane activity; Model membranes; QSAR*

INTRODUCTION

Current approaches to drug discovery include strategies to exploit certain essential proteins that play principle roles in cellular metabolism (i.e., enzymes, receptors, ion channels, etc.) as drug targets. The desirable therapeutic effects of drugs that affect these targets are achieved through the alteration of specific functions and/or metabolic pathways. However, a compound may produce a biological effect by altering the physico-chemical properties of the cell membrane rather than interacting selectively with a protein target. For instance, the biological activity of a number of food preservatives, antiseptics, disinfectants, peptides, and antibiotics is related to the nonspecific disruption of the cell membrane (1–3). Therefore, the testing of chemical agents for membrane activity is useful to separate biological activity due to membrane disruption from activity due to interaction with specific cellular protein targets.

Determining the membrane activity of chemical compounds using liposomes as a model was introduced by Bangham and co-workers (4,5). They investigated three aspects of the mechanism of action of anesthetics: (i) to reveal the manner in which water, small uncharged solutes, and ions cross lipid bilayers; (ii) to show that anesthetics release both protons and catecholamines from model transmitter vesicles; and (iii) to serve as a thermodynamic model. Liposomes have also been applied to the field of antibacterial research, where aminoglycosides have been demonstrated to induce aggregation of negatively-charged liposomes (6–8). Moreover, a correlation between aminoglycoside ototoxicity in guinea pigs and the perturbation of liposomes was found (7). Liposomes were also used to determine the effects of thallium ions on the H^+/OH^- and Cl^- permeabilities of liposomes prepared from brain myelin lipids (9). The results from these studies indicated that thallium ions catalyze an electroneutral Cl^-/OH^- exchange, e.g., antiport of different ion species, and raise the possibility that myelin may be a target for thallium. Although the potential of liposomes for drug screening purposes is well recognized, a systematic evaluation of this model against different compound classes is required to support the use of liposomes as a tool for profiling prospective drugs for membrane activity.

There are several ways that compounds may have an effect on biological membranes. For instance, membrane-active compounds may act as ionophores able to induce a specific ion permeability (e.g., valinomycin for K^+ and protonophoric uncouplers for H^+), or cause an ion antiport like nigericin which exchanges H^+ for K^+, or form ion channels as gramicidin. Other compounds may act as detergents, physically disrupting the membrane. The liposome system described in this work allows for the determination of all these membrane effects and opens the possibility of using liposomes in high-throughput screens to identify membrane-active compounds.

EXPERIMENTAL

Materials

Pork brain polar lipids and egg phosphatidylcholine were from Avanti Polar Lipids, Inc., Alabaster, AL. The lipids were dissolved in chloroform, and stored at $-70^{\circ}C$. Acridine orange, ammonium chloride, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), dimethyl-sulfoxide (DMSO), dithiothreitol (DTT), gramicidin, melittin, 2-[*N*-morpholino] ethanesulfonic acid (MES), 3-[*N*-morpholino] propanesulfonic acid (MOPS), nigericin, Tris, Triton X-100, and valinomycin were obtained from Sigma-Aldrich Co., St. Louis, MO. Octylglucoside (*n*-octyl-β-D-glucoside) was purchased from Anatrace, Inc., Maumee, OH. The Genesis collection of drugs and biologically-active compounds was obtained from Microsource Discovery Systems, Inc., Gaylordsville, CT. Aminoglycosides (amikacin, kanamycin, neomycin, spectinomycin, and tobramycin) were purchased from Sigma-Aldrich Co., St. Louis, MO.

Methods

Preparation of Liposomes

Liposomes were prepared as described (10) with modifications. An aliquot (600 μL) of the lipids

(16 mg) was mixed with 160 μL of the egg phosphatidylcholine (1.6 mg) in a 16-mm glass tube and dried under nitrogen gas stream. The residue was further dried under a vacuum for 3 hr (or overnight). An aliquot (600 μL) of 0.1 M MOPS buffer, pH 7.0, supplemented with 1.2% of octylglucoside and 1 mM DTT was added to the dried residue. The suspension was sonicated in a bath-type sonicator 1210 (Branson, Danbury, CT) for 10–15 min until clear. The clear suspension was then divided into two aliquots (0.3 mL each), and the aliquots were kept at −70°C. An aliquot of the suspension was thawed on ice before use and sonicated briefly in a Tekmar TM250B Sonic Disruptor (Heat Systems Ultrasonics, Farmingdale, NY) equipped with a microtip. A 300-μL aliquot of the suspension was mixed with 400 μL of 0.1 M MOPS buffer, pH 7.0, supplemented with 1.2% of octylglucoside and 1 mM DTT. The surface of the suspension was flushed with nitrogen, and the suspension was sonicated for 1 min in a 1210 bath-type sonicator (Branson Ultrasonics, Danbury, CT). After sonication, an aliquot of the suspension (0.7 mL) was diluted under vigorous stirring into 23.1 mL of the NH_4Cl buffer supplemented with 15 mM MES, pH 5.0, 0.15 M NH_4Cl, and 1 mM DTT, and the stirring continued for 20 min at room temperature. The liposomes were pelleted at 175,000**g** for 1 hr at 4°C. The supernatant was discarded, and the pellet resuspended in an aliquot (0.8 mL) of the NH_4Cl buffer by pipeting. The liposomes were frozen in dry ice/ethanol mixture and stored at −70°C.

Cryo-Transmission Electron Microscopy of Liposomes

For cryo-transmission electron microscopy (cryo-TEM), the liposome samples were presonicated and diluted as described above. Then, the samples were prepared in a controlled environment vitrification system (CEVS), which is described in detail elsewhere (11). A 3-μL drop of the liposome suspension was placed on a carbon-coated holey polymer support film mounted on a standard 300-mesh TEM grid (Ted Pella, Inc., Redding, CA). The drop was blotted with filter paper until it was reduced to a thin film (10–200 nm) spanning the holes (2–8 μ) of the support film. The liposome sample was then vitrified by rapidly plunging it through a synchronous shutter at the bottom of the CEVS into liquid ethane at its freezing point. The vitreous specimen was transferred under liquid nitrogen into a Philips CM120 transmission electron microscope for imaging. The temperature of the sample was kept under −175°C throughout the examination. The liposomes observed under this set of conditions were almost all unilamellar.

Measurement of Proton Gradient in Liposomes

Changes in intraliposomal pH were monitored using the proton gradient-sensitive probe acridine orange, whose fluorescence is quenched by the acidic internal compartments of the liposomes (12,13). The quenching occurs as free base of acridine orange ($pK_a = 10.45$) present in the medium diffuses very fast across the liposomal membrane and is protonated in the liposome interior (13). Increasing the concentration of acridine orange in the liposomes causes the aggregation of the dye molecules and, hence, the fluorescence quenching. As described in the literature (13), the quenching of the acridine orange fluorescence depends almost linearly on the pH gradient when the value of the pH gradient on the liposome membrane is above 1.7 pH units and the liposomes are prepared at pH 7.0.

Our experiments were performed as described (13) with some modifications. The value of the pH gradient was determined by two factors: (i) liposomes with interior pH 7.0 were diluted in a reaction buffer with pH 8.0; (ii) an excess concentration of ammonium sulfate was present inside the liposomes. It is known that ammonium inside the liposomes dissociates into neutral amine and proton. A subsequent efflux of the neutral amine from such ammonium-loaded liposomes causes acidification of the liposome interior and, therefore, generation of a pH gradient on the liposome membrane (13).

The measurements were performed in a spectrofluorimeter equipped with a 96-well reader set at 485 nm for the excitation and 520 nm for the emission. The liposome suspension was thawed on ice and sonicated for 1–2 sec using a sonicator equipped with a microtip. Then, an aliquot of the suspension (20 μL) was diluted in 1.98 mL of 15 mM MOPS buffer, pH 7.0. This, and all subsequent steps, were performed at room temperature. The aliquots (20 μL) of the liposome suspension were diluted with 180 μL of the reaction buffer (20 mM Tris-HCl, pH 8.0, 50 mM KCl, 50 mM NaCl, 1 mM $MgCl_2$,

and 2 μM acridine orange) supplemented with either a test compound in an organic solvent or the organic solvent itself in a 96-well plate. The plate was placed inside the spectrometer and measurements were made after a 5-min incubation. Each compound was tested in triplicate. Stock solutions of the test compounds were made either in DMSO or in ethanol at 200-fold higher concentration than the final concentration. The control fluorescence measurements were performed in a buffer supplemented with a solvent to account for a solvent effect on the fluorescence. Accordingly, an effect of a test compound was measured in a buffer supplemented with a given compound and a solvent. The fluorescence quenching by liposomes was determined by subtracting the level of the dye fluorescence observed in the presence of liposomes from the level of the dye fluorescence observed in the absence of liposomes. The membrane activity (% effect) of a given compound was calculated using Eq. (1):

$$\%\ \text{effect} = \frac{(F_0 - F_{0L}) - (F_C - F_{LC})}{(F_0 - F_{0L})} \times 100\% \qquad (1)$$

where F_0 = fluorescence in absence of lipsomes, F_{0L} = fluorescence in presence of liposomes, F_C = fluorescence in absence of liposomes in the presence of compound, F_{LC} = fluorescence in presence of liposomes in the presence of compound. Seven compounds (aklavin, hydroxyzine, gentian violet, protoporphyrin IX, dichlorophene, dihydroxyflavone, and rifampin) among the 198 tested interacted directly with acridine orange in the absence of liposomes, causing its quenching (not shown). Those colored and/or fluorescent compounds were excluded from the liposome assay.

Development and Interpretation of Quantitative Structure–Activity Relationship (QSAR) Models for Compound Activity

In order to study the liposome activity of the compounds on a molecular basis, a set of structures was selected to span 280 to 380 atomic mass units (amu) with a mean molecular weight of 332 amu for the whole data set. The two-dimensional (2D) structures for these compounds were entered into a database in SYBYL (Version 6.6, Tripos, Inc., St. Louis, MO) running on a Silicon Graphics Octane under the IRIX operating system (Version 6.5, Silicon Graphics, Inc., Mountain View, CA). Reasonable low-energy conformations were obtained by first converting all the 2D structures to 3D conformations using CONCORD (14), followed by strain-energy minimization using the Tripos force field (15), including electrostatic terms. The atomic partial charges used in the energy-minimization step were calculated in SYBYL using the Gasteiger–Huckel method (16). Once the energy-minimized structures had been obtained, they were transferred to the ADAPT system (17,18) for calculation of descriptors and development of QSAR models. The processes of descriptor calculation, analysis, and subsequent model development have been described previously (19). The atomic partial charges used in the calculation of the CPSA (20) and related HBOND descriptors (21) were those calculated in SYBYL using the Gasteiger–Huckel method as described above. Physical interpretation of the QSAR models was accomplished using partial least-squares analysis (22) as implemented in the SCAN program (Release 1, Minitab, Inc., State College, PA). Estimates of the octanol–water partition coefficient ($\log P$) were calculated using the C$\log P$ program (Version 4.62, Daylight Chemical Information Services, Inc., Mission Viejo, CA).

RESULTS

Proton Gradient Stability in Liposomes

The proton gradient in liposomes was stable in an equimolar mixture of sodium chloride and potassium chloride over a period of 30 min (not shown). The effect of DMSO on the liposomes was investigated due to the fact that small organic compounds may alter the permeability of membranes (5,6) and that DMSO is used as the solvent of choice to solubilize compounds of combinatorial libraries. Testing at different concentrations of DMSO revealed that a concentration of 0.5% caused small decreases (< 10%) in the proton gradient during the first 5 min of liposome exposure to the solvent, while higher DMSO concentrations and longer incubation times gave larger effects (not shown). Therefore, 0.5% DMSO was maintained in the screens presented below and measurements were made after a 5-min incubation of the liposomes with test compounds.

Liposomes described in this study were stable for months. Table 1 provides stability data for a typical

Table 1

Liposome Stability During the Study Period

Acridine Quenching in Response to the pH Gradient in Liposomes (%)	Date of Experiment (month/day in 1999)
82.9	6/11
82.2	6/11
78.9	6/14
75.2	6/14
76.4	6/25
75.0	6/25
74.6	7/1
79.0	7/1
77.3	7/2
75.4	7/2
80.0	7/7
73.7	7/7
79.9	7/8
80.5	7/8
79.2	7/10
75.6	7/10
78.4	8/12
71.2	8/12

batch of the liposomes. Thus, the freshly-prepared liposomes were dispensed in 0.8-mL aliquots and kept at -70°C during the study period. Typically, two aliquots of the liposomes were used during one day (see Table 1). The fluorescence quenching caused by the pH gradient in the liposomes was reduced by 14% over a period of two months. If the liposomes were kept at -70°C for five months, the quenching was reduced by 23% (not shown). Such liposomes were discarded.

The pH gradient in the liposomes was completely dissipated by the following compounds: ionophoric antibiotics (tyrothricin, gramicidin, nigericin, and valinomycin); protonophores (dicumarol, CCCP); ammonium chloride (not shown). The activity of the ionophoric antibiotics required the presence of K^+ ions in the medium. The activity of valinomycin was additionally enhanced in the presence of protonophores (not shown). This corresponds to the ability of ionophoric antibiotics and protonophores to dissipate ion gradients on membranes by catalyzing coupled ion fluxes, while preserving the physical integrity of the membrane (23–25). In this respect, ionophores differed from detergents (triton X-100) and mellitin, that dissipated the pH gradient in the liposomes in salt-free media.

Classification of Drugs and Biologically-Active Compounds According to Their Membrane Activity

A collection of 198 compounds was tested on the liposomes. This collection encompassed representatives of several drug classes: antibacterial agents designed for systemic use (beta-lactams, quinolones, macrolides, aminoglycosides, vancomycin, tetracyclines), cardiovascular (CV) agents, central nervous system (CNS) drugs, anti-inflamatory drugs, and a number of topical drugs. Each compound was tested at a single concentration corresponding to 10 μg/mL. This concentration was chosen as it is within the range of sustained serum levels of representative marketed antibiotics (26,27).

The effects of this set of compounds varied from a lack of effect to the complete dissipation of the pH gradient. These effects were classified as follows: strong effects (>50%), moderate effects (20–50%), and no effect (<20%). Compounds with a strong effect represented 13% of the 198 compounds tested. Compounds with a moderate activity encompassed only 9% of the compounds, while the majority of compounds (78%) had no effect on the liposomes.

Comparison of Membrane Activity of Systemic and Topical Drugs

The set of compounds tested encompassed 179 systemic drugs and 10 topical drugs. Only 9% of systemic drugs caused a strong effect and 9% had a moderate effect, while a large majority (88%) had no effect. On the contrary, the majority (68%) of topical drugs were active in the liposomes, with 50% having a strong effect and 18% having a moderate effect (for instance, polymixin B and cloxyquin). About 32% of topical agents (for instance, chloroxylenol, nystatin, benzethonium chloride, thermosal, and chlorhexidine) were inactive against liposomes, while some topical antifungals (econazole, clotrimazole, sulcanazole, haloprogin) all caused a strong effect (Table 2).

Table 2

Activity of Different Classes of Systemic Drugs in the Liposome Model

Drug Classes Tested	Effects in Liposomes		
	Strong	Moderate	No Effect
Cardiovascular (16)			
Amiodarone, fendiline, flunarizine, prazosin, suloctidil (five compounds total)	+		
Chrysin		+	
Dichlorophenamide, danthron, khellin, minoxidil, methoxamine, nicardipine, norepinephrine, peruvoside, propanolol, rescinamine (10 compounds total)			+
Central nervous system (21)			
Clemastine, ethopropazine, pimozide, promethazine, trifluoperazine (five compounds total)	+		
Alverine citrate, chlorpromazine, clomipramine, dichlorothiokynurenic acid, meclizine, pimethixene, proadifen, trihexyphenidyl (eight compounds total)		+	
Caffeine, chlorcyclizine, droperidol, impramine, kappa agonist ICI-204,448, mebeverine, mebhydrolin, melatonin (eight compounds total)			+
Anti-inflamatory (10)			
Nefopam	+		
Aspirin, acetaminophen, drofenine, fenbufen, fenoprofen, ibuprofen, indoprofen, ketoprofen (nine compounds total)			+
Antimicrobials (113)			
Antimycin A, chloramphenicol palmitate, erythromycin propionate, meclocycline, methacycline, oxacillin (six compounds total)		+	
Aminoglycosides, antifungals, antivirals, beta-lactams, macrolides, quinolones, tetracyclines (107 compounds total)			+

Membrane Activity of Systemic Drugs

Classes of systemic drugs tested in the liposome model were the following: CV drugs (16 compounds); CNS drugs (21 compounds); anti-inflammatory drugs (10 compounds); antimicrobials (113 compounds).

Compounds active in the liposomes were represented among drugs that target cardiovascular or central nervous systems (Table 2). For instance, five CV drugs (amiodarone, fendiline, flunarizine, prazosin, suloctidil) and five CNS drugs (clemastine, ethopropazine, pimozide, promethazine, trifluoperazine) had a strong effect on the liposomes. Among 10 anti-inflamatory agents tested, only one compound (nefopam) had a strong effect, while the rest (nine compounds) had no effect (see Table 2). For moderate effects, there was one CV drug (chrysin) and eight CNS drugs (alverine, chlorpromazine, clomipramine, dichlorothiokynurenic acid, meclizine, pimethixene, proadifen, trimexyphenidyl), while 10 CV drugs and eight CNS drugs did not affect the liposomes (see Table 2).

The majority of systemic antimicrobials (107 compounds) did not affect the proton gradient in liposomes at all (see Table 2). Five antibiotics (chloramphenicol, erythromycin, methacycline, meclocycline, oxacillin) and an antifungal drug (antimycin A) caused a moderate effect.

All five aminoglycosides tested were among the compounds that did not affect liposomes. In this regard, it appears that either the liposomes used in this study are not suitable for detection of the effects of aminoglycosides, or the changes in the binding of 1-anilino-8-naphthalene sulfonate to the liposomes described earlier (7) do not reflect the status of membrane permeability to ions.

Evaluation of Physiological Relevance of the Liposome Model

If the liposome model has a physiological relevance, then therapeutic drug concentrations must be lower than the concentrations that affect liposomes. Accordingly, adverse physiological effects are expected within the range of concentrations that damage the liposome membrane.

As indicated above, some compounds with activity against targets in both the cardiovascular and the central nervous systems had strong effects on the liposomes. To address this issue, a set of compounds (amiodarone, clemastine, flunarizine, pimozide, tamoxifen, trifluoperazine) were titrated on the liposomes to determine their range of effective concentrations (see Table 3). These were then compared with the plasma concentrations reported in the literature (26,27). The data presented in Table 3 indicate that typical plasma concentrations for flunarizine, pimozide, tamoxifen, and trifluoperazine are significantly lower than those that affect liposomes. But for two drugs, amiodarone and clemastine, therapeutic concentrations are close to the concentrations exhibiting membrane activity (Table 3). For instance, the steady-state plasma concentrations of amiodarone (1000–2500 ng/mL) are within the range of the concentrations that affect the liposomes (1020–1700 ng/mL). In this regard, it is relevant to mention that adverse physiological reactions to the drug are reported (26,27) at plasma concentrations above 2500 ng/mL. The high end of the plasma concentration of clemastine is reported to be 1390 ng/mL, while an effective concentration range of this compound against the liposomes is 2070–3910 ng/mL (Table 3). Thus, the plasma concentration of clemastine is only two- to threefold lower than the concentration found to be active in the liposomes.

Mechanism of Membrane Activity

Different dependence of the activity of ionophoric antibiotics and detergents on the presence of monovalent inorganic ion species in the medium (see "Proton Gradient Stability in Liposomes") was used to address the mechanism of membrane activity of compounds presented in Table 3. The experiments were performed both in the medium devoid of inorganic ions and in the presence of salts. The comparison of the observed EC50 values (see Table 3) indicates that most compounds have a detergent-like membrane activity which does not require the presence of inorganic ions. One compound, pimozide, was about twofold more active in the presence of inorganic ions. But that is

Table 3

Comparison of Membrane Activity of Systemic Drugs in the Liposomes with Their Plasma Concentrations. Their Activity in the Liposomes Was Determined in the 1 to 40,000 ng/mL Concentration Range as Described in Materials and Methods. Ionic Buffer: 20 mM Tris-HCl, pH 8.0, 50 mM KCl, 50 mM NaCl, 1 mM $MgCl_2$, and 2 μM Acridine Orange. Non-ionic buffer: 20 mM Tris-HCl, pH 8.0, and 2 μM Acridine Orange. EC50 Values Relate to the Concentrations of Compounds That Caused 50% Effect in the Assay. C log P Is the Calculated log P for the Compound

Compound	C log *P*	Concentrations Effective in Liposomes, EC50 (ng/mL)		Plasma Concentrations in Humans (ng/mL) (26,27)
		Ionic Buffer	Non-ionic Buffer	
Amiodarone	8.93	1020	1700	Single oral dose: 150–700 Steady state: 1000–2500 Adverse effects: above 2500
Clemastine	5.57	3910	2070	Single oral dose: 920–1390
Flunarizine	7.25	1430	1430	Single oral dose: 81 Steady state: 60–120
Pimozide	6.95	10,200	4620	Single oral dose: 4–19 Steady state: 50
Tamoxifen citrate	6.82	2820	1690	Single oral dose: 17–70 Steady state: 120–340
Trifluoperazine	5.18	5040	5040	Single oral dose: 0.53–3.09

different from the activity of typical ionophores, which depends completely on the presence of inorganic ions. Therefore, we regard the dependency of the pimozide activity on salts to be too small to be related to specific ion fluxes across the membrane.

Quantitative Structure–Activity (QSAR) Model for Liposome Activity

The initial model for liposome activity included four structural descriptors. The complete model accounted for 76.4% of the variance in the training set data ($R^2 = 0.764$). The analysis of the model by the partial least squares (PLS) method (22) showed that two descriptors, molecular weight and the weighted-path count starting with oxygen atoms (WTPT-4) (28), accounted for 60.1% of the variance. Example structures that illustrate this trend are shown in Fig. 1. Based on the structure–activity trend observed in the first component of the PLS analysis, the two descriptors appear to be coding for the hydrophobicity of the molecules in question. In this trend, large, hydrophobic molecules show greater liposome activity than small, hydrophilic molecules.

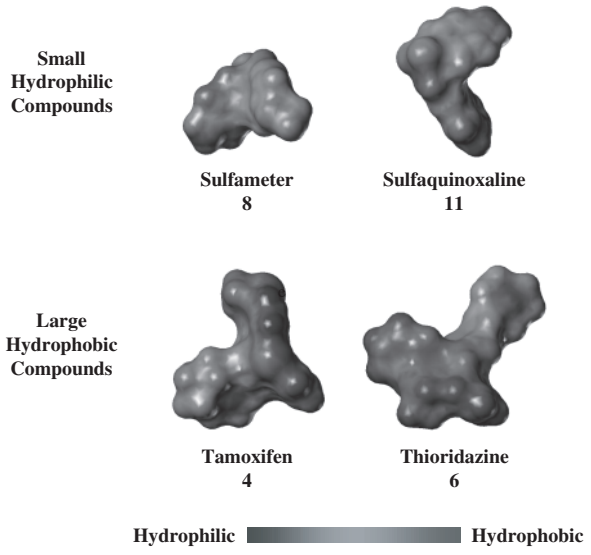

Figure 1. Example structures of compounds that illustrate the primary structure–property QSAR trend for activity in liposomes. The molecules are represented using the solvent-accessible surface area that has been colored to represent the hydrophobic and hydrophilic properties of the various regions of the molecules. *(See color insert at end of issue.)*

The comparison of calculated $\log P$ with liposome activity of the 31 compounds in the data set is shown in Fig. 2. This plot shows a non-linear pattern, as noted in the first PLS component of the initial QSAR model. The plot further suggests that there are two subsets of compounds in the data set. The first includes compounds exhibiting a $\log P$ of 2.5 or below. These compounds show little liposome activity at all. The second set includes compounds exhibiting a $\log P$ above 2.5. In this case, increases in $\log P$ above 2.5 appear to yield liposome activity for these molecules (Fig. 2).

The relationship between structure, $\log P$, and the observed liposome activity was studied in more detail. First, the data set was modified by removing from consideration any compound with a calculated $\log P$ of 2.5 or less. A new four-variable QSAR model was generated as before, using the remaining 25 structures, this time allowing $\log P$ to be considered as a descriptor. The model yielded an R^2 of 0.837. Subsequent PLS analysis showed that the first two components alone accounted for 82.7% of the variance in the data set. The first component (see Fig. 3) accounted for 55.5% of the variance, with $\log P$ being the single most important descriptor. The second component accounted for an additional 27.2% of the variance, and involved primarily one other descriptor (FPSA-1, fractional positive surface area-1) (19). The purpose of the

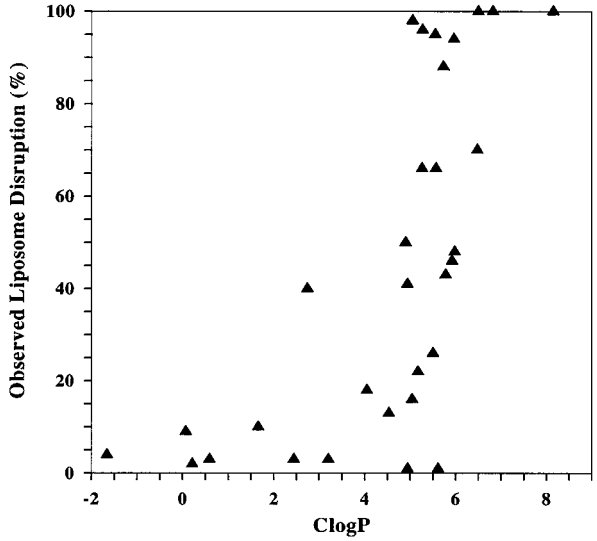

Figure 2. The relationship of calculated $\log P$ (C$\log P$) and the observed activity in liposomes.

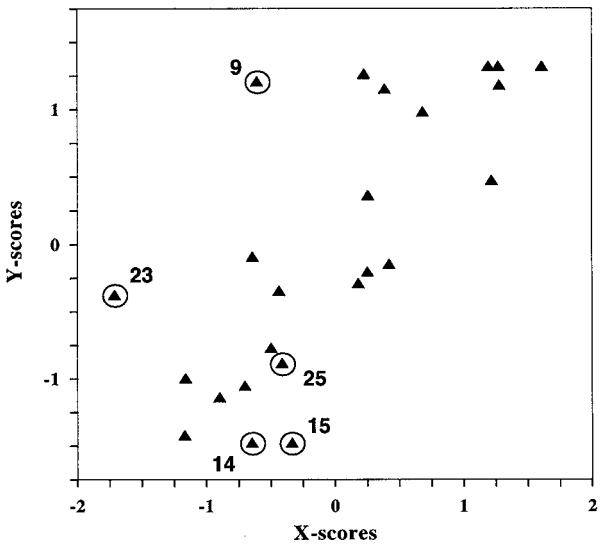

Figure 3. Score plot for the first PLS component obtained for the second QSAR model that incorporated C log *P* as a descriptor. In this plot, compounds **14**, **15**, and **25** represent aliphatic amines whose log *P* values are overestimated. Compounds **9** and **23** are compounds that produced errors in the C log *P* calculation that appears to have resulted in an underestimation of the log *P* values. In both cases, the second component of the model appears to be necessary to provide corrections to the calculated log *P* values.

second component appears to be to correct for errors in the calculated log *P* values for the outlying compounds in the first component.

These outliers are identified in Fig. 3. The three aliphatic tertiary amines (**14**, **15**, and **25**) were overestimated by the first component, and a correction was required in the second component because these compounds are protonated and thus exhibit a greater hydrophilicity and a lower log *P* at physiological pH. Also, two other compounds (**9** and **23**, see Fig. 3) also generated an error code during the log *P* calculation due to an underestimation of the log *P* for these compounds. Thus, the second component was used to correct for errors in the original log *P* estimates.

DISCUSSION

The membrane activity of systemically-delivered antibiotics (cell-wall synthesis inhibitors, DNA replication inhibitors, protein synthesis inhibitors, etc.), as well as sets of drugs that have targets in the cardiovascular or the central nervous systems, or act as anti-inflamatory agents, was examined.

The majority of systemic antibiotics, represented by 107 chemically-diverse compounds, demonstrated no effect on the liposomes (Table 2). Thus, the lack of membrane activity may be used as a guidance principle to design drugs with antimicrobial activity due to the inhibition of a bacterial enzyme rather than disruption of the function of the cell membrane.

Membrane-active (moderate to strong effects on the liposomes) compounds have been detected among drugs that target the cardiovascular or the central nervous systems (Table 3). According to the liposome model, therapeutic concentrations of these compounds should be below those that alter the liposome membrane. For most compounds the experimental data reported in the literature support this prediction (see Table 3).

However, damage of the cell membrane is expected in vivo for the drugs whose concentration in plasma reaches a level at which they affect the liposomes. In this regard, it is interesting to note that both amiodarone and clemastine have therapeutic concentrations close to the concentrations at which membrane activity is detected and that adverse reactions for amiodarone, and even lethality due to clemastine overdose, have been reported at concentrations that cause a strong effect on the liposomes (Table 3). This may suggest that toxicity of amiodarone and clemastine has a membrane-activity component, but much more work would be needed to establish the relationship between membrane activity determined in vitro and the development of adverse drug reactions in vivo.

Due to the diversity of the structures of the compounds studied, computer modeling was limited to a homogeneous set of compounds with molecular weight about 332 amu. Therefore, modeling of the membrane-activity properties of large ionophoric antibiotics, such as, for instance, gramicidin and valinomycin, was beyond the scope of this study. The structure–property relationship obtained for the homogeneous set of compounds shows that the calculated log *P* values provide an estimate of their membrane activity. This is intuitively appealing, because it seems likely that as the compounds become more hydrophobic, as indicated by increasing log *P* values, they tend to partition into the liposome, which may produce a disruption of the barrier function of the liposome membrane.

More subtle relationships may be found among members of a structurally-related series, and this is the focus of future work.

In conclusion, the liposome system described here is able to detect compounds with multiple mechanisms of action, e.g., compounds that physically disrupt biological membranes (detergents and mellitin), as well as compounds that specifically alter membrane permeability to ions (e.g., ionophores, antiporters, and ion channels). As examples, membrane activity was detected for several classes of compounds: (i) ionophores that induce specific ion permeability, e.g., valinomycin for K^+ and protonophoric uncouplers for H^+; (ii) ion antiporters that exchange H^+ for other ions, e.g., nigericin; (iii) agents that form low specificity ion channels in the membrane, e.g., gramicidin; and (iv) detergents and other membrane-disrupting agents including some systemic drugs (see above). The assay has been developed in a format suitable for high-throughput screening, which may provide information critical in helping us to better understand the specificity of drug interaction(s) with their molecular targets and biological membranes. Due to the specificity, sensitivity, stability, and high throughput, the liposome system may prove to be an important tool for the discovery of drug-like molecules, in particular, antimicrobial drugs directed against specific cellular targets.

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