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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 : A61K 49/02, G01N 33/544 — A1

(11) International Publication Number: **WO 87/ 07150**

(43) International Publication Date: 3 December 1987 (03.12.87)

(21) International Application Number: PCT/US87/00965

(22) International Filing Date: 28 April 1987 (28.04.87)

(31) Priority Application Number: 869,047

(32) Priority Date: 30 May 1986 (30.05.86)

(33) Priority Country: US

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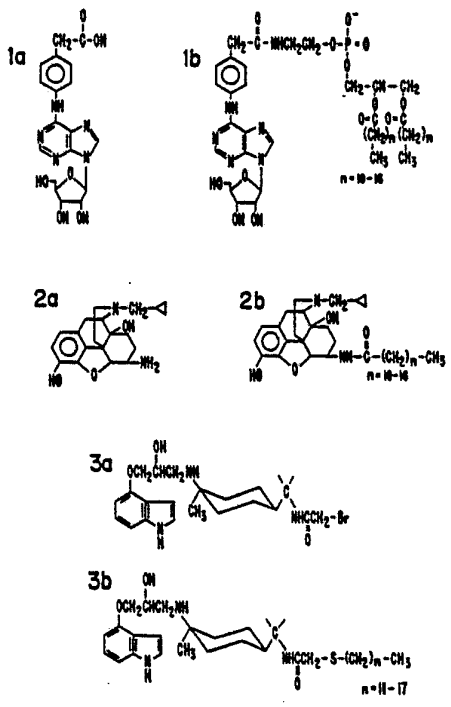
(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BG, BR, CH, CH (European patent), DE, DE (European patent), DK, FI, FR (European patent), GB, GB (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, MW, NL, NL (European patent), NO, RO, SD, SE, SE (European patent), SU.

Published
With international search report.

(54) Title: RECEPTOR-TARGETED DRUG DELIVERY SYSTEMS

(57) Abstract

Drug carrying such as liposomes and nanoparticles may be targeted to specific tissues or organs of the body by covalent attachment of therapeutic and pharmacologic ligands which retain high affinity for their receptors as well as retaining pharmacological activity. The ligands may be conjugated to monomers before they are assembled into the liposome or nanoparticle type structure. Drugs which may be carried by these carrying agents may be therapeutic or diagnostic materials.



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RECEPTOR-TARGETED DRUG DELIVERY SYSTEMS

TECHNICAL FIELD OF THE INVENTION

The present invention relates to the field of human diagnostics and therapeutics. Specifically, the invention relates to the targeting of drugs to particular body locations.

BACKGROUND OF THE INVENTION

Liposomes are synthetic lipid vesicles, which can encapsulate a variety of sizes of molecules in their internal spaces. Liposomes have been widely studied as carriers for delivering substances to cells and tissues in the body. (Gregoriadis, Liposome Technology, Vol. I, II, III, CRC Press, Boca Raton, 1985.) Nanoparticles, like liposomes, also

have been studied for use as drug carriers. Nanoparticles are made of synthetic polymeric substances such as polycyanoacrylate, polyanhydrides of aromatic and aliphatic dicarboxylic acids, polymethylcyanoacrylate and the like. They may also be formed of proteins such as albumin and gelatin. (Polymeric Nanoparticles and Microspheres, Boca Raton, CRC Press, 1986.) Nanoparticles may entrap or adsorb the drugs which they carry.

Because it is often desired that a substance be localized within the body, means have been sought to target liposomes to particular tissues or organs of the body. One targeting means which has been explored employs antibodies attached to liposomal surfaces. This approach requires that there be tissue or organ-specific antibodies available as reagents. However, in many cases such antibodies are not available.

The present invention provides an alternative means of targeting drug carrying agents which does not require tissue-specific antibodies. The present invention utilizes the specific interactions of therapeutic and functional pharmacological agents and their cell surface receptors.

Cells of many types, diseased as well as healthy, display recpectors on their surfaces which specifically interact with hormones, drugs, neurotransmitters and biologically active peptides. These receptors are expressed in a characteristic but heterogenous pattern among the cells of an organism. (Handbook of Chemical Anatomy, Vol. VIII, Classical Neurotransmitter and Neurotransmitter Receptors in the CNS, Elsevier, Amsterdam 1984.) For example, the receptors for beta-adrenergic ligands are predominantly found in the lung, heart and adipose tissue, whereas opiate receptors are found in the gut, spinal cord, and on lymphocytes. Furthermore, certain cancerous tissues

characteristically express receptors, for example, brain glioma expresses beta-adrenergic receptors (Receptor Binding Techniques, Society for Neuroscience, Washington, D.C. 1980) and bronchogenic carcinoma expresses receptors for certain biologically active peptides (Peptides, Vol. 4, pages 683-686, 1983.)

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a receptor-targeted drug delivery system.

It is an object of the present invention to provide a therapeutic method of treating humans having diseased organs or tissues.

It is an object of the present invention to provide a diagnostic method for determining whether a human has diseased organs or tissues.

It is another object of the invention to provide processes for making the receptor-targeted drug delivery system.

These and other objects are achieved by the present invention which provides in one embodiment a receptor-targeted drug delivery system comprising:

a polymeric drug carrying agent selected from the group consisting of liposomes and nanoparticles;

a ligand capable of binding a receptor, said ligand being covalently attached to said carrying agent and being a therapeutic and functional pharmacological agent; and

a drug, said drug being associated with said drug carrying agent.

In another embodiment a therapeutic method of treating a human having a diseased organ or tissue is provided, comprising: administering an effective amount of the receptor-targeted drug delivery system of the present invention to the human to affect the metabolism of cells expressing receptors for the ligand on their surfaces or nearby cells, said cells being among those of the diseased organ or tissue.

In yet another embodiment a diagnostic method for determining whether a human has a diseased organ or tissue is provided, comprising: administering the receptor-targeted drug delivery system to the human; and noninvasively detecting the localization of said drug.

In addition, processes are taught for making the receptor-targeted drug delivery system of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts schemes for the synthesis of ligand-lipid conjugates.

Figure 2 shows examples of ligands with reactive functional groups and ligand-lipid conjugates made therefrom.

Figure 3 shows the affinity of ligand-lipid conjugates for their receptors.

Figure 4 reports data demonstrating the formation of stearyl-pindolol-containing liposomes and the encapsulation of a fluorescent solute.

Figure 5 shows the stability of encapsulated solutes within liposomes composed of ligand-lipid conjugates, as measured by ^{45}Ca-EDTA release.

Figure 6 reports data showing that ligand-lipid conjugates are incorporated into the structure of the liposomes.

Figure 7 shows the affinity of adenosine phosphatidyl ethanolamine-bearing liposomes for adenosine receptors.

Figure 8 shows the affinity of stearyl-pindolol-bearing liposomes for beta-adrenergic receptors.

Figure 9 reports data showing the delivery of methotrexate to cells with beta-adrenergic receptors by ligand targeted liposomes containing methotrexate.

DETAILED DESCRIPTION OF THE INVENTION

It is a discovery of the present invention that ligands having therapeutic and functional pharmacologic activity and capable of binding a receptor can be covalently joined to polymeric carrying agents to form ligand-polymeric carrying agent conjugates which retain their ability to bind to cell surface receptors. Drugs thus associated with these ligand-polymeric carrying agents are thereby delivered to cells bearing the corresponding cell surface receptor.

Carrying agents contemplated for use in the present invention are polymeric substances, although the polymers need not be covalent polymers. For example, liposomes are suitable polymeric carrying agents whose monomer molecules are generally lipids or phospholipids which are noncovalently joined. Alternatively, nanoparticles may be used as polymeric carrying agents. Nanoparticles are covalently linked

polymers of monomers such as acrylates, cyanoacrylates, aromatic and aliphatic dicarboxylic acids and the like. The nanoparticles may be either homopolymers or heteropolymers. The size of the polymeric carrying agents may range from about 20 nm to about 10 u in diameter. Any size which would enable the polymeric carrying agents to circulate in the vasculature or other body compartment is suitable for most applications. However, for topical and other means of administration, even larger particles could be used.

Liposomes may be formed using standard methods such as the reverse evaporation method (REV) of Papahadjopolous (Proceedings of the National Academy of Sciences U.S.A., Vo. 75, pgs. 4194-4198, 1978), the dehydration-rehydration method (DRV) of Gregoriadas (U.K. Patent Application 8321012, 1983), sonication to form small unilamellar vesicles (SUV) (Biochemistry Vol. 8, pg. 344, 1967) or other suitable methods.

The lipids employed in such liposomes will generally be naturally occurring lipids such as phosphatidylcholine, phosphatidylserine, sphingomyelin, cholesterol, their synthetic analogs, and mixtures thereof.

Nanoparticles may be formed using standard procedures taught in the art. For example, Brasseur et al. European Journal of Cancer, Vol. 16, pgs. 1441-1445, 1980, teaches preparation of nanoparticles from methylcyanoacrylate by means of polymerization and filtration through a fritted glass filter. (See, for other examples, Couvreur, et al. Journal of Pharmacy and Pharmacology, Vol. 31, pgs. 331-332, 1979, and Leong et al. Journal of Biomedical Materials Research, Vol. 20, pgs. 51-64, 1986)

The ligands which are covalently attached to the polymeric carrying agents may be any that are therapeutic and functional pharmacologic

agents, and which bind to receptors on the surface of cells. As used herein, the term therapeutic and functional pharmacologic agent means natural or synthetic substances which produce biological effects by stimulating or blocking specific cell surface receptors. The substances may be of varied chemical structures, such as peptides, or other organic chemical structures. Desirably the avidity of such binding is K_D less than 10^{-5}M and preferably the binding would be even stronger, with K_D less than 10^{-8}M. The ligands may be, for example, hormones, drugs, neurotransmitters or biologically active peptides or their chemical derivatives or analogs. (See, Burger, A., Medicinal Chemistry, Third Ed., Vol. 2, John Wiley & Sons, New York 1970 and Snyder, S. Science, Vol. 209, pgs. 976-983, 1980.) Specific examples are beta-adrenergic compounds, insulin, growth hormone, analogs of adenosine, and opiate drugs. Receptors for these ligands are known,

and some are discussed, for example, in Snyder, Science, Vol. 224, pgs. 22-31 (1984).

Typically, analogs of naturally occurring or synthetic ligands which retain the properties of specificity of binding to the ligand receptors and functionality as pharmacologic agents are used. Such analogs may be designed with appropriate reactive groups for coupling to lipids or to other carrying agent monomers. See, for example, Snyder, Journal of Medicinal Chemistry, Vol. 26, pgs. 1667-1672, 1973.

It is important that conjugates formed by covalently joining the ligand and polymeric carrying agent retain binding and pharmacologic functionality characteristics. Covalently joined ligands can be routinely tested to insure that these properties are retained.

The preferred way to insure retention of receptor-binding and pharamcologic activity is to provide a ligand having a unique

functional group for joining to the polymeric carrying agent. Such functional groups should not be constituents of the binding or active portion of the ligand. Covalent ligand-monomer conjugates of defined chemical structure, composition and purity are synthesized according to techniques known in the art. In any event, the ligand should preferably have no more than about 10, and more preferably no more than about 2, identical functional groups. If there are multiple similarly reactive groups, then means must be taken to separate the reaction products. Once separated, each can be chemically characterized and tested for retention of binding to cell surface receptors. Such means of separation and testing are standard procedures which are known in the art, and certain techniques will be preferred depending on the compound. As the number of similarly reactive functional groups increases, however, such separation and testing become more difficult,

and obtaining substantially pure receptor-targeted carriers becomes significantly more complicated.

The ligands may be covalently attached to the polymeric carrying agents either before or after polymerization, that is to say the ligand may be attached to carrying agent monomer molecules or to the subsequently prepared polymeric carrying agent. If first attached to monomers, then the ligand-monomer conjugates may be used alone or preferably are admixed with other free monomers and processed to form the polymeric carrying agent. Of course, the monomers used to prepare the conjugate need not be the same as the free monomers used when subsequently polymerizing the carrying agent. In fact, other monomers generally will be preferred in order to permit separate optimization of the preparation and properties of conjugates and polymeric carrying agents. The ratio of free monomers to ligand-monomer conjugates may

vary. Preferably, at least one ligand-monomer will be incorporated per subsequently prepared carrying agent polymer. In the case of liposomes as the carrying agent, it is preferred that the amount of ligand-monomer conjugates be from about 0.5 mol% to about 10 mol%.

Generally, covalent coupling of ligands to polymeric carrying agents is accomplished using standard techniques of organic chemistry (See Figure 1). For example, a ligand bearing a carboxylic acid functional group can be coupled to a lipid bearing a primary amino group, resulting in an amide ligand-lipid conjugate; or the carboxyl group-containing ligand can be coupled to an hydroxyl-containing lipid, resulting in an ester ligand-lipid conjugate. For example, lipids which may be coupled to carboxylic acid-containing ligands include the naturally occurring stearylamine, phosphatidyl ethanolamine, sphinganine, and ceramide, as well as paraffin alcohols and other

synthetic lipids. Generally such reactions require a carbodiimide reagent as a stoichiometric reagent. Conditions for these reactions are well known in organic chemistry and vary with the specific reactants. Alternatively, an amino group-containing ligand can be coupled to a fatty acid or its active ester or acid chloride, again yielding an amide ligand-lipid conjugate; or a ligand bearing an hydroxyl group can be reacted with a fatty acid to yield an ester linked ligand-lipid conjugate. Yet another means of coupling would involve nucleophilic substitution of electrophilic ligands, for example by thiol derivatives of lipids or phospholipids. Lipids which may be coupled to nucleophilic ligands include phosphatidyl serine, fatty acids preferably having from 12 to 18 carbon atoms, and their active esters or acid chlorides (X). Standard reaction conditions are used depending upon the reactants. Lipids for coupling to electrophilic

ligands include thiol derivatives of phosphatidyl ethanolamine or other lipid. Such lipids have been described (Nature 288, 602-604, 1980).

As recognized by one skilled in the art, similar reactions also are used to join ligands to synthetic monomers to form nanoparticles and to join ligands to pre-formed liposomes and nanoparticles. As a specific example of ligand-targeted nanoparticles, polyalkyl cyanoacrylate or aerylamide nanoparticles may be attached to ligands by copolymerizing the acrylate monomer with a small amount (0.1 to 5%) of an active ester of acrylic acid, as described in Methods in Enzymology, vol. LXXXIII, pp. 306-310, 1982. After formation of polymer and processing to form nanoparticles, an amine containing ligand is reacted with the matrix and the ligand-polymer is purified by standard techiques. Alternatively, a ligand-monomer conjugate is prepared using an amine containing ligand and the active acryloyl ester. This

compound is then copolymerized with the acrylic acid monomer to form targeted nanoparticles. The specific reactions to be employed will vary with the compounds used and are known in the art.

The drug which is chosen to be associated with (carried by) the carrying agent may or may not interact with the same cell surface receptor as does the ligand which is covalently bound to the outer surface of the carrying agent. For example, insulin could be used to target liposomes, by being covalently bound to the liposomes, and could also be delivered by the liposomes to the target tissue or organ. Association of the drug to the carrying agent can occur, for example, by the drug filling interstitial spaces of the carrying agent, such that the carrying agent physically entraps the drug, or by covalent, ionic, or hydrogen bonding, or by means of adsorption by non-specific bonds. Whatever the mode of association, the drug must retain its

therapeutic or diagnostic properties. The drugs may be solid or liquid, hydrophobic, hydrophilic or both.

The dosages of drug associated with the carrying agent which are to be administered are those required to deliver an effective amount of the drug to the target tissue or organ. Such amounts are determined clinically. Such amounts are determined clinically. The same considerations of toxicity and efficacy apply for the carrying agent-associated drug as for the free drug. In general lower dosages may suffice as compared to administration of free drug, because the carrying agent will stabilize the drug in the body, making it more resistant to degradation. In addition, the enhanced delivery efficiency due to the targeting of the drug will provide the same effective concentration of a drug to a localized area of the body even when using a lower total dosage as computed per body weight.

In general, two classes of drugs are contemplated for use in the present invention: bioaffecting molecules and diagnostic molecules. Bioaffecting molecules are any which affect cell and body functions, either positively or negatively. This class includes cytotoxics, cytostatics, hormones, neurotransmitters, biologically active peptides and the like. Diagnostic molecules are those which can be detected in the body without recourse to invasive procedures such as surgery. Such molecules include fluorescent compounds, radiolabeled compounds, X-ray opaque dyes, ferromagnetic compounds, and the like. A compendium of drugs which may be used is found in Gilman et al., Goodman and Gilman's The Pharmacologic Basis of Therapeutics, MacMillan, New York, 1980.

Administration of the receptor-targeted drug delivery system may be by any of the various means known in the art. Such means include but are not limited to: nebulizers, eye drops, topically, by implant, intraperitoneally, intramuscularly, intravenously, and orally.

The following examples are not meant to limit the scope of the invention, but only to more fully illustrate the invention.

Example 1.

Synthesis of Adenosine-Phosphatidyl Ethanolamine (Adenosine-PE)

Coupling of N^6-(4-carboxymethyl)phenyladenosine (1a in figure 2) and distearyl phosphatidyl ethanolamine (DSPE) was performed as follows. To 1a (0.123 mmol) in dimethyl formamide (DMF) (10 ml) at 4°C were added sequentially with stirring hydroxybenzyltriazole and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.15 mmol of each). After 30 min the solution was warmed to 23°C and DSPE (0.125 mmol) and triethylamine (15 mmol) dissolved in $CHCl_3$ were added. The reaction was stirred under an argon atmosphere for 16 h. The solvents were evaporated, and the isolated oil product was dissolved in a mixture of

$CHCl_3$/MeOH (2/1) and extracted three times with ice-cold one-tenth saturated $NaHCO_3$, and then with water. The resulting white powder was chromatographed over silica gel using $CHCl_3$/MeOH/water (60/30/5) as solvent to yield 33 mg pure adenosine-phosphatidylethanolamine (1b) (23% final yield). The product was a ninhydrin negative, phosphate positive spot (R_f 0.44) on thin-layer chromatography using silica gel developed in $CHCl_3$/MeOH/water (60/30/5.) It forms a white emulsion in aqueous solutions. Proton nmr (d_6DMSO) δ ppm 8.54 and 8.39 (each s,1H,adenine), 8.21 (t,1H, amide), 7.82 and 7.20 (each d, 2H,aromatic), 5.93 (d,1H, ribose-C_1), 0.85-1.49 (m,alkyl). UV (95% ethanol) 303C(log e = 4.36). $C_{55}H_{99}O_{13}N_6P_1$, predicted 1130, $[M+H]^+$ (FAB) 1131.

Example 2.

Synthesis of Palmityl-naltrexamine

Acylation of 6-beta -naltrexamine (2a.) with palmityl chloride was performed as follows. Naltrexamine free base (0.24 mmol) in tetrahydrofuran (THF) (2 ml) was cooled to -10°C and palmityl chloride (0.26 mmol) was added. The reaction was stirred for 2 h and then at 23°C for 16h. After evaporation of the solvent and dissolution in methanol, NH_4OH (2 drops) was added and stirred 16 h. The solvents were evaporated and the oil was chromatographed on silica gel with a $CHCl_3$ wash and then eluted with ethylacetate/methanol/ammonia (100/10/3). The peak fractions contained about 80% yield of palmityl-naltrexamine (2b in figure 2.) $C_{34}H_{51}N_2O_4$ calculated 551.8.

Example 3.

Synthesis of Stearyl-Pindolol

Alkylation of stearyl mercaptan with bromoacetylindolementhane (3a in Figure 2) was performed as follows. The Na-salt of stearyl mercaptan (0.12 mmol) was prepared in $NaOCH_3$ in methanol (5 ml). Toluene (5 ml) was added and 3a in methanol (0.12 mmol) was added. The reaction mixture was stirred at room temperature under an argon atmosphere for 24 h. After evaporation and dissolution in $CHCl_3$, the material was extracted with water, dried over Na_2SO_3 and evaporated to yield a crude oil. The product was isolated from silica column chromatography using $CHCl_3$/methanol (9/1) solvent. Proton nmr ($CDCl_3$ ppm 7.17 - 6.55 (m,6H, aromatic), 4.12-4.03 (m,3H,-$C\underline{H}_2C\underline{H}$(OH)-), 3.60 (broad s, 2H,-NH), 3.05 (s, 2H, -$COCH_2S$-), 1.24-0.85 (m,55H, cycloheaxane and -CH_2- and -CH_3). UV (MeOH) 265 (6865), 287 (3430). $C_{41}H_{71}O_3N_2S_1$, predicted 672.07, $[M+H]^+$ (FAB), 687.

Example 4.

Ligand-Lipid Incorporated into Liposomes

SUV (small unilamellar vesicles) were formed by the technique of Biochemistry, 8, pg. 344, 1967, using 9.5 umol phosphatidyl choline (labeled with tritium), 10 umol cholesterol and 0.5 umol of conjugate 1b (Figure 2.) The resulting lipsomes were purified by chromatography over Sephadex™ G25. Fractions containing ^{3}H-phosphatidyl choline were extracted with $CHCl_3$/MeOH (2/1) and water. The organic layer was dried and resuspended in 95% ethanol and the UV spectrum was obtained. It revealed a peak at 303 nm corresponding to quantitative recovery of (1b.)

Example 5

Affinity of Ligand-Carrying Agent Monomer Conjugates for Receptors

The affinity of ligand-carrying agent monomers conjugates for membrane receptors was determined by comparing their ability to inhibit radiolabeled ligands from binding to their specific receptors. Stearyl-pindolol (3b) shows an IC_{50} (concentration which produces 50% inhibition) for the beta-adrenergic receptor of about 500 nM and in the presence of the solubilizer HPBCD about 20nM. The adenosine-lipid conjugate shows an IC_{50} of about 50 nM for adenosine (A_1) receptors.

Standard receptor binding assays were performed with ^{125}I-cyanopindolol (50 pM) (compound A) and rat lung membrane homogenates and with ^{3}H-l-PIA phenyl isopropyl adenosine (compound B) (2 nM) and calf cortex membrane homogenates. The results are shown in Figures 3A

and 3B, respectively. Varying concentrations of ligand-lipid conjugates or other compounds were mixed with the radio-ligand before the addition of membranes. Specific binding is defined by the binding inhibited by the addition of 1uM pindolol in assays of compound A and 10uM 1-PIA (phenylisopropyl adenosine) in assays of compound B. Percent binding is defined as the binding at a given concentration of inhibitor compared to specific binding.

Example 6.

Formation of Stearyl-Pindolol Liposomes and Encapsulation of Solute

Small unilamellar vesicles (SUV) as described above were prepared using distearyl phosphatidyl choline (20 umol) and stearyl pindolol (0.2 umol) with cholesterol (20 umol). Trace quantities of ^{3}H-phosphatidyl choline were used as a marker of the lipid membranes.

Carboxyfluorescein (0.15 M) was included in the aqueous phase. Equal quanities of liposomes were loaded on a molecular sieve column (Sephadex G25, 1.5 x 30 cm). Fractions were assayed for ^{3}H and fluorescence. The fluorescence measurements plotted in Figure 4 were obtained after releasing encapsulated dye with Triton X-100 (1%). Over 90% of the fluorescence was quenched for both types of liposomes.

Example 7.

Stable Encapsulation of Solutes into Liposomes Composed of Ligand-Lipid Conjugates.

Phosphatidyl choline: cholesterol REV liposomes (as described by Papahadjopolous, Proceedings of the National Academy of Science, USA. 75, pp. 4194-4198, 1978) with or without 1 mol% stearyl pindolol were prepared containing ^{45}Ca-EDTA. The liposomes were purified by repeated centrifugation and then dialyzed to remove unencapsulated ^{45}Ca-EDTA.

Aliquots of the liposomes were placed into dialysis bags at 4°C or 37°C and transferred to new dialysate at the intervals plotted in Figure 5. Released ^{45}Ca is defined as the radioactivity measured in the dialysate.

Example 8.

Incorporation of Stearyl-pindolol Conjugate into Liposomes

Phosphatidyl choline: cholesterol SUV liposomes were prepared using 5 mol% stearyl-pindolol and trace ^{3}H-phosphatidyl choline and chromatographed over Sephadex G25. The fractions containing the liposomes were pooled and rechromatographed and the resulting fractions were assayed for ^{3}H and quantities of stearyl-pindolol using a radioreceptor assay (Life Sciences 23, 2031-2038, 1978). Results are shown in Figure 6.

Example 9.

Affinity of the Adenosine-Targeted Liposomes for the Adenosine (A_1) Receptor

SUV liposomes prepared as described above composed of distearyl phosphatidyl choline and cholesterol and supplemented with either adenosine-phosphtidyl ethanolamine (adenosine-PE) 1b, biotin-PE or stearylpindolol (3b) at 1 mol% were prepared and purified. The liposomes were added to standard receptor binding assays using ^{3}H-l-PIA (phenyl isopropyl adenosine) and calf cortex membranes with l-PIA (10 mM) used as blank. Figure 7 shows the inhibition of receptor binding to ^{3}H-l-PIA which is caused by the targeted liposomes.

Liposomes containing adenosine-PE but not stearyl-pindolol or biotin-PE compete effectively for adenosine A_1 receptors.

Example 10.

Affinity of Stearyl-Pindolol Liposomes for the Beta-Adrenergic Receptor

SUV liposomes were prepared as described above with egg PC (phosphatidyl choline) and cholesterol and varying concentrations of stearyl-pindolol (0-10 mol%) as indicated. Varying quantities of liposomes were added to standard receptor binding assays using ^{125}I-cyanopindolol and rat lung membranes. Liposomes containing stearyl-pindolol inhibit binding in a dose-dependent manner whereas liposomes lacking stearyl-pindolol do not. Maximal inhibition obtained by the liposomes was only approximately 50% of specific ^{125}I-cyanopindolol binding, perhaps due to geometric constraints on the accessibility of liposomes to membrane receptors.

Example 11.

Delivery of Methotrexate to Cells with B-Adrenergic Receptors by Targeted Liposomes.

Distearyl phosphatidyl choline (DSPC) and cholesterol liposomes containing methotrexate supplemented with nothing, stearyl-pindolol or adenosine-PE at 1 mol% were added to N18 tg2 mouse neuroblastoma for 48 h. In control experiments free methotrexate was used. In two experiments, before addition of methotrexate targeted stearyl-pindolol liposomes, either free pindolol (10 mM) or empty stearyl-pindolol liposomes were added. Figure 9 shows the results.

While specific embodiments have been given above, they do not limit the scope of the invention which is defined by the appended claims.

CLAIMS

1. A receptor-targeted drug system, comprising:

a polymeric drug carrying agent selected from the group consisting of liposomes and nanoparticles,

a ligand capable of binding a receptor, said ligand being covalently attached to said carrying agent, and being a therapeutic and functional pharmacological agent; and

a drug, said drug being associated with said drug carrying agent.

2. The receptor-targeted drug delivery system of claim 1, wherein said drug affects the cell and body functions.

3. The receptor-targeted drug delivery system of claim 1, wherein the localization of said drug can be detected noninvasively.

4. The receptor-targeted drug delivery system of claim 2, wherein the drug is cytotoxic.

5. The receptor-targeted drug delivery system of claim 2, wherein the drug is cytostatic.

6. The receptor-targeted drug delivery system of claim 2, wherein the drug is a hormone.

7. The receptor-targeted drug delivery system of claim 2, wherein the drug is a neurotransmitter.

8. The receptor-targeted drug delivery system of claim 2, wherein the drug is a neuropeptide.

9. The receptor-targeted drug delivery system of claim 3, wherein the drug is fluorescent-labelled.

10. The receptor-targeted drug delivery system of claim 3, wherein the drug is radio-labelled.

11. The receptor-targeted drug delivery system of claim 3, wherein the drug is opaque to X-rays.

12. The receptor-targeted drug delivery system of claim 1, wherein said ligand and said drug are both capable of binding the same receptor.

13. The receptor-targeted drug delivery system of claim 1, wherein the drug carrying agent is a liposome.

14. The receptor-targeted drug delivery system of claim 1, wherein the drug carrying agent is a nanoparticle.

15. A therapeutic method of treating a human having a diseased organ or tissue, comprising:

administering an effective amount of the drug delivery system of claim 2 to the human to affect the metabolism of the cells expressing the receptors on their surfaces, said cells being in the diseased organ or tissue.

16. A diagnostic method for determining whether a human has a diseased organ or tissue, comprising:

administering the drug delivery system of claim 3 to the human; and

noninvasively detecting the localization of said drug.

17. A process for making the receptor-targeted drug delivery system of claim 1, comprising:

covalently joining the ligand to the polymeric drug carrying agent, to form ligand-carrying agent conjugates wherein substantially all of said conjugates retain the ability to bind receptors;

mixing the ligand-carrying agent conjugates with the drug under conditions to associate the drug with the carrying agent.

18. The process of claim 17, wherein said drug becomes associated with said carrying agent by means of physical entrapment.

19. The process of claim 17, wherein said drug becomes associated with said carrying agent by means of adsorption.

20. A process for making the receptor-targeted drug delivery system of claim 1, comprising:

covalently joining the ligand to drug carrying agent monomer molecules to form ligand-monomer conjugates, wherein substantially all of said conjugates retain the ability to bind receptors;

assembling said ligand-monomer conjugates in the presence of drug under conditions to form drug-associated ligand-polymeric carrying agents.

21. The process of claim 20, wherein said drug is associated with said ligand-polymer carrying agents by means of entrapment.

22. The process of claim 20, wherein said drug is associated with said ligand-polymer carrying agents by means of adsorption.

23. The receptor-targeted drug delivery system of claim 13 wherein the ligand is N^6-p-carboxymethyl phenyladenosine.

24. The receptor-targeted drug delivery system of claim 13 wherein the ligand is 6-beta-naltrexamine.

25. The receptor-targeted drug delivery system of claim 13 wherein the ligand is bromoacetyl indole menthane.

26. The receptor-targeted drug delivery system of claim 13 wherein the ligand is capable of binding to the beta-adrenergic receptor.

27. The receptor-targeted drug delivery system of claim 13 wherein the ligand is capable of binding to the adenosine receptor.

28. The receptor-targeted drug delivery system of claim 13 wherein the ligand is capable of binding to the opiate receptor.

FIG. 1

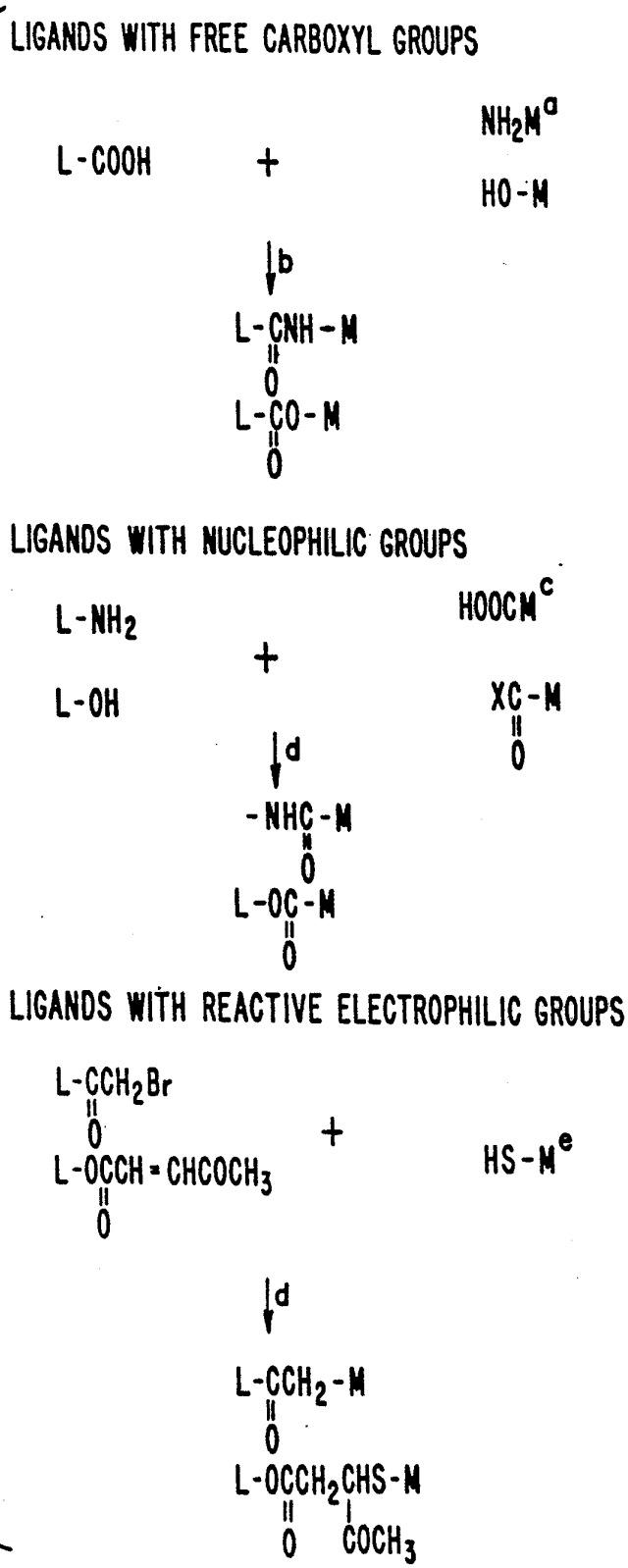

LIGANDS WITH FREE CARBOXYL GROUPS
L-COOH
+
NH2M[a]
HO-M
b
L-CNH-M
O
L-CO-M
O
LIGANDS WITH NUCLEOPHILIC GROUPS
L-NH2
+
L-OH
HOOCM[c]
XC-M
O
d
-NHC-M
O
L-OC-M
O
LIGANDS WITH REACTIVE ELECTROPHILIC GROUPS
L-CCH2Br
O
L-OCCH=CHCOCH3
O
+
HS-M[e]
d
L-CCH2-M
O
L-OCCH2CHS-M
O
COCH3

FIG. 2

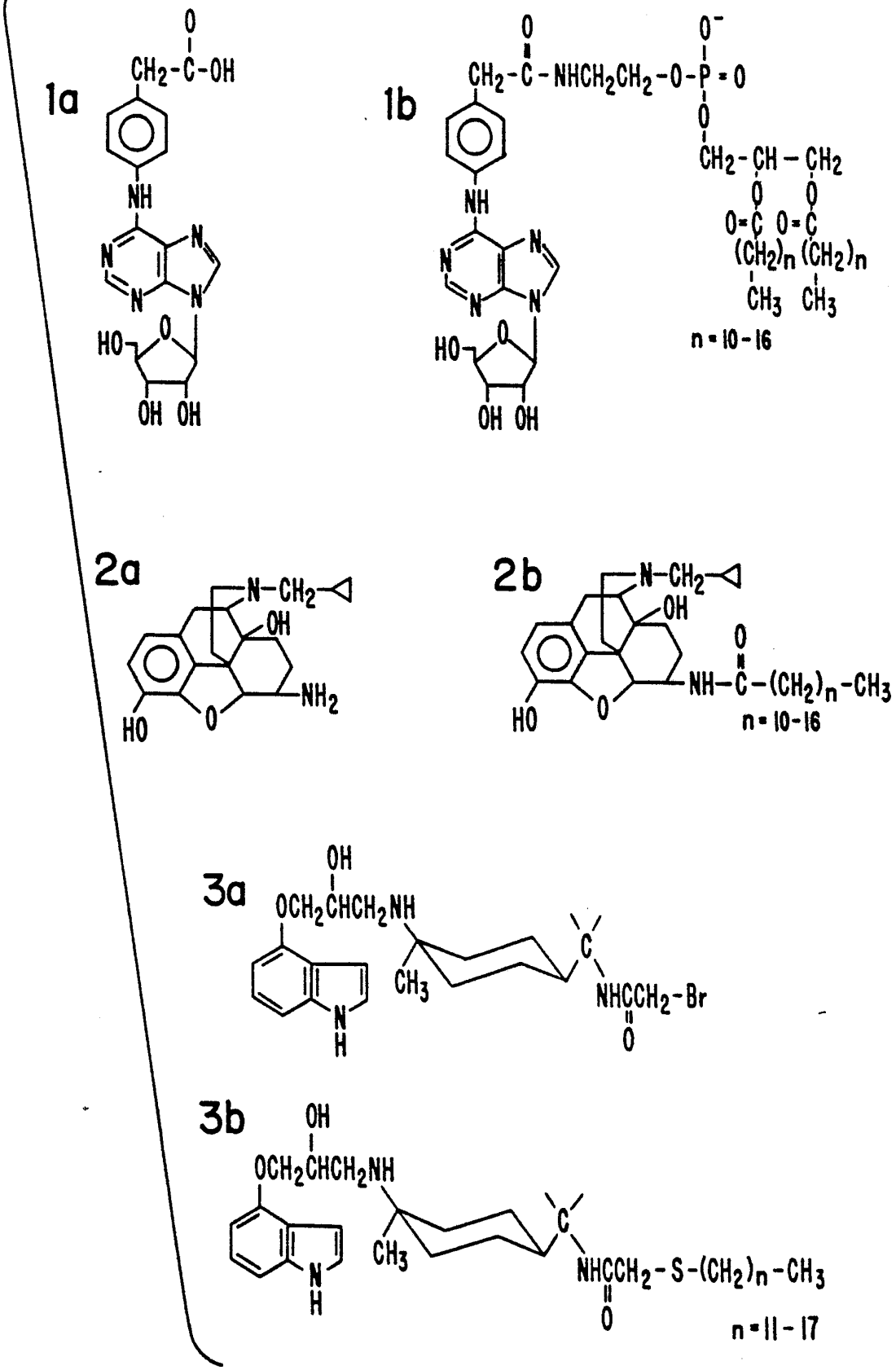


FIG. 3a

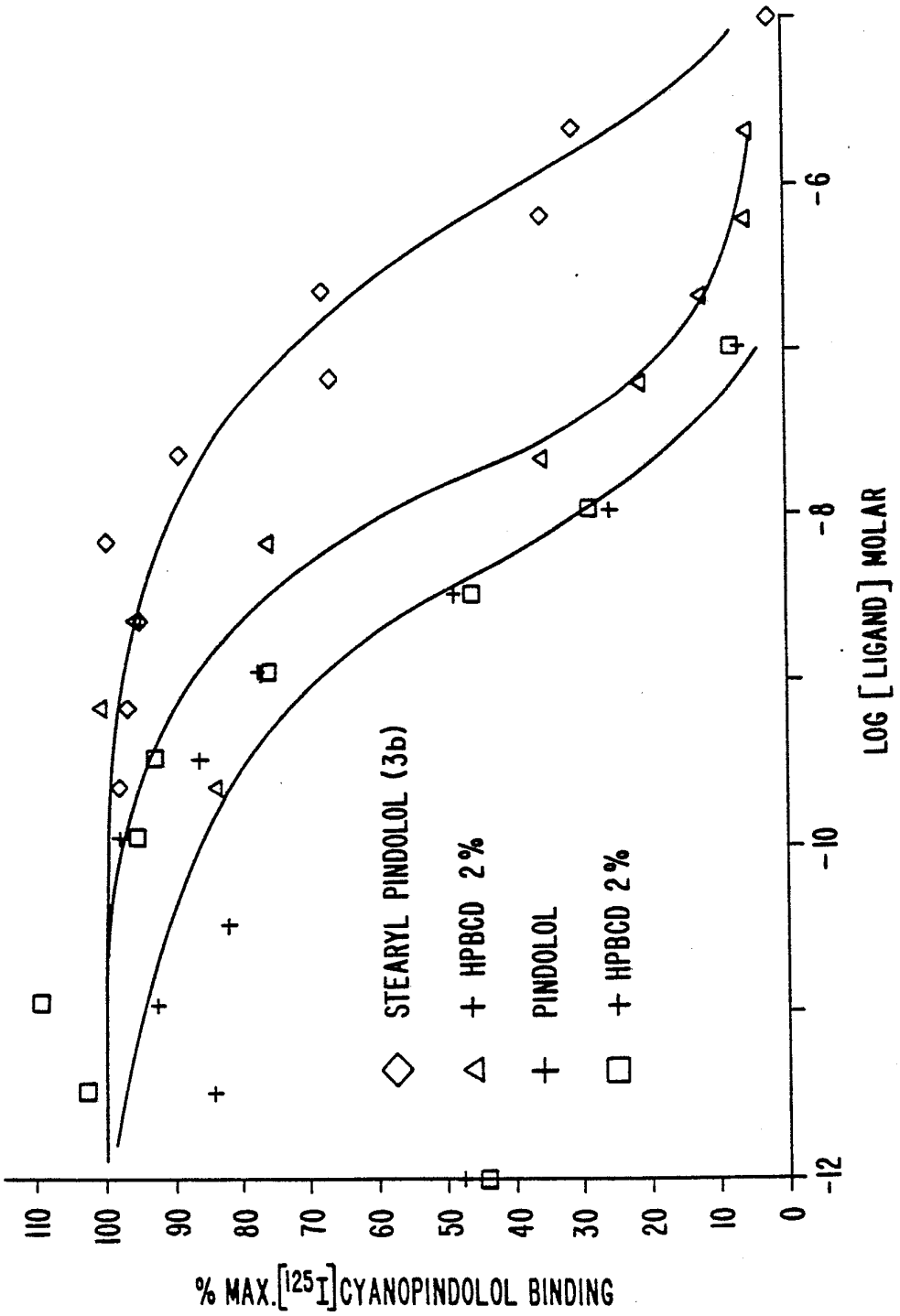

STEARYL PINDOLOL (3b)
+ HPBCD 2%
PINDOLOL
+ HPBCD 2%
% MAX.[125I]CYANOPINDOLOL BINDING
LOG [LIGAND] MOLAR
110
100
90
80
70
60
50
40
30
20
10
0
-6
-8
-10
-12

FIG. 3b

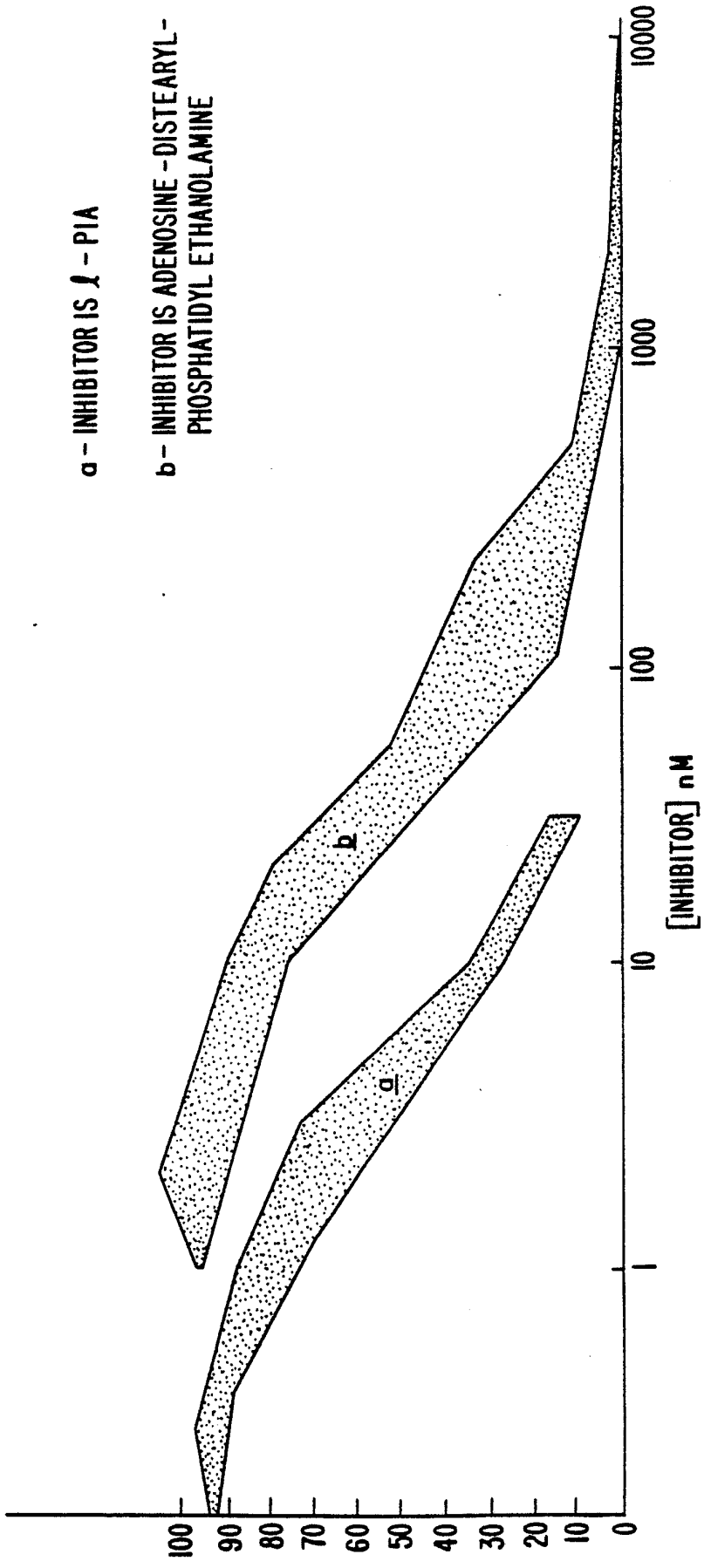

INHIBITION OF BINDING TO CALF CORTEX MEMBRANES
a - INHIBITOR IS L - PIA
b - INHIBITOR IS ADENOSINE - DISTEARYL - PHOSPHATIDYL ETHANOLAMINE
a
b
100
90
80
70
60
50
40
30
20
10
0
% MAX. ^{3}H-L-PIA BINDING
1
10
100
1000
10000
[INHIBITOR] nM

FIG. 4

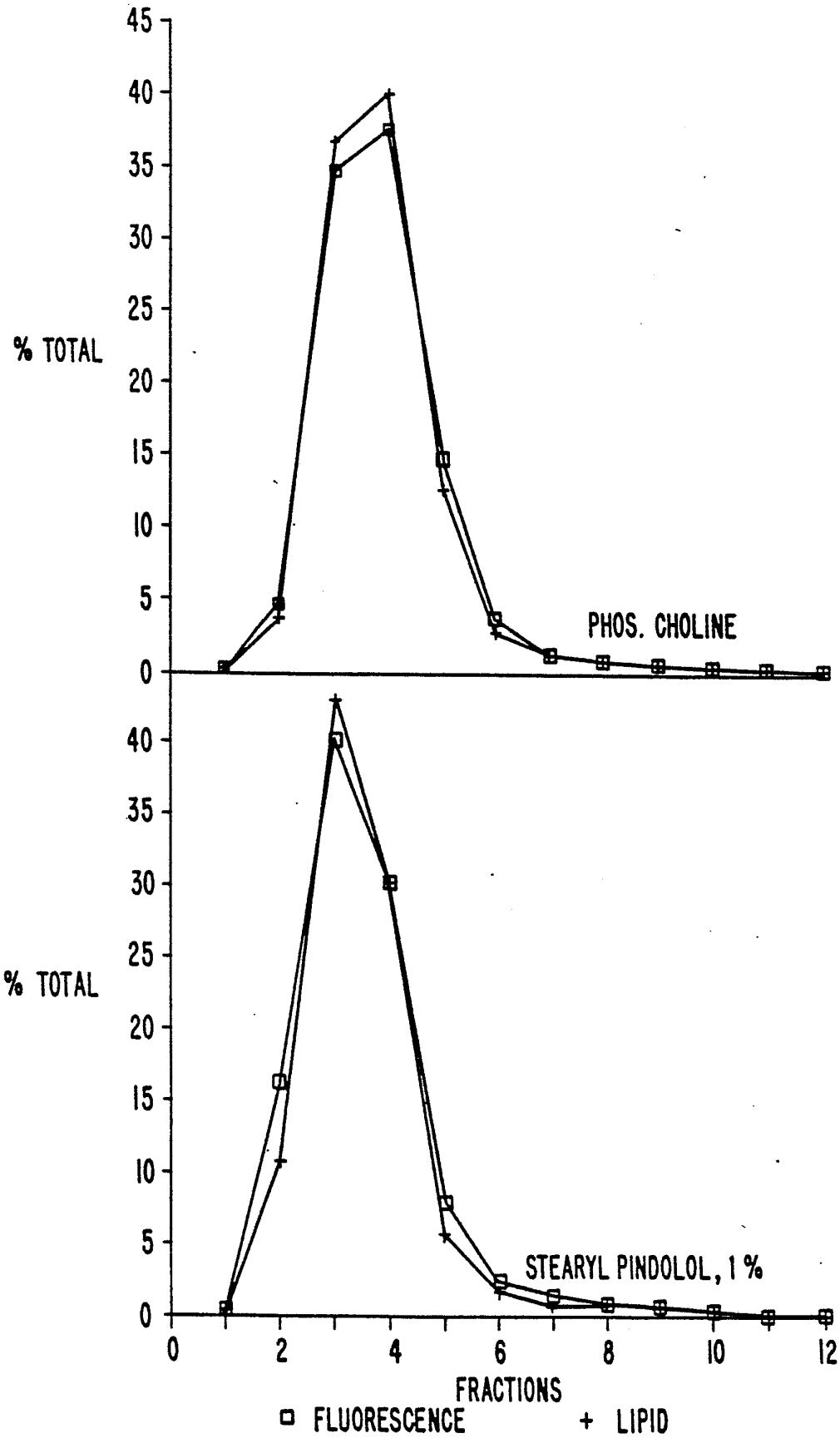

% TOTAL
PHOS. CHOLINE
% TOTAL
STEARYL PINDOLOL, 1 %
FRACTIONS
FLUORESCENCE
LIPID

FIG. 5

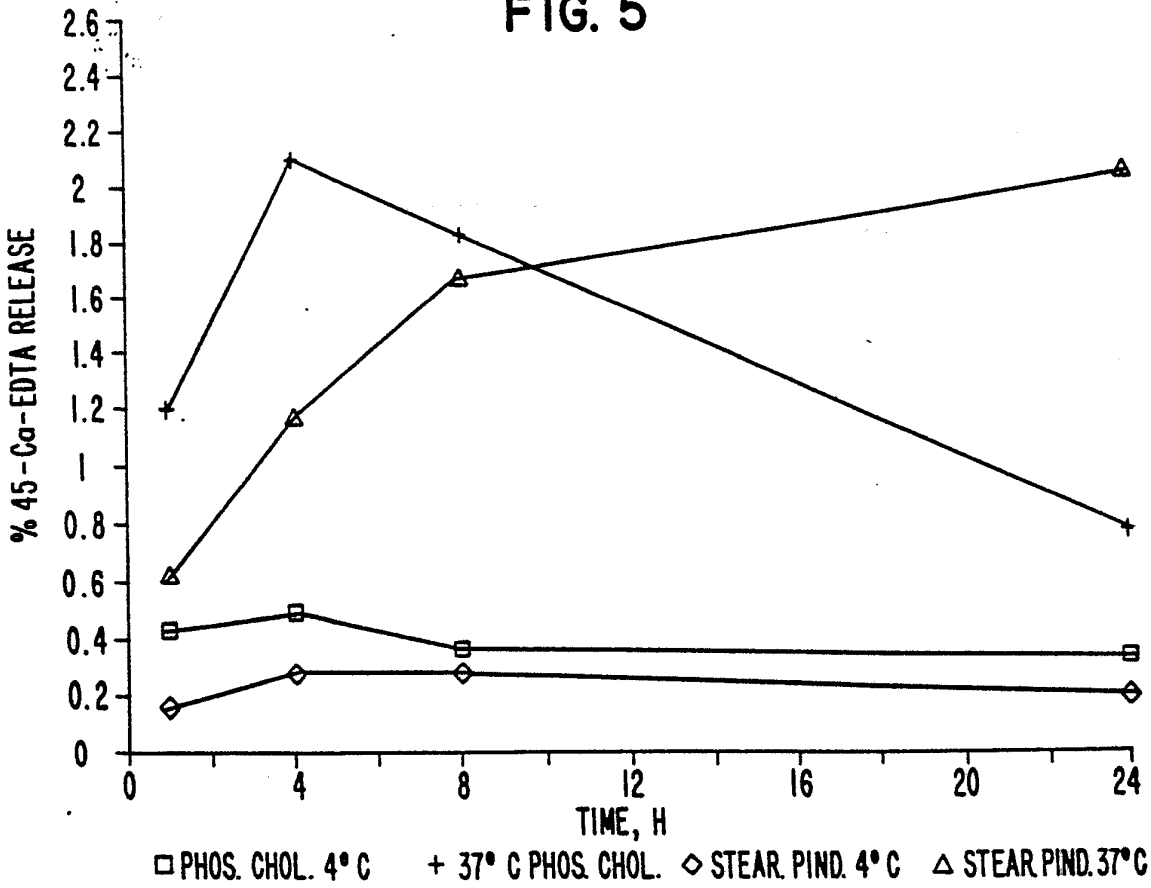

% 45-Ca-EDTA RELEASE
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
4
8
12
16
20
24
TIME, H
□ PHOS. CHOL. 4° C
+ 37° C PHOS. CHOL.
◇ STEAR. PIND. 4° C
△ STEAR PIND. 37°C

FIG. 6

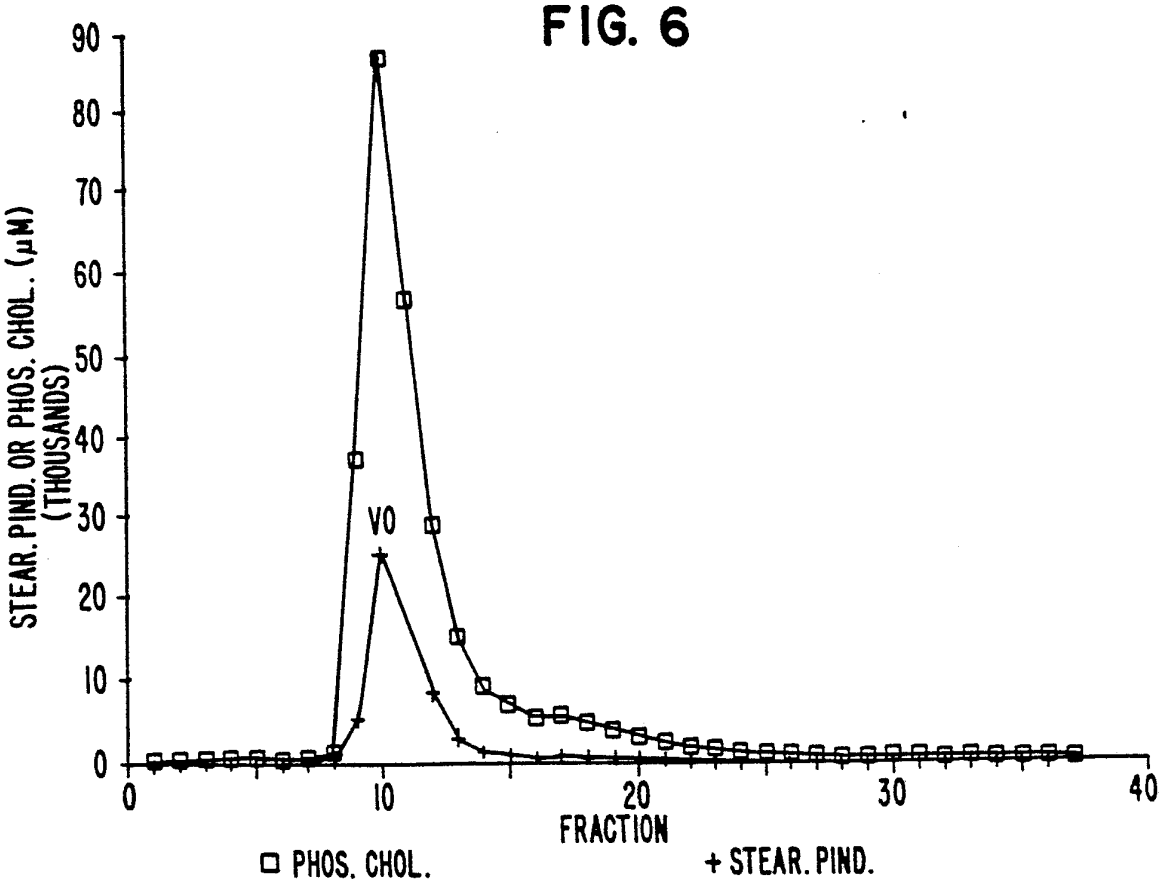

STEAR. PIND. OR PHOS. CHOL. (μM)
(THOUSANDS)
90
80
70
60
50
40
30
20
10
0
VO
0
10
20
30
40
FRACTION
□ PHOS. CHOL.
+ STEAR. PIND.

FIG. 7

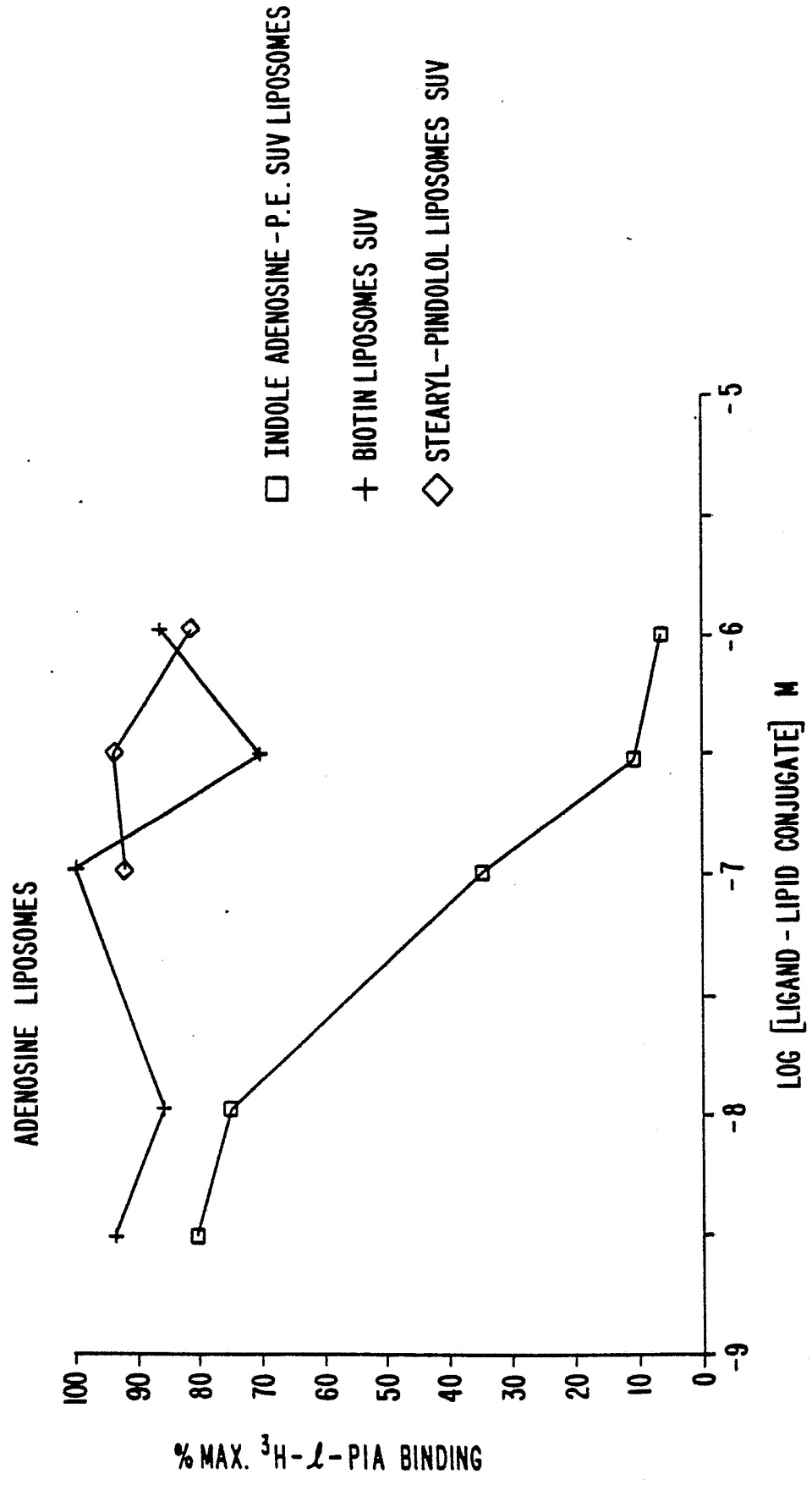

ADENOSINE LIPOSOMES
□ INDOLE ADENOSINE-P.E. SUV LIPOSOMES
+ BIOTIN LIPOSOMES SUV
◇ STEARYL-PINDOLOL LIPOSOMES SUV
% MAX. ^{3}H-l-PIA BINDING
100
90
80
70
60
50
40
30
20
10
0
-9
-8
-7
-6
-5
LOG [LIGAND-LIPID CONJUGATE] M

FIG. 8

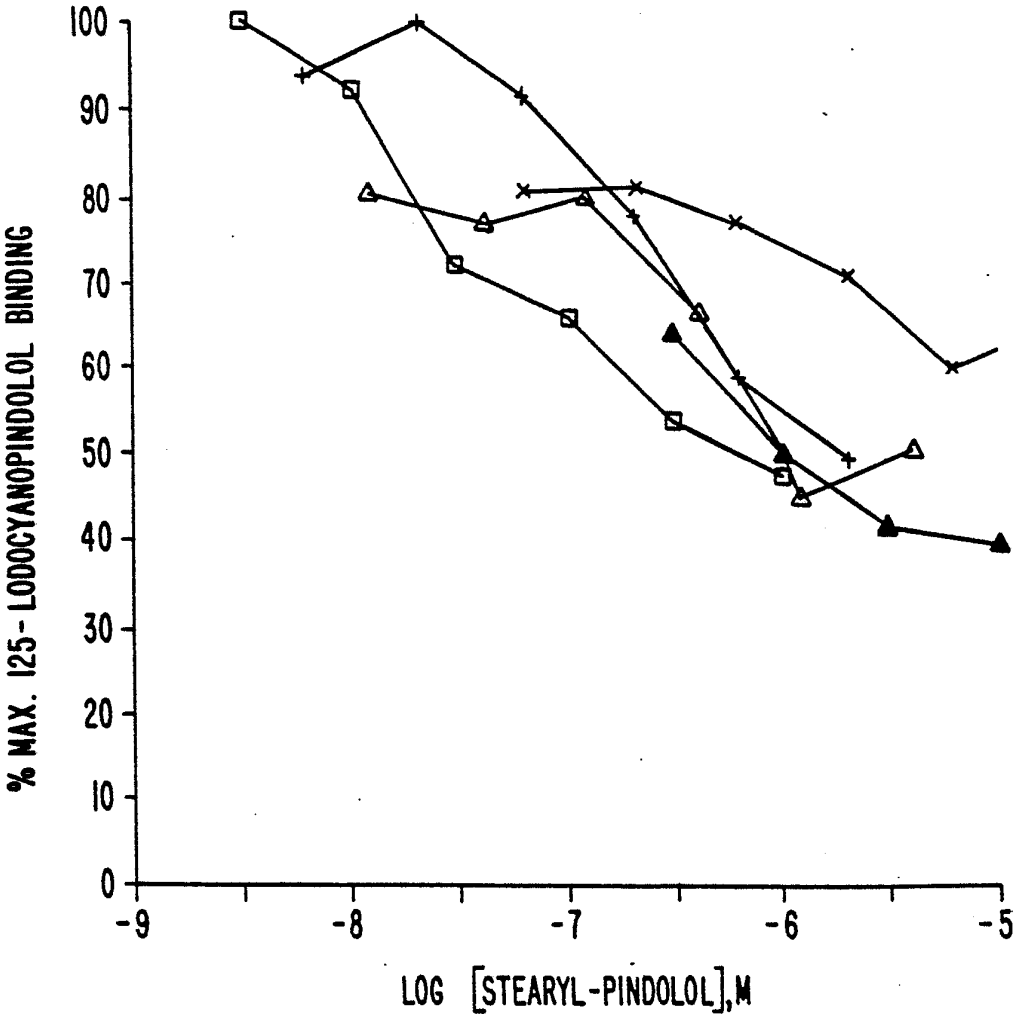
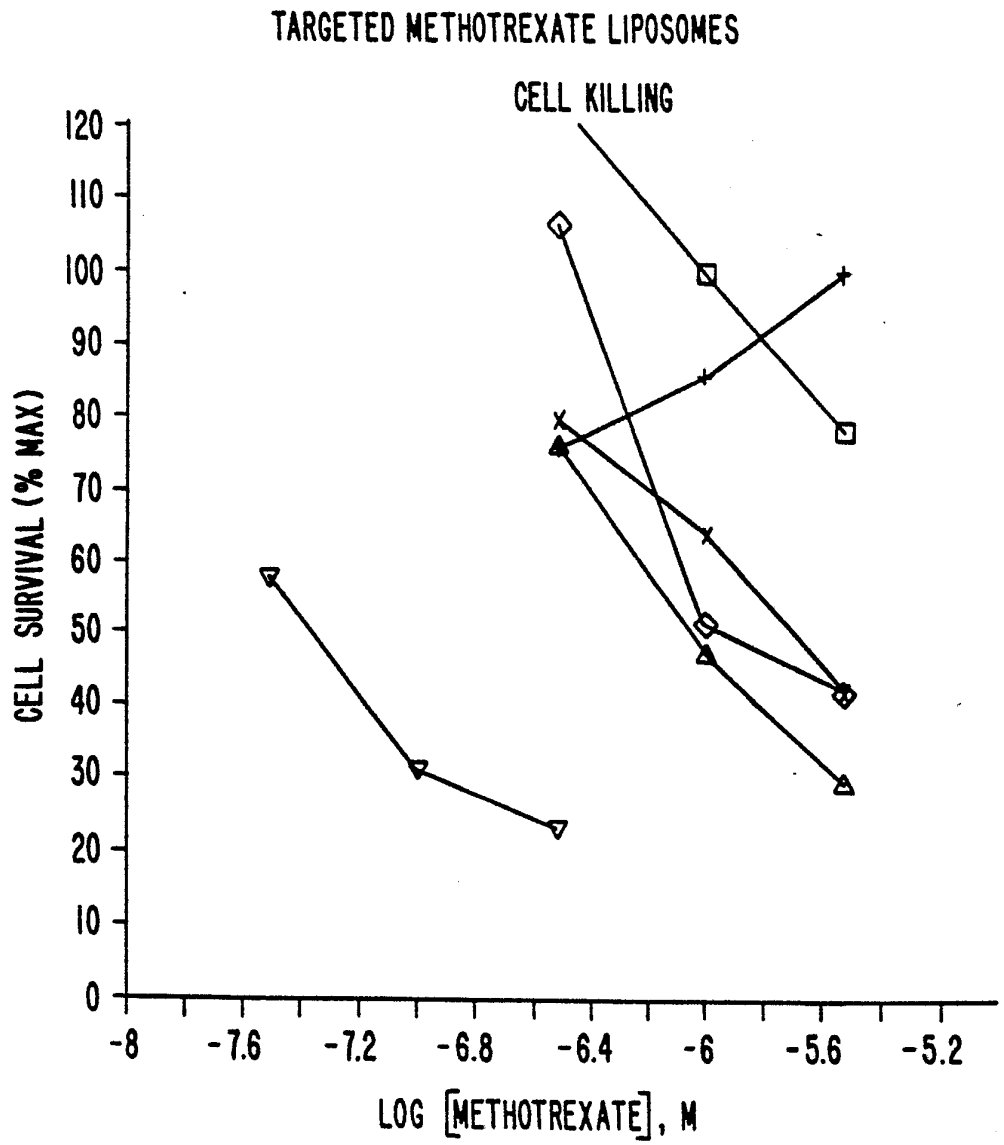

% MAX. 125-LODOCYANOPINDOLOL BINDING
100
90
80
70
60
50
40
30
20
10
0
-9
-8
-7
-6
-5
LOG [STEARYL-PINDOLOL],M
□ 0.5 % STEARYL-PINDOLOL
+ 1 % " "
△ 2 % " "
▲ 5 % " "
× 10 % STEARYL-PINDOLOL

FIG. 9


TARGETED METHOTREXATE LIPOSOMES
CELL KILLING
CELL SURVIVAL (% MAX)
120
110
100
90
80
70
60
50
40
30
20
10
0
-8
-7.6
-7.2
-6.8
-6.4
-6
-5.6
-5.2
LOG [METHOTREXATE], M
DISTEARYL-P.C. SUV UNTARGETED, METHOTREXATE
INDOLE ADENOSINE P.C. SUV, METHOTREXATE
STEARYL-PINDOLOL-P.C. SUV, METHOTREXATE
" + PINDOLOL 10mM
" + STEARYL PINDOLOL-P.C. SUV EMPTY
FREE METHOTREXATE NO LIPOSOMES

INTERNATIONAL SEARCH REPORT

International Application No PCT/US87/00965

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[4] A 61K 49/02; G01N 33/544

US. Cl. 424/1.1,9; 436/528, 529

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	424/1.1, 9, 450, 501; 436/528, 529, 532, 533

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X Y	US, A 4,544,545, published 1 October 1985, Ryan et al. see Col. 2, lines 40-68, Col. 3, lines 1-51	1-13,15,16,26-28 23-25
Y	US, A, 4,460,560, published 17 July 1984, Tokes et al see Col. 2, lines 15-37	14, 20-22
Y	US, A, 4,565,696, published 21 January 1986, Heath et al. see Col. 5, lines 3-20	17-19
Y	US, A, 4,377,567 published 22 March 1983, Geho, see Col. 4, lines 49-57	14
A	US, A, 3,857,931 published 31 December 1974, Hager	
A	US, A, 4,140,662, published 20 February 1979, Reckel et al.	
A	US, A, 4,283,382, published 11 August 1981, Frank et al.	
A	US, A, 4,429,008, published 31 January 1984 Martin et al.	

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
15 July 1987	29 JUL 1987
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	John S. Maples

Form PCT/ISA/210 (second sheet) (October 1981)