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[54] CALCIUM RECEPTOR-ACTIVE MOLECULES

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[58] **Field of Search** 564/337, 366, 564/374, 384, 389; 514/654

[56] **References Cited**

U.S. PATENT DOCUMENTS

2,276,618	3/1942	Kulz et al. .	
2,930,731	3/1960	Heinzelmann	167/52
2,949,359	8/1960	Blout	96/66
3,202,711	8/1965	Fruhstorfer et al. .	
3,262,977	7/1966	Harsanyi et al. .	
3,493,662	2/1970	Duerr	424/330
3,536,712	10/1970	Keck et al. .	
3,689,524	9/1972	Jack et al. .	
3,842,067	10/1974	Sarantakis	
3,862,925	1/1975	Sarantakis .	
3,972,859	8/1976	Fujino et al. .	
4,000,197	12/1976	Barfknecht et al. .	
4,014,937	3/1977	Richardson .	
4,098,890	7/1978	Molloy .	
4,105,602	8/1978	Colescott et al. .	

(List continued on next page.)

FOREIGN PATENT DOCUMENTS

621300	3/1962	Belgium .
1065857	4/1991	China .
217009	1/1984	Czechoslovakia .
0009702	9/1979	European Pat. Off. .
0005848	12/1979	European Pat. Off. .
0007204	1/1980	European Pat. Off. .
0009702	4/1980	European Pat. Off. .
0015505	9/1980	European Pat. Off. .
0408284	1/1981	European Pat. Off. .
0023385	2/1981	European Pat. Off. .
0 044 158 A1	1/1982	European Pat. Off. .
0101069	8/1983	European Pat. Off. .
0092787	11/1983	European Pat. Off. .
0101069	2/1984	European Pat. Off. .
0200101	12/1986	European Pat. Off. .

(List continued on next page.)

OTHER PUBLICATIONS

Takenaka, et al., J. Chem. Soc. Perkin Trans 2:EN; 95–99 (1978), Induced Circular Dichroism of chiral Amine–Benzoylbenzoic Acid Systems.

Lavanchy, Archives Des Sciences (Geneva), 11, 252–255 (1958).

Walker, et al., J. Med. Chem., 9(4), 624–30 (1966), Synthesis of Varied Heterocyclic and Substituted Aryl Alkyl Secondary Amines, Related Schiff Bases, and Amides.

Burke, et al., J. Org. Chem. 28, 1098–1100 (1963), Mono–1, 3–benzoxazines from Hydroquinone.

Giovambattista, et al., Ciencia e invest. (Buenos Aires) 14, 34–5 (1968), Investigaciones Recientes.

West, et al., J. Am. Pharm. Assoc. 46, 58–61 (1957), A pharmacological Study of a Series of Aralkylamines.

Arjona et al., "Sterochemistry of the reduction of the imino group. IV. Sterochemistry of the reduction of N–(1–phenylethyl)–1–alkyl–1–arylmethanimines," *An. Quim. Ser. C* 81(1):23–29 (1985).

Freifelder, "Selective Hydrogenolysis. Dehalogenation in the Presence of N–Benzyl Linkage," *J. Org. Chem.* 31(11):3875–3877 (1966).

Grethe et al., "Syntheses in the Isoquinoline Series. Synthesis of 2,3–Dihydro–4(1H)–isoquinolones," *J. Org. Chem.* 33(2):491–494 (1968).

Hashimoto et al., "Highly Diastereoselective Addition of Organometallic Reagents to Chiral Imines Derived from 1–(2–Methoxyphenyl)ethylamine," *Synlett Letters* pp. 961–962 (1995).

Hiroi et al., "A Highly Efficient and Recyclable Chiral Director for Asymmetric Synthesis of Sulfoxides," *Chemistry Letters* pp. 1595–1598 (1980).

(List continued on next page.)

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[57] **ABSTRACT**

The present invention relates to the different roles inorganic ion receptors have in cellular and body processes. The present invention features: (1) molecules which can modulate one or more inorganic ion receptor activities, preferably the molecule can mimic or block an effect of an extracellular ion on a cell having an inorganic ion receptor, more preferably the extracellular ion is Ca^{2+} and the effect is on a cell having a calcium receptor; (2) inorganic ion receptor proteins and fragments thereof, preferably calcium receptor proteins and fragments thereof; (3) nucleic acids encoding inorganic ion receptor proteins and fragments thereof, preferably calcium receptor proteins and fragments thereof; (4) antibodies and fragments thereof, targeted to inorganic ion receptor proteins, preferably calcium receptor protein; and (5) uses of such molecules, proteins, nucleic acids and antibodies.

103 Claims, 85 Drawing Sheets

U.S. PATENT DOCUMENTS

4,242,355	12/1980	Nedelec et al. .	
4,289,787	9/1981	Molloy et al. .	
4,360,511	11/1982	Baldwin et al. .	
4,391,826	7/1983	Mills et al. .	
4,487,965	12/1984	Himmele et al. .	
4,587,253	5/1986	Halczenko et al. .	
4,591,605	5/1986	Ray .	
4,608,391	8/1986	Ginos et al. .	
4,609,494	9/1986	Baldwin .	
4,647,446	3/1987	Sargent et al. .	
4,661,635	4/1987	Carson .	
4,675,321	6/1987	Baldwin .	
4,677,101	6/1987	Claremon et al. .	
4,728,660	3/1988	Haynes et al. .	
4,769,483	9/1988	Lombardi et al. .	
4,797,411	1/1989	Crugnola et al. .	
4,808,718	2/1989	Hartman et al. .	
4,839,369	6/1989	Youssefyeh et al. .	
4,916,145	4/1990	Tilley et al. .	
4,925,664	5/1990	Jackson et al. .	
4,925,873	5/1990	Freidhoff .	
4,945,050	7/1990	Sanford et al. .	
4,967,003	10/1990	Rentzea et al. .	
4,987,071	1/1991	Cech et al. .	
4,988,730	1/1991	Korbonits et al. .	
4,992,378	2/1991	Kelly et al. .	
5,001,251	3/1991	MacManus et al. .	
5,011,834	4/1991	Weber et al. .	
5,021,599	6/1991	Beer et al. .	
5,030,576	7/1991	Dull et al. .	
5,034,514	7/1991	Nitecki et al. .	
5,045,466	9/1991	Morrison .	
5,053,337	10/1991	Weinshank et al. .	
5,064,657	11/1991	Jackson et al. .	
5,073,648	12/1991	Hagishita et al.	564/374
5,075,338	12/1991	Knoll et al.	514/654
5,082,837	1/1992	Palfreyman et al. .	
5,298,660	3/1994	Yoneyoshi et al. .	
5,334,628	8/1994	Maeda et al. .	
5,403,861	4/1995	Goldin et al. .	
5,504,253	4/1996	Van Wagenen et al. .	
5,510,519	4/1996	Yoneyoshi et al. .	
5,633,404	5/1997	Van Wagenen et al. .	

FOREIGN PATENT DOCUMENTS

0253327	1/1988	European Pat. Off. .
0270376	6/1988	European Pat. Off. .
0289287	11/1988	European Pat. Off. .
0309100	3/1989	European Pat. Off. .
0384740	8/1990	European Pat. Off. .
0395357	10/1990	European Pat. Off. .
0455510	3/1991	European Pat. Off. .
0443606	8/1991	European Pat. Off. .
0224163	10/1991	European Pat. Off. .
0508307	10/1992	European Pat. Off. .
1231690	1/1967	Germany .
1231690	12/1967	Germany .
2541184	4/1976	Germany .
2825961	3/1980	Germany .
53-90272	8/1978	Japan .
59-50358	3/1984	Japan .
2200658	8/1990	Japan .
1079091	9/1967	United Kingdom .
1109924	4/1968	United Kingdom .
1448437	7/1974	United Kingdom .
1464209	9/1977	United Kingdom .
2113089	8/1983	United Kingdom
2213818	8/1989	United Kingdom .
8204052	11/1982	WIPO .
8906135	7/1989	WIPO .
8909834	10/1989	WIPO .
9100853	1/1991	WIPO .
9109594	7/1991	WIPO .
9113077	9/1991	WIPO .
9207829	5/1992	WIPO .
9214709	9/1992	WIPO .
9304373	3/1993	WIPO .
9310073	5/1993	WIPO .
9315044	5/1993	WIPO .
9313052	8/1993	WIPO .
9418959	9/1994	WIPO .
9511221	4/1995	WIPO .
9518134	6/1995	WIPO .
9518134	7/1995	WIPO .
9521815	8/1995	WIPO .

OTHER PUBLICATIONS

Hiroi et al., "Studies on Chiral Organo–Sulfur Compounds. I. Asymmetric Synthesis of Sulfoxides with Optically Active o–Aminoalkylphenol Derivatives," *Chem. Pharm. Bull.* 31:3471–3485 (1983).

Kametani et al., "Studies on the Synthesis of Heterocyclic Compounds. Part 687. Asymmetric Synthesis of Salsolidine," *J. Chem. Soc. Perkin Trans. 1* pp. 579–581 (1977).

Kang et al., "Rhodium(I)–catalysed Asymmetric Hydrogenation of Imines," *J. Chem. Soc. Chem. Commun.* pp. 1466–1467 (1988).

Kienzle et al., "1,5–Dihydroimidazoquinazolinones as blood platelet aggregation inhibitors," *Eur. J. Med. Chem.—Chem. Ther.* 17:547–556 (1982).

Kozlov et al., "Reductive animation of 1–acetylcyclohexene by nitriles," *Vestsi Akad. Navuk BSSR, Ser. Khim. Navuk* pp. 55–58 (1977).

Majewski and MacKinnon, "Enantioselective deprotonation of protected 4–hydroxycylohexanones," *Can. J. Chem.* 72:1699–1704 (1994).

Mori et al., "Formic Acid Reduction. XI. Reduction of Schiff Bases," *Chem. Pharm. Bull.* 19:1722–1727 (1971).

Muff et al., "Regulation of Hormone Secretion and Cytosolic Ca^{2+} by Extracellular Ca^{2+} in Parathyroid Cells and C–Cell: Role of Voltage–Sensitive Ca^{2+} Channels," *Archives of Biochemistry and Biophysics* 265:128–135 (1988).

Standridge et al., "Phenylalkylamines with Potential Psychotherapeutic Utility. 2. Nuclear Substituted 2–Amino–1–phenylbutanes," *J. Med. Chem.* 23:154–162 (1980).

Standridge et al., "Phenylalkylamines with Potential Psychotherapeutic Utility. 1. 2–Amino–1–(2, 5–dimethoxy–4–methylphenyl) butane," *J. Med. Chem.* 19:1400–1404 (1976).

Wang and Bäckvall, "Ruthenium–catalysed Transfer Hydrogenation of Imines by Propan–2–ol," *J. Chem. Soc. Commun.* pp. 980–982 (1992).

Majewski et al., "1,3–Dioxan–5–ones: synthesis, deprotonation, and reactions of their lithium enolates," *Can. J. Chem.* 73:1616–1625 (1995).

Majewski et al., "Synthesis of Butenolides via Enantioselective Deprotonation of Protected 4–Hydroxycyclohexanone," *Tetrahedron Asymmetry* 6:1837–1840 (1995).

Merck Index, 11th Edition, Monograph No. 2993, p. 2997 (1989).

Polniaszek and Kaufman, "Steroselective Nucleophilic Additions to the Carbon–Nitrogen Double Bond. 2. Chiral Iminium Ions Derived from "Second Generation" Chiral Amines," *J. Am. Chem. Soc.* 111:4859–4863 (1989).

Anderson and Santi, "Phenylalanyl Transfer Ribonucleic Acid Synthetase from *Escherichia coli* B. Potent Inhibition by Analogues of N–Benzyl–2–phenylethylamine," *J. Med. Chem.* 19:1270–1275 (1976).
Boyd et al., "Dynamic Sterochemistry of Imines and Derivatives. Part 18. Photosynthesis and Photoracemization of Optically Active Oxaziridines," *J. Chem. Soc. Perkin Trans. I* 4:849–855 (1985).
Fraser et al., "Substitution α to the Nitrogen in Dibenzylamine via Carbanion Intermediates," *Can. J. Chem.* 51:1109–1115 (1973).
Gracheva et al., "Stereodirection of Ketimine Reduction Reactions," *Zhural Organicheskoi Khimii* 9(6):1235–1239 (1973).
Gracheva et al., "The Stereoselectivity of the Reactions of Schiff Bases with Organomagnesium Compounds," *Zhural Organicheskoi Khimii* 10(3):557–561 (1974).
Hu et al., "Lithium hydride elimination in the reactions of organolithium compounds with imines: synthesis of secondary amines with branched groups," *C.R. Acad. Sci. Paris Ser C* 284(4):195–198 (1977).
Hutton et al., "Organic Reagents for the Precipitation of Nitrate Ion. Part I. N–Substituted 1–naphthylmethylamines," *J. Chem. Soc. (A)* 11:1573–1579 (1966).
Joshi and Mehrotra, "Reductive Coupling In Substituted Imines with Aluminium–Amalgam in Moist Ether," *Nat. Acad. Sci. Letters (India)* 3:268–272 (1980).
Ikegami and Yamada, "Chemistry of Sodium Borohydride and Diborane. II. Reduction of Schiff Bases with Diborane in Tetrahydrofuran," *Chem. Pharm. Bull.* 14(12):1389–1399 (1966).
Katritsky et al., "Convenient Preparations of Imines and Symmetrical Secondary Amines Processing Primary or Secondary Alkyl Groups," *Synthesis* 9:703–708 (1991).
Langlois et al., "Asymmetric synthesis of amines by hydrosilylation of imines catalyzed by a chiral complex of rhodium," *Tetrahedron Lett.* 49:4865–4868 (1973).
Neuvonen and Pihlaja, "Studies on the Benzoxazine Series. Part 3—Preparation and ^{13}C NMR Structural Study of γ Effects of Some N–Substituted, 3,4–Dihydro–2H–1,3–benzoxazines," *Magnetic Resonance in Chemistry* 28:239–245 (1990).
Polniaszek and Dillard, "Diastereoselective Addition of Organometallic Reagents to Chiral Immune Ions: Synthesis of (S)–(+)–Cryptostyline I," *Tetrahedron Lett.* 31:797–800 (1990).
Larsson et al., "Paradoxical effects of K^+ and D–600 on parathyroid hormone secretion and cytoplasmic Ca^{2+} in normal bovine and pathological human parathyroid cells," *Biochem. Biophys. Acta* 847:263–269 (1985).
Kim et al., "Studies on the Structural Requirements for the Activity of the Skeletal Muscle Dihydropyridine Receptor/Slow Ca^{2+} Channel," *J. Biol. Chem.* 265:11858–11863 (1990).
Mikami et al., "Primary Structure and functional expression of the cardiac dihydropyridine–sensitive calcium channel," *Nature* 340:230–233 (1989).
Clifton et al., "Arylethanolamines derived from salicylamide with alpha– and beta–adrenoceptor blocking activities. Preparation of labetalol, its enantiomers and related salicylamides," *J. Med. Chem.* 25:670–679 (1982).
Danks, "Reaction of Hydride Transfer Reducing Agents with (1–heterodiene) tricarbonyliron(0) Complexes and the Synthesis of Saturated Amines and Alcohols," *Tetrahedron Lett.* 35:4177–4178 (1994).
Duelfer et al., "Synthesis of 14C–dilevalol," *J. Labelled Comp[d. Radiopharm* 25:855–863 (1988).
Lensink and De Vries, "Diastereoselective hydrogenation and kinetic resolution of imines using rhodium/diphosphine catalyzed hydrogenation," *Tetrahedron: Asymmetry* 4:215–222 (1993).
Capuano et al., "Characterization of the Human Calcium Receptor Gene," *J. Bone and Mineral Research* 9(1):S145 at 98 (1994).
Fox et al., "A First Generation Calcimimetic Compound (NPS R–568) That Acts on the Parathyroid Cell Calcium Receptor: A Novel Therapeutic Approach for Hyperparathyroidism," *J. Bone and Mineral Research* 8(1):S181 at 260 (1993).
Fox et al., "NPS R–568 Inhibits Parathyroid Hormone Secretion and Stimulates Calcitonin Secretion in Hyperparathyroid Rats with Chronic Renal Failure," *J. American Society of Nephrology* 4:719 at 69P (1993).
Fox et al., "NPS R–568 Acts on Calcium Receptors to Inhibit Parathyroid Hormone and Stimulate Calcitonin Secretion: A Novel Therapeutic Approach for Hyperparathyroidism," *J. American Society of Nephrology* 4:719 at 120P (1993).
Fox et al., "Parathyroid Gland Calcium Receptor Gene Expression is Unaffected by Chronic Renal Failure or Low Dietary Calcium in Rats," *J. Am. Soc. Nephrology* 5:879 at 90P (1994).
Fox et al., "Physiologically Relevant PTH Levels are Anabolic on Bone in Ovariectomized Rats," *Bone* 16:194S at 434 (1995).
Garrett et al., "Cloning and Expression of a G–Protein–Coupled Calcium Receptor From a Human Parathyroid Adenoma," *J. Bone and Mineral Research* 8(1):S148 at 125 (1993).
Hammerland et al., "Mechanism of Action of the Calcimimetic Compounds NPS R–467 and NPS R–568 in Xenopus Oocytes Expressing a Bovine Parathyroid Cell Calcium Receptor," *J. Bone and Mineral Research* 8(1):S133 at 65 (1993).
Harootunian et al., "Effects of Calcitonin and Extracellular Calcium on Cytosolic Levels of Cyclic AMP and Ca^{2+} in Rabbit Osteoclasts," *J. Bone and Mineral Research* 9(1):S246 at B66 (1994).
Heath et al., "Inhibition of Human Parathyroid Hormone Secretion In Vivo by NPS R–568, a Calcimimetic Drug that Targets the Parathyroid Cell–Surface Calcium Receptor," *Bone* 16:85S at 23 (1995).
Hung et al., "Coupling of the Porcine Calcitonin Receptor to Cytosolic Ca^{2+} and cAMP Levels in Xenopus Oocytes," *J. Bone and Mineral Research* 9(1):S410 at C400 (1994).
Nemeth, E., "Ca^{2+} Receptor–Dependent Regulation of Cellular Functions," *News in Physiological Sciences* 10:1–15 (1995).
Nemeth et al., "Screening of compounds with potential action against calcium receptors and their use in therapy of disorders of calcium metabolism," *Chemical Abstracts* 122(1):P1057y (1995).
Rogers et al., "Localization of Calcium Receptor mRNA in Rat Thyroid and Parathyroid Glands Using In Situ Hybridization Histochemistry," *J. Bone and Mineral Research* 9(1):S409 at C390 (1994).
Rogers et al., "Calcium Receptor Expression in the Parathyroid Glands of Vitamin D–Deficient Rats is not Regulated by Plasma Caclium and $1,25(OH)_2D_3$," *J. Bone and Mineral Research* 9(1):S409 at C392 (1994).

Rogers et al., "The Calcimimetic Compound NPS467 Reduces Plasma Calcium in a Dose-Dependent and Stero-Specific Manner," *J. Bone and Mineral Research* 8(1):S180 at 254 (1993).

Steffey and Nemeth, "Extracellular Calcium-Sensing Mechanisms on Osteoclasts and Parathyroid Cells are Pharmacologically Distinct," *J. Bone and Mineral Research* 8(1):S384 at 1071 (1993).

Steffey et al., "Calcimimetics: Structually and Mechanistically Novel Compounds that Inhibit Hormone Secretion From Parathyroid Cells," *J. Bone and Mineral Research* 8(1):S175 at 236 (1993).

Abe et al., "Molecular Characterization of a Novel Metabotropic Glutamate Receptor mGluR5 Coupled to Inositol Phosphate/Ca^{2+} Signal Transduction," *J. Biol. Chem.*, 267:13361–13368 (1992).

Barney et al., "A Convenient Synthesis of Hindered Amines and α-Trifluoromethylamines from Ketones," *Tetrahedron Letters*, 31:5547–5550 (1990).

Batra and Alenfall, "Effects of Diverse Categories of Drugs on Human Colon Tumour Cell Proliferation," *Anticancer Research* 11:1221–1224 (1991).

Becalski et al., "Catalytic asymmetric hydrogenation of imines. Use of rhodium(I)/phosphine comploexes and characterization of rhodium(I)/imine complexes," *Chemical Abstracts* 116:558 at Abstract No. 14742U (1992).

Bertz et al., "Asymmetric Induction with Amidocuprates," *J. Org. Chem.* 51:4953–4959 (1986).

Bringmann et al., "Enantiomerically Pure-N-Boc Protected β-Keto-γ-Amino Acid Esters from Simple Keto Precursors: A Novel, Stereocontrolled Approach to Statine Derivatives with Any Desired Configuration," *Synlett* pp. 253–255 (May 1990).

Bringmann et al., "The Enantioselective Synthesis of Optically Active, Benzene Nucleus-Substituted 1-Phenylethylamines from the Corresponding Acetophenones," *Liebigs Ann. Chem.* pp. 795–805 (1990).

Brinster et al., "Factors affecting the efficiency of introducing foreign DNA into mice by microinjecting eggs," *Proc. Natl. Acad. Sci. USA*, 82:4438–4442 (1985).

Brown, "Extracellular Ca^{2+} Sensing, Regulation of Parathyroid Cell Function and Role of Ca^{2+} and Other Ions as Extracellular (First) Messengers," *Physiological Reviews* 71:371–411 (1991).

Brown et al, "Cloning and characterization of an extracellular Ca^{2+} sensing receptor from bovine parathryoid," *Nature* 366:575–580 (1993).

Brown et al., "A Comparison of the Effects of Divalent and Trivalent Cations on Parathyroid Hormone Release, 3',5'-Cyclic-Adenosine Monophosphate Accumulation, and the Levels of Inositol Phosphates in Bovine Parathyroid Cells," *Endocrinology* 127:1064–1071 (1990).

Brown et al., "High Extracellular Ca^{2+} and Mg^{2+} Stimulate Accumulation of Inositol Phosphates in Bovine Parathyroid Cells," *FEBS Letters* 218:113–118 (1987).

Brown et al., "Neomycin Mimics the Effects of High Extracellular Calcium Concentrations on Parathyroid Function in Dispersed Bovine Parathyroid Cells," *Endocrinology*, 128:3047–3054 (1991).

Brown et al., "Polyarginine, Polylysine, and Protamine Mimic the Effects of High Extracellular Calcium Concentrations on Dispersed Bovine Parathyroid Cells," *J. Bone and Mineral Res.*, 6:1217–1225 (1991).

Buck and Axel, "A Novel Multigene Family May Encode Odorant Receptors: A Molecular Basis for Odor Recognition," *Cell*, 65:175–187 (1991).

Capecchi, "Altering the Gonome by Homologous Recombination," *Science*, 244:1288–1292 (1989).

Chemical Abstracts Formula Index, vol. 110 p. 537F (1989).

Chemical Abstracts Formula Index, vol. 110 p. 1793F (1989).

Chen et al., "Divalent Cations Suppress 3,5'-Adenosine Monophosphate Accumulation by Stimulating a Pertussis Toxin-Sensitive Guanine Nucleotide-Binding Protein in Cultured Bovine Parathyroid Cells," *Endocrinology* 124:233–239 (1989).

Chen et al., "Effects of Fluoride on Parathyroid Hormone Secretion and Intracellular Second Messengers in Bovine Parathyroid Cells," *Journal of Bone and Mineral Research* 3:279–288 (1988).

Chen et al., "Injection of Bovine Parathyroid Poly(A)$^+$ RNA into Xenopus Oocytes Confers Sensitivity to High Extracellular Calcium," *J. Bone Min. Res.* 9:293–300 (1994).

Chen and Brown, "The Diltiazem Analog TA-3090 Mimics the Actions of High Extracellular Ca^{2+} on Parathyroid Function in Dispersed Bovine Parathyroid Cells," *J. Bone and Mineral Res.*, 5:581–587 (1990).

Cooper et al., "Receptors," *The Biochemical Basis of Neuropharmacology*, 4th Edition, eds. J.R. Cooper, F.E. Bloom, R.H. Roth (New York:Oxford University Press, 1982) pp. 61–76.

Davies and Ichihara, "Asymmetric Synthesis of R-β-Amino Butanoic Acid and S-β-Tyrosine: Homochiral Lithium Amide Equivalents for Michael Additions to α,β-Unsaturated Esters," *Tetrahedron: Asymmetry* 2:183–186 (1991).

De Feo et al., "Natriuretic Peptide Receptors Regulate Endothelin Synthesis and Release From Parathyroid Cells," *Proc. Natl. Acad. Sci. USA* 88:6496–6500 (1991).

Dottavio-Martin and Ravel, "Radiolabeling of Proteins by Reductive Alkylation with [^{14}C]Formaldehyde and Sodium Cyanoborohydride," *Analytical Biochem.*, 87:562–565 (1978).

Ferguson and Williams, "Cell-Surface Anchoring of Proteins Via Glycosylphosphatidylinositol Structures," *Ann. Rev. Biochem.*, 57:285–320 (1988).

Fox et al., "Prevention of Hypocalcemia Prolongs the Plasma Parathyroid Hormone and Calcitonin Responses to the Calcimimetic Compound NPS R-568 in Rats," *J. Bone Min. Res.* 9(1):S409 at C396 (1994).

Fuji et al., "Endothelin as an Autocrine Factor in the Regulation of Parathyroid Cells," *Proc. Natl. Acad. Sci. USA* 88:4235–4239 (1991).

Fuleihan et al., "Effects of the Lectin Concanavalin-A on the Regulation of Second Messengers and Parathyroid Hormone Release by Extracellular Ca^{2+} in Bovine Parathyroid Cells," *Endocrinology* 128:2931–2936 (1991).

Fuleihan and Brown, "Effect on the Lectin Concanavalin-A on Calcium-Regulated Adenosine 3', 5'-Monophosphate Accumulation in Bovine Parathyroid Cells," *Endocrinology* 126:1996–2002 (1990).

Garrett et al., "Expression of the Parathyroid Calcium Receptor Gene in C-Cells," *J. Bone Min. Res.* 9(1):S409 at C398 (1994).

Goldberger and Anfinsen, "The Reversible Masking of Amino Groups in Ribonuclease and Its Possible Usefulness in the Synthesis of the Protein," *Biochemistry*, ed. H. Neurath (Easton, PA:Mack Printing Company, 1962) 1:401–405.

Gross and Witkop, "Selective Cleavage of the Methionyl Peptide Bonds in Ribonuclease with Cyanogen Bromide," *J. Amer. Chem. Soc.*, 83:1510–1511 (1961).

Hammer et al., "Spontaneous Inflammatory Disease in Transgenic Rats Expressing HLA–B27 and Human β_2m: An Animal Model of HLA–B27–Associated Human Disorders," *Cell*, 63:1099–1112 (1990).

Hawkins et al., "The Effects of High Extracellular Ca^{2+} and Mg^{2+} Concentrations on the Levels of Inositol 1,3,4,5–Tetrakisphosphate in Bovine Parathyroid Cells," *Endocrinology* 124:838–844 (1989).

Hediger, "High Resolution Preparative Gel Electrophoresis of DNA Fragments and Plasmid DNA Using a Continuous Elution Apparatus," *Analytical Biochem.*, 159:280–286 (1986).

Holtje et al., "Conformational Analysis on Calcium Channel Active Diphenylalkylamines, Diphenylbutylpiperidines, Phenylalkylamines, and Perhexiline," *Quantitative Structure–Activity Relationships* 8:259–265 (1989).

Houdebine and Chourrout, "Transgenesis in Fish," *Experientia*, 47:891–897 (1991).

Hutchison et al., "Mutagenesis at a Specific Position in a DNA Sequence," *J. Biol. Chem.*, 253:6551–6560 (1978).

Jansen et al, "Immunotoxins: Hybrid Molecules Combining High Specificity and Potent Cytotoxicity," *Immunological Rev.*, 62:185–216 (1982).

Jasys et al., "The Total Synthesis of Argiotoxins 636, 659 and 673," *Tetrahedron Letters*, 29:6223–6226 (1988).

Joyner et al., "Production of a mutation in mouse En–2 gene by homologous recombination in embryonic stem cells," *Nature*, 338:153–156 (1989).

Juaristi et al., "Use of N,N^1–Dimethylpropyleneura (DMPU) as Solvent in the Efficient Preparation of Enantiomerically Pure Secondary Amines," *Synthesis* pp. 1243–1246 (Dec. 1993).

Katz et al., "Structure–Function Relationships for the Effects of Various Aminoglycoside Antibotics on Dispersed Bovine Parathyroid Cells," *Endocrinology* 131:903–910 (1992).

Kifor et al., "Phorbol Esters Modulate the High Ca^{2+}–Stimulated Accumulation of Inositol Phosphates in Bovine Parathyroid Cells," *Journal of Bone and Mineral Research* 5:1003–1011 (1990).

Kifor and Brown, "Relationship between Diacylglycerol Levels and Extracellular Ca^{2+} in Dispersed Bovine Parathyroid Cells," *Endocrinology* 123:2723–2729 (1988).

Killen and Lindstrom, "Specific Killing of Lymphocytes that Cause Experimental Autoimmune Myasthenia Gravis by Ricin Toxin–Acetylcholine Receptor Conjugates," *J. of Immunology*, 133:2549–2553 (1984).

Koenig et al., "Polyamines Mediate Androgenic Stimulation of Clacum Fluxes and Membrane Transport in Rat Heart Myocytes," *Circulation Research* 64:415–426 (1989).

Komeyoshi and Kudo, "Optically active amines and their manufacture, intermediates and uses," *Chemical Abstracts* 121:1060 at Abstract No. 230462Y (1994).

Lensink and de Vries, "Improving Enantioselectivity by Using a Mono–Sulphonated Diphosphine as Ligand for Homogeneous Imine Hydrogenation," *Tetrahedron:Asymmetry* 3:235–238 (1992).

Leszkovszky et al., "The Pharmacology of Diphenylalkyl Derivatives," *Acta Physiologica Academiae Scientiarum Hungaricae Tomus* 29:283–297 (1966).

Levine, *Pharmacology: Drug Actions and Reactions*, Little Brown and Company, Inc. pp. 192–196 (1990).

Lindley, "A New Synthetic Substrate for Trypsin and its Application to the Determination of the Amino–acid Sequence of Proteins," *Nature*, 178:647–658 (1956).

Lopez–Barneo and Armstrong, "Depolarizing Response of Rat Parathyroid Cells to Divalent Cations," *J. Gen. Physiol.* 82:269–294 (1983).

Mattson et al., "An Improved Method for Reductive Alkylation of Amines Using Titanium(IV) Isopropoxide and Sodium Cyanoborohydride," *J. Org. Chem.*, 55:2552–2554 (1990).

Merck Index, 11th Edition, Monograph No. 3916, p. 623 1992.

Merck Index, 11th Edition, Monograph No. 8699, pp. 420, 1379 1992.

Merrifield, "Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide," *J. Amer. Chem. Society*, 85:2149–2154 (1963).

Mithal et al., "Highly Purified Sheep C–Cells Express an Extracellular Ca^{2+} Receptor Similar to that Present in Parathyroid," *J. Bone Min. Res.* 9(1):S282 at B209 (1994).

Nason et al., "Synthesis of Neurotoxic Nephlla Spider Venoms: NSTX–3 and JSTX–3," *Tetrahedron Letters*, 30:2337–2340 (1989).

Nelson et al., "Detection of Acridinium Esters by Chemiluminescence," *Nonisotopic DNA Probe Techniques*, ed. L.J. Kricka (San Diego:Academic Press, Inc. 1992) pp. 275–310.

Nemeth, "Evidence for the Presence of a Novel Ca^{2+}–Binding Protein (Ca^{2+} Receptor) on the Surface of Parathyroid Cells," *Calcium–Binding Proteins in Health and Disease*, eds. A.W. Norman, T.C. Vanaman, A.R. Means (San Diego:Academic Press, Inc. 1987) pp. 36–38.

Nemeth, "Regulation of cytosolic calcium by extracellular divalent cations in C–cells and parathyroid cells," *Cell Calcium* 11:323–327 (1990).

Nemeth and Carafoli, "The role of extracellular calcium in the regulation of intracellular calcium and cell function," *Cell Calcium* 11:319–321 (1990).

Nemeth and Scarpa, "Cytosolic Ca^{2+} and the Regulation of Secretion in Parathyroid Cells," *FEBS Letters* 203:15–19 (1986).

Nemeth and Scarpa, "Rapid Mobilization of Cellular Ca^{2+} in Bovine Parathyroid Cells Evoked by Extracellular Divalent Cations–Evidence for a Cell Surface Calcium Receptor," *J. Biol. Chem.*, 262(11):5188–5196 (1987).

Nemeth and Scarpa, "Receptor–Dependent Mobilization of Cellular Ca^{2+} and the Regulation of Hormone Secretion in Parathyroid Cells," *Calcium Regulation and Bone Metabolism: Basic and Clinical* 9:167–171 (1987).

Nemeth and Scarpa, "Spermine Evokes the Rapid Mobilization of Cellular Ca^{2+} in Parathyroid Cells," *Calcium–Binding Proteins in Health and Disease*, eds. A.W. Norman, T.C. Vanaman, A.R. Means (San Diego:Academic Press, Inc. 1987) pp. 33–35.

Opie, "Calcium Channel Antagonists Part V: Second–Generation Agents," *Cardiovascular Drugs and Therapy* 2:191–203 (1988).

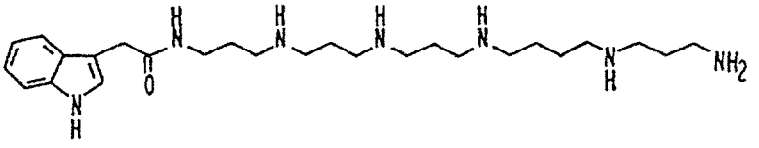
Parker et al., "Targeted gene walking polymerase chain reaction," *Nucleic Acids Research*, 19:3055–3060 (1991).

Pilch and Czech, "Affinity Cross–Linking of Peptide Hormones and Their Receptors," *Membranes, Detergents, and Receptor Solubilization*, eds. J.C. Venter, L.C. Harrison (New York:Alan R. Liss, Inc. 1984) pp. 161–175.

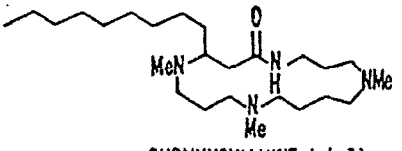
Pursel et al., "Genetic Engineering of Livestock," *Science*, 244:1281–1288 (1989).

Racke, "Functional Expression of the Parathyroid Cell Calcium Receptor in Xenopus oocytes," *FEBS Lett.* 333:132–136 (1993).
Racke et al., "Functional Expression of the Parathyroid Cell Calcium Receptor in Xenopus Oocytes," *J. of Bone and Mineral Res.,* Supplement 1, 6(S1):S118 (1991).
Schaefer et al., "Polyamine Toxins from Spiders and Wasps," *The Alkaloids,* 45:1–125 (1994).
Seely et al., "The Calcium Channel Blocker Diltizaem Lowers Serum Parathyroid Hormone Levels in vivo and in vitro," *Journal of Clinical Endocrinology and Metabolism,* 68:1007–1012 (1989).
Shoback and Chen, *J. Bone Mineral Res.* 9:293 (1994).
Shoback and Chen, "Injection of Poly $(A)^+$ RNA from Bovine Parathyroid Tissue into Xenopus Oocytes Confers Sensitivity to Extracellular Calcium," *J. of Bone and Mineral Res.,* Supplement 1, 6(S1):S135 (1991).
Simons et al., "Gene Transfer into Sheep," *Bio/Tech.,* 6:179–183 (1988).
Triggle et al., "Ca^{2+} Channel Ligands: Structure–Function Relationships of the 1,4–Dihydropyridines," *Medicinal Research Review,* vol. 9, No. 1, pp. 123–180 (1989).
Van Niel and Pandit, "NADH Models XXI. Steroselective Reduction of Chiral Imines with Hantzsch Ester," *Tetrahedron* 41:6065–6011 (1985).
Witkop, "Nonenzymatic Methods for the Preferential and Selective Cleavage and Modification of Proteins," *Advances in Protein Chemistry,* eds. C.B. Anfinsen, K. Bailey, M.L. Anson, J.T. Edsall (New York:Academic Press 1961) 16:221–321.
Zaidi, "'Calcium Receptors' on Eukaryotic Cells with Special Reference to the Osteoclast," *Bioscience Reports* 10:493–507 (1990).
Zaidi, "Intracellular calcium in the control of osteoclast function. II. Paradoxical elevation of cytosolic free calcium by verapamil," *Biochem. and Biophys. Res. Communications,* 167:807–812 (1990).
Kienzle et al., "1,5–Dihydroimidazoquinazolinones as blood platelet aggregation inhibitors," *Eur. J. Med. Chem.—Chem. Ther.* 17:547–556 (1982).
Paulsen–Sorman et al., "Cytochrome P–455 nm Complex Formation in the Metabolism of Phenylalkylamines. 8. Stereoselectivity in Metabolic Intermediary Complex Formation with a Series of Chiral 2–Substituted 1–Phenyl–2–aminoethanes," *J. Med. Chem.* 27:342–346 (1984).
Yamaguchi et al., "Asymmetric Reduction with Chiral Reagents from Lithium Aluminum Hydride and (S)–(153)–N–(o–Substituted benzyl)–α–phenylethylamines," *J. Org. Chem.* 42:1578–1581 (1977).
Schwartz and Hu, "Synthesis of Hindered Secondary Amines via Grignard Reagent Addition to Ketonitrones," *Tetrahedron Lett.* 13:1689–1692 (1992).
Van Dijk and Moed, "Synthesis of β–Phenylethyloamine Derivatives X^{1*} N–(Hydroxy– and Methoxy–Aralkyl) Derivatives," *Recl. Trav. Chim. Pays–Bas* 92:1281–1297 (1973).
Yamaguchi et al., "Asymmetric Reduction with Chiral Reagents from Lithium Aluminum Hydride and (S)–(–)–N–(o–Substituted benzyl)–α–phenylethylamines," *J. Org. Chem.* 42:1578–1581 (1977).

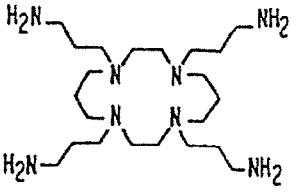
FIG. 1a.



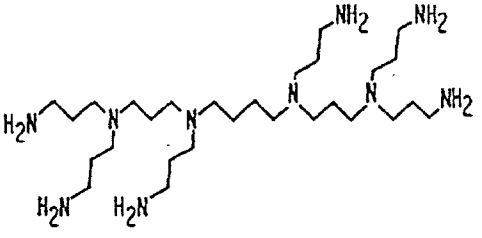
NPS 019



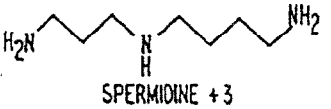
BUDMUNCHIAMINE A (+3)



NPS 383 (+8;EC_{50}=150μM)

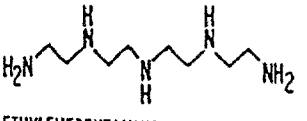


NPS 381 (+10 EC_{50}=100μM)

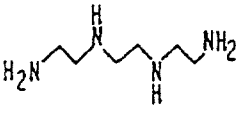


SPERMIDINE +3

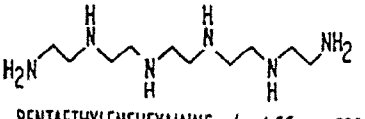
1,12-DIAMINODODECANE -2
(DADD)



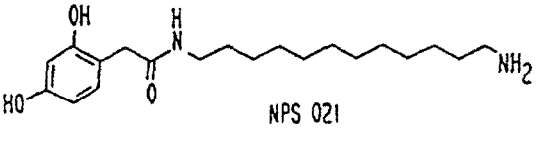
TETRAETHYLENEPENTAMINE (+5;EC_{50}=2500μM)



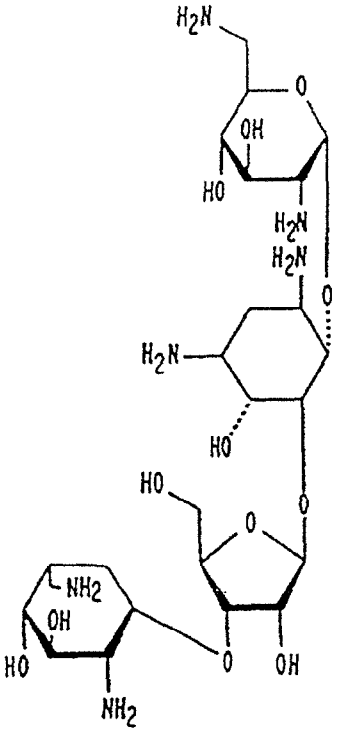
TRIETHYLENETETRAMINE (+4;EC_{50}=8000μM)



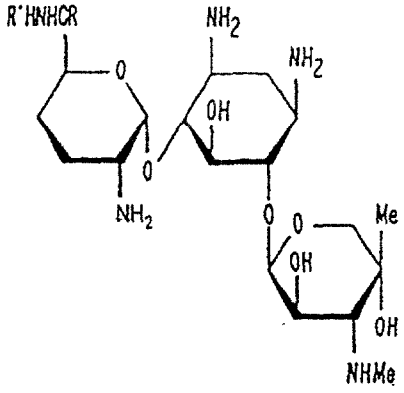
PENTAETHYLENEHEXAMINE (+6;EC_{50}=500μM)



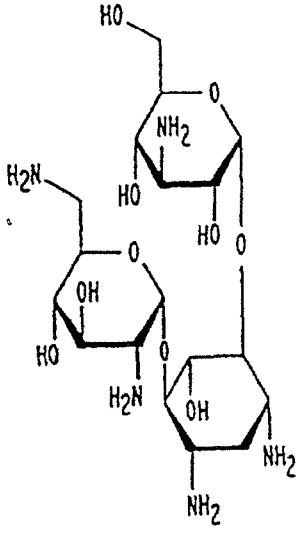
NPS 021



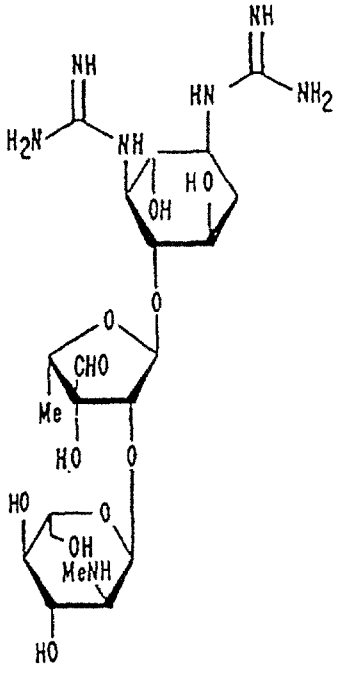
Neomycin B (+6)



Gentamicin (Complex +5)
C1 R = R' = Me
C2 R = Me; R' = H
C1aR = R' = H

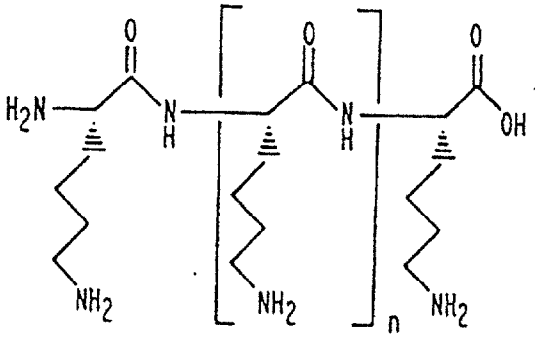


Bekanamycin (+5)

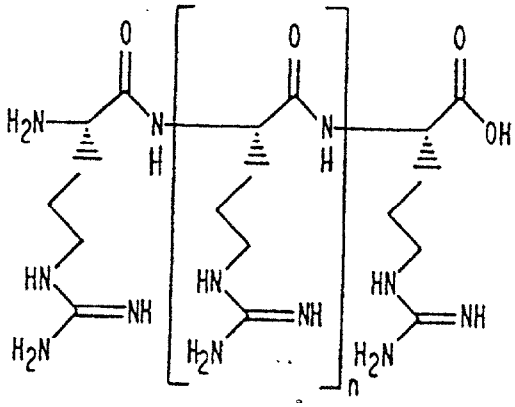


Streptomycin (+3)

FIG. 1b.



poly-Lys (27 kD) n=210 +210
poly-Lys (14 kD) n=110 +110
poly-Lys (38 kD) n=55 +55

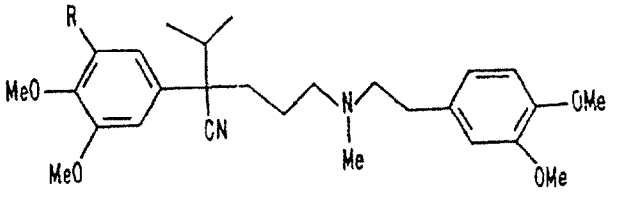


poly-Arg (100 kD) n=640 +640
poly-Arg (40 kD) n=256 +256

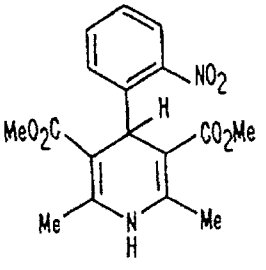
PROTAMINE +21

H N-Pro-Arg-Arg-Arg-Ser-Ser-Ser-Arg-Pro-Val-Arg-Arg-Arg-Arg-Arg-
Pro-Arg-Val-Ser-Arg-Arg-Arg-Arg-Arg-Arg-Gly-Gly-Arg-Arg-Arg-Arg-OH

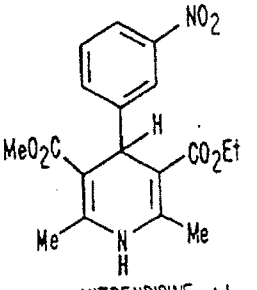
FIG. 1c.



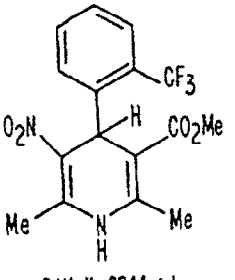
VERAPAMIL R=H +1
D-600 R=OMe +1



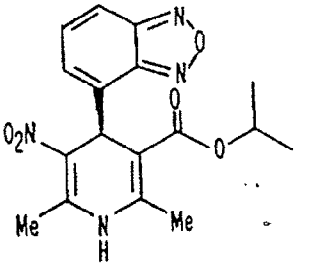
NIFEDIPINE +1



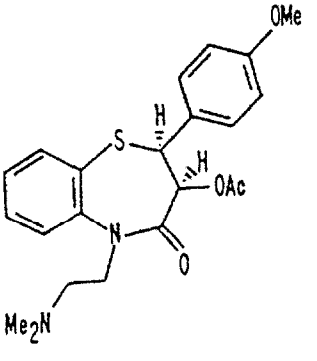
NITRENDIPINE +1



BAY K 8644 +1

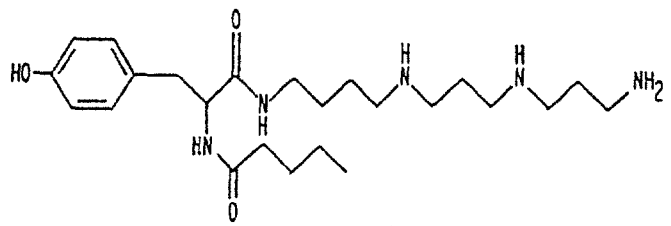


202-791 +1

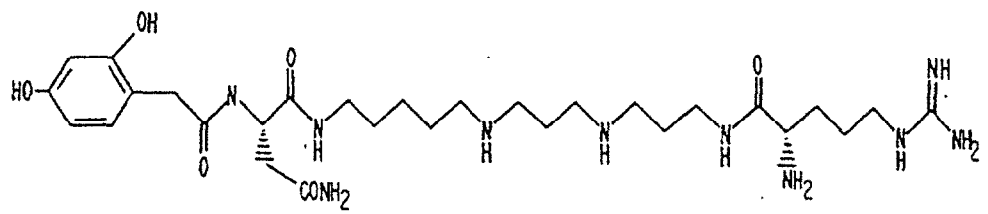


DILTIAZEM +1

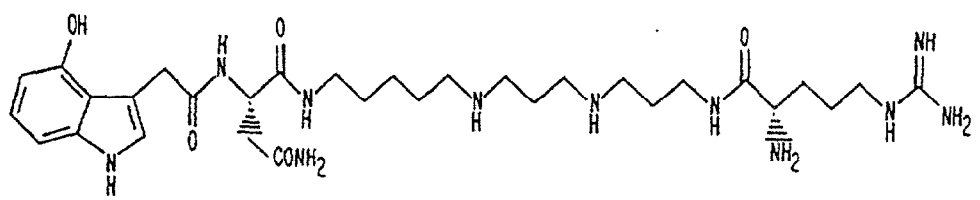
FIG. 1d.



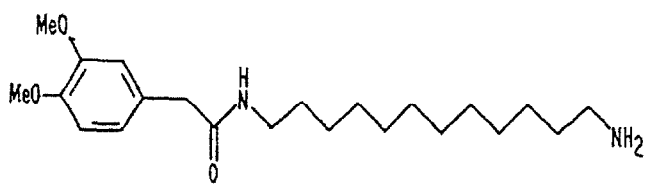
PHILANTHOTOXIN 433 +3



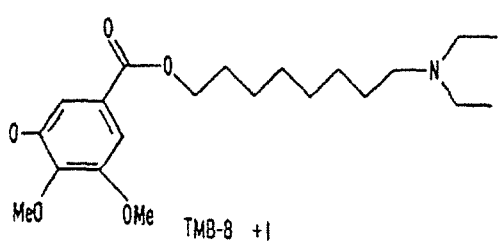
ARGIOTOXIN 636 +4



ARGIOTOXIN 659 +4

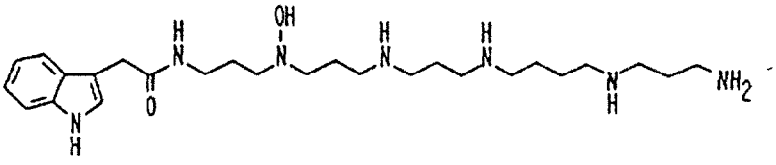


NPS 384

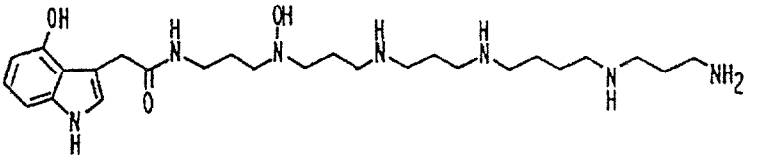


TMB-8 +1

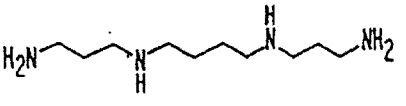
FIG. 1e.



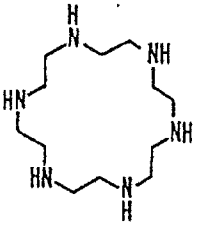
AGA 489



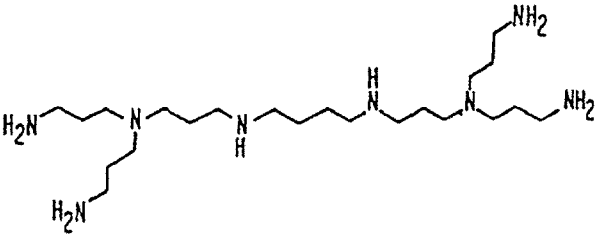
AGA 505



SPERMINE 141.148 μM



HEXACYCLEN 20.9 μM



NPS 382 55.0 μM

FIG. 1f.

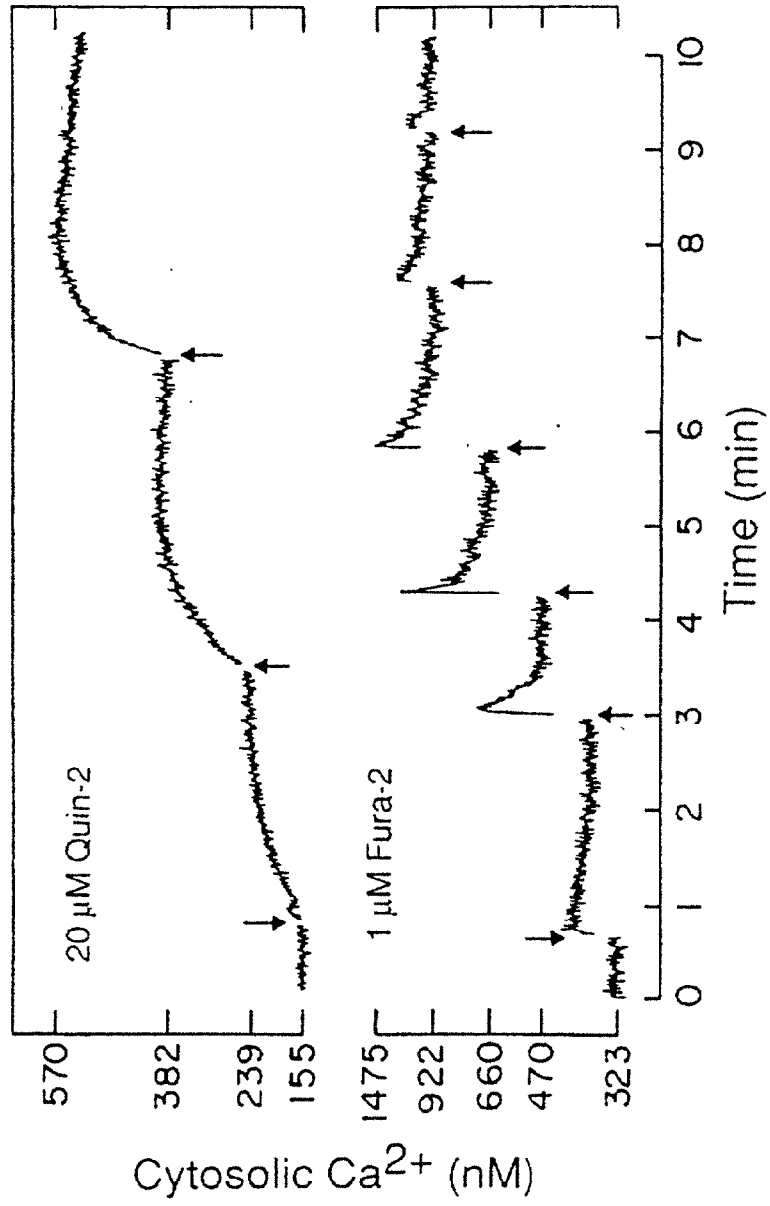

20 μM Quin-2
1 μM Fura-2
570
382
239
155
1475
922
660
470
323
Cytosolic Ca2+ (nM)
0
1
2
3
4
5
6
7
8
9
10
Time (min)

FIG. 2.

FIG. 3a.

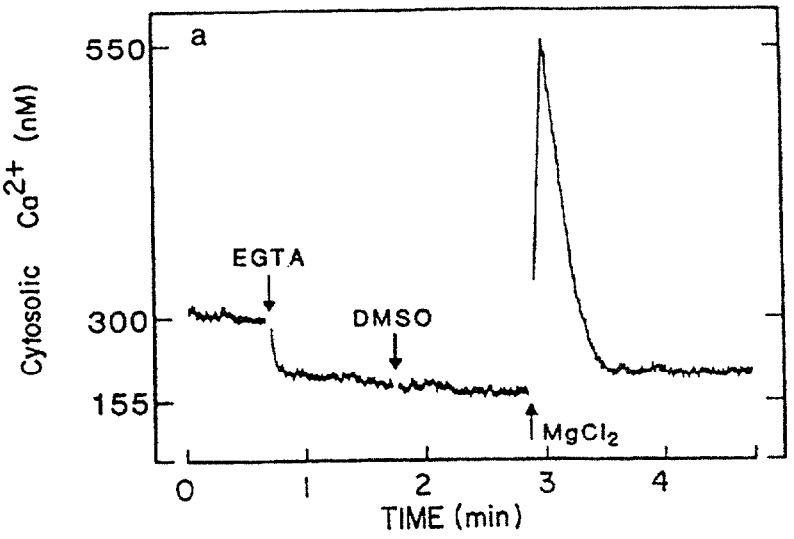


FIG. 3b.

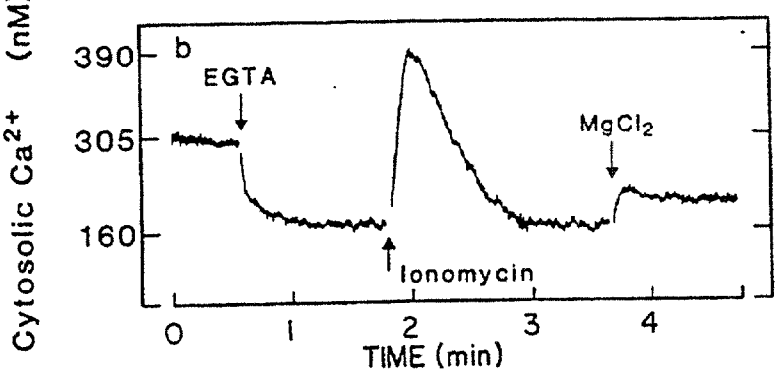


FIG. 3c.

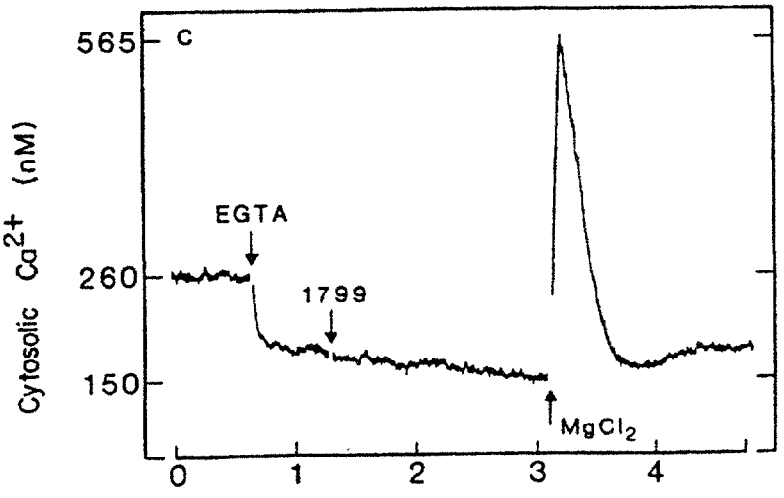


FIG. 4a.

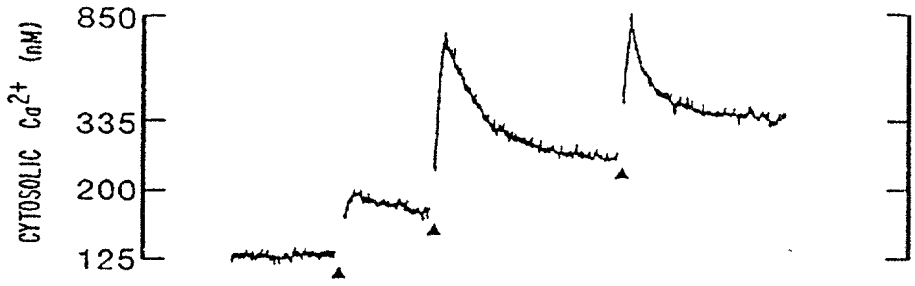


FIG. 4b.

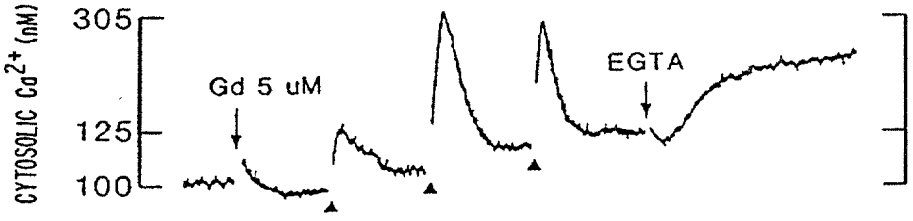
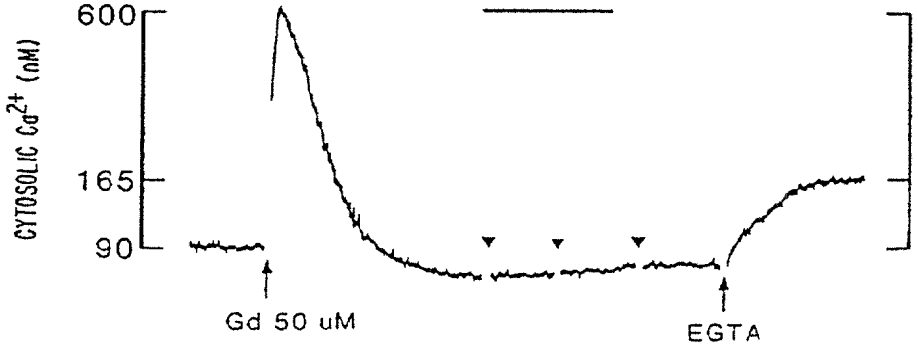


FIG. 4c.



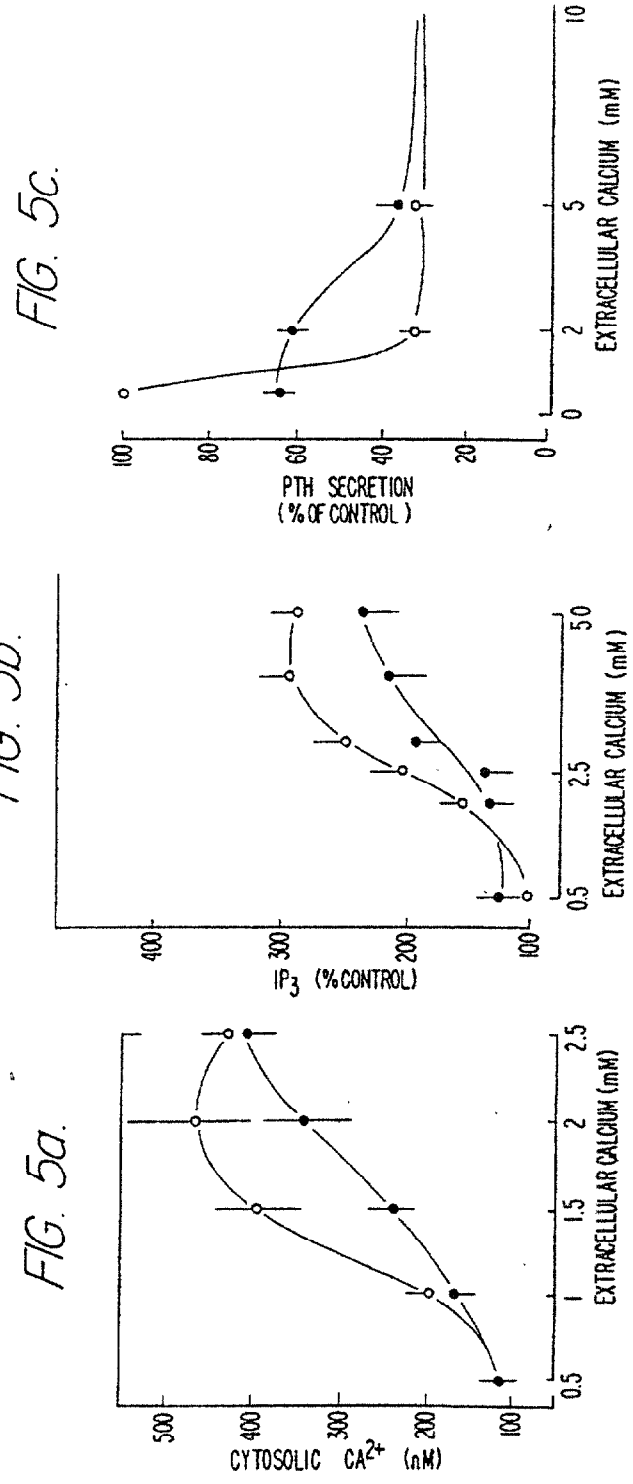


FIG. 5a.
CYTOSOLIC CA2+ (nM)
500
400
300
200
100
0.5
1
1.5
2
2.5
EXTRACELLULAR CALCIUM (mM)
FIG. 5b.
IP3 (%CONTROL)
400
300
200
100
0.5
2.5
5.0
EXTRACELLULAR CALCIUM (mM)
FIG. 5c.
PTH SECRETION
(% OF CONTROL)
100
80
60
40
20
0
0
2
5
10
EXTRACELLULAR CALCIUM (mM)

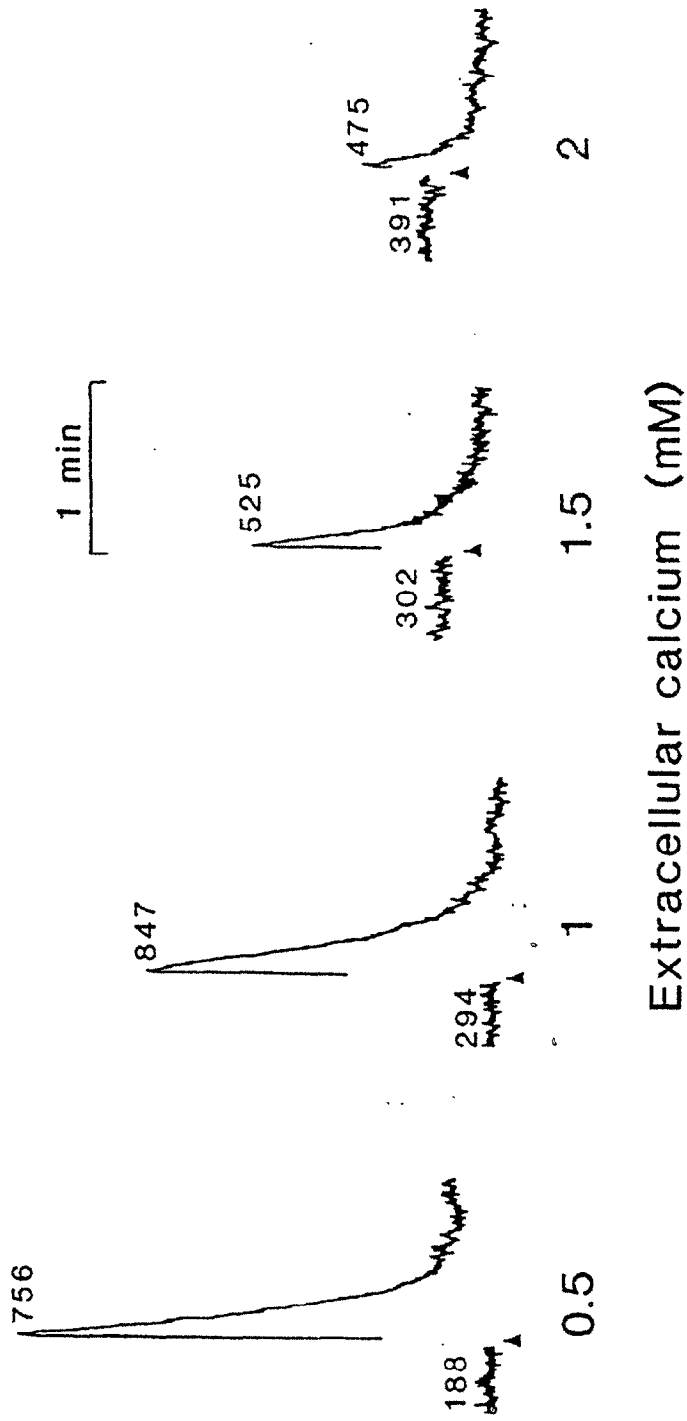

1 min
756
188
0.5
847
294
1
525
302
1.5
475
391
2
Extracellular calcium (mM)

FIG. 6.

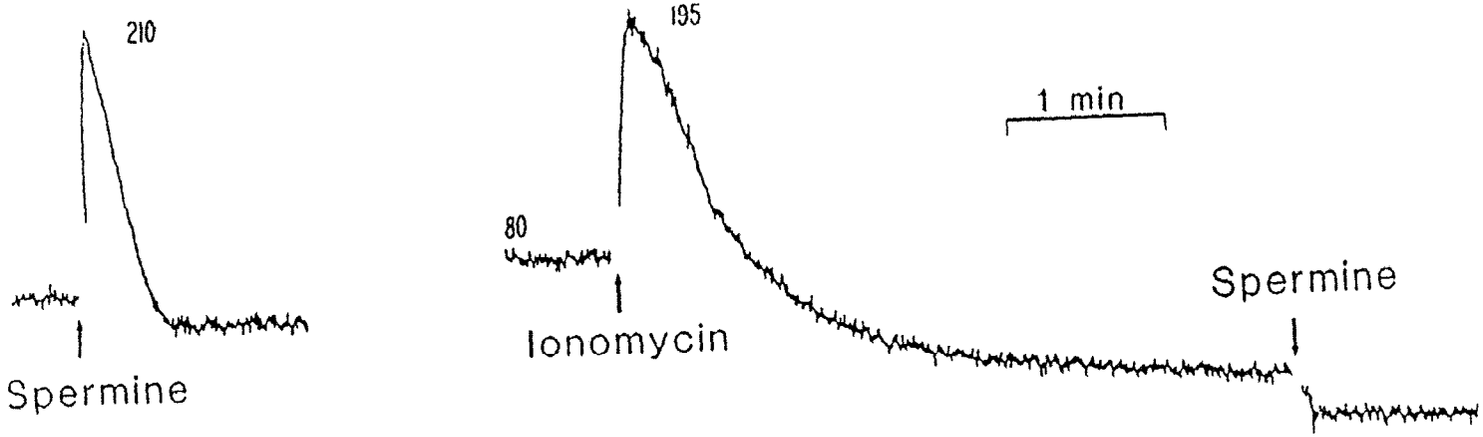


FIG. 7.

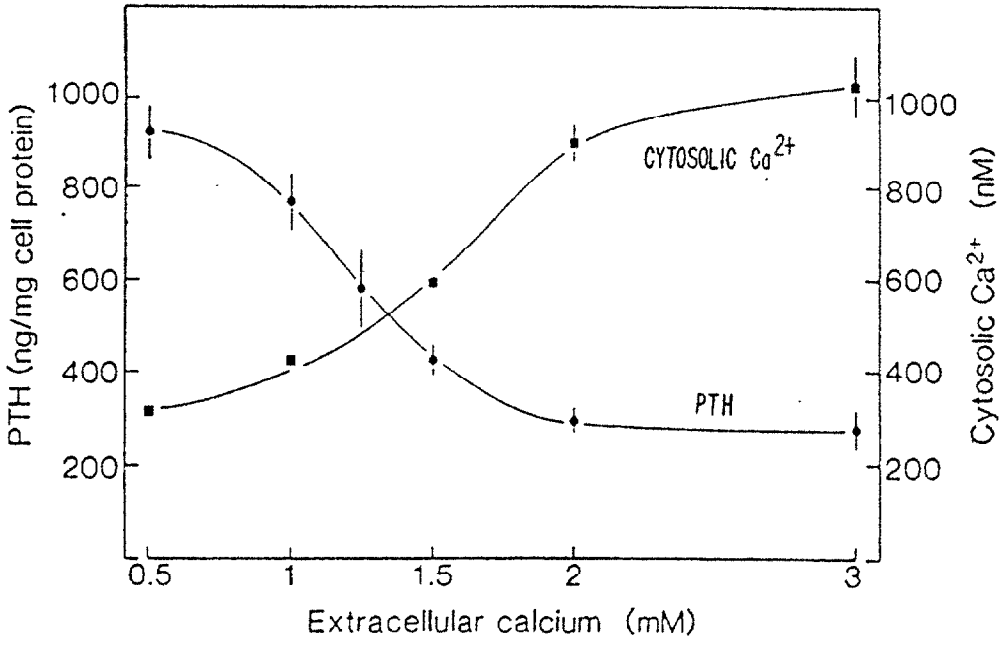

PTH (ng/mg cell protein)
1000
800
600
400
200
CYTOSOLIC Ca2+
PTH
Cytosolic Ca2+ (nM)
1000
800
600
400
200
0.5
1
1.5
2
3
Extracellular calcium (mM)

FIG. 8a.

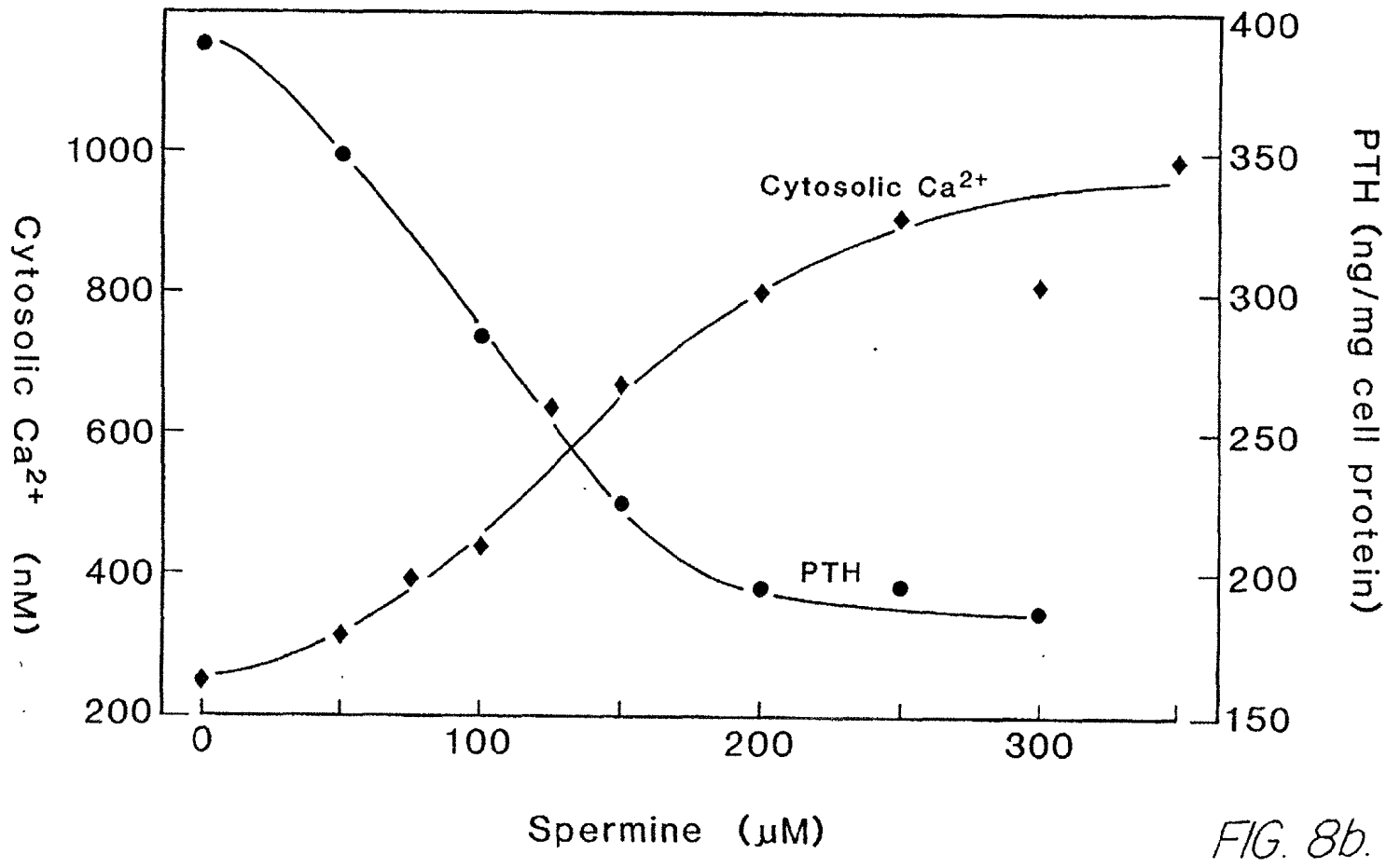

Cytosolic Ca2+ (nM)
1000
800
600
400
200
PTH (ng/mg cell protein)
400
350
300
250
200
150
Cytosolic Ca2+
PTH
0
100
200
300
Spermine (μM)

FIG. 8b.

FIG. 9a.

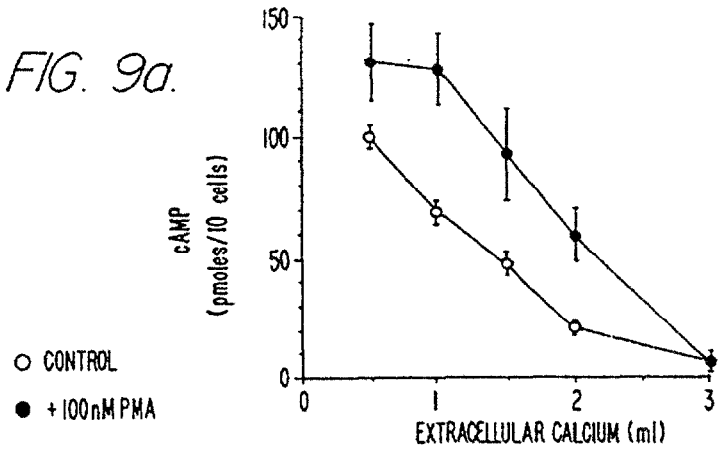


FIG. 9b.

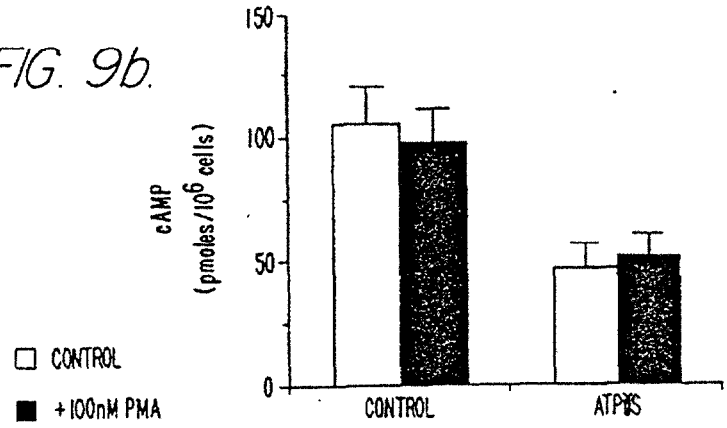


FIG. 9c.

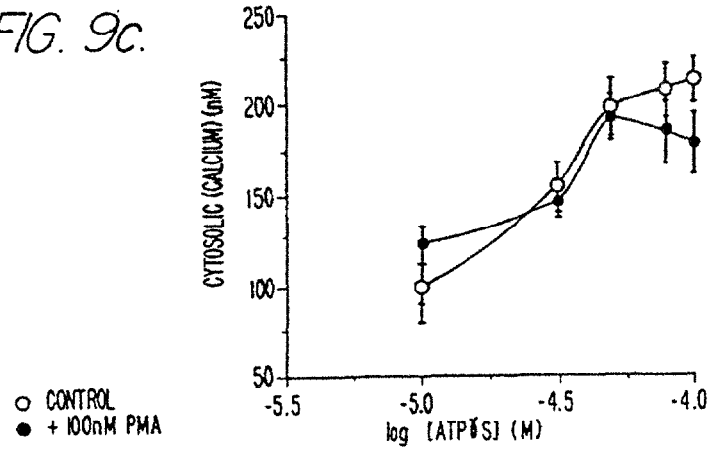


FIG. 10a.

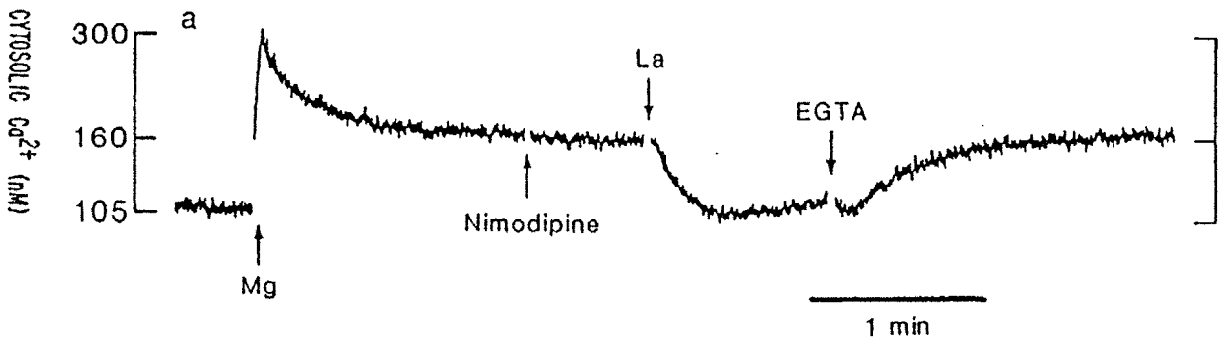
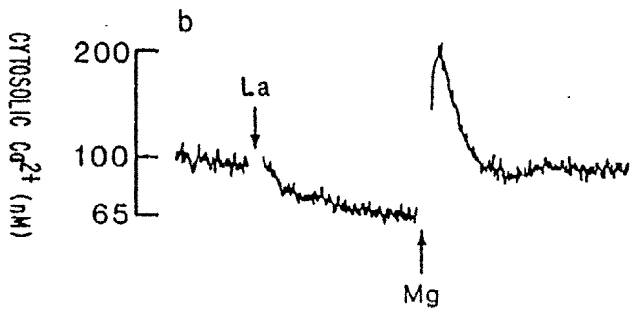


FIG. 10b.



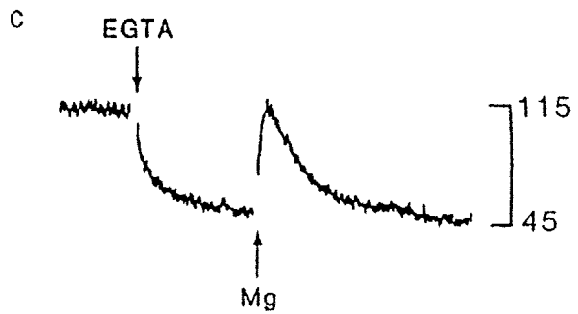


FIG. 10c.

FIG. 11a.

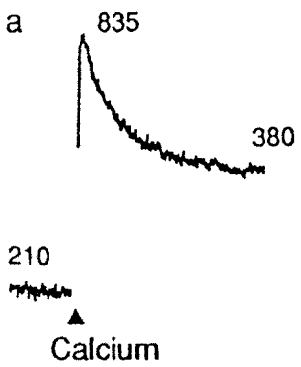


FIG. 11b.

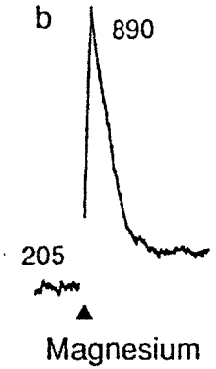


FIG. 11c.

FIG. 11d.

FIG. 11e.

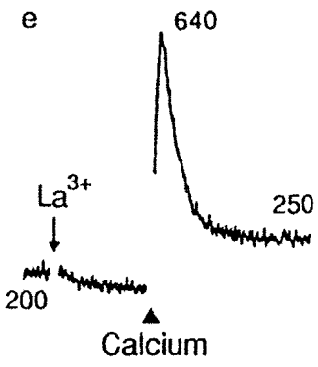


FIG. 11f.

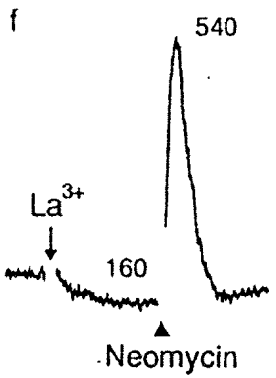


FIG. 11g.

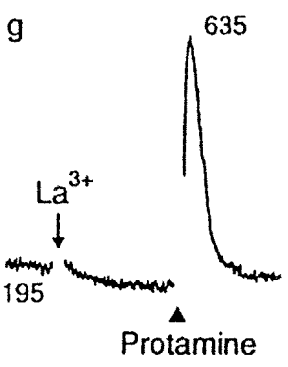


FIG. 11h.

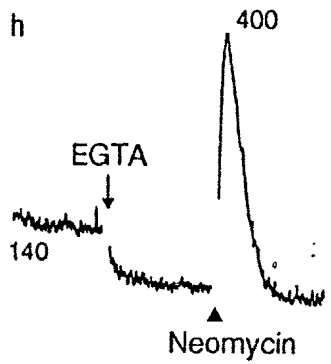


FIG. 11i.

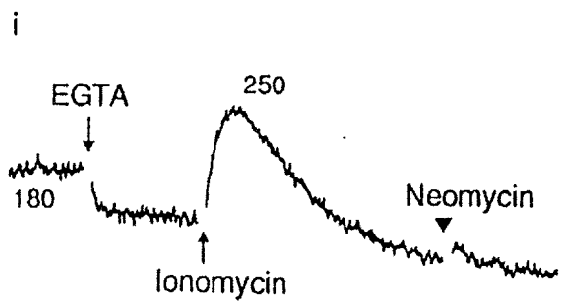


FIG. 12.

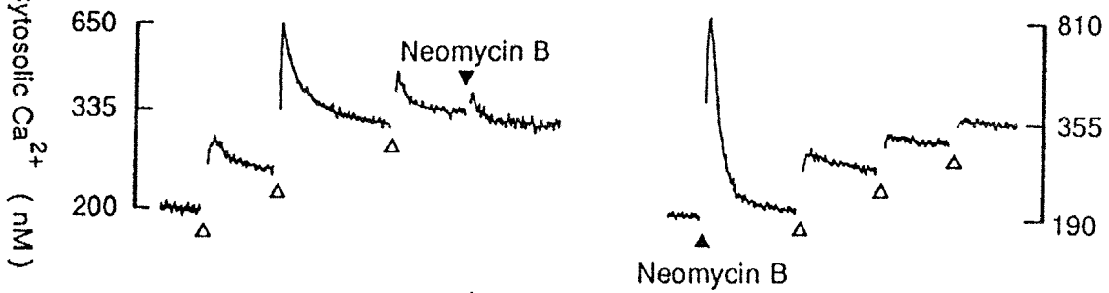


FIG. 13a.

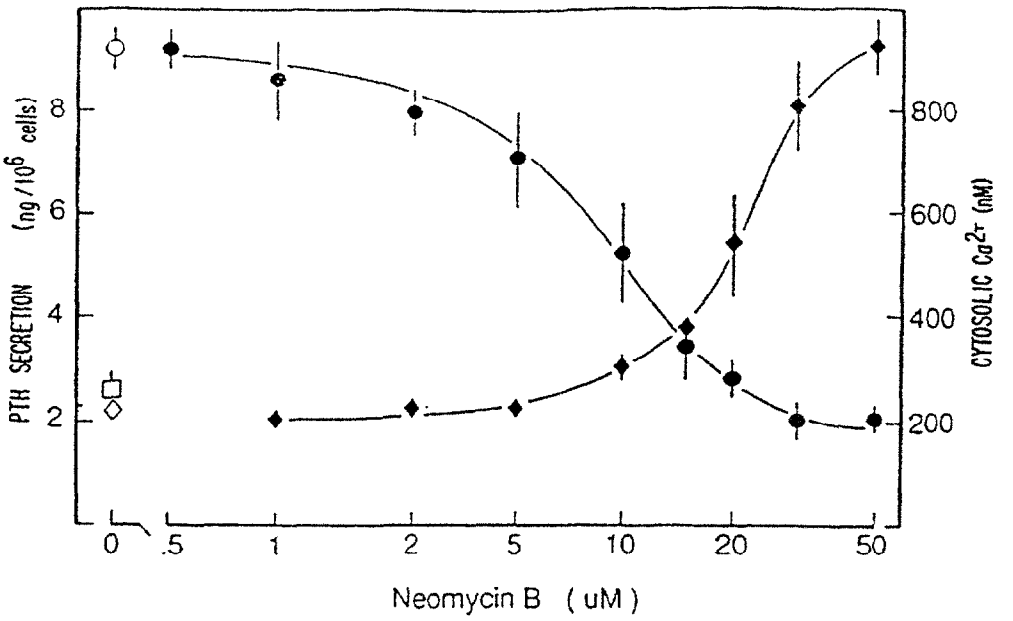
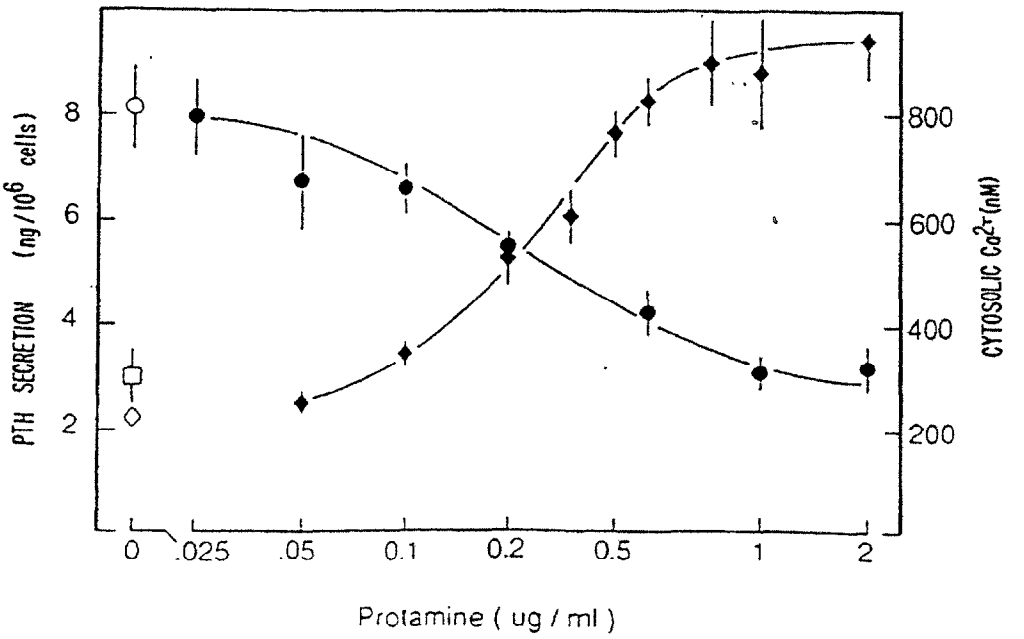


FIG. 13b.



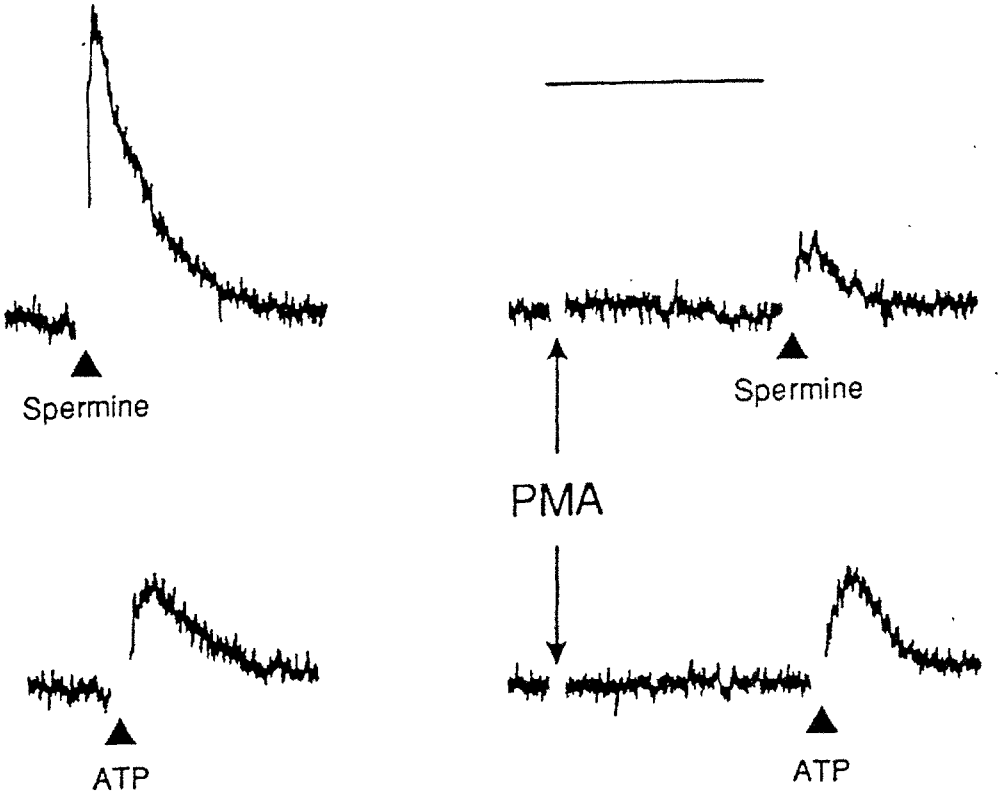
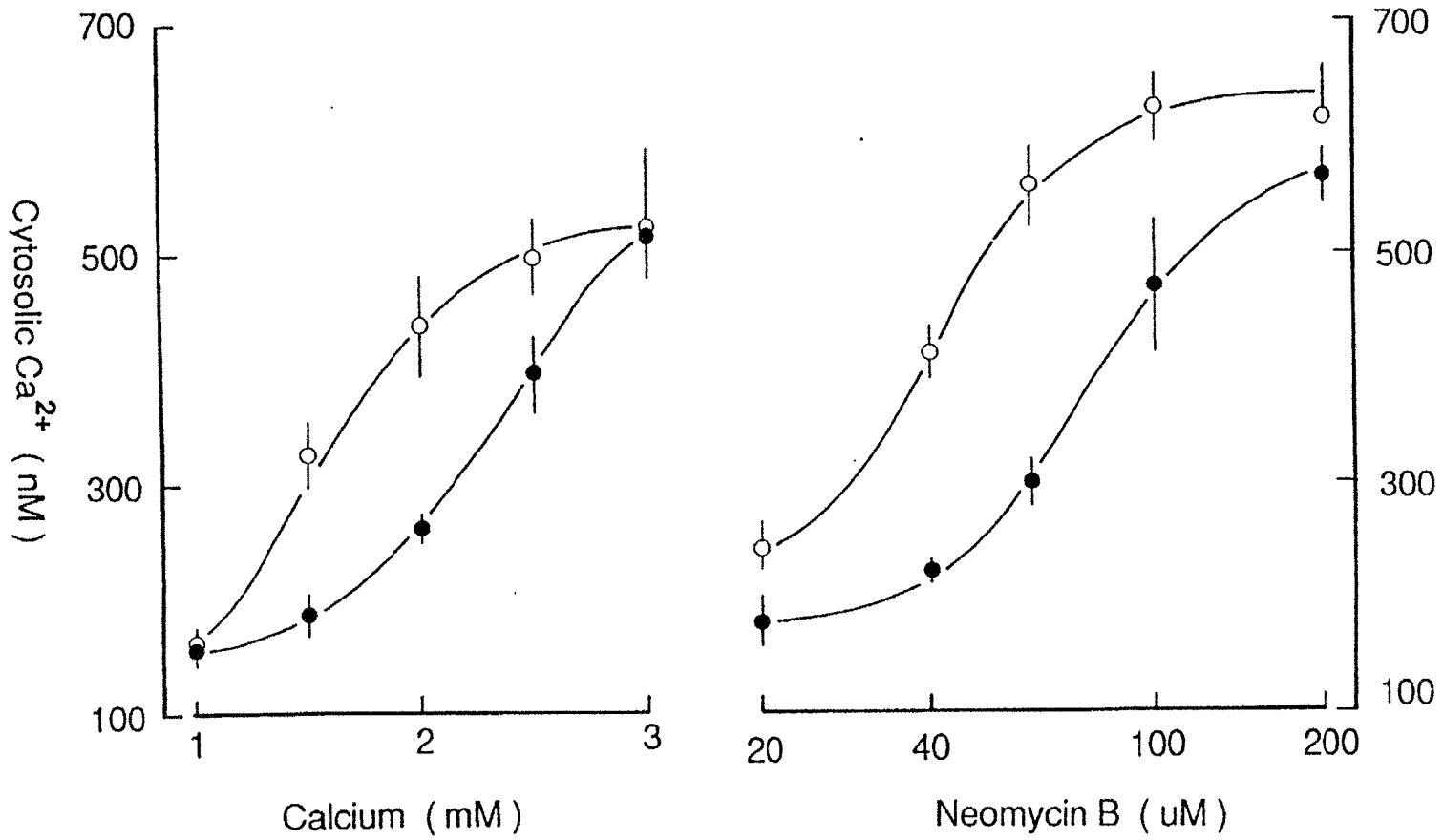


FIG. 14.

FIG. 15.


Cytosolic Ca^{2+} (nM)
700
500
300
100
1
2
3
Calcium (mM)
20
40
100
200
Neomycin B (uM)

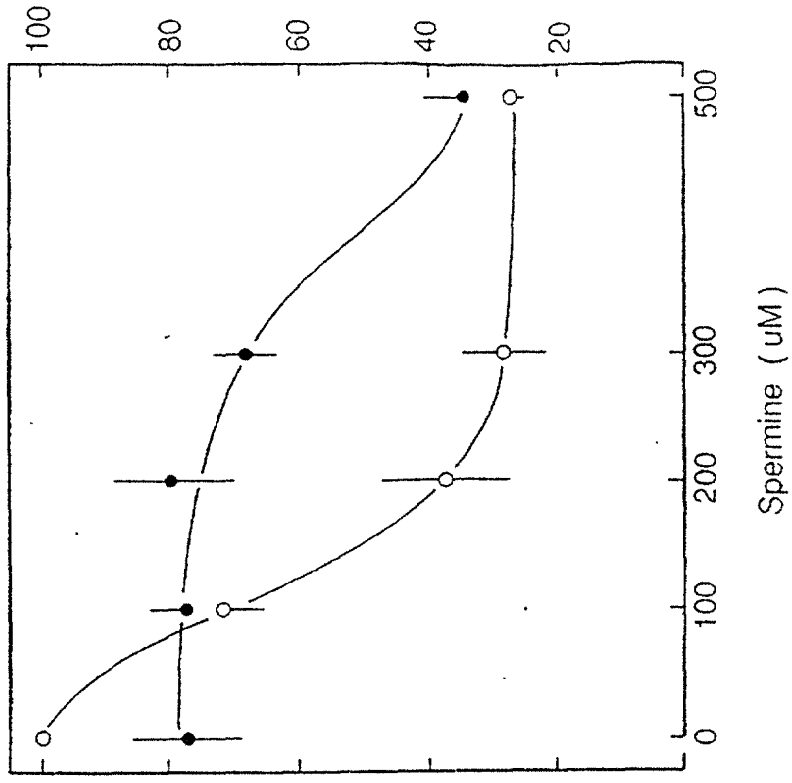


FIG. 16b.

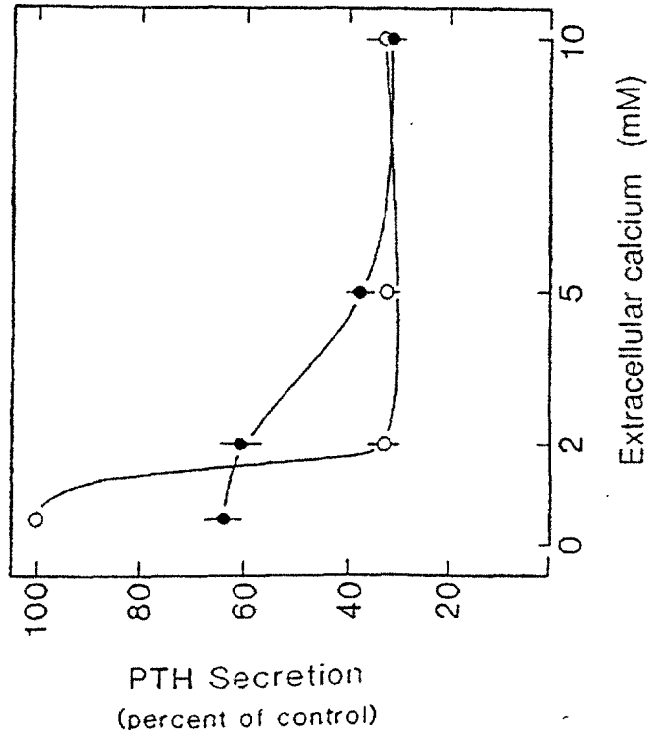


FIG. 16a.

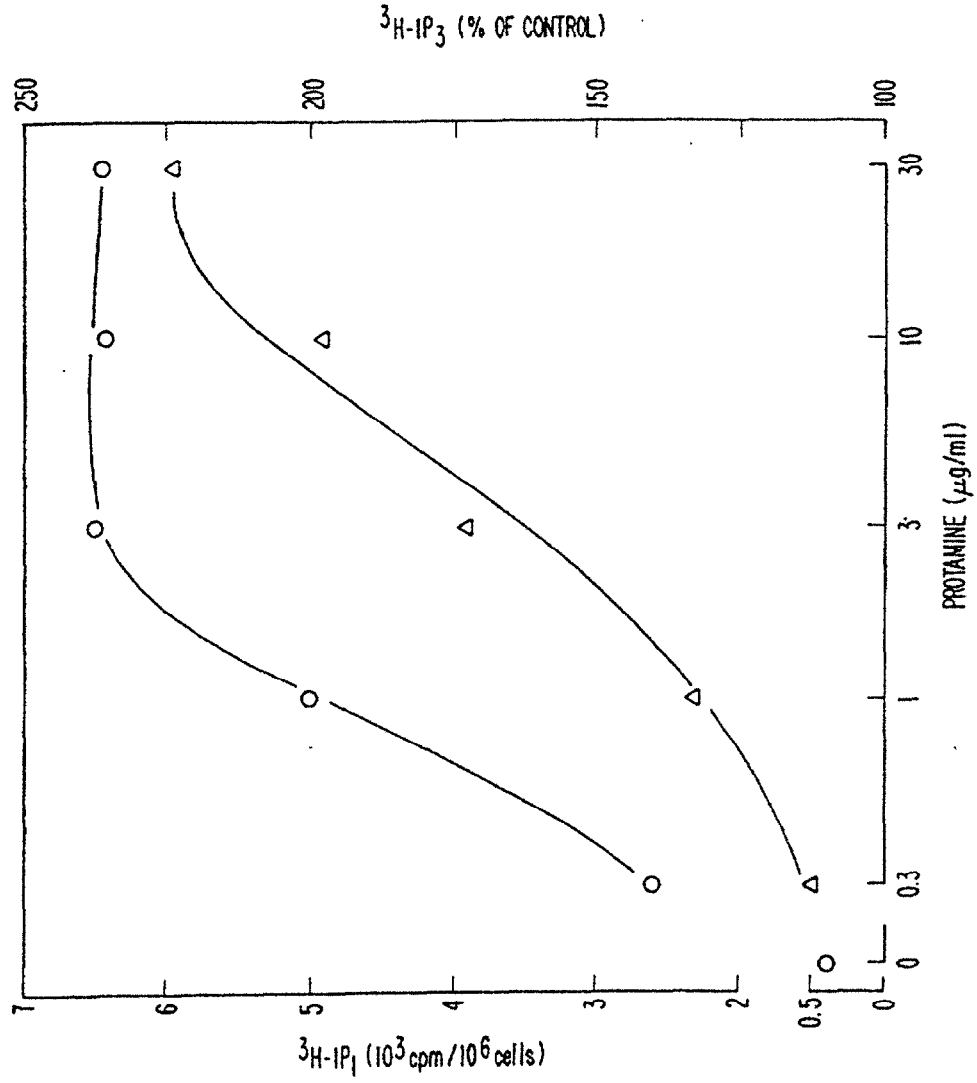

^{3}H-IP$_3$ (% OF CONTROL)
250
200
150
100
^{3}H-IP$_1$ (10^3cpm/10^6cells)
7
6
5
4
3
2
0.5
0
0
0.3
1
3
10
30
PROTAMINE (μg/ml)

FIG. 17.

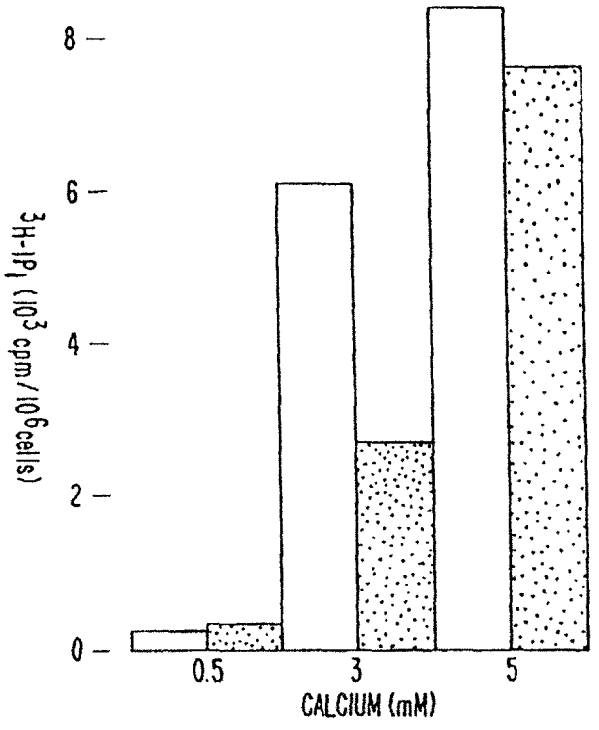


FIG. 18a.

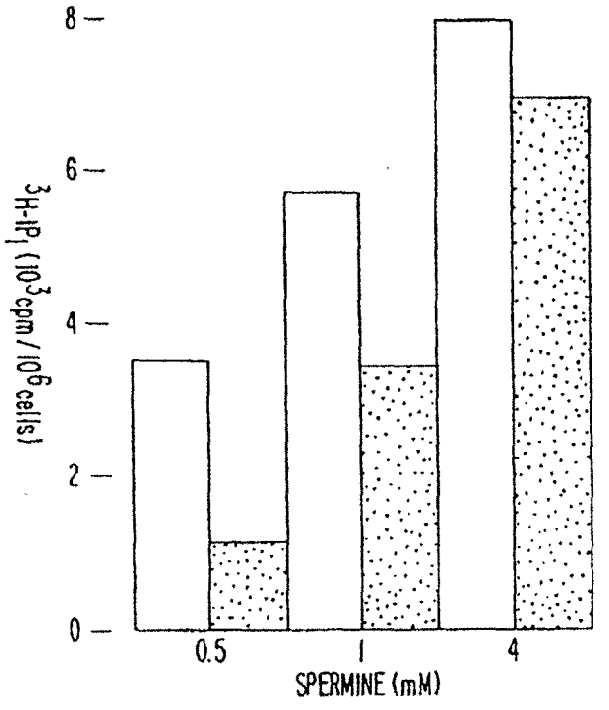


FIG. 18b.

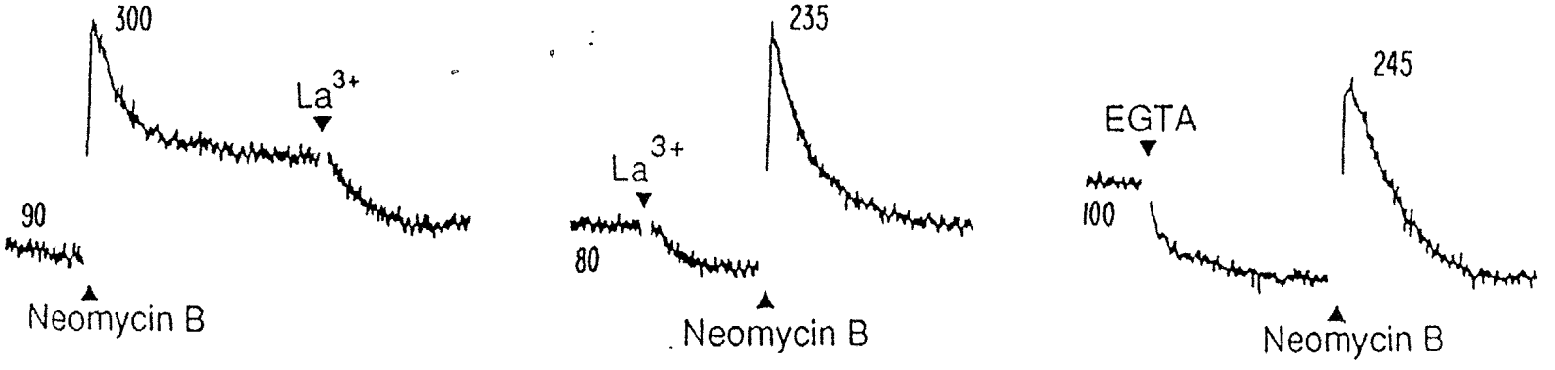

300
La3+
90
Neomycin B
235
La3+
80
Neomycin B
245
EGTA
100
Neomycin B

FIG. 19.

FIG. 20a.

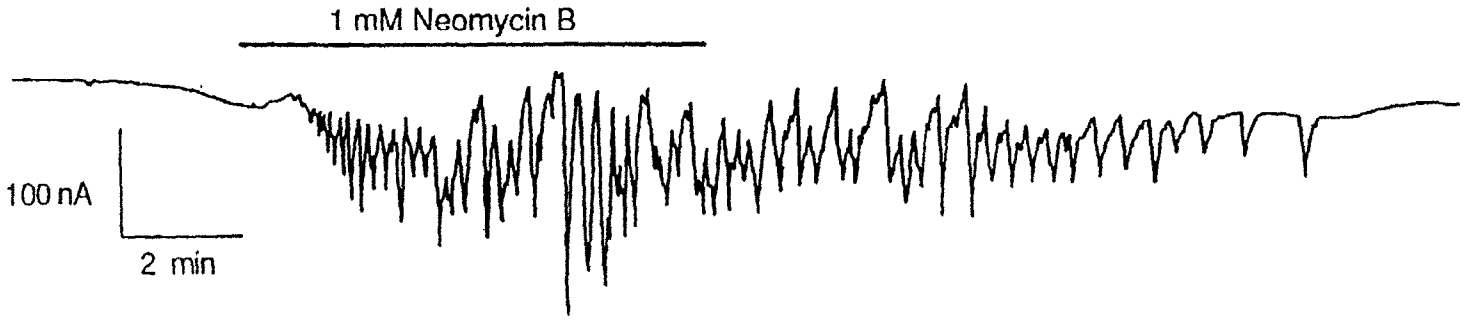


FIG. 20b.

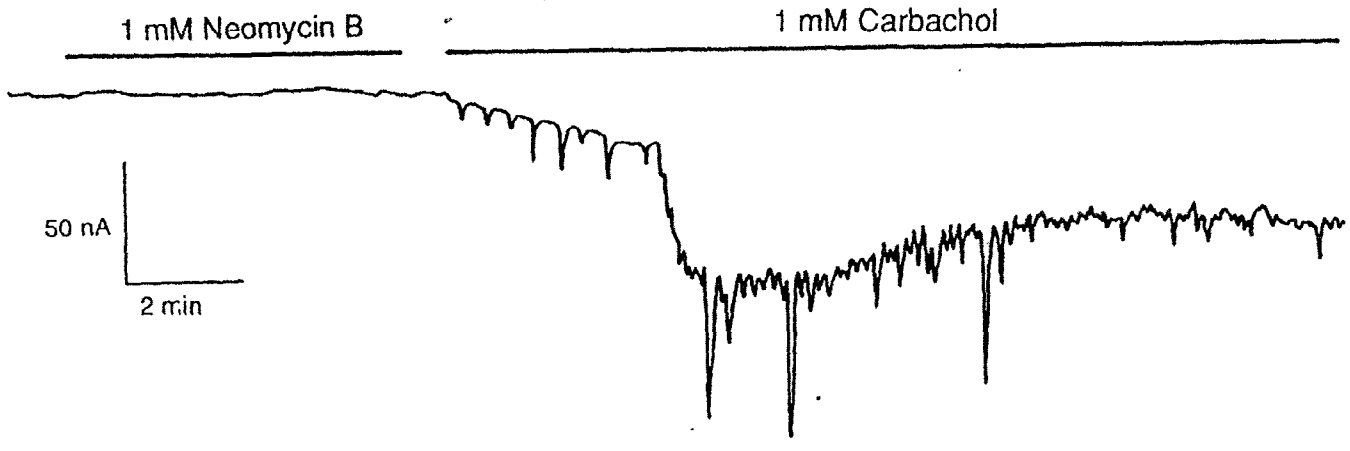


FIG. 21.

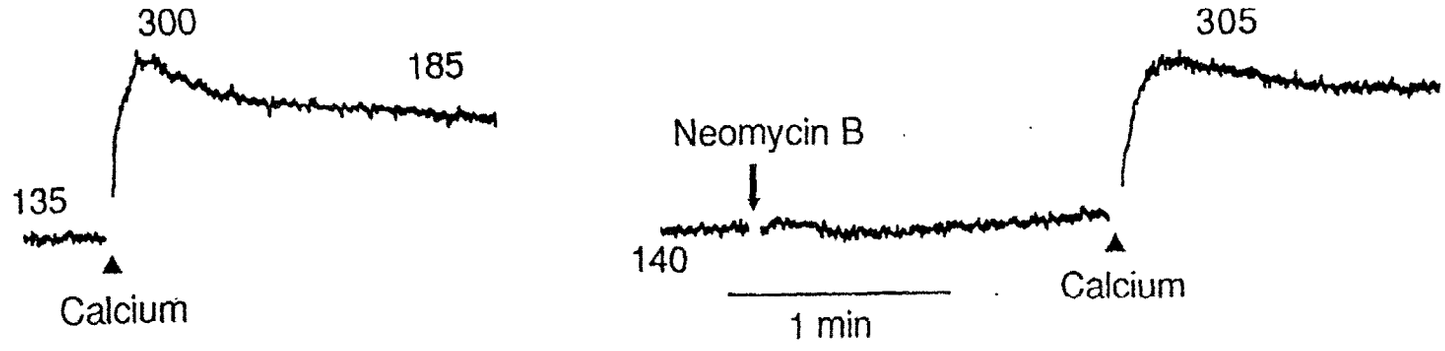


FIG. 22.

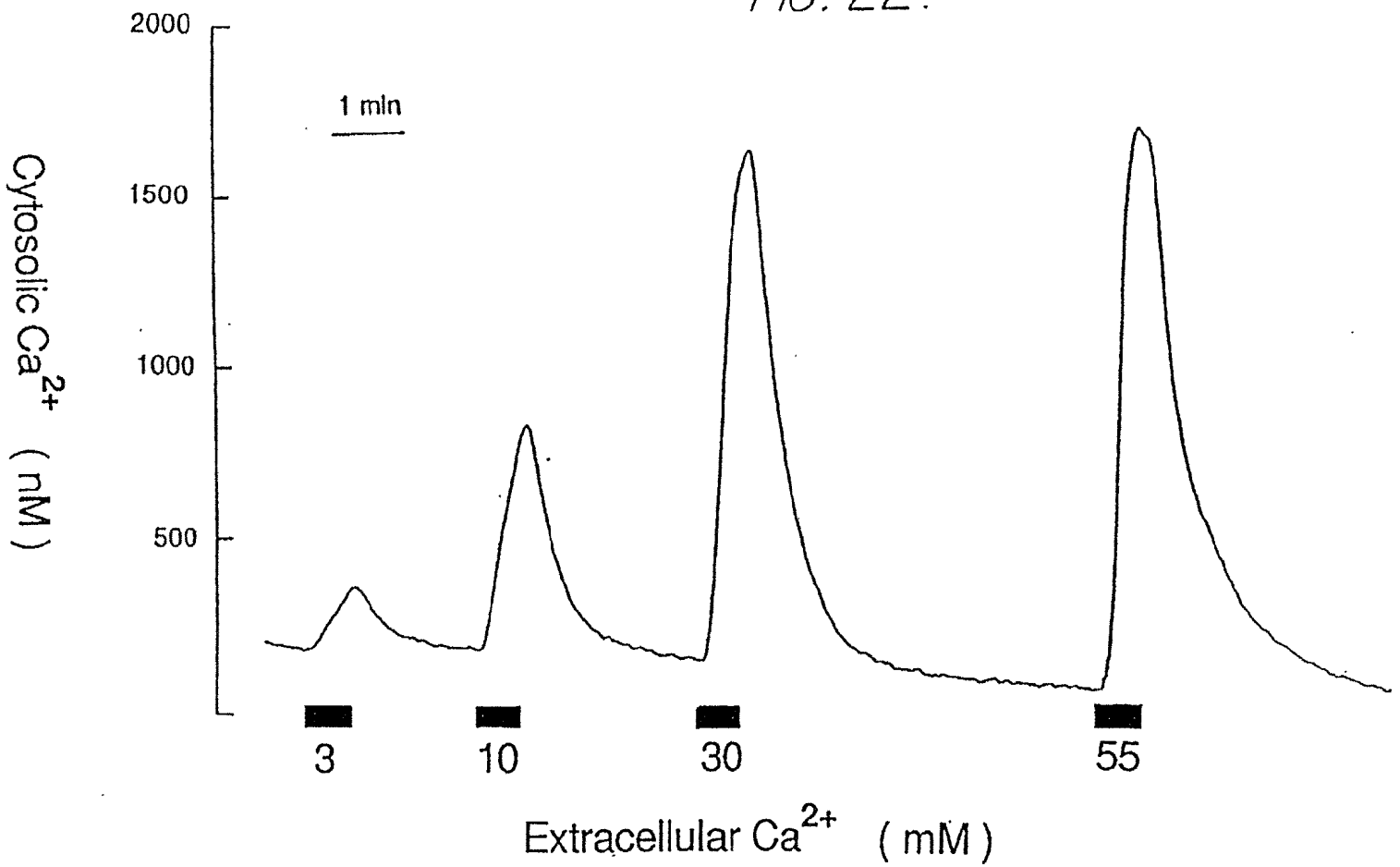
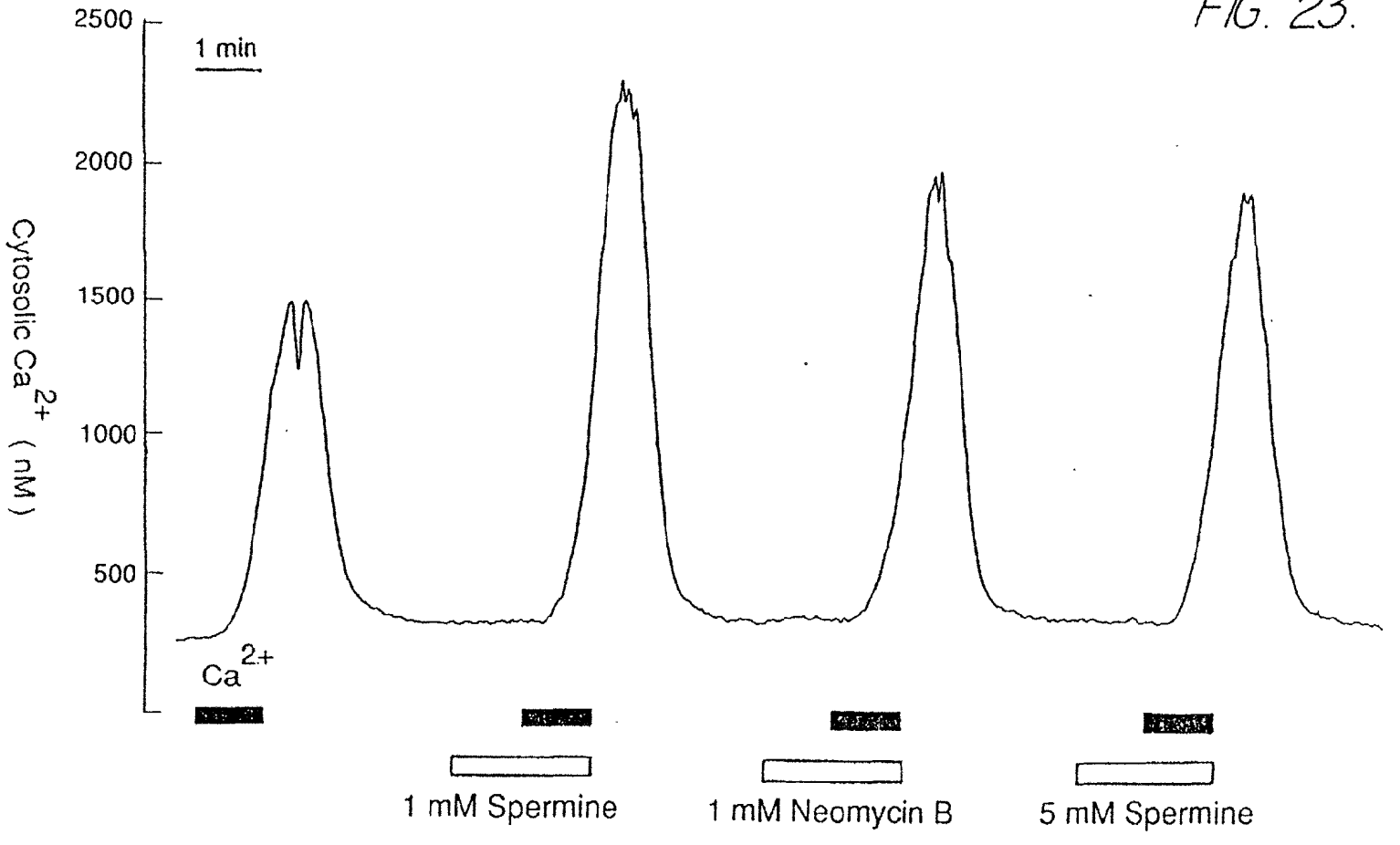

2000
1500
1000
500
1 min
Cytosolic Ca^{2+} (nM)
3
10
30
55
Extracellular Ca^{2+} (mM)

FIG. 23.


2500
2000
1500
1000
500
Cytosolic Ca^{2+} (nM)
1 min
Ca^{2+}
1 mM Spermine
1 mM Neomycin B
5 mM Spermine

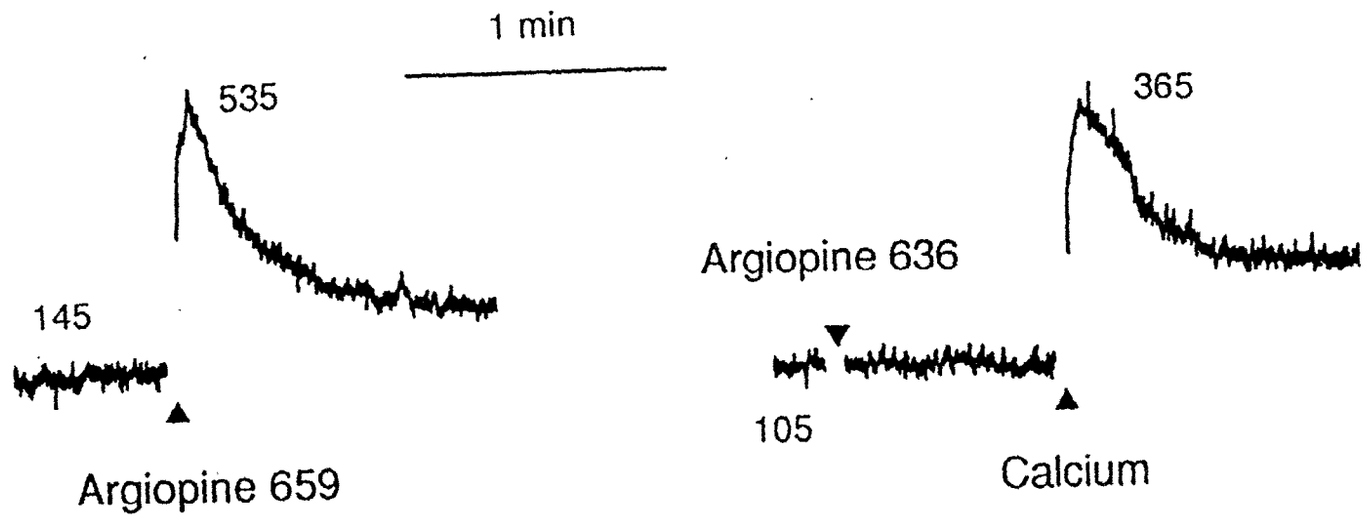


FIG. 24.

FIG. 25a.

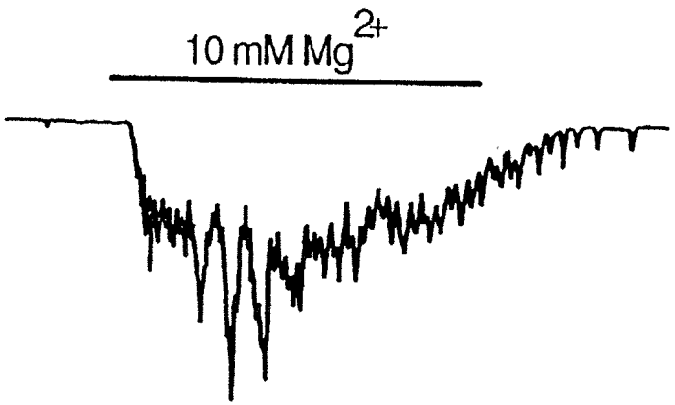


FIG. 25b.

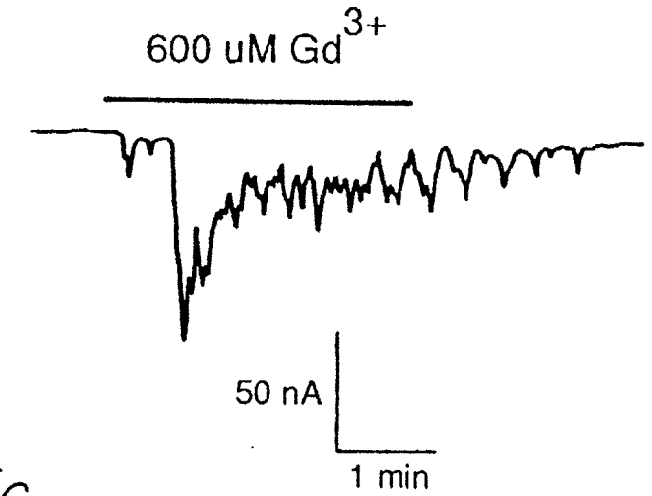


FIG. 25c.

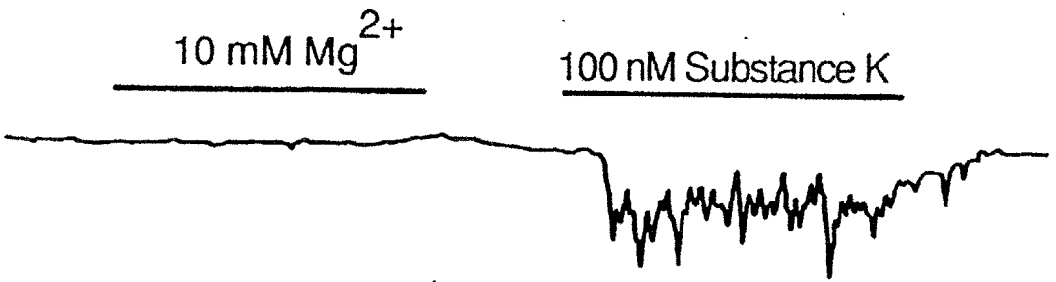


FIG. 26.

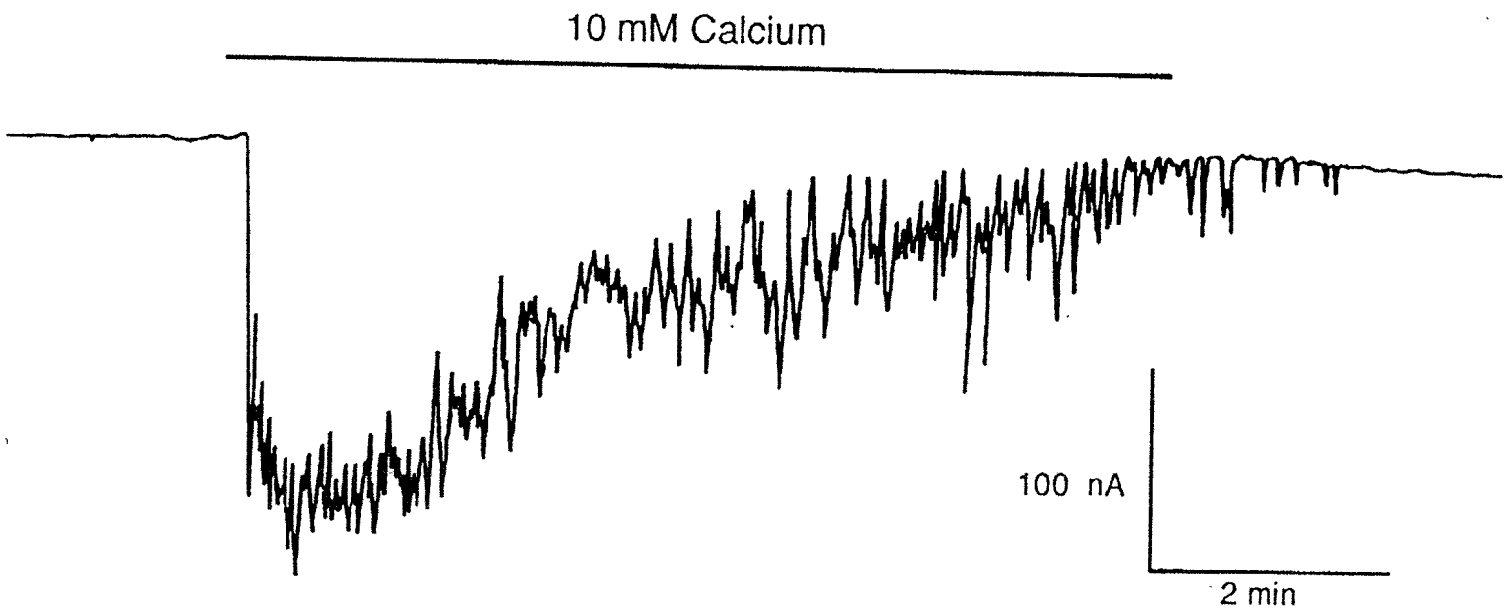
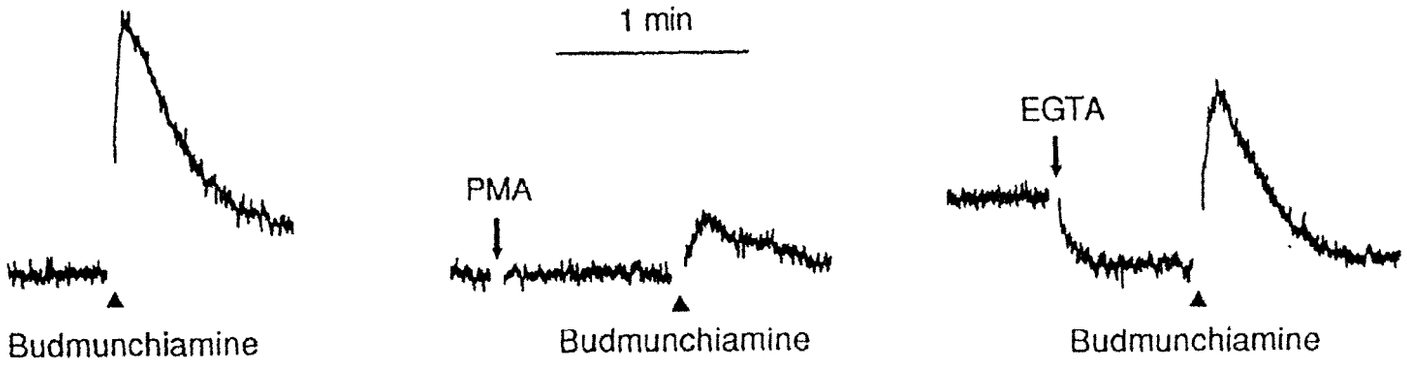
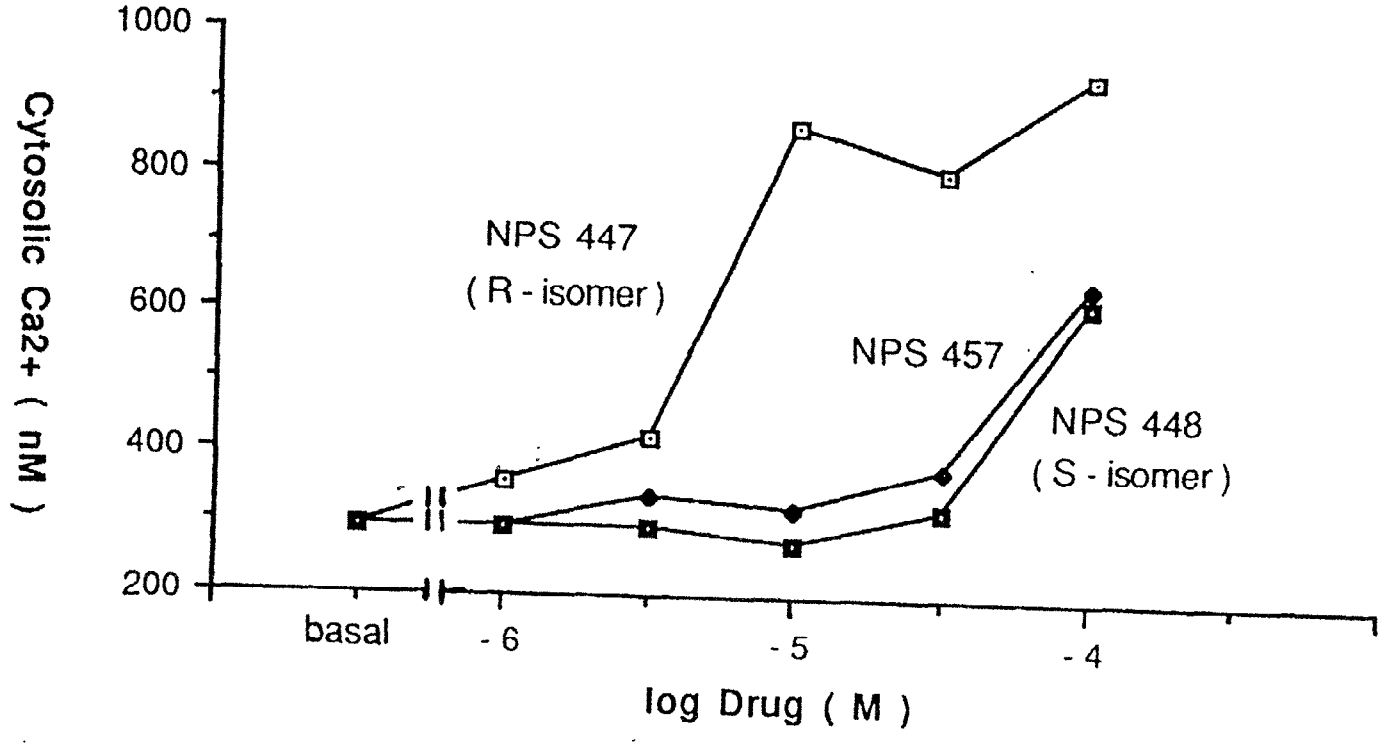


FIG. 27



US006011068 file:\\\cadmrfs01\firmdata\lp\FoleyPat\PatentDocuments\US006011068.CPC]

FIG. 28a.


1000
800
600
400
200
Cytosolic Ca2+ (nM)
NPS 447
(R - isomer)
NPS 457
NPS 448
(S - isomer)
basal
- 6
- 5
- 4
log Drug (M)

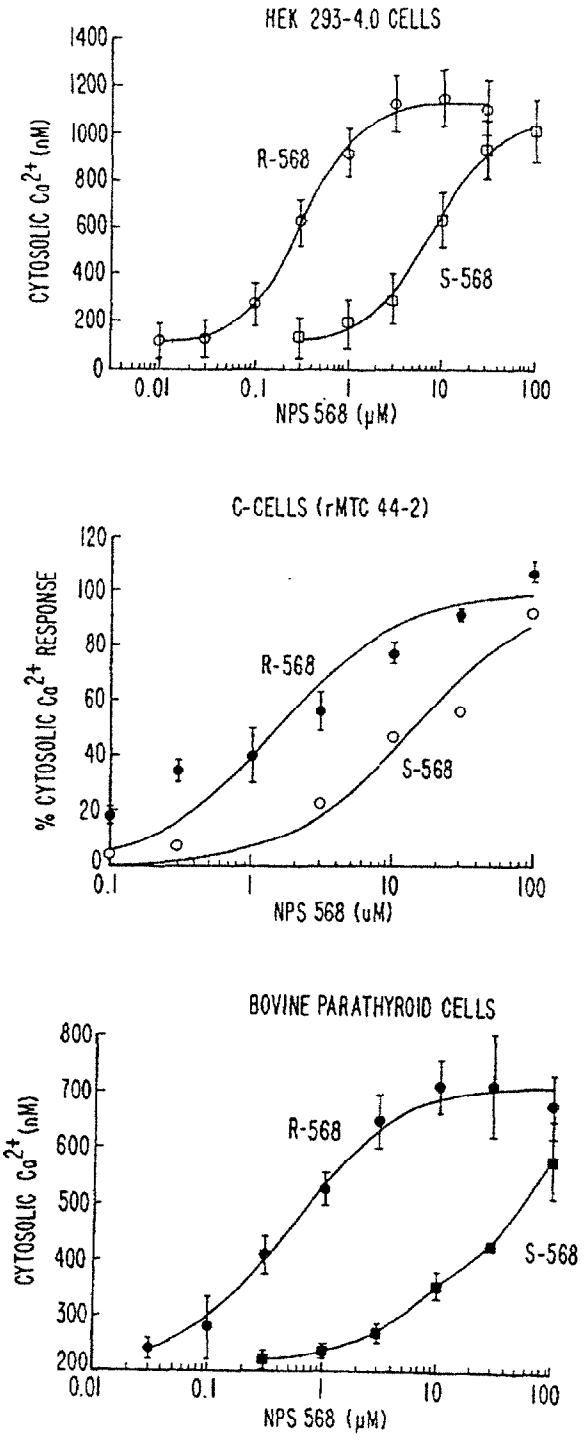

HEK 293-4.0 CELLS
CYTOSOLIC Ca2+ (nM)
R-568
S-568
NPS 568 (μM)
C-CELLS (rMTC 44-2)
% CYTOSOLIC Ca2+ RESPONSE
R-568
S-568
NPS 568 (uM)
BOVINE PARATHYROID CELLS
CYTOSOLIC Ca2+ (nM)
R-568
S-568
NPS 568 (μM)

FIG. 28b.

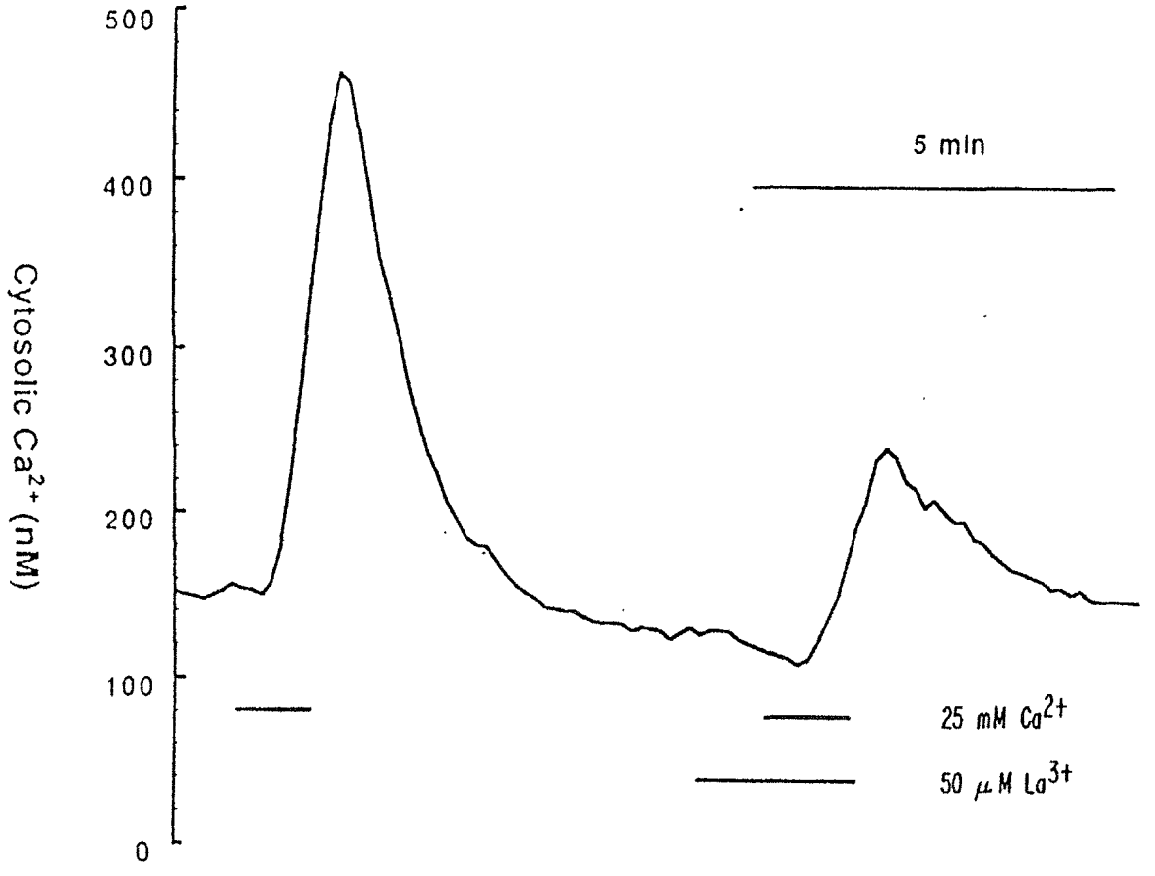


FIG. 29.

FIG. 30a.

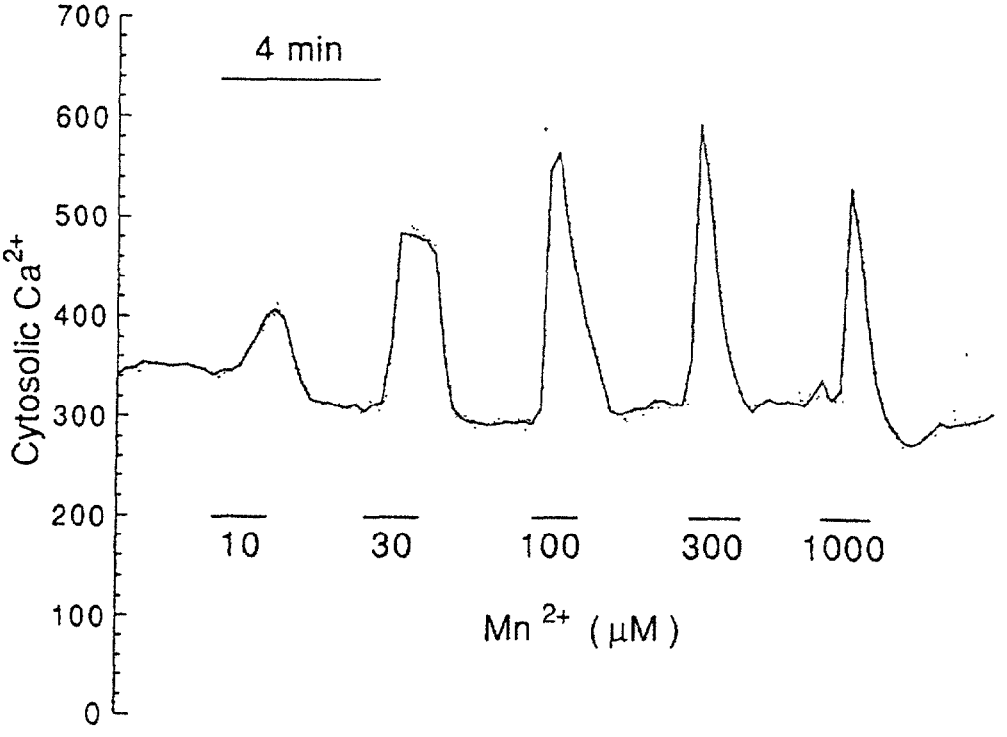
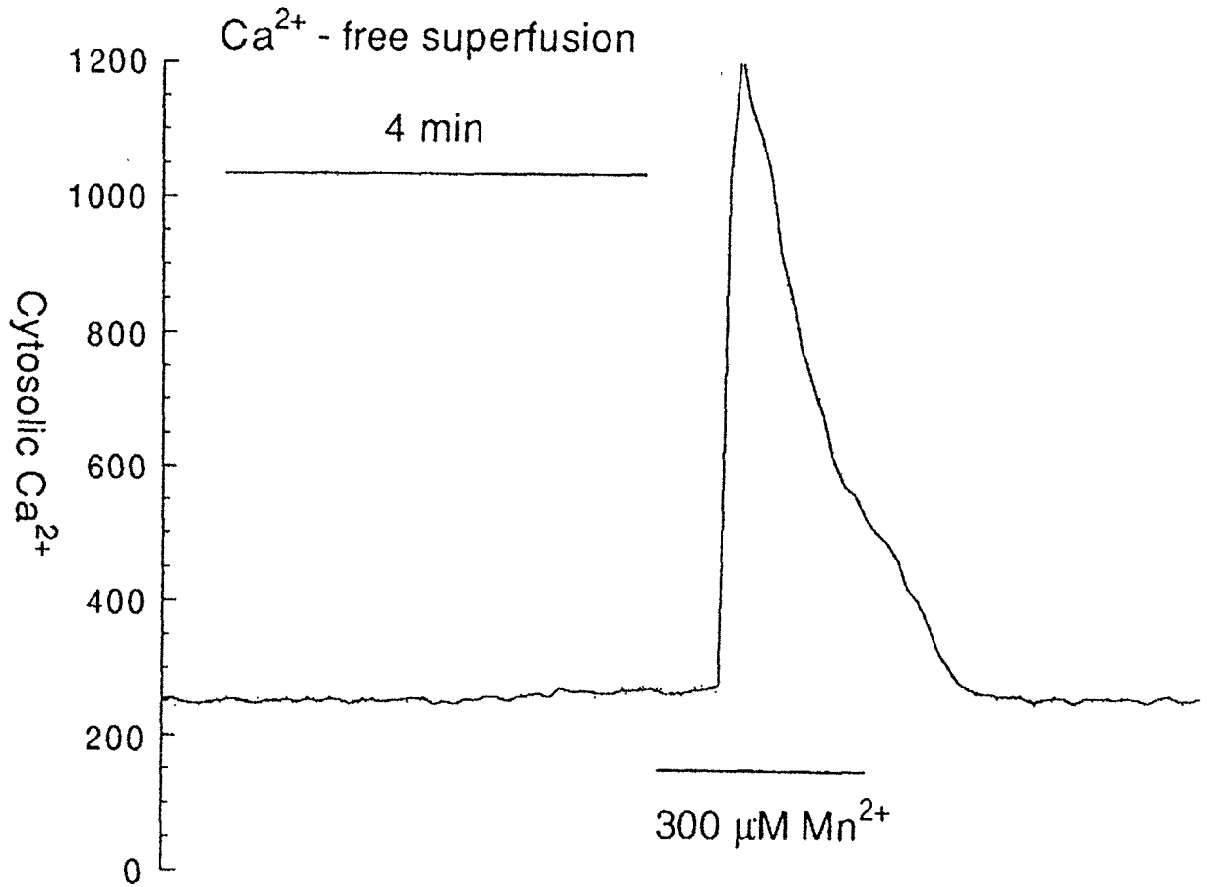

700
600
500
400
300
200
100
0
4 min
Cytosolic Ca2+
10
30
100
300
1000
Mn 2+ (μM)

FIG. 30b.


Ca2+ - free superfusion
4 min
300 μM Mn2+
Cytosolic Ca2+
1200
1000
800
600
400
200
0

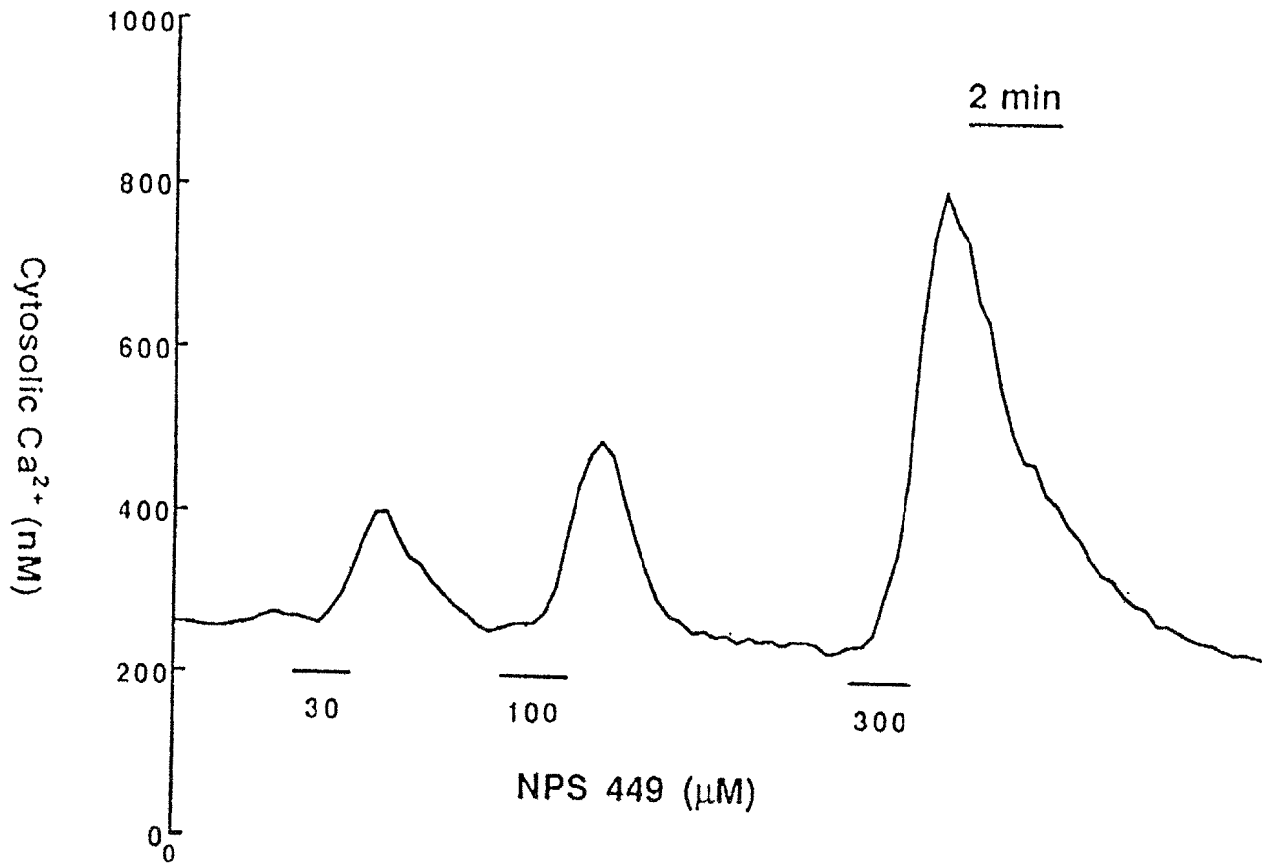

1000
800
600
400
200
0
0
Cytosolic Ca2+ (nM)
2 min
30
100
300
NPS 449 (μM)

FIG. 31a.

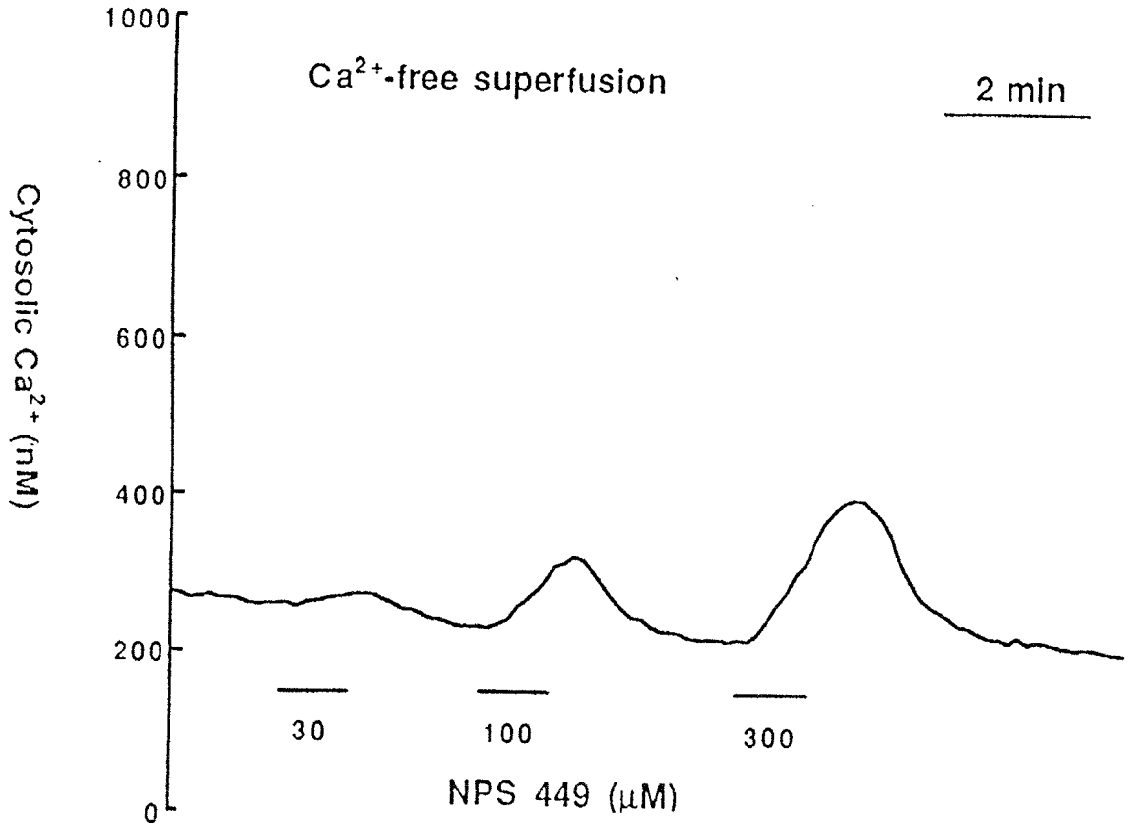

Ca2+-free superfusion
2 min
Cytosolic Ca2+ (nM)
1000
800
600
400
200
0
30
100
300
NPS 449 (μM)

FIG. 31b.

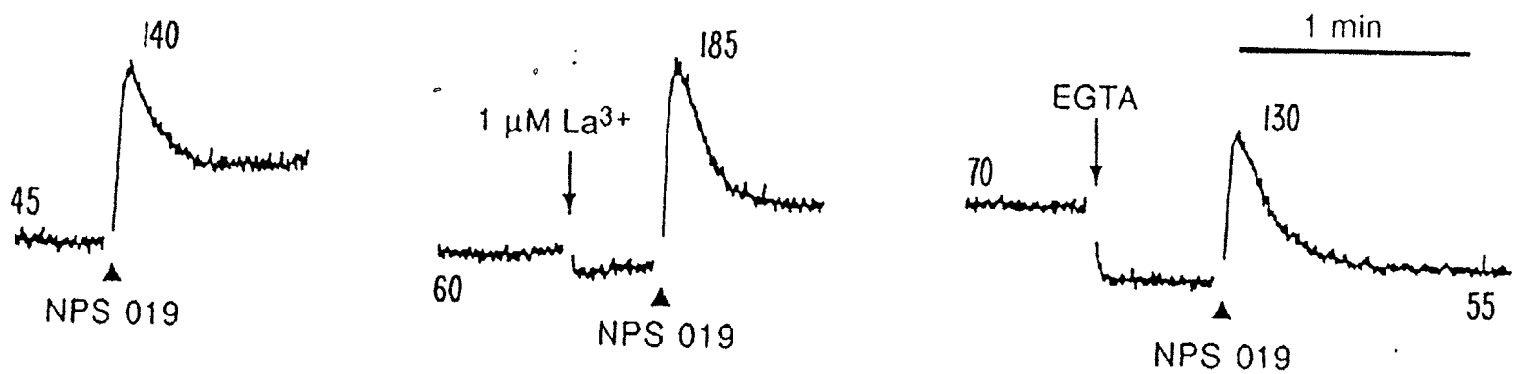


FIG. 32.

FIG. 33.

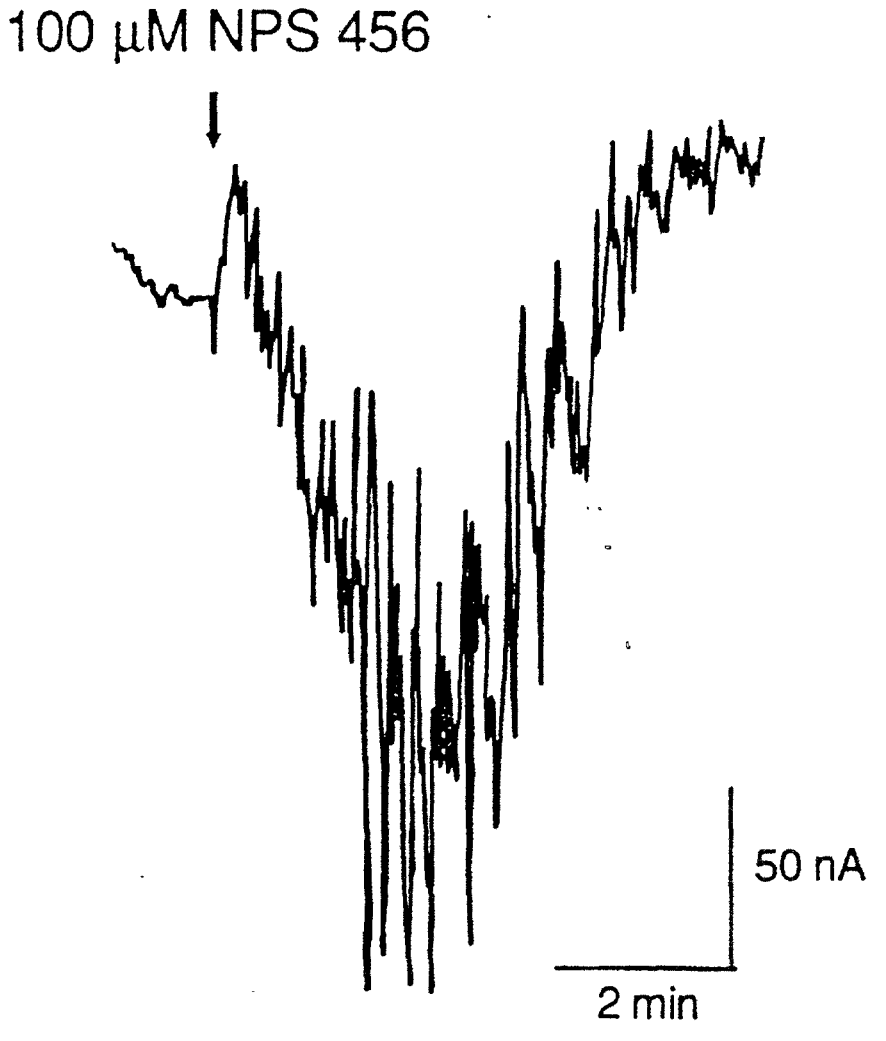
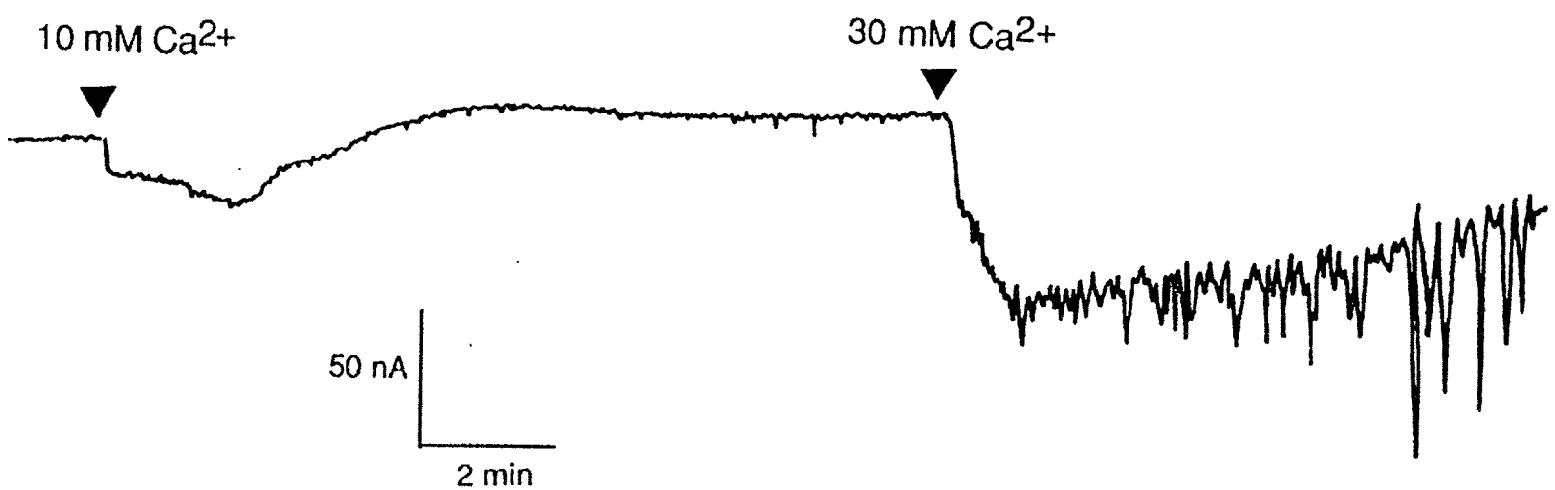


FIG. 34.



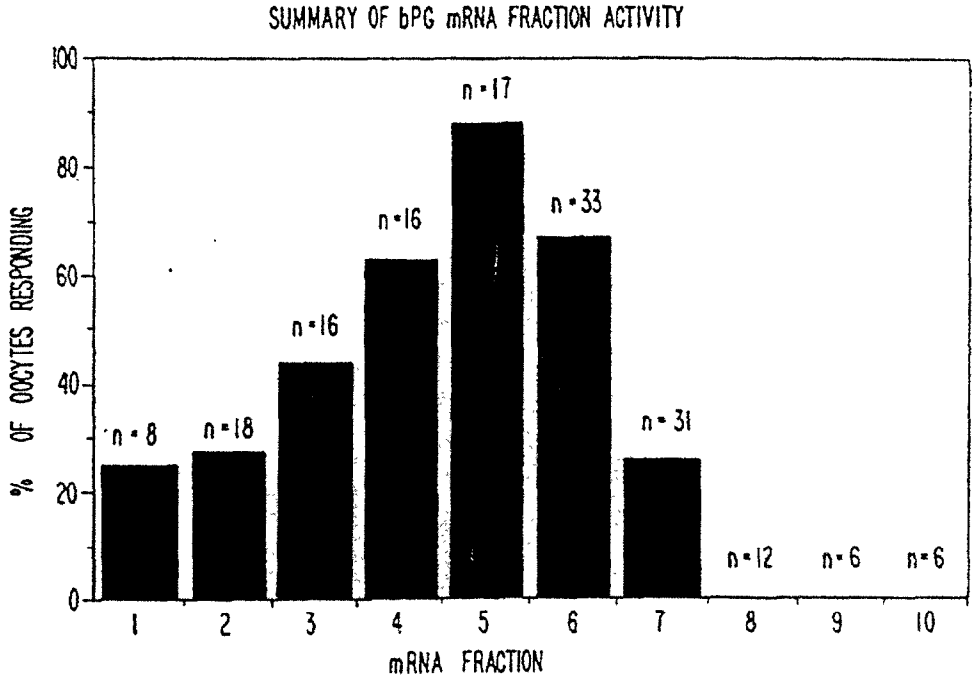

SUMMARY OF bPG mRNA FRACTION ACTIVITY
% OF OOCYTES RESPONDING
100
80
60
40
20
0
n = 8
n = 18
n = 16
n = 16
n = 17
n = 33
n = 31
n = 12
n = 6
n = 6
1
2
3
4
5
6
7
8
9
10
mRNA FRACTION

FIG. 35.

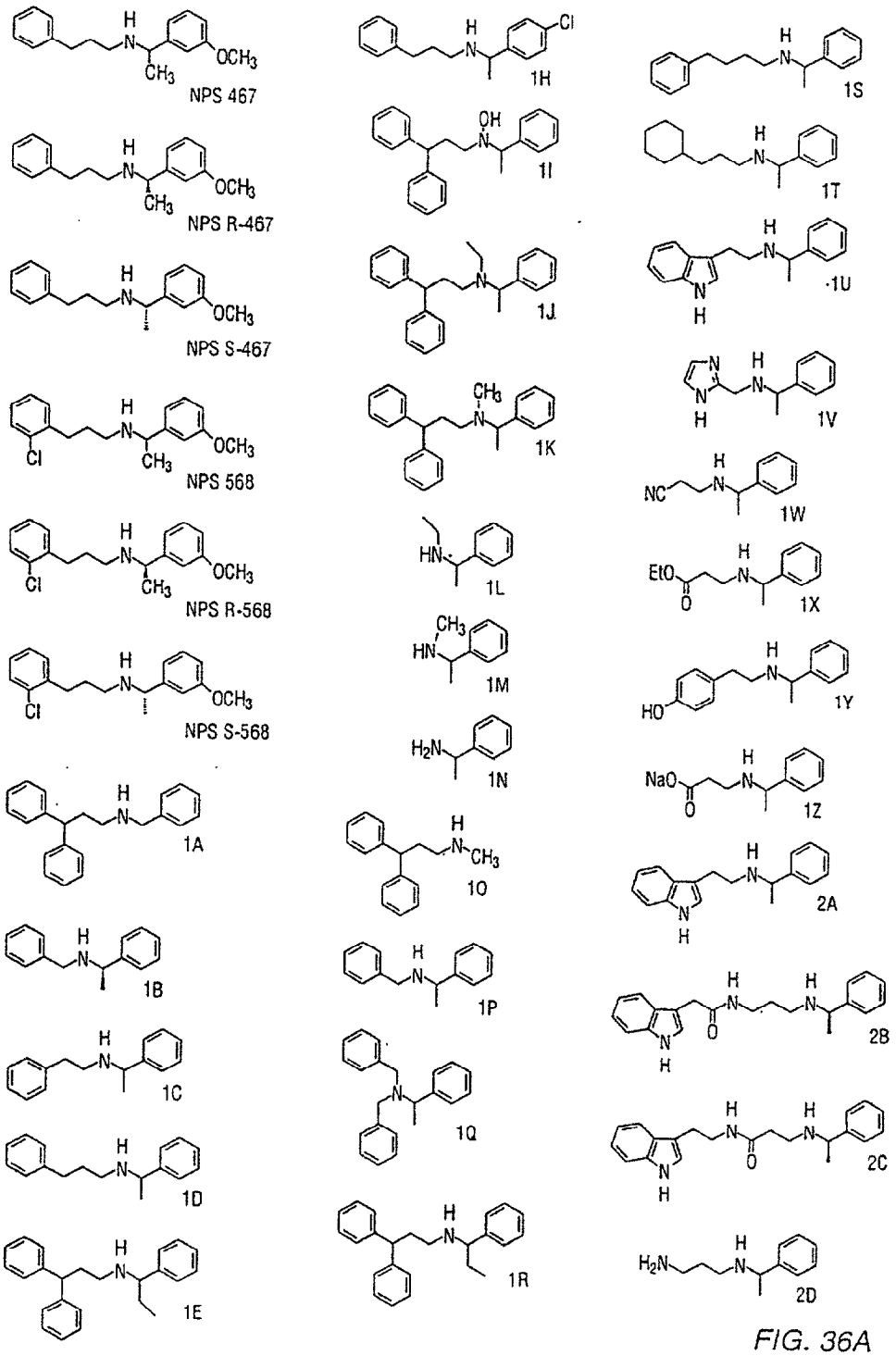

NPS 467
NPS R-467
NPS S-467
NPS 568
NPS R-568
NPS S-568
1A
1B
1C
1D
1E
1H
1I
1J
1K
1L
1M
1N
1O
1P
1Q
1R
1S
1T
1U
1V
1W
1X
1Y
1Z
2A
2B
2C
2D

FIG. 36A

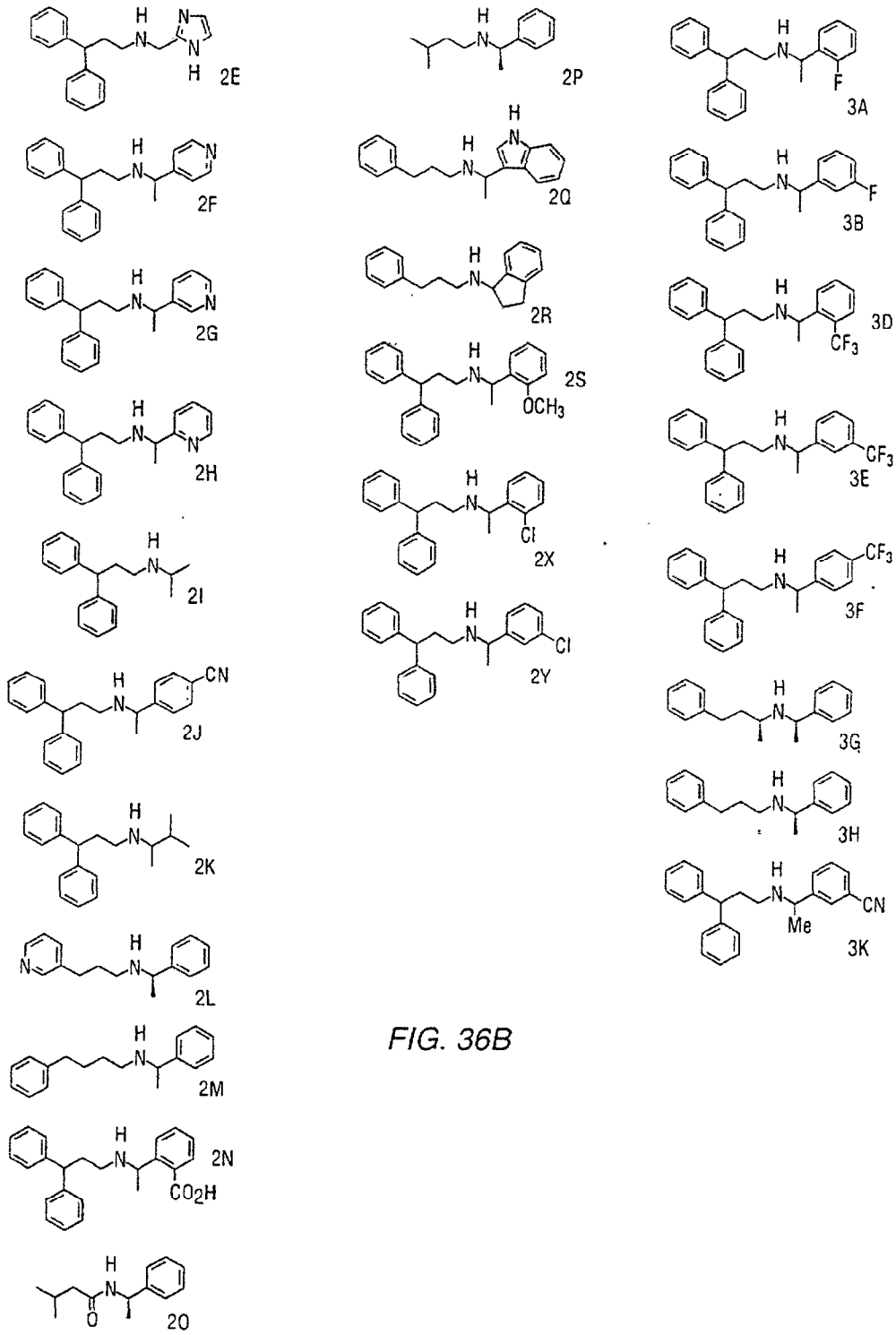
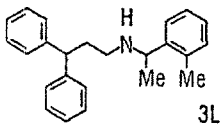
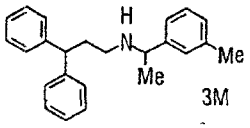
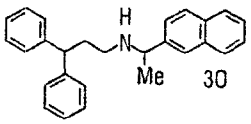
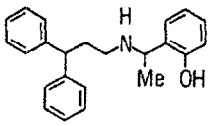

2E
2F
2G
2H
2I
2J
2K
2L
2M
2N
2O
2P
2Q
2R
2S
2X
2Y
3A
3B
3D
3E
3F
3G
3H
3K

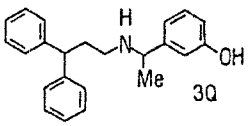
FIG. 36B

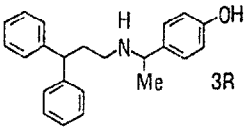

3L

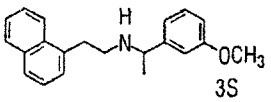

3M

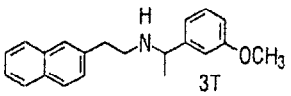

3O

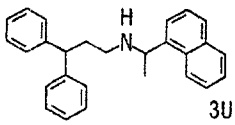

3P

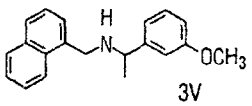

3Q

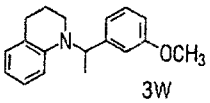

3R

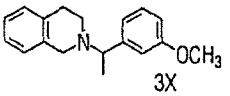

3S

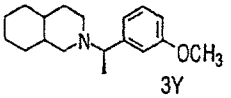

3T

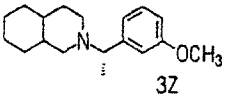

3U

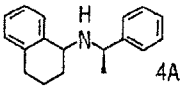

3V

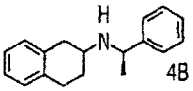

3W

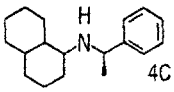

3X

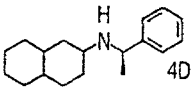

3Y

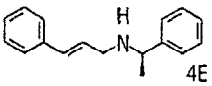

3Z

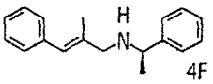

4A

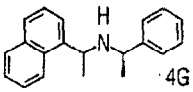

4B

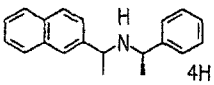

4C

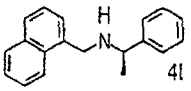

4D

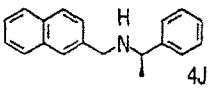

4E

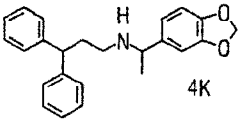

4F

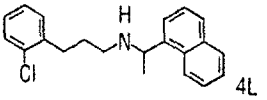

4G

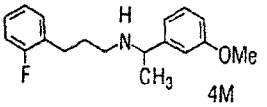

4H

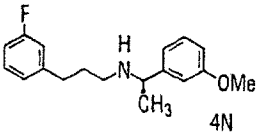

4I

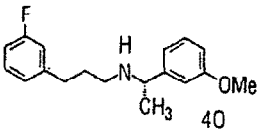

4J

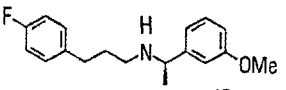

4K

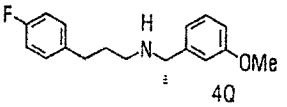

4L

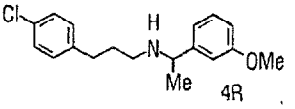

4M

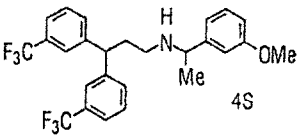

4N

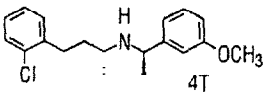

4O

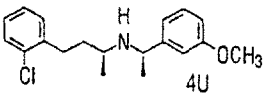

4P

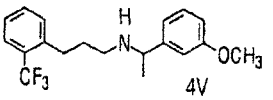

4Q


4R


4S


4T


4U


4V

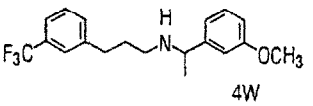

4W

FIG. 36C

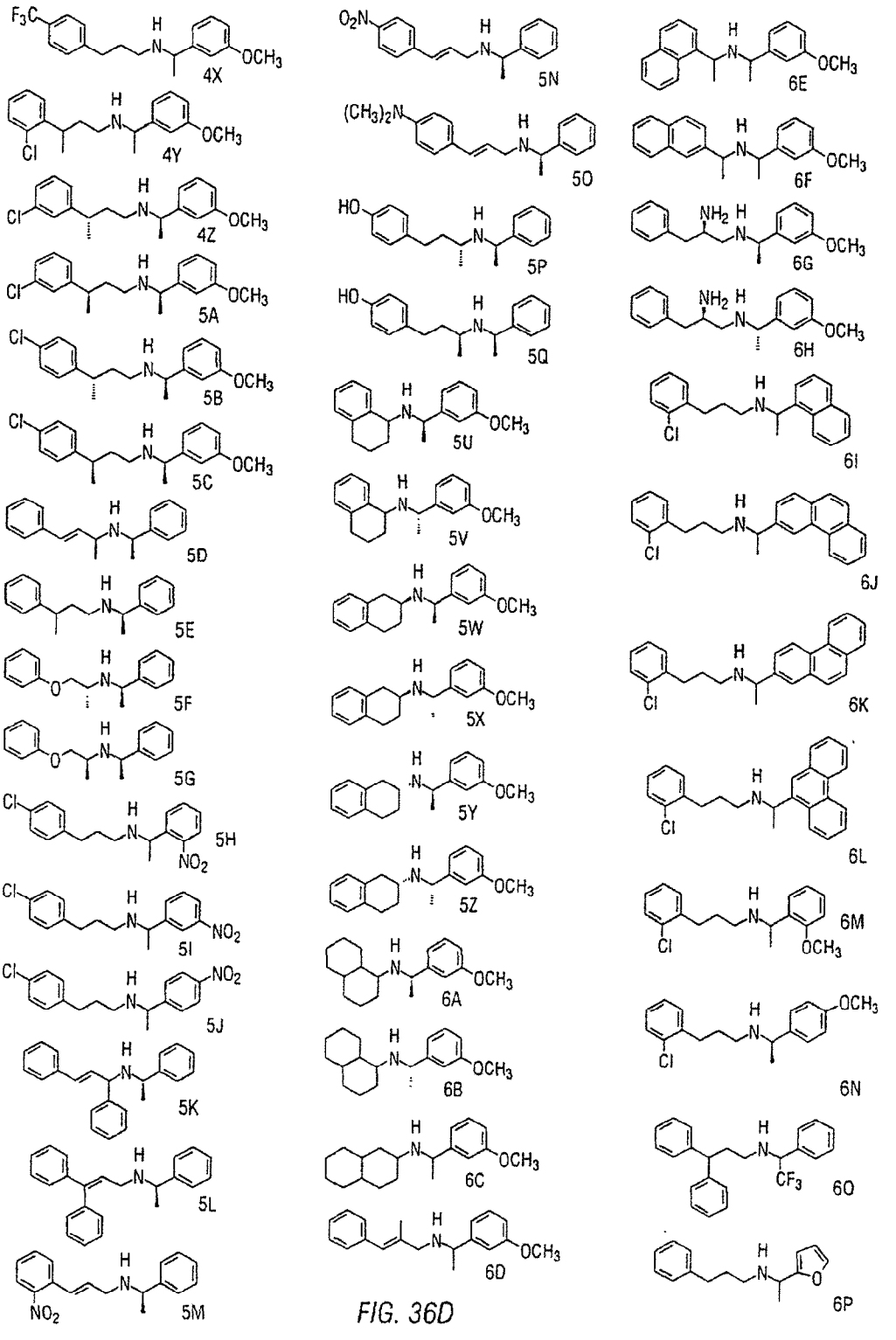

4X
4Y
4Z
5A
5B
5C
5D
5E
5F
5G
5H
5I
5J
5K
5L
5M
5N
5O
5P
5Q
5U
5V
5W
5X
5Y
5Z
6A
6B
6C
6D
6E
6F
6G
6H
6I
6J
6K
6L
6M
6N
6O
6P

FIG. 36D

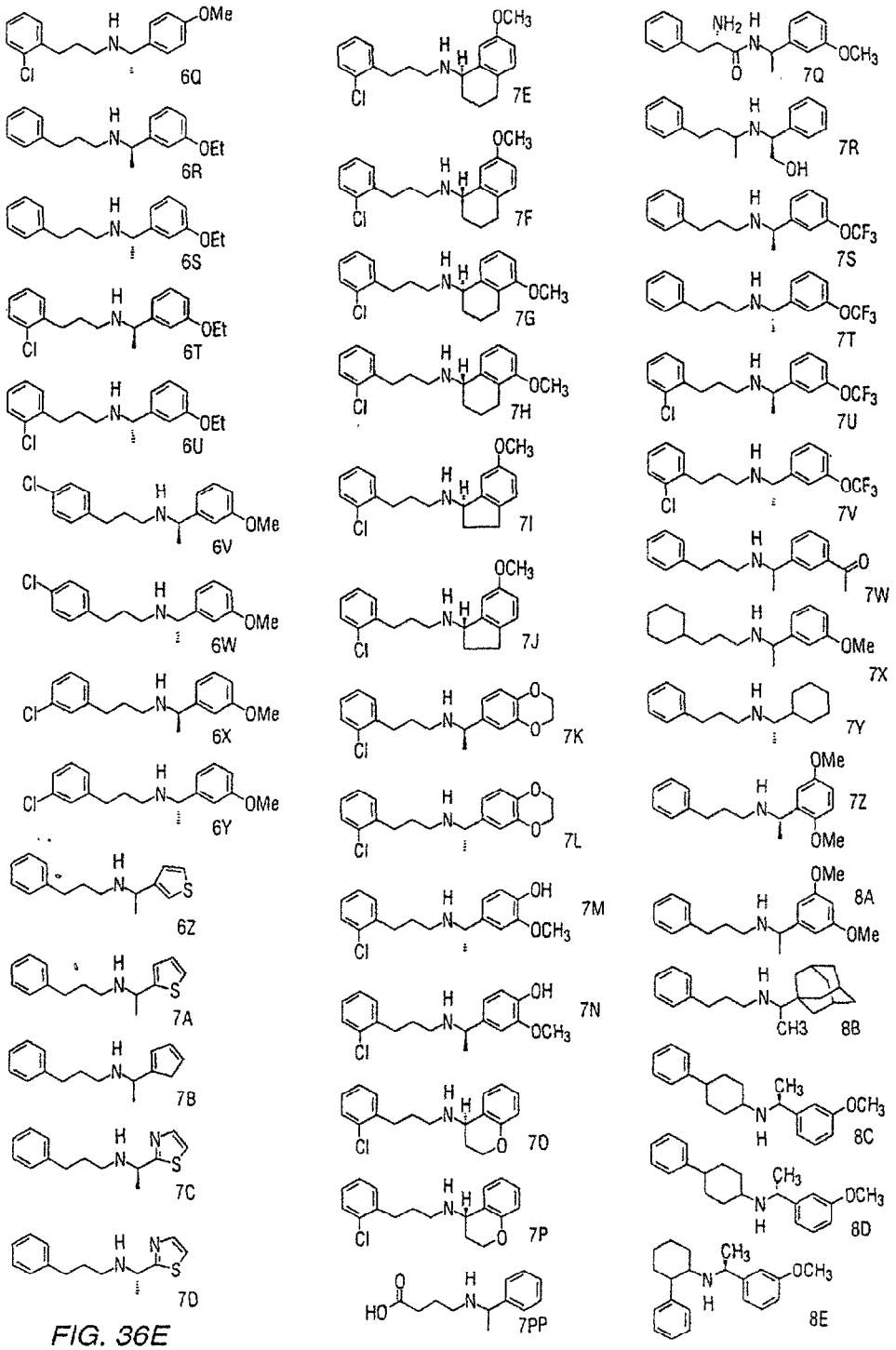

6Q
6R
6S
6T
6U
6V
6W
6X
6Y
6Z
7A
7B
7C
7D
7E
7F
7G
7H
7I
7J
7K
7L
7M
7N
7O
7P
7PP
7Q
7R
7S
7T
7U
7V
7W
7X
7Y
7Z
8A
8B
8C
8D
8E

FIG. 36E

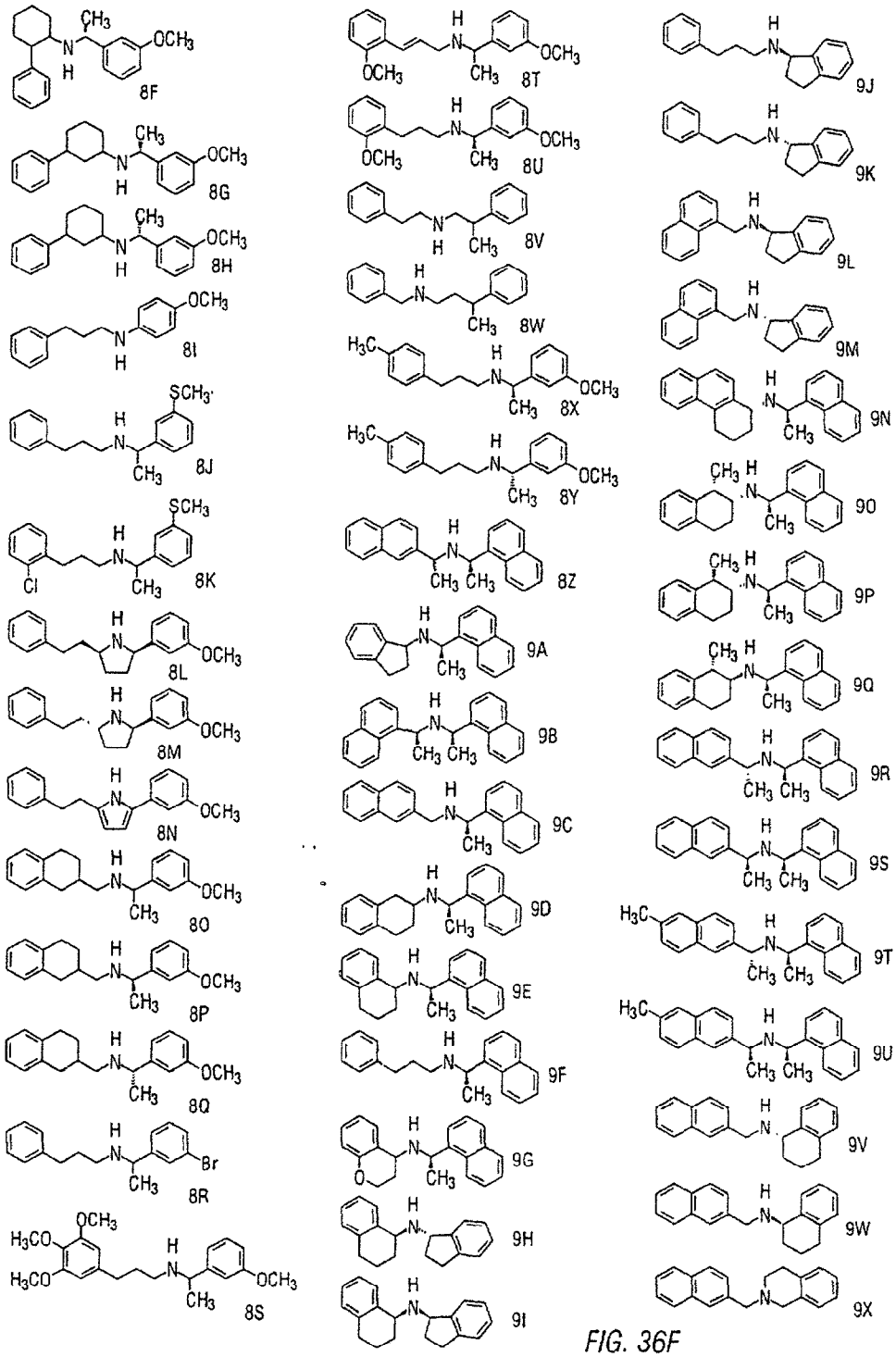

8F
8G
8H
8I
8J
8K
8L
8M
8N
8O
8P
8Q
8R
8S
8T
8U
8V
8W
8X
8Y
8Z
9A
9B
9C
9D
9E
9F
9G
9H
9I
9J
9K
9L
9M
9N
9O
9P
9Q
9R
9S
9T
9U
9V
9W
9X

FIG. 36F

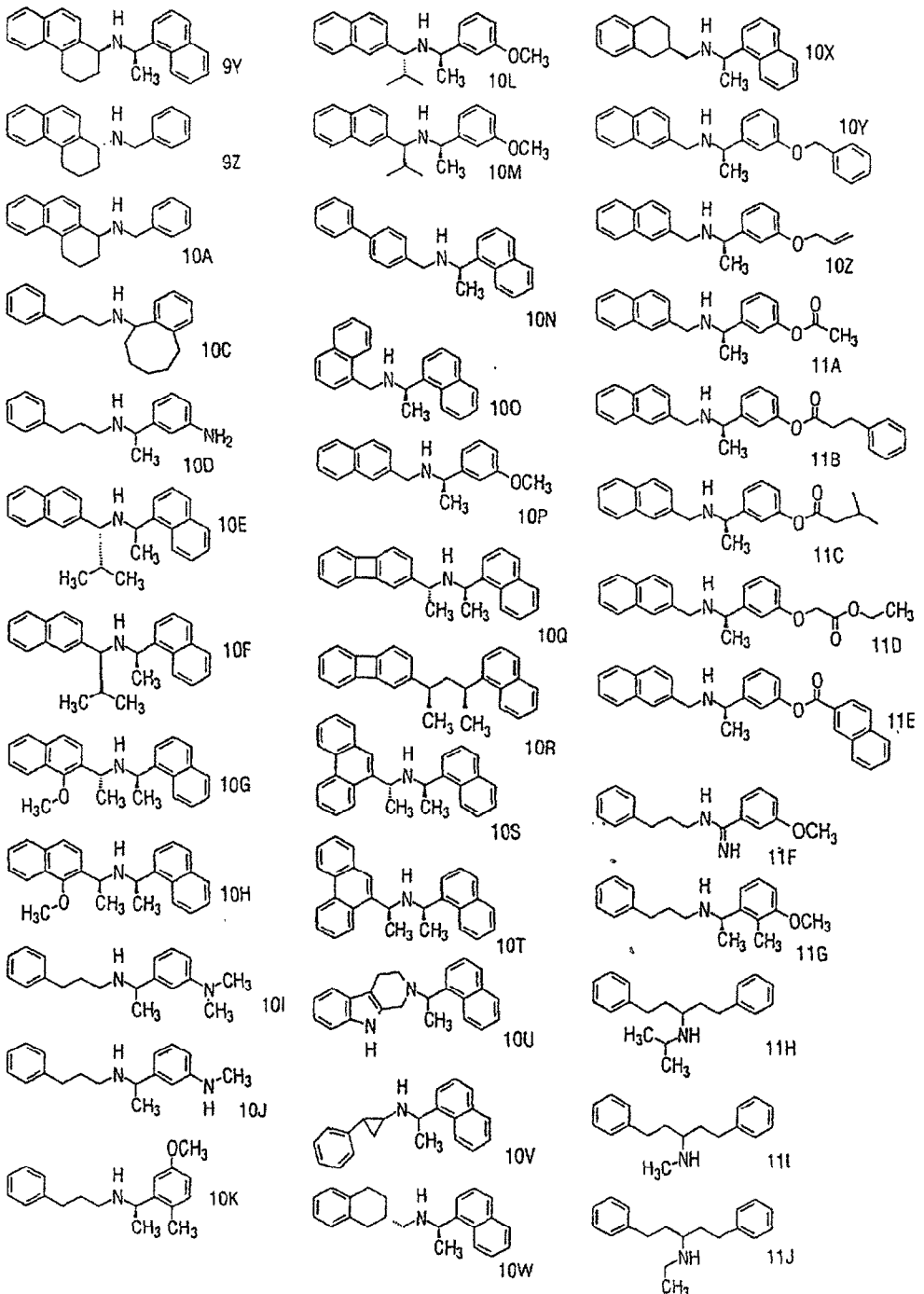

9Y
9Z
10A
10C
10D
10E
10F
10G
10H
10I
10J
10K
10L
10M
10N
10O
10P
10Q
10R
10S
10T
10U
10V
10W
10X
10Y
10Z
11A
11B
11C
11D
11E
11F
11G
11H
11I
11J

FIG. 36G

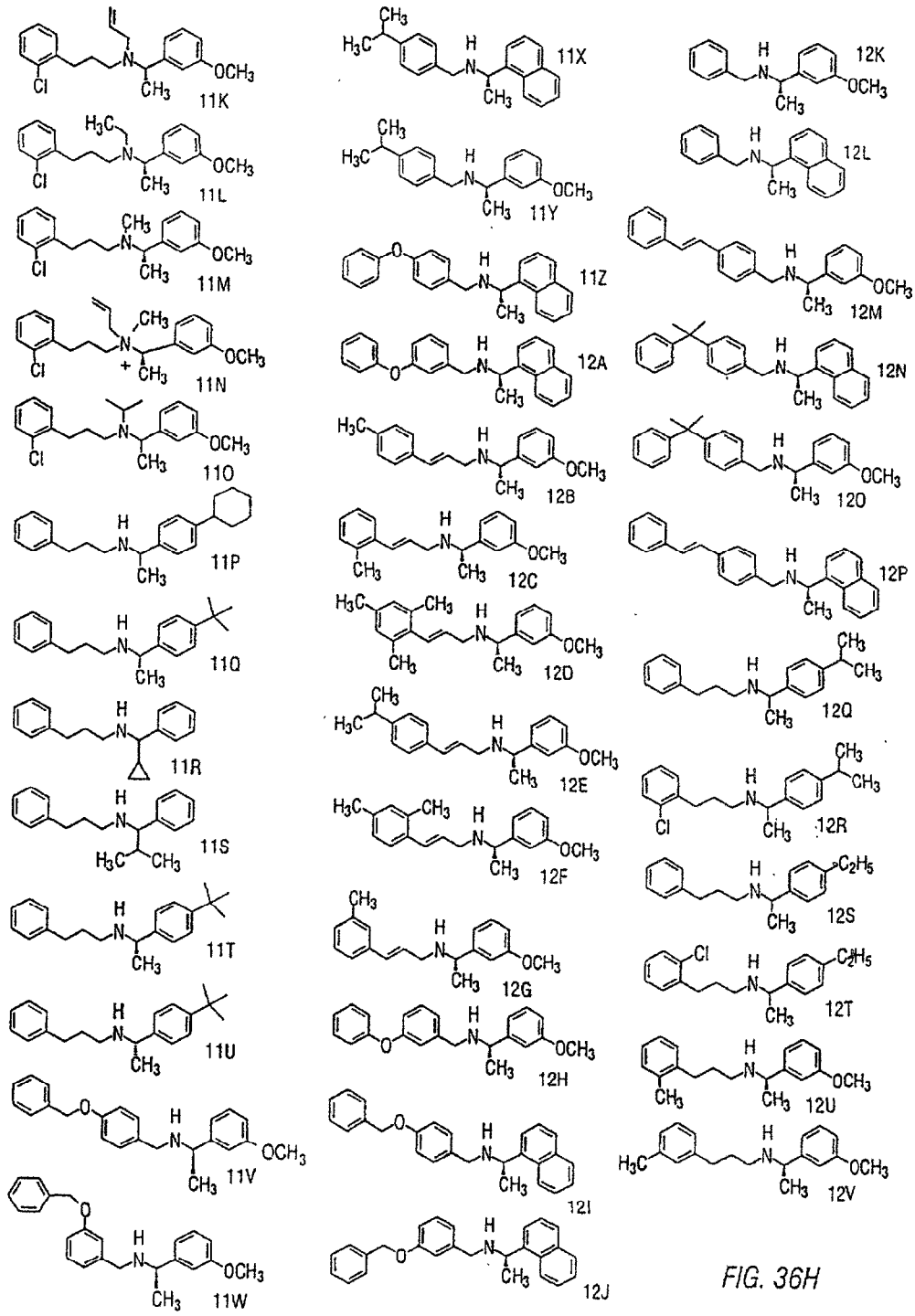

11K
11L
11M
11N
11O
11P
11Q
11R
11S
11T
11U
11V
11W
11X
11Y
11Z
12A
12B
12C
12D
12E
12F
12G
12H
12I
12J
12K
12L
12M
12N
12O
12P
12Q
12R
12S
12T
12U
12V

FIG. 36H

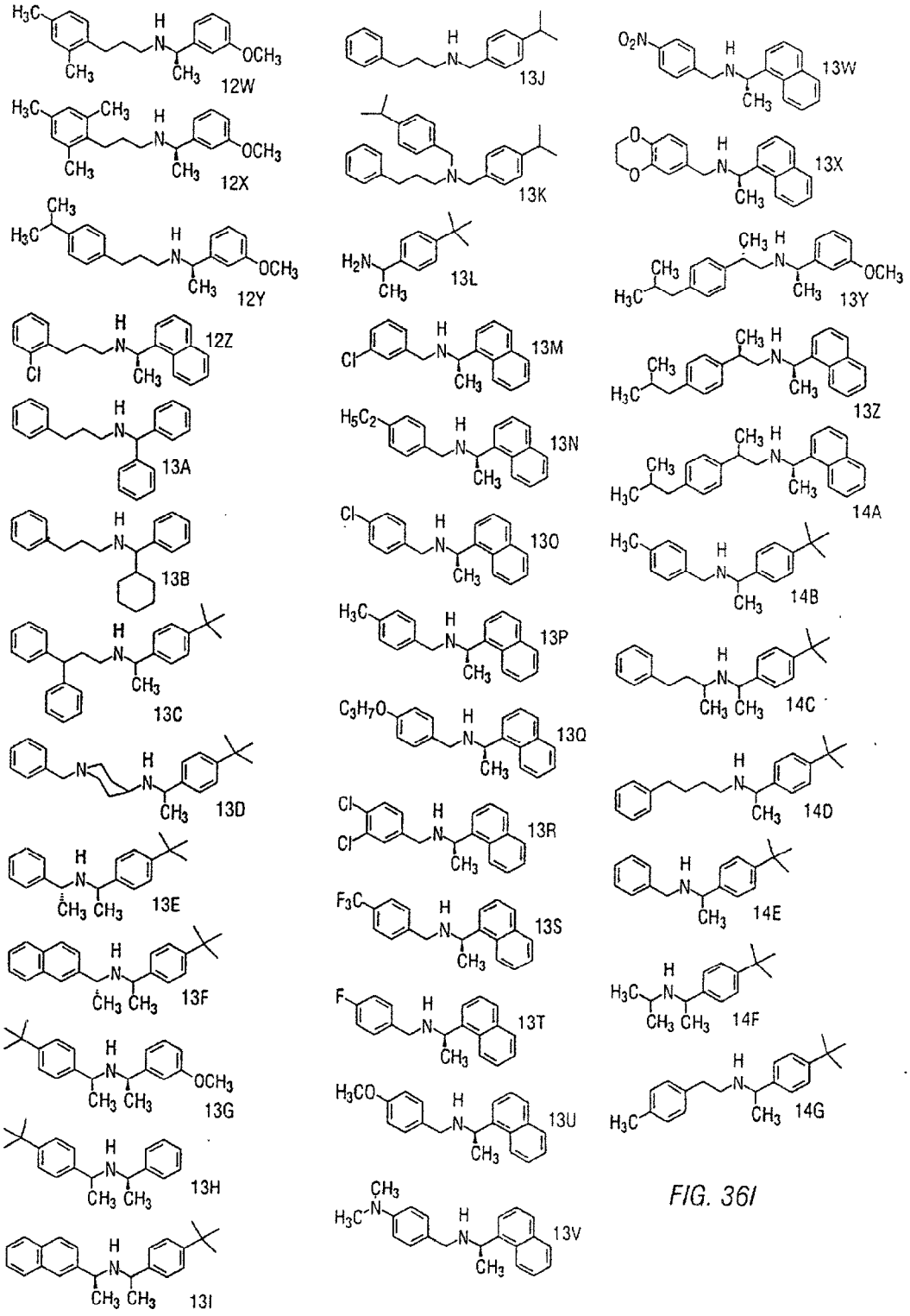

12W
12X
12Y
12Z
13A
13B
13C
13D
13E
13F
13G
13H
13I
13J
13K
13L
13M
13N
13O
13P
13Q
13R
13S
13T
13U
13V
13W
13X
13Y
13Z
14A
14B
14C
14D
14E
14F
14G

FIG. 36I

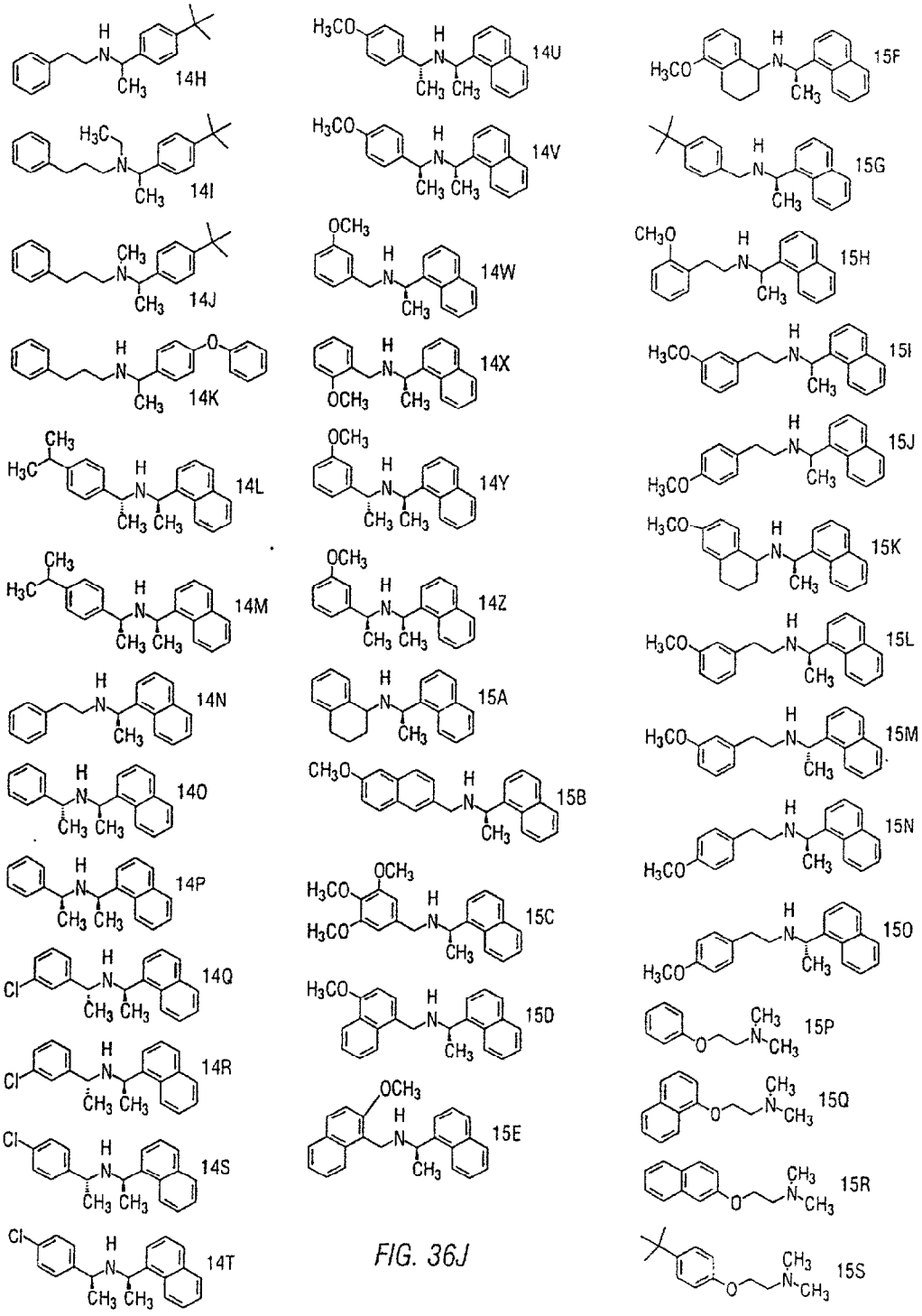

14H
14I
14J
14K
14L
14M
14N
14O
14P
14Q
14R
14S
14T
14U
14V
14W
14X
14Y
14Z
15A
15B
15C
15D
15E
15F
15G
15H
15I
15J
15K
15L
15M
15N
15O
15P
15Q
15R
15S

FIG. 36J

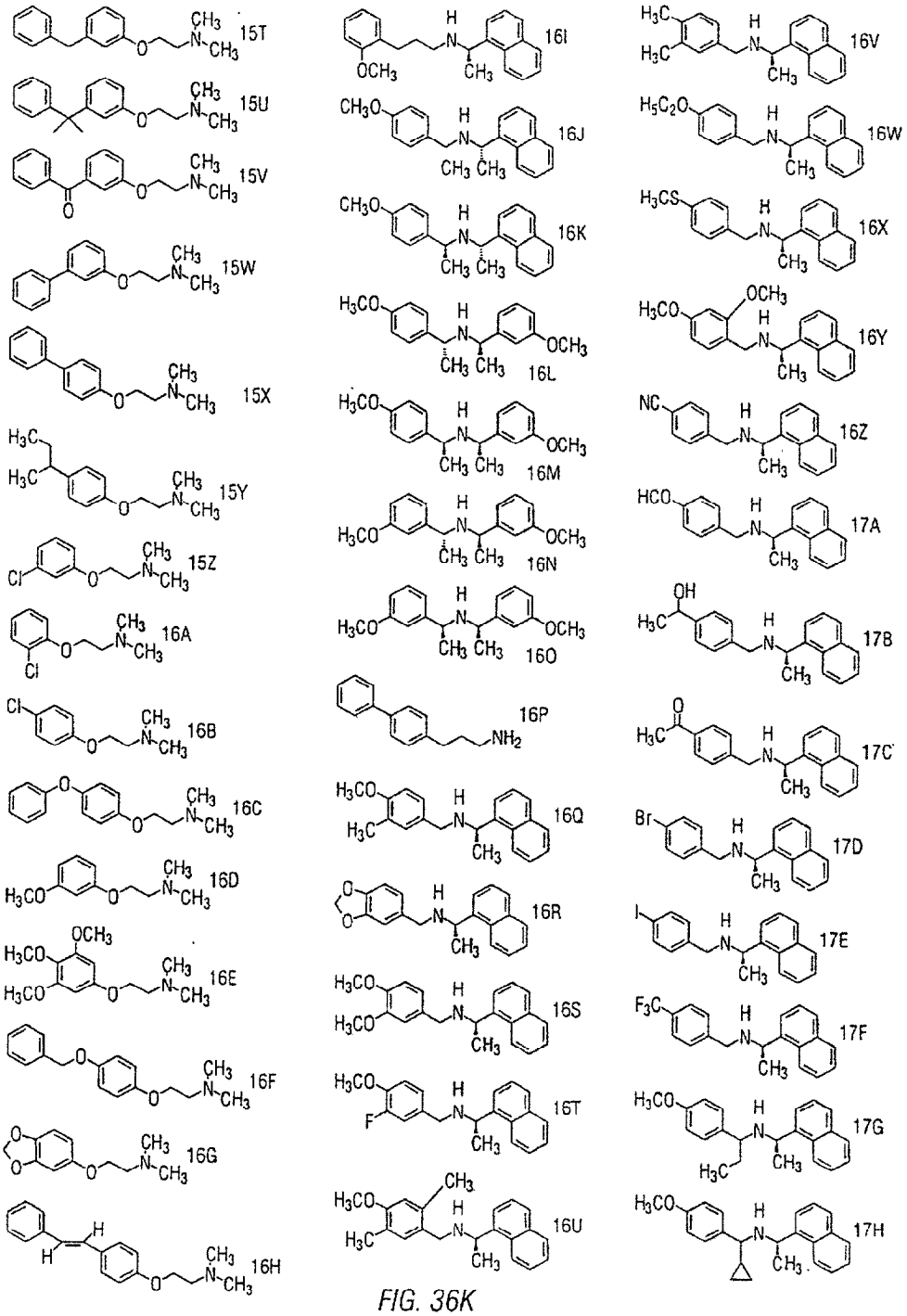

15T
15U
15V
15W
15X
15Y
15Z
16A
16B
16C
16D
16E
16F
16G
16H
16I
16J
16K
16L
16M
16N
16O
16P
16Q
16R
16S
16T
16U
16V
16W
16X
16Y
16Z
17A
17B
17C
17D
17E
17F
17G
17H

FIG. 36K

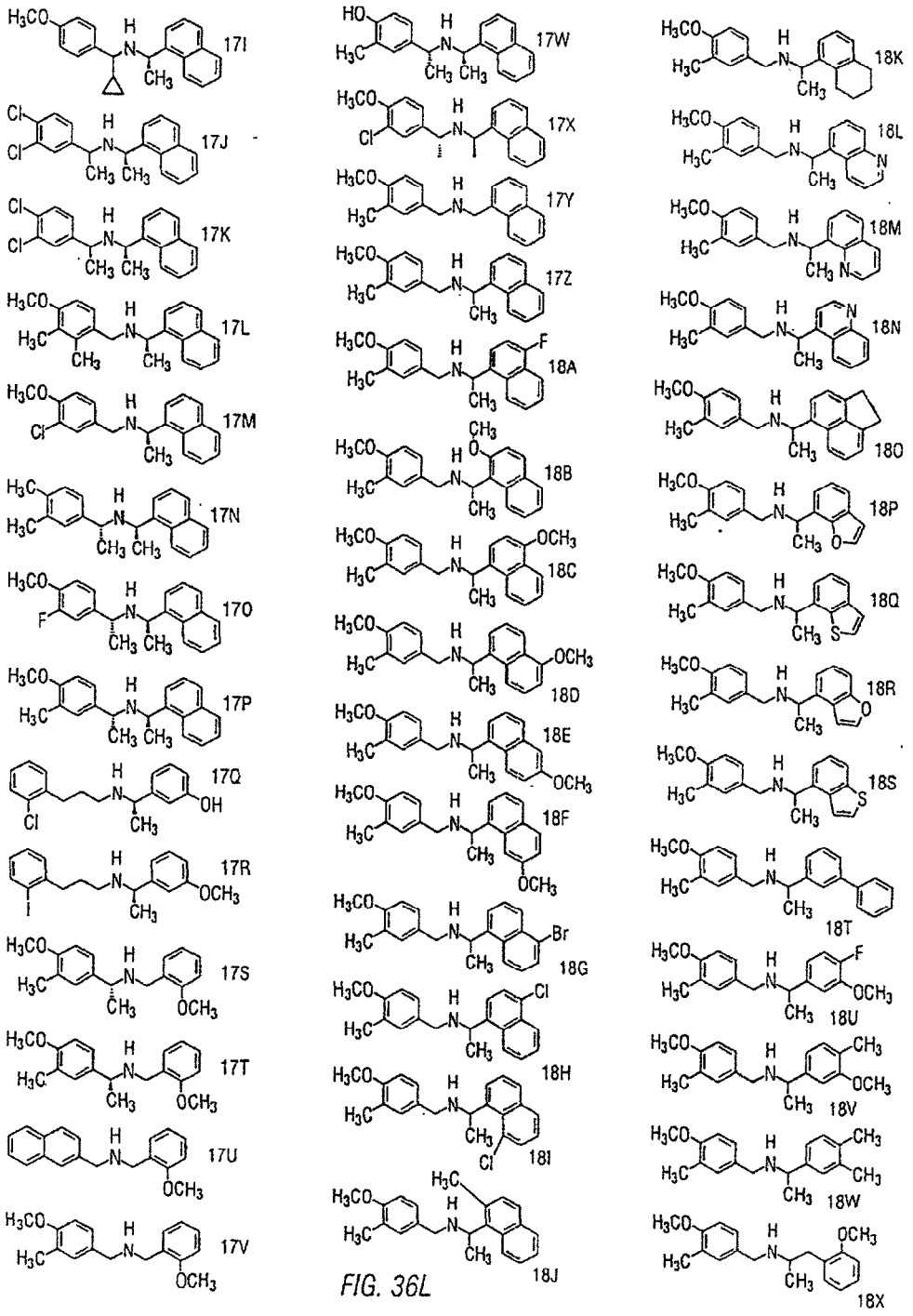

17I
17J
17K
17L
17M
17N
17O
17P
17Q
17R
17S
17T
17U
17V
17W
17X
17Y
17Z
18A
18B
18C
18D
18E
18F
18G
18H
18I
18J
18K
18L
18M
18N
18O
18P
18Q
18R
18S
18T
18U
18V
18W
18X

FIG. 36L

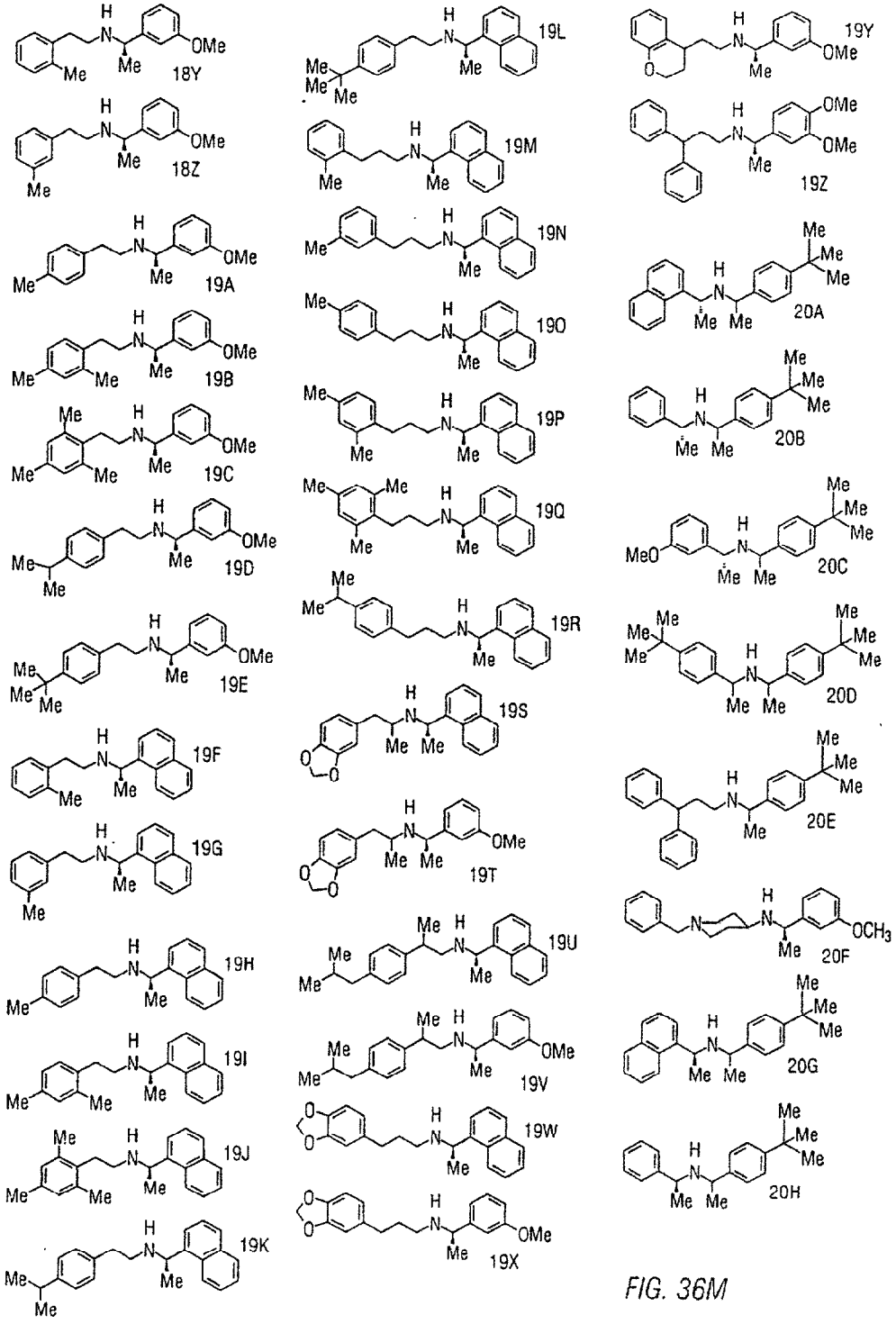

18Y
18Z
19A
19B
19C
19D
19E
19F
19G
19H
19I
19J
19K
19L
19M
19N
19O
19P
19Q
19R
19S
19T
19U
19V
19W
19X
19Y
19Z
20A
20B
20C
20D
20E
20F
20G
20H

FIG. 36M

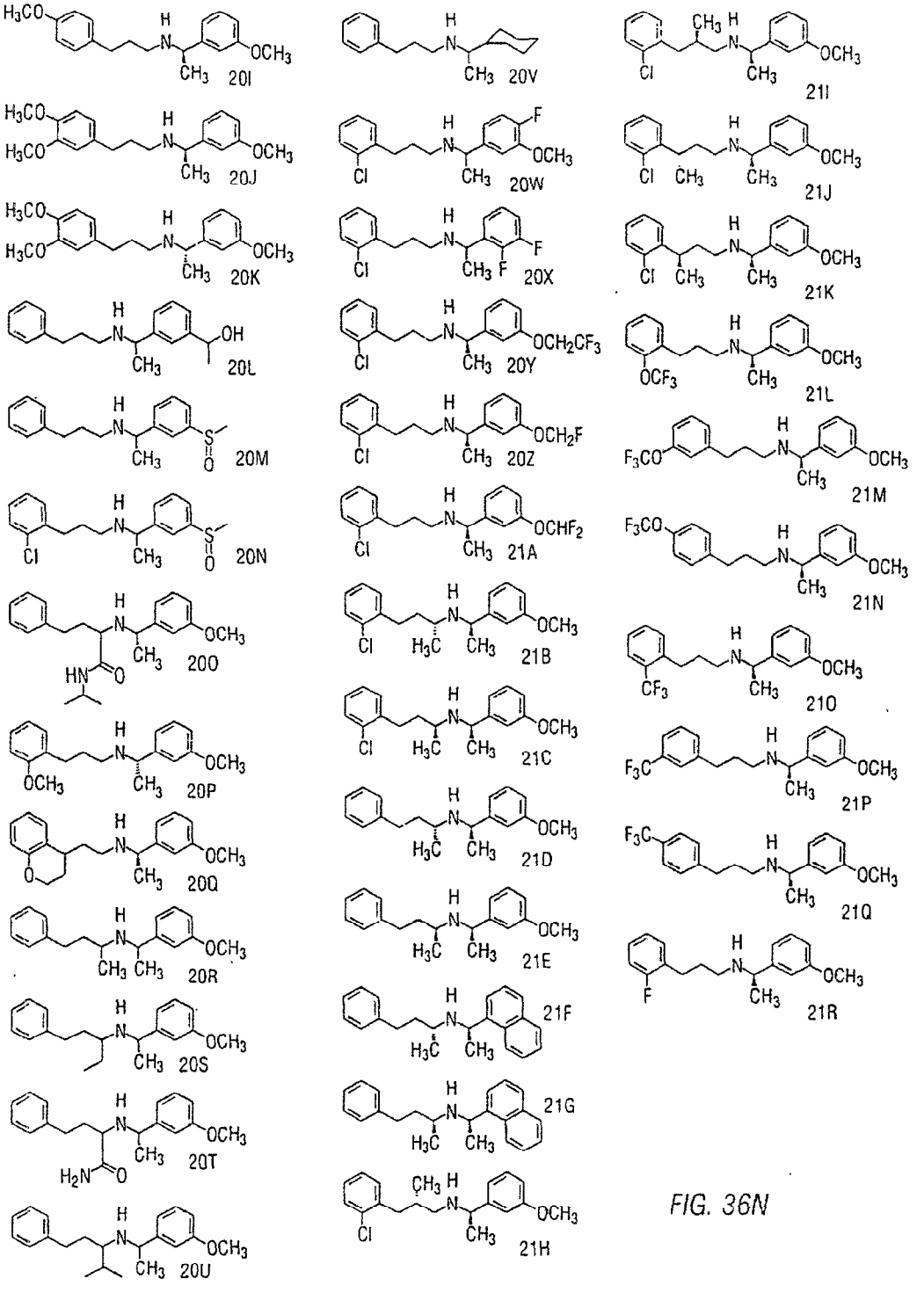

20I
20J
20K
20L
20M
20N
20O
20P
20Q
20R
20S
20T
20U
20V
20W
20X
20Y
20Z
21A
21B
21C
21D
21E
21F
21G
21H
21I
21J
21K
21L
21M
21N
21O
21P
21Q
21R

FIG. 36N

FIG. 37a.

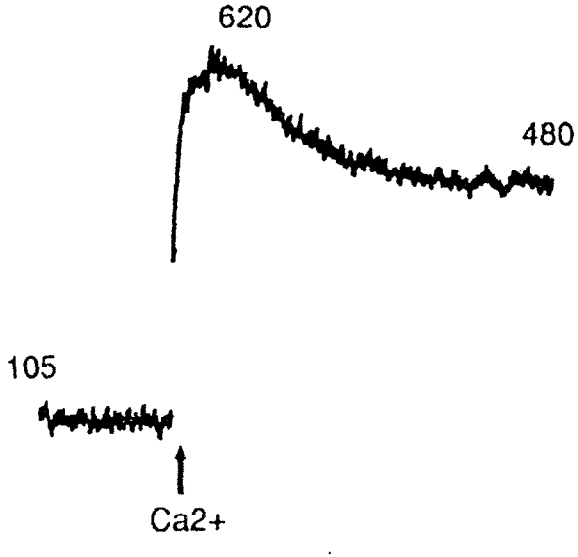


FIG.

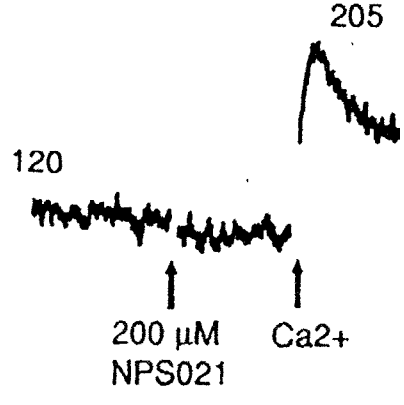
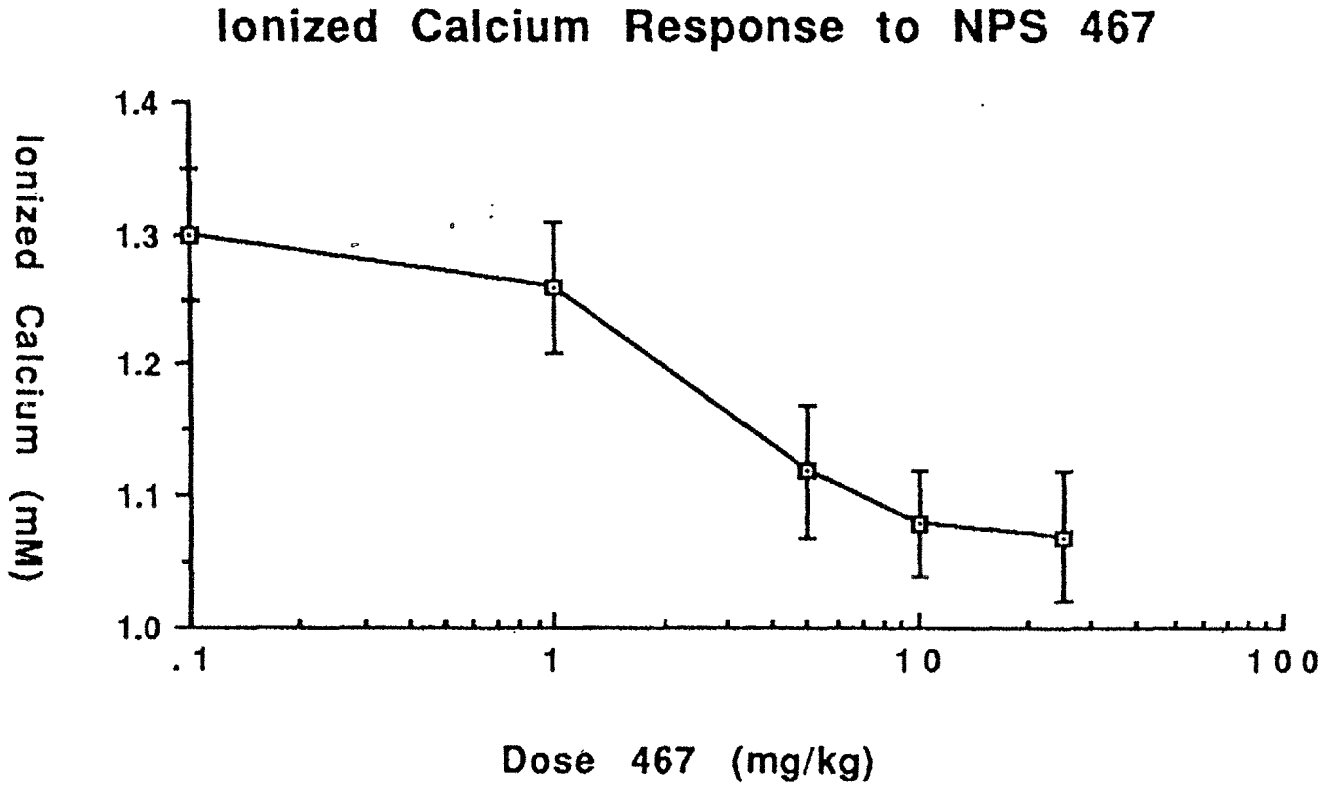


FIG. 38.


Ionized Calcium Response to NPS 467
Ionized Calcium (mM)
1.4
1.3
1.2
1.1
1.0
.1
1
10
100
Dose 467 (mg/kg)

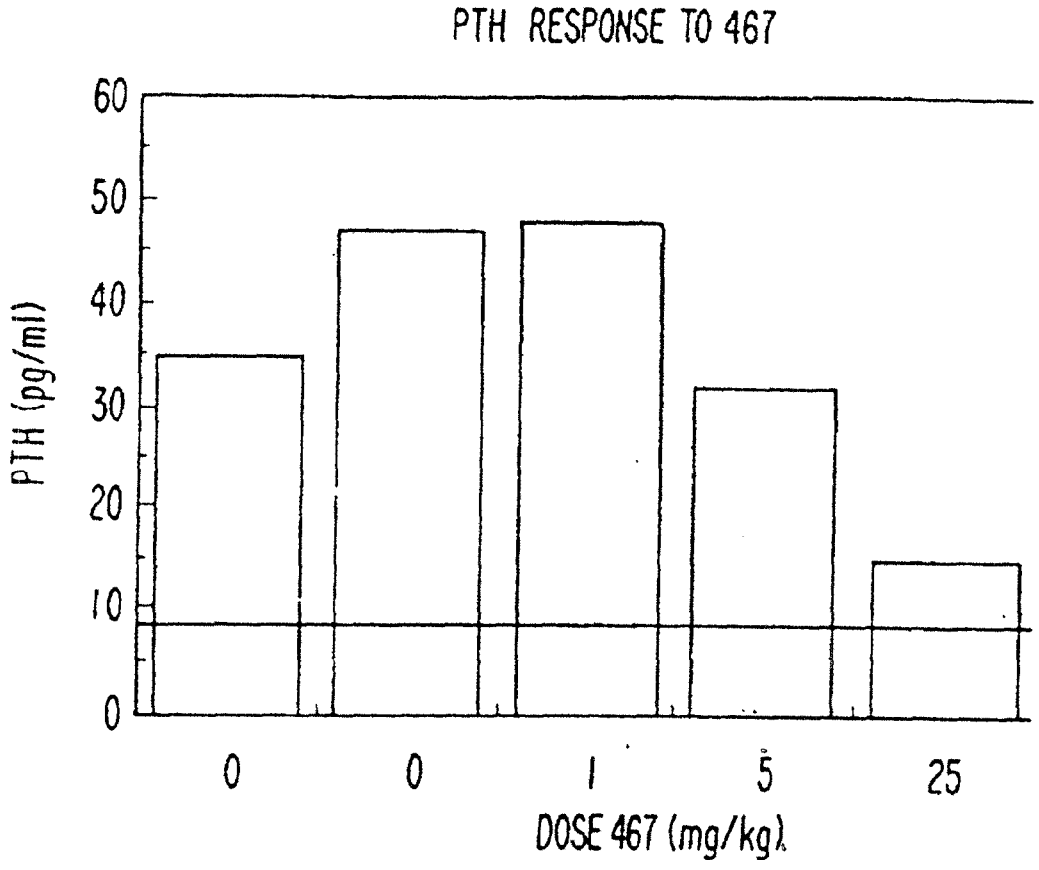
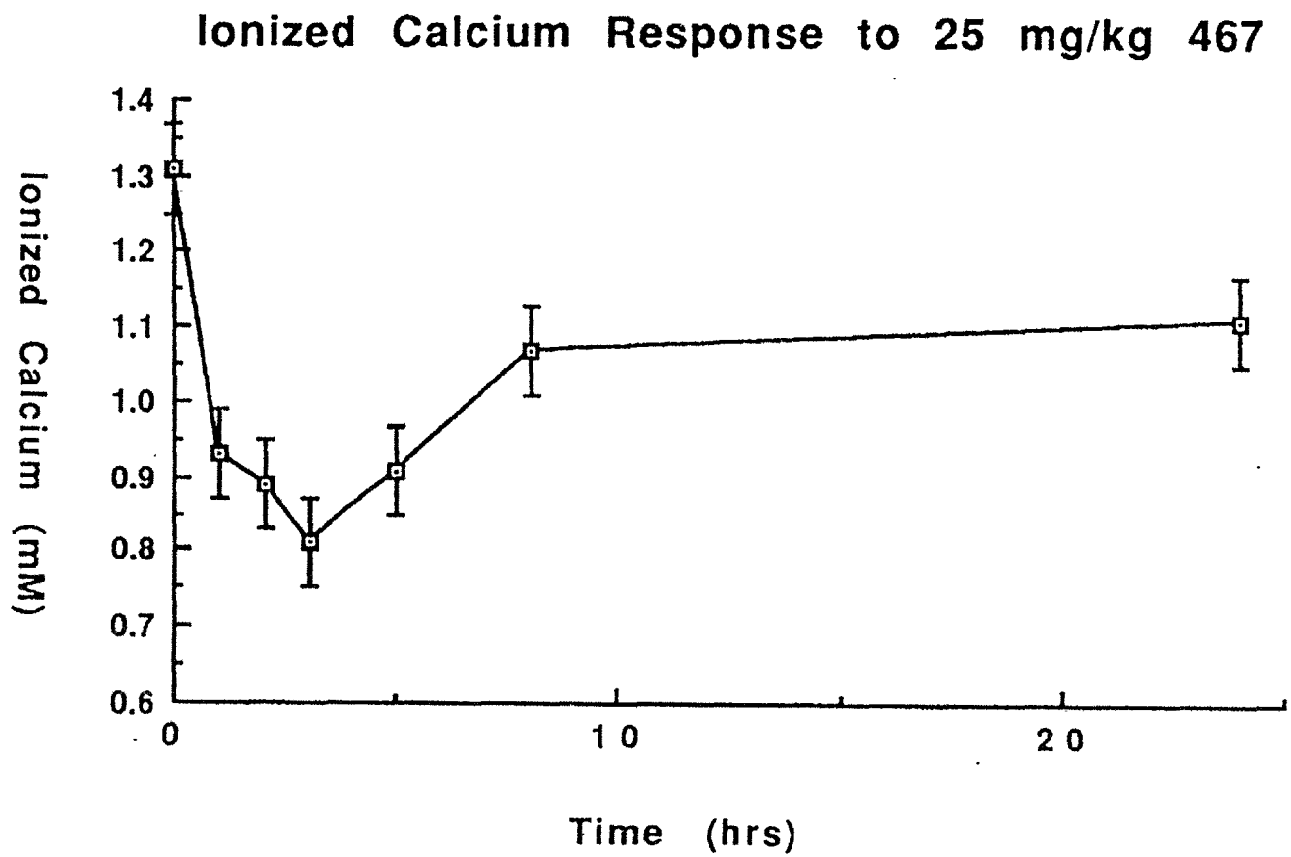

PTH RESPONSE TO 467
PTH (pg/ml)
60
50
40
30
20
10
0
0
0
1
5
25
DOSE 467 (mg/kg)

FIG. 39.

FIG. 40.


Ionized Calcium Response to 25 mg/kg 467
Ionized Calcium (mM)
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0
10
20
Time (hrs)

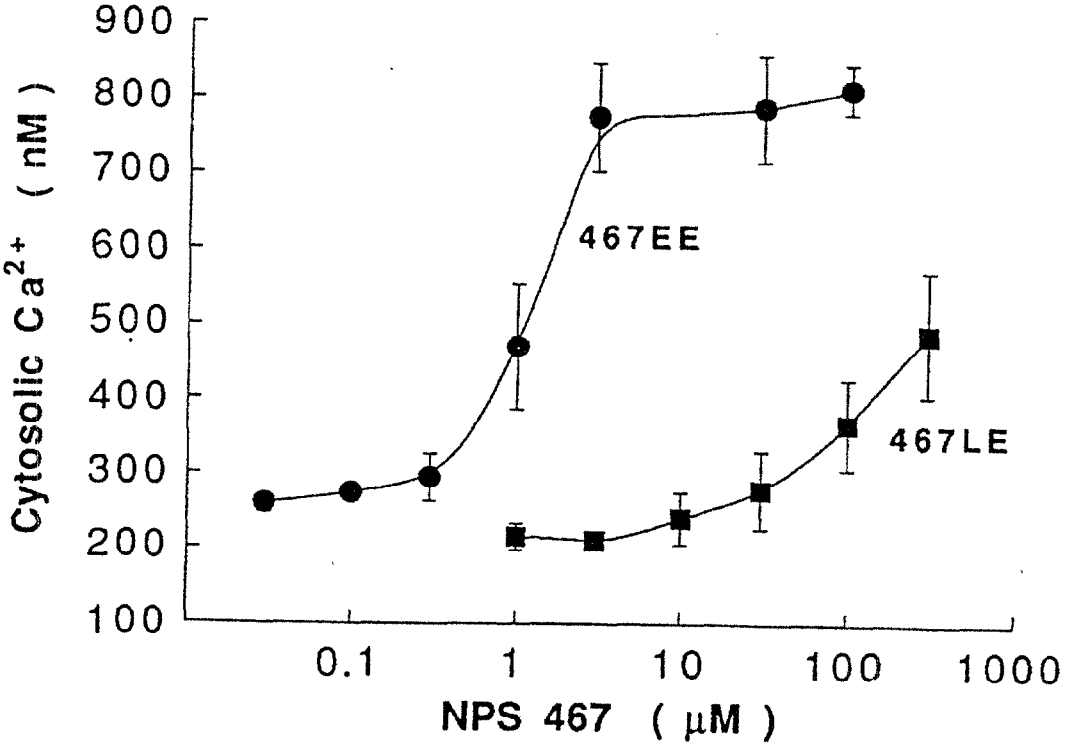

900
800
700
600
500
400
300
200
100
Cytosolic Ca2+ (nM)
467EE
467LE
0.1
1
10
100
1000
NPS 467 (μM)

FIG. 41.

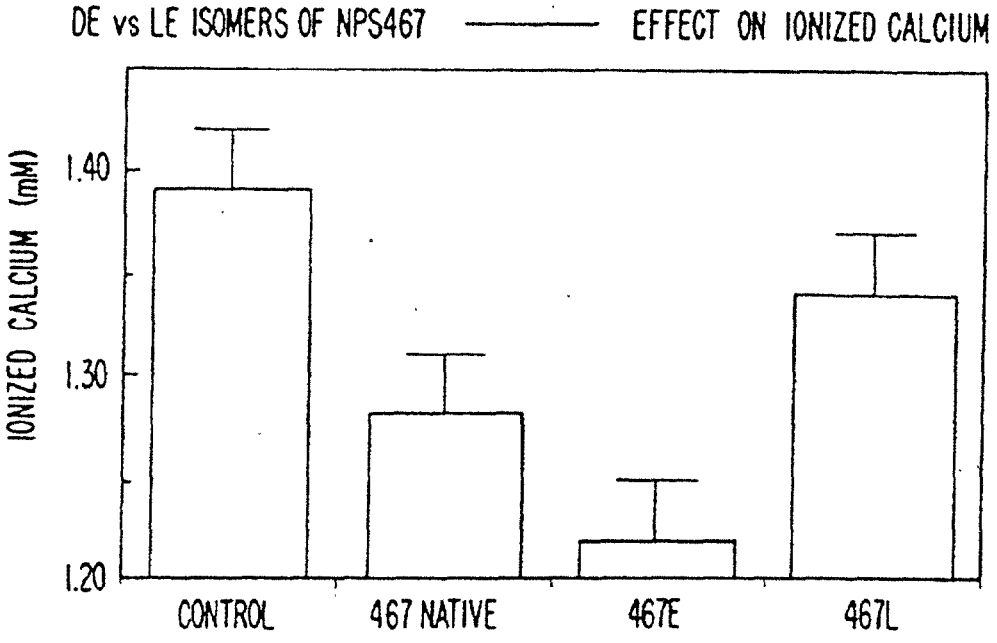

DE vs LE ISOMERS OF NPS467
EFFECT ON IONIZED CALCIUM
IONIZED CALCIUM (mM)
1.40
1.30
1.20
CONTROL
467 NATIVE
467E
467L

FIG. 42.

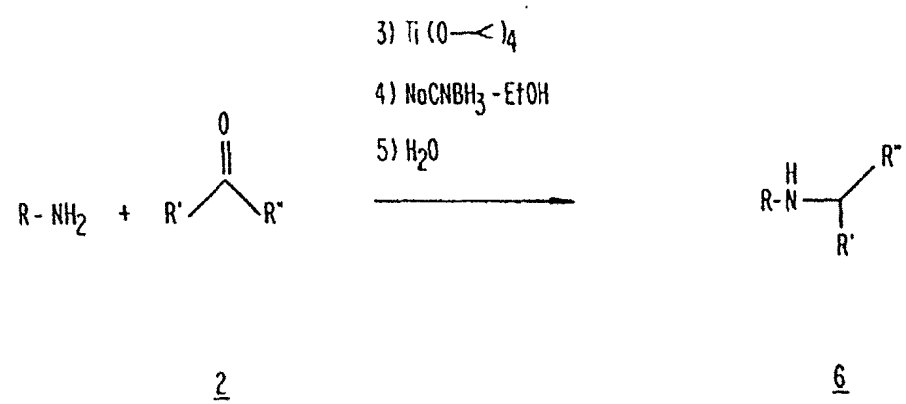


FIG. 43a.

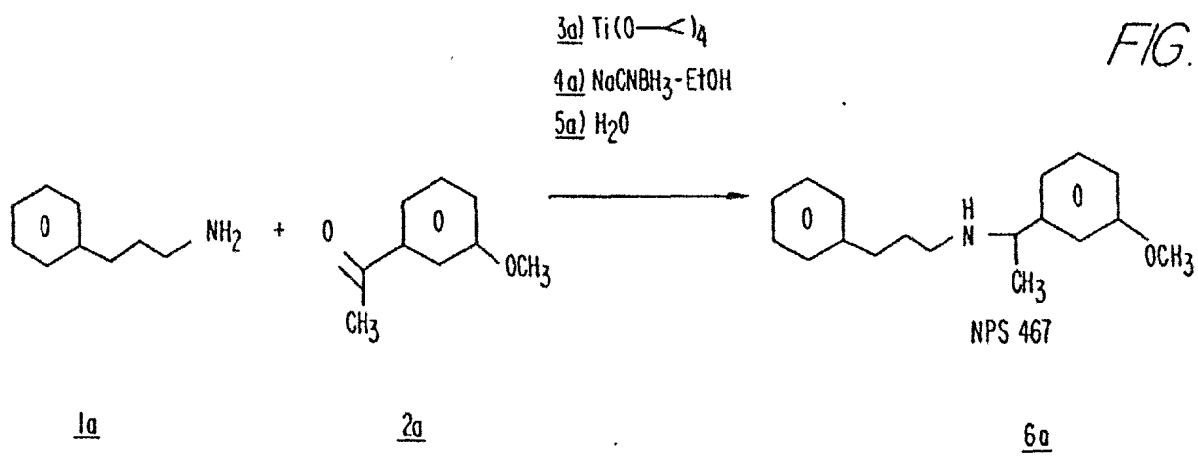


FIG. 43b.

FIG. 44.

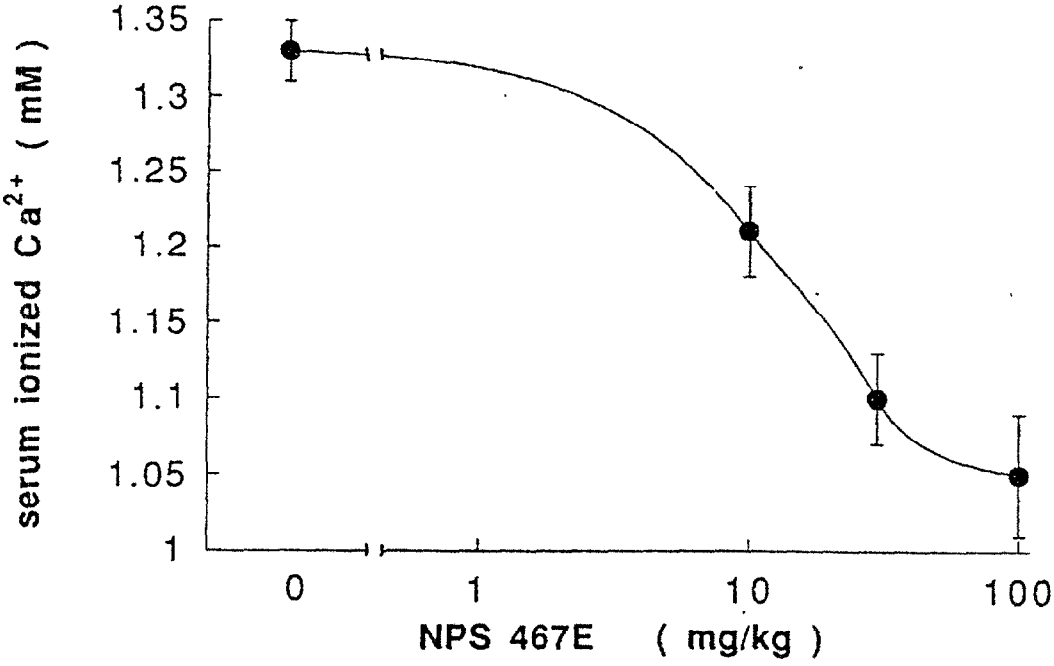
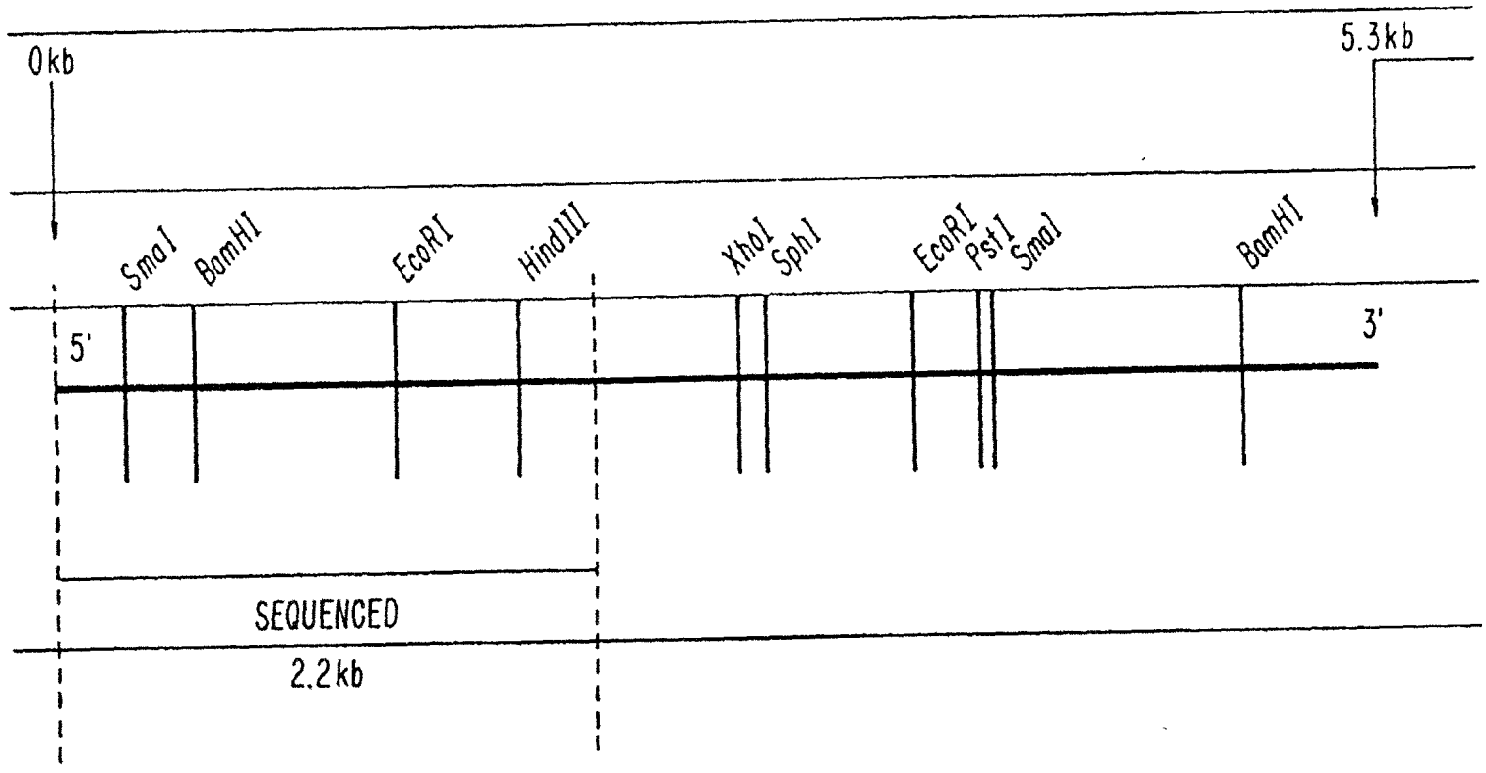

serum ionized Ca^{2+} (mM)
1.35
1.3
1.25
1.2
1.15
1.1
1.05
1
0
1
10
100
NPS 467E (mg/kg)

FIG. 45.

BoPCaRI cDNA RESTRICTION MAP



US006011068 [file:/\\cadmrfs01\fir:rmdata\lpl\Foley\Patt\PatentDocuments\US006011068.CPC]

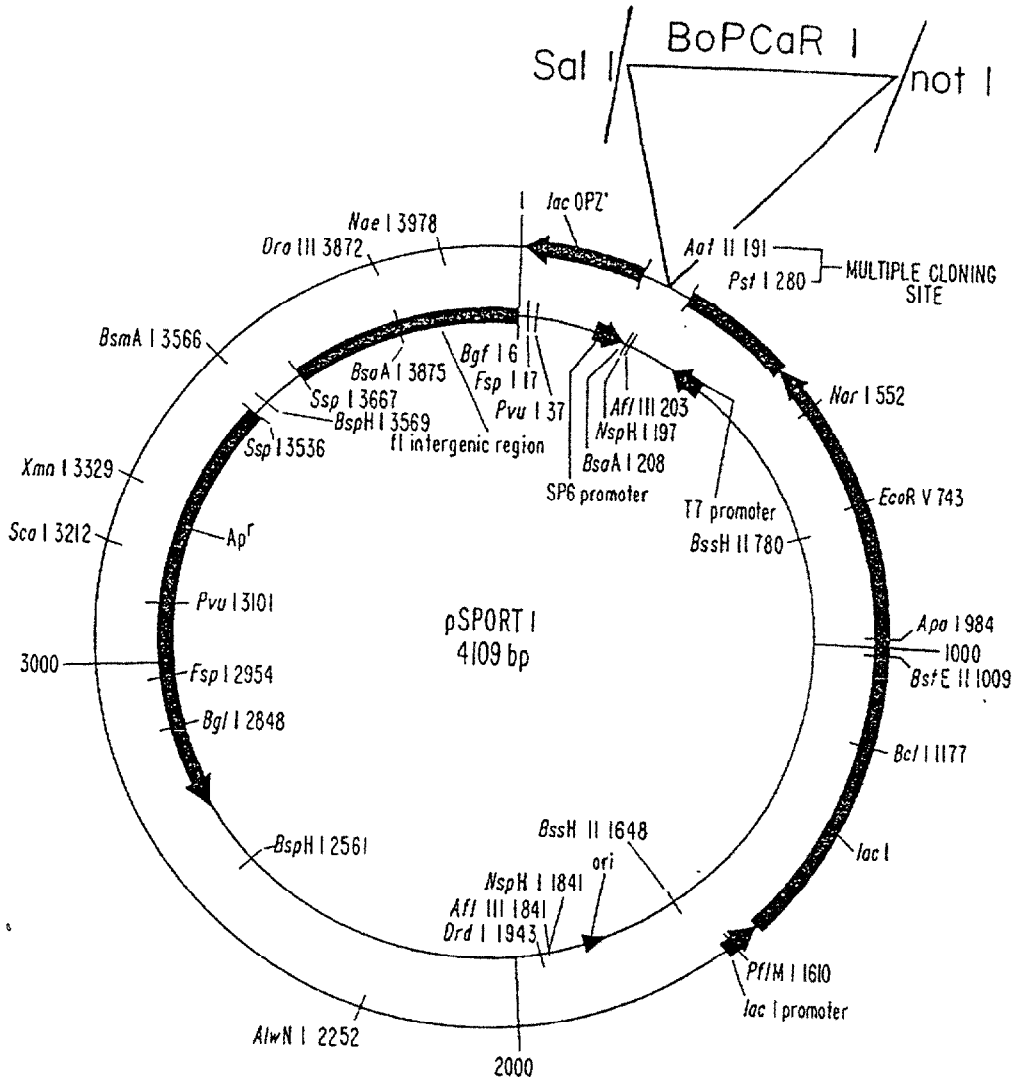

Sal I
BoPCaR I
not I
lac OPZ'
Aat II 191
Pst I 280
MULTIPLE CLONING SITE
Nae I 3978
Dra III 3872
BsmA I 3566
BsaA I 3875
Bgf I 6
Fsp I 17
Pvu I 37
Ssp I 3667
BspH I 3569
f1 intergenic region
Ssp I 3536
Afl III 203
NspH I 197
BsaA I 208
SP6 promoter
T7 promoter
BssH II 780
Nar I 552
EcoR V 743
Xma I 3329
Sca I 3212
Apr
Pvu I 3101
pSPORT I
4109 bp
Apa I 984
1000
BstE II 1009
3000
Fsp I 2954
Bgl I 2848
Bcl I 1177
BspH I 2561
BssH II 1648
ori
NspH I 1841
Afl III 1841
Drd I 1943
lac I
PflM I 1610
lac I promoter
AlwN I 2252
2000

FIG. 46.

```
         10        20        30        40        50        60        70
123456789012345678901234567890123456789012345678901234567890123456789012345
CGGAAAAAAAAAAAAAGTTCCCCACTCTAGTACAGAGAAGGTTGGCAGAGTCGTAAGCCCCCAACCTCTTAAACT    75

TCTCTGCATCTCCAAGGAGAAGGAGGGAAGAGGGGTTCTTTCCGACCTGAGGAGCTGGATCTGGGGTCCGAGAAC   150

CCCAAGGTAGCACCGGAAAGAACAGCACAGGAGGCGAGAGCGTGGCCGGTGGCCGGGAGAACCAGACCCGACGCG   225

CGGTCCTCGGCGCCGGGGTCCCGGGGACTCAGCTCAGCACGACTGGGAAGCCGAAAGTACTACACACGGTCTCTG   300

CATGATGTGACTTCTGAAGACTCAAGAGCCACCCACTTCACTAGTCTGCAATGGAGAAGGCAGAAATGGAAAGTC   375

AAACCCCACGGTTCCATTCTATTAATTCTGTAGACATGTGCCCCCACTGCAGGGAGTGAGTCGCACCAAGGGGGA   450

AAGTCCTCAGGGGCCCCCAGACCACCAGCGCTTGAGTCCCTCTTCCTGGAGAGAAAGCAGAACTATGGCACTTTA   525
                                                                MetAlaLeuTy

TAGCTGCTGTTGGATCCTCTTGGCTTTTTCTACCTGGTGCACTTCCGCCTATGGGCCTGACCAGCGAGCCCAAAA   600
rSerCysCysTrpIleLeuLeuAlaPheSerThrTrpCysThrSerAlaTyrGlyProAspGlnArgAlaGlnLy

GAAAGGGGACATTATCCTCGGGGGGCTCTTTCCTATTCATTTTGGGGTTGCAGTGAAAGATCAGGATCTAAAGTC   675
sLysGlyAspIleIleLeuGlyGlyLeuPheProIleHisPheGlyValAlaValLysAspGlnAspLeuLysSe

GAGGCCGGAGTCCGTGGAGTGTATCAGGTATAATTTCCGAGGATTTCGCTGGTTACAAGCTATGATATTTGCCAT   750
rArgProGluSerValGluCysIleArgTyrAsnPheArgGlyPheArgTrpLeuGlnAlaMetIlePheAlaIl

AGAGGAAATAAACAGCAGTCCAGCCCTTCTTCCCAACATGACCCTGGGATACAGGATATTCGACACTTGTAACAC   825
eGluGluIleAsnSerSerProAlaLeuLeuProAsnMetThrLeuGlyTyrArgIlePheAspThrCysAsnTh

CGTCTCTAAAGCCTTGGAGGCCACCCTGAGTTTTGTGGCCCAGAACAAAATTGACTCTTTGAACCTTGATGAGTT   900
rValSerLysAlaLeuGluAlaThrLeuSerPheValAlaGlnAsnLysIleAspSerLeuAsnLeuAspGluPh

CTGCAACTGCTCAGAGCACATCCCCTCTACCATCGCAGTGGTGGGAGCTACTGGCTCGGGCATCTCCACAGCAGT   975
eCysAsnCysSerGluHisIleProSerThrIleAlaValValGlyAlaThrGlySerGlyIleSerThrAlaVa

GGCCAACCTGCTGGGGCTCTTCTACATCCCCCAGGTCAGCTATGCCTCCTCCAGCAGACTCCTCAGCAACAAGAA  1050
lAlaAsnLeuLeuGlyLeuPheTyrIleProGlnValSerTyrAlaSerSerSerArgLeuLeuSerAsnLysAs

TCAATTCAAGTCCTTCCTCCGCACCATACCCAATGATGAACACCAGGCCACGGCCATGGCTGACATCATCGAGTA  1125
nGlnPheLysSerPheLeuArgThrIleProAsnAspGluHisGlnAlaThrAlaMetAlaAspIleIleGluTy

CTTCCGCTGGAACTGGGTGGGCACAATTGCAGCTGACGATGACTATGGCCGGCCAGGGATCGAGAAGTTTCGAGA  1200
rPheArgTrpAsnTrpValGlyThrIleAlaAlaAspAspAspTyrGlyArgProGlyIleGluLysPheArgGl

GGAAGCTGAGGAGAGGGACATCTGCATCGACTTCAGCGAGCTCATCTCCCAATACTCTGATGAGGAAAAGATCCA  1275
uGluAlaGluGluArgAspIleCysIleAspPheSerGluLeuIleSerGlnTyrSerAspGluGluLysIleGl

GCAGGTGGTGGAGGTGATCCAGAATTCCACCGCCAAAGTCATTGTCGTCTTCTCCAGCGGCCCAGACCTGGAACC  1350
nGlnValValGluValIleGlnAsnSerThrAlaLysValIleValValPheSerSerGlyProAspLeuGluPr
```

FIG. 47a.

```
         10        20        30        40        50        60        70
123456789012345678901234567890123456789012345678901234567890123456789012345
CCTCATCAAAGAGATCGTCCGGCGCAATATCACAGGCAGGATCTGGCTGGCCAGCGAGGCCTGGGCCAGCTCTTC   1425
oLeuIleLysGluIleValArgArgAsnIleThrGlyArgIleTrpLeuAlaSerGluAlaTrpAlaSerSerSe

CCTGATTGCTATGCCCGAGTATTTCCATGTGGTCGGAGGCACCATTGGGTTTGGTTTGAAAGCTGGGCAGATCCC   1500
rLeuIleAlaMetProGluTyrPheHisValValGlyGlyThrIleGlyPheGlyLeuLysAlaGlyGlnIlePr

AGGCTTCCGGGAATTCCTGCAGAAAGTCCACCCCAGGAAGTCTGTCCACAATGGTTTTGCCAAGGAGTTTTGGGA   1575
oGlyPheArgGluPheLeuGlnLysValHisProArgLysSerValHisAsnGlyPheAlaLysGluPheTrpGl

AGAAACATTTAACTGCCACCTGCAAGAGGGTGCTAAAGGCCCATTACCGGTGGACACCTTCCTGAGAGGTCACGA   1650
uGluThrPheAsnCysHisLeuGlnGluGlyAlaLysGlyProLeuProValAspThrPheLeuArgGlyHisGl

AGAAGGAGGTGCCAGGTTAAGCAACAGTCCCACTGCCTTCCGACCTCTGTGCACTGGGGAGGAGAACATCAGCAG   1725
uGluGlyGlyAlaArgLeuSerAsnSerProThrAlaPheArgProLeuCysThrGlyGluGluAsnIleSerSe

TGTCGAGACTCCTTACATGGATTATACACATTTACGGATATCCTACAACGTCTACTTAGCCGTCTACTCCATTGC   1800
rValGluThrProTyrMetAspTyrThrHisLeuArgIleSerTyrAsnValTyrLeuAlaValTyrSerIleAl

TCATGCCCTACAAGATATATACACCTGCATACCTGGGAGAGGGCTCTTCACCAACGGTTCCTGCGCAGATATCAA   1875
aHisAlaLeuGlnAspIleTyrThrCysIleProGlyArgGlyLeuPheThrAsnGlySerCysAlaAspIleLy

GAAGGTTGAAGCTTGGCAGGTCCTGAAACACCTGCGGCACCTAAATTTTACCAGCAATATGGGGGAGCAAGTAAC   1950
sLysValGluAlaTrpGlnValLeuLysHisLeuArgHisLeuAsnPheThrSerAsnMetGlyGluGlnValTh

TTTCGATGAATGTGGAGACCTGGCAGGGAACTATTCCATCATCAACTGGCACCTCTCCCCAGAGGACGGCTCCAT   2025
rPheAspGluCysGlyAspLeuAlaGlyAsnTyrSerIleIleAsnTrpHisLeuSerProGluAspGlySerIl

AGTGTTTAAGGAAGTTGGATATTACAATGTCTATGCCAAGAAAGGAGAGAGACTCTTCATCAATGATGAAAAAAT   2100
eValPheLysGluValGlyTyrTyrAsnValTyrAlaLysLysGlyGluArgLeuPheIleAsnAspGluLysIl

TCTGTGGAGTGGATTCTCAAGGGAGGTGCCTTTCTCCAACTGCAGTCGAGACTGCCTGGCAGGGACCAGGAAAGG   2175
eLeuTrpSerGlyPheSerArgGluValProPheSerAsnCysSerArgAspCysLeuAlaGlyThrArgLysGl

AATCATTGAGGGGGAGCCCACCTGCTGCTTTGAGTGTGTGGAATGTCCTGATGGGGAGTACAGCGACGAGACAGA   2250
yIleIleGluGlyGluProThrCysCysPheGluCysValGluCysProAspGlyGluTyrSerAspGluThrAs

TGCAAGTGCCTGTGATAAGTGCCCTGATGACTTCTGGTCCAATGAGAACCACACTTCCTGCATCGCCAAGGAGAT   2325
pAlaSerAlaCysAspLysCysProAspAspPheTrpSerAsnGluAsnHisThrSerCysIleAlaLysGluIl

CGAGTTTCTGTCGTGGACCGAGCCCTTCGGGATCGCACTCACGCTCTTTGCTGTGCTGGGCATTTTCCTCACAGC   2400
eGluPheLeuSerTrpThrGluProPheGlyIleAlaLeuThrLeuPheAlaValLeuGlyIlePheLeuThrAl

CTTCGTGCTGGGCGTCTTCATCAAGTTCCGCAACACGCCCATCGTCAAGGCCACCAACCGGGAGCTCTCCTATCT   2475
aPheValLeuGlyValPheIleLysPheArgAsnThrProIleValLysAlaThrAsnArgGluLeuSerTyrLe

CCTTCTCTTCTCCCTGCTCTGCTGCTTCTCCAGCTCCCTGTTCTTCATCGGGGAGCCCCAGGACTGGACGTGCCG   2550
uLeuLeuPheSerLeuLeuCysCysPheSerSerSerLeuPhePheIleGlyGluProGlnAspTrpThrCysAr

CCTGCGCCAGCCGGCCTTTGGCATCAGCTTCGTGCTCTGCATCTCGTGCATCCTGGTGAAAACCAATCGGGTCCT   2625
gLeuArgGlnProAlaPheGlyIleSerPheValLeuCysIleSerCysIleLeuValLysThrAsnArgValLe

CCTGGTGTTTGAGGCCAAGATTCCCACCAGCTTCCACCGGAAGTGGTGGGGGCTCAACCTGCAGTTCCTGCTGGT   2700
uLeuValPheGluAlaLysIleProThrSerPheHisArgLysTrpTrpGlyLeuAsnLeuGlnPheLeuLeuVa
```

FIG. 47b.

```
         10        20        30        40        50        60        70
123456789012345678901234567890123456789012345678901234567890123456789012345
CTTCCTCTGCACCTTCATGCAGATTGTCATCTGTGCCATTTGGCTCAATACAGCGCCCCCCTCGAGCTACCGCAA   2775
lPheLeuCysThrPheMetGlnIleValIleCysAlaIleTrpLeuAsnThrAlaProProSerSerTyrArgAs

CCACGAGCTGGAGGACGAGATCATCTTCATCACCTGCCACGAGGGCTCGCTCATGGCGCTGGGCTTCCTGATCGG   2850
nHisGluLeuGluAspGluIleIlePheIleThrCysHisGluGlySerLeuMetAlaLeuGlyPheLeuIleGl

CTACACCTGCTTGCTGGCCGCCATCTGCTTCTTCTTCGCCTTCAAGTCCCGGAAGCTGCCAGAGAACTTCAATGA   2925
yTyrThrCysLeuLeuAlaAlaIleCysPhePhePheAlaPheLysSerArgLysLeuProGluAsnPheAsnGl

AGCCAAGTTCATCACCTTCAGCATGCTCATCTTCTTCATCGTCTGGATCTCTTTCATCCCCGCCTACGCCAGCAC   3000
uAlaLysPheIleThrPheSerMetLeuIlePhePheIleValTrpIleSerPheIleProAlaTyrAlaSerTh

TTACGGCAAGTTCGTCTCTGCCGTGGAGGTGATCGCCATCCTGGCGGCCAGCTTTGGCTTGCTGGCCTGTATCTT   3075
rTyrGlyLysPheValSerAlaValGluValIleAlaIleLeuAlaAlaSerPheGlyLeuLeuAlaCysIlePh

CTTCAACAAGGTCTACATCATCCTCTTCAAGCCTTCCCGGAACACCATCGAGGAGGTGCGCTGCAGCACCGCGGC   3150
ePheAsnLysValTyrIleIleLeuPheLysProSerArgAsnThrIleGluGluValArgCysSerThrAlaAl

ACACGCCTTCAAGGTGGCCGCCCGAGCCACGCTGCGCCGCAGCAACGTCTCCCGCCAGCGGTCCAGCAGCCTAGG   3225
aHisAlaPheLysValAlaAlaArgAlaThrLeuArgArgSerAsnValSerArgGlnArgSerSerSerLeuGl

GGGCTCCACGGGATCCACCCCCTCCTCCTCCATCAGCAGCAAGAGCAACAGCGAGGACCCGTTCCCTCAGCAGCA   3300
yGlySerThrGlySerThrProSerSerSerIleSerSerLysSerAsnSerGluAspProPheProGlnGlnGl

GCCGAAGAGGCAGAAGCAGCCGCAGCCGCTGGCCCTGAGCCCGCACAACGCGCAGCAGCCACAGCCGCGGCCACC   3375
nProLysArgGlnLysGlnProGlnProLeuAlaLeuSerProHisAsnAlaGlnGlnProGlnProArgProPr

CTCGACCCCACAGCCGCAGCCACAGTCGCAGCAGCCGCCCCGATGCAAGCAGAAGGTCATCTTCGGCAGCGGCAC   3450
oSerThrProGlnProGlnProGlnSerGlnGlnProProArgCysLysGlnLysValIlePheGlySerGlyTh

CGTCACCTTCTCGCTGAGCTTTGACGAGCCTCAGAAGACCGCCGTGGCTCACAGGAATTCCACGCACCAGACCTC   3525
rValThrPheSerLeuSerPheAspGluProGlnLysThrAlaValAlaHisArgAsnSerThrHisGlnThrSe

CCTGGAGGCCCAGAAAAACAATGACGCCCTGACCAAACACCAGGCGTTGCTCCCGCTGCAGTGCGGAGAGACGGA   3600
rLeuGluAlaGlnLysAsnAsnAspAlaLeuThrLysHisGlnAlaLeuLeuProLeuGlnCysGlyGluThrAs

CTCAGAATTGACCTCCCAGGAGACAGGCCTGCAGGGCCCTGTGGGTGAGGACCACCAGCTAGAGATGGAGGACCC   3675
pSerGluLeuThrSerGlnGluThrGlyLeuGlnGlyProValGlyGluAspHisGlnLeuGluMetGluAspPr

CGAAGAGATGTCCCCGGCACTTGTAGTGTCTAATTCCCGGAGCTTTGTCATCAGTGGCGGAGGCAGCACTGTTAC   3750
oGluGluMetSerProAlaLeuValValSerAsnSerArgSerPheValIleSerGlyGlyGlySerThrValTh

GGAAAACATGCTGCGTTCTTAAAAGGGAAGGAGAAAGCCAGTTCAGGGGGAATCCAGGCAGTTTCCCCGGGATGA   3825
rGluAsnMetLeuArgSer

CCTTCTCCAAAGGGATGAGGAACTGCCCCCCCACCCCCACCCCCTTCCTCCAGGAAGGAGGGATAAGACCCACCA   3900

AATGCTTGGAACTTAGTTGCACTGCTATAAACGACAGTGAATGAAATAATGTCCCCCTTAAAATTAAAAAGAGGG   3975

GAGCGGTGTGCTTCTGTGGTTAGGTTTATCAGAGTGCTGAGATCCCTATAGTCAGGTTCGCCTTTCCTATCCCTG   4050
```

FIG. 47c.

```
         10        20        30        40        50        60        70
123456789012345678901234567890123456789012345678901234567890123456789012345
CTTCCATTCTCCTCTTCTGTTCTATCCCATCCAACAGTCCAGAGATAAAACCATGGCTTTAAGATACCCACCTAT   4125

TCCCCCTAGGGTCTTATTTGTTGTTTTTGTTGCTGTTGTTTTGGTTTGATTTTTGTTTTTAATGTTGAAACGTCT   4200

GCCCTGAACTTTGCAGACAGCCTGGTCCAAAAACAAACCTGTGCAGAGTGACAGGACCTCCTATGGGCACCACTA   4275

GAGTTGAGTGCGAAAGACAGAATGTCGCCAGCGCTGCCCAACACCTTGACAGTGGGAAGAACTTGAAATGTCCAG   4350

AGCTGTAAGATGAATGTGTCCCCTCCTATTTATGAAAAATGTTAAATATGTGGTTTCCTACTTGCTGCTGCTGTC   4425

ACGTGACATGGAGAAGGTTAGCATCCATCCTCCAGCAGTATGTCTGATCTTGTCCAGAGTGTGATGGTGATGCCA   4500

CGTTTAGATTCCAATATCTCAGGAATCACCTCAGCCTGCATGAATCCAATGAGCTGTATCTGTAATTAATATTGT   4575

CATATGTAGCTTTATCCTTAAGAAAATGTGTTTGTTTTAATAGTCCGTGGAAAATATAAGCTGGAAAAAATGTCC   4650

CAGTCTGGTTGATATAAGGCAGTATTATTGAGTCCCGTTTTCTTTGCCCGCCCCACCACCCACACCCCAATGAGC   4725

TAAGCCCTAAATGAGCCCTTTCAGGGCCCAGGGATCCAGAAGCTCCCTCTTTCTCCACCCCAAACGCTTCCTGAA   4800

GTCAGATCCATGCCTTTCCCTGTGAAGAATAAGCTCCCAGTCTCTGACCTCCTACCAGTTTCTGGGGTAAGAACA   4875

CGTGGTTCCAAGAGAGCTCTCATGGGATATTACTCTTGGCACCCCCCAATGCCATACTTAGGTTCCCTCCAGCAG   4950

TGGGATCTGCCCATGGGTAGTTACAAGATTGAACGTTGAATGGCATACTGCTGAACAGTCAGTTCTGGAGCTAGA   5025

GAGGCCTGGGGTCAAGTGCTGGGTTTGTCACTCACAAGTTGGGTGACCACAGGCAGGGAACCTTGACCTCACTCA   5100

GCCCCAGCTTCTTTGTGTCTAAAATGGAGGTAATAATCATCCTTTTCCCGCAGAGCTCTTATGTGGGTTAAATGA   5175

GATAAATGTATGTAAAGTATTTTAGCATGGTGCCTAGCCCATAGTAAGCACGCAATAAATATTAGTTAATATTAA   5250

AAAAAAAAAAAAAAAAAAAAAAAAA                                                     5275
```

FIG. 47d.

```
         10        20        30        40        50        60        70
123456789012345678901234567890123456789012345678901234567890123456789012345
GCTGCTGTGGCCGGACCCGAAGGCGGGCGCCGGGAGCGCAGCGAGCCAGACGCGCCTCTCCAAGACCGTGACCTT    75

GGCATAGGGAGCGGGGCTGCGCGCAGTCCTGAGATCAGACCAGAGCTCATCCTCGTGGAGACCCACGGCCGAGGG   150

GCCGGAGCTGCCTCTGTGCGAGGGAGCCCTGGCCGCGGCGCAGAAGGCATCACAGGAGGCCTCTGCATGATGTGG   225

CTTCCAAAGACTCAAGGACCACCCACATTACAAGTCTGGATTGAGGAAGGCAGAAATGGAGATTCAAACACCACG   300

TCTTCTATTATTTTATTAATCAATCTGTAGACATGTGTCCCCACTGCAGGGAGTGAACTGCTCCAAGGGAGAAAC   375

TTCTGGGAGCCTCCAAACTCCTAGCTGTCTCATCCCTTGCCCTGGAGAGACGGCAGAACCATGGCATTTTATAGC   450
                                                                MetAlaPheTyrSer

TGCTGCTGGGTCCTCTTGGCACTCACCTGGCACACCTCTGCCTACGGGCCAGACCAGCGAGCCCAAAAGAAGGGG   525
CysCysTrpValLeuLeuAlaLeuThrTrpHisThrSerAlaTyrGlyProAspGlnArgAlaGlnLysLysGly

GACATTATCCTTGGGGGGCTCTTTCCTATTCATTTTGGAGTAGCAGCTAAAGATCAAGATCTCAAATCAAGGCCG   600
AspIleIleLeuGlyGlyLeuPheProIleHisPheGlyValAlaAlaLysAspGlnAspLeuLysSerArgPro

GAGTCTGTGGAATGTATCAGGTATAATTTCCGTGGGTTTCGCTGGTTACAGGCTATGATATTTGCCATAGAGGAG   675
GluSerValGluCysIleArgTyrAsnPheArgGlyPheArgTrpLeuGlnAlaMetIlePheAlaIleGluGlu

ATAAACAGCAGCCCAGCCCTTCTTCCCAACTTGACGCTGGGATACAGGATATTTGACACTTGCAACACCGTTTCT   750
IleAsnSerSerProAlaLeuLeuProAsnLeuThrLeuGlyTyrArgIlePheAspThrCysAsnThrValSer

AAGGCCTTGGAAGCCACCCTGAGTTTTGTTGCTCAAAACAAAATTGATTCTTTGAACCTTGATGAGTTCTGCAAC   825
LysAlaLeuGluAlaThrLeuSerPheValAlaGlnAsnLysIleAspSerLeuAsnLeuAspGluPheCysAsn

TGCTCAGAGCACATTCCCTCTACGATTGCTGTGGTGGGAGCAACTGGCTCAGGCGTCTCCACGGCAGTGGCAAAT   900
CysSerGluHisIleProSerThrIleAlaValValGlyAlaThrGlySerGlyValSerThrAlaValAlaAsn

CTGCTGGGGCTCTTCTACATTCCCCAGGTCAGTTATGCCTCCTCCAGCAGACTCCTCAGCAACAAGAATCAATTC   975
LeuLeuGlyLeuPheTyrIleProGlnValSerTyrAlaSerSerSerArgLeuLeuSerAsnLysAsnGlnPhe

AAGTCTTTCCTCCGAACCATCCCCAATGATGAGCACCAGGCCACTGCCATGGCAGACATCATCGAGTATTTCCGC  1050
LysSerPheLeuArgThrIleProAsnAspGluHisGlnAlaThrAlaMetAlaAspIleIleGluTyrPheArg

TGGAACTGGGTGGGCACAATTGCAGCTGATGACGACTATGGGCGGCCGGGGATTGAGAAATTCCGAGAGGAAGCT  1125
TrpAsnTrpValGlyThrIleAlaAlaAspAspAspTyrGlyArgProGlyIleGluLysPheArgGluGluAla

GAGGAAAGGGATATCTGCATCGACTTCAGTGAACTCATCTCCCAGTACTCTGATGAGGAAGAGATCCAGCATGTG  1200
GluGluArgAspIleCysIleAspPheSerGluLeuIleSerGlnTyrSerAspGluGluGluIleGlnHisVal

GTAGAGGTGATTCAAAATTCCACGGCCAAAGTCATCGTGGTTTTCTCCAGTGGCCCAGATCTTGAGCCCCTCATC  1275
ValGluValIleGlnAsnSerThrAlaLysValIleValValPheSerSerGlyProAspLeuGluProLeuIle

AAGGAGATTGTCCGGCGCAATATCACGGGCAAGATCTGGCTGGCCAGCGAGGCCTGGGCCAGCTCCTCCCTGATC  1350
LysGluIleValArgArgAsnIleThrGlyLysIleTrpLeuAlaSerGluAlaTrpAlaSerSerSerLeuIle
```

FIG. 48a.

```
         10        20        30        40        50        60        70
123456789012345678901234567890123456789012345678901234567890123456789012345
GCCATGCCTCAGTACTTCCACGTGGTTGGCGGCACCATTGGATTCGCTCTGAAGGCTGGGCAGATCCCAGGCTTC   1425
AlaMetProGlnTyrPheHisValValGlyGlyThrIleGlyPheAlaLeuLysAlaGlyGlnIleProGlyPhe

CGGGAATTCCTGAAGAAGGTCCATCCCAGGAAGTCTGTCCACAATGGTTTTGCCAAGGAGTTTTGGGAAGAAACA   1500
ArgGluPheLeuLysLysValHisProArgLysSerValHisAsnGlyPheAlaLysGluPheTrpGluGluThr

TTTAACTGCCACCTCCAAGAAGGTGCAAAAGGACCTTTACCTGTGGACACCTTTCTGAGAGGTCACGAAGAAAGT   1575
PheAsnCysHisLeuGlnGluGlyAlaLysGlyProLeuProValAspThrPheLeuArgGlyHisGluGluSer

GGCGACAGGTTTAGCAACAGCTCGACAGCCTTCCGACCCCTCTGTACAGGGGATGAGAACATCAGCAGTGTCGAG   1650
GlyAspArgPheSerAsnSerSerThrAlaPheArgProLeuCysThrGlyAspGluAsnIleSerSerValGlu

ACCCCTTACATAGATTACACGCATTTACGGATATCCTACAATGTGTACTTAGCAGTCTACTCCATTGCCCACGCC   1725
ThrProTyrIleAspTyrThrHisLeuArgIleSerTyrAsnValTyrLeuAlaValTyrSerIleAlaHisAla

TTGCAAGATATATATACCTGCTTACCTGGGAGAGGGCTCTTCACCAATGGCTCCTGTGCAGACATCAAGAAAGTT   1800
LeuGlnAspIleTyrThrCysLeuProGlyArgGlyLeuPheThrAsnGlySerCysAlaAspIleLysLysVal

GAGGCGTGGCAGGTCCTGAAGCACCTACGGCATCTAAACTTTACAAACAATATGGGGGAGCAGGTGACCTTTGAT   1875
GluAlaTrpGlnValLeuLysHisLeuArgHisLeuAsnPheThrAsnAsnMetGlyGluGlnValThrPheAsp

GAGTGTGGTGACCTGGTGGGGAACTATTCCATCATCAACTGGCACCTCTCCCCAGAGGATGGCTCCATCGTGTTT   1950
GluCysGlyAspLeuValGlyAsnTyrSerIleIleAsnTrpHisLeuSerProGluAspGlySerIleValPhe

AAGGAAGTCGGGTATTACAACGTCTATGCCAAGAAGGGAGAAAGACTCTTCATCAACGAGGAGAAAATCCTGTGG   2025
LysGluValGlyTyrTyrAsnValTyrAlaLysLysGlyGluArgLeuPheIleAsnGluGluLysIleLeuTrp

AGTGGGTTCTCCAGGGAGCCACTCACCTTTGTGCTGTCTGTCCTCCAGGTGCCCTTCTCCAACTGCAGCCGAGAC   2100
SerGlyPheSerArgGluProLeuThrPheValLeuSerValLeuGlnValProPheSerAsnCysSerArgAsp

TGCCTGGCAGGGACCAGGAAAGGGATCATTGAGGGGGAGCCCACCTGCTGCTTTGAGTGTGTGGAGTGTCCTGAT   2175
CysLeuAlaGlyThrArgLysGlyIleIleGluGlyGluProThrCysCysPheGluCysValGluCysProAsp

GGGGAGTATAGTGATGAGACAGATGCCAGTGCCTGTAACAAGTGCCCAGATGACTTCTGGTCCAATGAGAACCAC   2250
GlyGluTyrSerAspGluThrAspAlaSerAlaCysAsnLysCysProAspAspPheTrpSerAsnGluAsnHis

ACCTCCTGCATTGCCAAGGAGATCGAGTTTCTGTCGTGGACGGAGCCCTTTGGGATCGCACTCACCCTCTTTGCC   2325
ThrSerCysIleAlaLysGluIleGluPheLeuSerTrpThrGluProPheGlyIleAlaLeuThrLeuPheAla

GTGCTGGGCATTTTCCTGACAGCCTTTGTGCTGGGTGTGTTTATCAAGTTCCGCAACACACCCATTGTCAAGGCC   2400
ValLeuGlyIlePheLeuThrAlaPheValLeuGlyValPheIleLysPheArgAsnThrProIleValLysAla

ACCAACCGAGAGCTCTCCTACCTCCTCCTCTTCTCCCTGCTCTGCTGCTTCTCCAGCTCCCTGTTCTTCATCGGG   2475
ThrAsnArgGluLeuSerTyrLeuLeuLeuPheSerLeuLeuCysCysPheSerSerSerLeuPhePheIleGly

GAGCCCCAGGACTGGACGTGCCGCCTGCGCCAGCCGGCCTTTGGCATCAGCTTCGTGCTCTGCATCTCATGCATC   2550
GluProGlnAspTrpThrCysArgLeuArgGlnProAlaPheGlyIleSerPheValLeuCysIleSerCysIle

CTGGTGAAAACCAACCGTGTCCTCCTGGTGTTTGAGGCCAAGATCCCCACCAGCTTCCACCGCAAGTGGTGGGGG   2625
LeuValLysThrAsnArgValLeuLeuValPheGluAlaLysIleProThrSerPheHisArgLysTrpTrpGly

CTCAACCTGCAGTTCCTGCTGGTTTTCCTCTGCACCTTCATGCAGATTGTCATCTGTGTGATCTGGCTCTACACC   2700
LeuAsnLeuGlnPheLeuLeuValPheLeuCysThrPheMetGlnIleValIleCysValIleTrpLeuTyrThr
```

FIG. 48b.

```
         10        20        30        40        50        60        70
123456789012345678901234567890123456789012345678901234567890123456789012345
GCGCCCCCCTCAAGCTACCGCAACCAGGAGCTGGAGGATGAGATCATCTTCATCACGTGCCACGAGGGCTCCCTC   2775
AlaProProSerSerTyrArgAsnGlnGluLeuGluAspGluIleIlePheIleThrCysHisGluGlySerLeu

ATGGCCCTGGGCTTCCTGATCGGCTACACCTGCCTGCTGGCTGCCATCTGCTTCTTCTTTGCCTTCAAGTCCCGG   2850
MetAlaLeuGlyPheLeuIleGlyTyrThrCysLeuLeuAlaAlaIleCysPhePhePheAlaPheLysSerArg

AAGCTGCCGGAGAACTTCAATGAAGCCAAGTTCATCACCTTCAGCATGCTCATCTTCTTCATCGTCTGGATCTCC   2925
LysLeuProGluAsnPheAsnGluAlaLysPheIleThrPheSerMetLeuIlePhePheIleValTrpIleSer

TTCATTCCAGCCTATGCCAGCACCTATGGCAAGTTTGTCTCTGCCGTAGAGGTGATTGCCATCCTGGCAGCCAGC   3000
PheIleProAlaTyrAlaSerThrTyrGlyLysPheValSerAlaValGluValIleAlaIleLeuAlaAlaSer

TTTGGCTTGCTGGCGTGCATCTTCTTCAACAAGATCTACATCATTCTCTTCAAGCCATCCCGCAACACCATCGAG   3075
PheGlyLeuLeuAlaCysIlePhePheAsnLysIleTyrIleIleLeuPheLysProSerArgAsnThrIleGlu

GAGGTGCGTTGCAGCACCGCAGCTCACGCTTTCAAGGTGGCTGCCCGGGCCACGCTGCGCCGCAGCAACGTCTCC   3150
GluValArgCysSerThrAlaAlaHisAlaPheLysValAlaAlaArgAlaThrLeuArgArgSerAsnValSer

CGCAAGCGGTCCAGCAGCCTTGGAGGCTCCACGGGATCCACCCCCTCCTCCTCCATCAGCAGCAAGAGCAACAGC   3225
ArgLysArgSerSerSerLeuGlyGlySerThrGlySerThrProSerSerSerIleSerSerLysSerAsnSer

GAAGACCCATTCCCACGGCCCGAGAGGCAGAAGCAGCAGCAGCCGCTGGCCCTAACCCAGCAAGAGCAGCAGCAG   3300
GluAspProPheProArgProGluArgGlnLysGlnGlnGlnProLeuAlaLeuThrGlnGlnGluGlnGlnGln

CAGCCCCTGACCCTCCCACAGCAGCAACGATCTCAGCAGCAGCCCAGATGCAAGCAGAAGGTCATCTTTGGCAGC   3375
GlnProLeuThrLeuProGlnGlnGlnArgSerGlnGlnGlnProArgCysLysGlnLysValIlePheGlySer

GGCACGGTCACCTTCTCACTGAGCTTTGATGAGCCTCAGAAGAACGCCATGGCCCACAGGAATTCTACGCACCAG   3450
GlyThrValThrPheSerLeuSerPheAspGluProGlnLysAsnAlaMetAlaHisArgAsnSerThrHisGln

AACTCCCTGGAGGCCCAGAAAAGCAGCGATACGCTGACCCGACACCAGCCATTACTCCCGCTGCAGTGCGGGGAA   3525
AsnSerLeuGluAlaGlnLysSerSerAspThrLeuThrArgHisGlnProLeuLeuProLeuGlnCysGlyGlu

ACGGACTTAGATCTGACCGTCCAGGAAACAGGTCTGCAAGGACCTGTGGGTGGAGACCAGCGGCCAGAGGTGGAG   3600
ThrAspLeuAspLeuThrValGlnGluThrGlyLeuGlnGlyProValGlyGlyAspGlnArgProGluValGlu

GACCCTGAAGAGTTGTCCCCAGCACTTGTAGTGTCCAGTTCACAGAGCTTTGTCATCAGTGGTGGAGGCAGCACT   3675
AspProGluGluLeuSerProAlaLeuValValSerSerSerGlnSerPheValIleSerGlyGlyGlySerThr

GTTACAGAAAACGTAGTGAATTCATAAAATGGAAGGAGAAGACTGGGCTAGGGAGAATGCAGAGAGGTTTCTTGG   3750
ValThrGluAsnValValAsnSer

GGTCCCAGGGATGAGGAATCGCCCCAGACTCCTTTCCTCTGAGGAAGAAGGGATAATAGACACATCAAATGCCCC   3825

GAATTTAGTCACACCATCTTAAATGACAGTGAATTGACCCATGTTCCCTTTAAAATTAAAAAAAAGAAGAGCCTT   3900

GTGTTTCTGTGGTTGCATTTGTCAAAGCATTGAGATCTCCACGGTCAGATTTGCTGTTCACCCACATCTAATGTC   3975

TCTTCCTCTGTTCTATCCCACCCAACAGCTCAGAGATGAAACTATGGCTTTAAACTACCCTCCAGAGTGTGCAGA   4050
```

FIG. 48c.

```
         10        20        30        40        50        60        70
123456789012345678901234567890123456789012345678901234567890123456789012345
CTGATGGGACATCAAATTTGCCACCACTAGAGCTGAGAGTCTGAAAGACAGAATGTCACCAGTCCTGCCCAATGC   4125

CTTGACAACAGACTGAATTTTAAATGTTCACAACATAAGGAGAATGTATCTCCTCCTATTTATGAAAACCATATG   4200

ATATTTTGTCTCCTACCTGCTGCTGCTATTATGTAACATCCAGAAGGTTTGCACCCCTCCTATACCATATGTCTG   4275

GTTCTGTCCAGGACATGATACTGATGCCATGTTTAGATTCCAGGATCACAAGAATCACCTCAAATTGTTAGGAAG   4350

GGACTGCATAAACCAATGAGCTGTATCTGTAATTAATATTCCTATATGTAGCTTTATCCTTAGGAAAATGCTTCT   4425

GTTGTAATAGTCCATGGACAATATAAACTGAAAAATGTCAGTCTGGTTTATATAAGGCAGTATTATTGAGCTCTA   4500

TTTCCCCACCCCACTATCCTCACTCCCATAAGCTAAGCCTTATGTGAGCCCCTTCAGGGACTCAAGGGTCCAGAA   4575

GTCCCTCCCATCTCTACCCCAAAGAATTCCTGAAGCCAGATCCACCCTATCCCTGTACAGAGTAAGTTCTCAATT   4650

ATTGGCCTGCTAATAGCTGCTAGGGTAGGAAAGCGTGGTTCCAAGAAAGATCCACCCTCAAATGTCGGAGCTATG   4725

TTCCCTCCAGCAGTGGTATTAATACTGCCGGTCACCCAGGCTCTGGAGCCAGAGAGACAGACCGGGGTTCAAGCC   4800

ATGGCTTCGTCATTTGCAAGCTGAGTGACTGTAGGCAGGGAACCTTAACCTCTCTAAGCCACAGCTTCTTCATCT   4875

TTAAAATAAGGATAATAATCATTCCTTCCCCTCAGAGCTCTTATGTGGATTAAACGAGATAATGTATATAAAGTA   4950

CTTTAGCCTGGTACCTAGCACACAATAAGCATTCAATAAATATTAGTTAATATTAT                      5014
```

FIG. 48d.

```
         10        20        30        40        50        60        70
123456789012345678901234567890123456789012345678901234567890123456789012345
CAACAGGCACCTGGCTGCAGCCAGGAAGGACCGCACGCCCTTTCGCGCAGGAGAGTGGAAGGAGGGAGCTGTTTG    75

CCAGCACCGAGGTCTTGCGGCACAGGCAACGCTTGACCTGAGTCTTGCAGAATGAAAGGCATCACAGGAGGCCTC   150

TGCATGATGTGGCTTCCAAAGACTCAAGGACCACCCACATTACAAGTCTGGATTGAGGAAGGCAGAAATGGAGAT   225

TCAAACACCACGTCTTCTATTATTTTATTAATCAATCTGTAGACATGTGTCCCCACTGCAGGGAGTGAACTGCTC   300

CAAGGGAGAAACTTCTGGGAGCCTCCAAACTCCTAGCTGTCTCATCCCTTGCCCTGGAGAGACGGCAGAACCATG   375
                                                                        Met

GCATTTTATAGCTGCTGCTGGGTCCTCTTGGCACTCACCTGGCACACCTCTGCCTACGGGCCAGACCAGCGAGCC   450
AlaPheTyrSerCysCysTrpValLeuLeuAlaLeuThrTrpHisThrSerAlaTyrGlyProAspGlnArgAla

CAAAAGAAGGGGGACATTATCCTTGGGGGGCTCTTTCCTATTCATTTTGGAGTAGCAGCTAAAGATCAAGATCTC   525
GlnLysLysGlyAspIleIleLeuGlyGlyLeuPheProIleHisPheGlyValAlaAlaLysAspGlnAspLeu

AAATCAAGGCCGGAGTCTGTGGAATGTATCAGGTATAATTTCCGTGGGTTTCGCTGGTTACAGGCTATGATATTT   600
LysSerArgProGluSerValGluCysIleArgTyrAsnPheArgGlyPheArgTrpLeuGlnAlaMetIlePhe

GCCATAGAGGAGATAAACAGCAGCCCAGCCCTTCTTCCCAACTTGACGCTGGGATACAGGATATTTGACACTTGC   675
AlaIleGluGluIleAsnSerSerProAlaLeuLeuProAsnLeuThrLeuGlyTyrArgIlePheAspThrCys

AACACCGTTTCTAAGGCCTTGGAAGCCACCCTGAGTTTTGTTGCTCAAAACAAAATTGATTCTTTGAACCTTGAT   750
AsnThrValSerLysAlaLeuGluAlaThrLeuSerPheValAlaGlnAsnLysIleAspSerLeuAsnLeuAsp

GAGTTCTGCAACTGCTCAGAGCACATTCCCTCTACGATTGCTGTGGTGGGAGCAACTGGCTCAGGCGTCTCCACG   825
GluPheCysAsnCysSerGluHisIleProSerThrIleAlaValValGlyAlaThrGlySerGlyValSerThr

GCAGTGGCAAATCTGCTGGGGCTCTTCTACATTCCCCAGGTCAGTTATGCCTCCTCCAGCAGACTCCTCAGCAAC   900
AlaValAlaAsnLeuLeuGlyLeuPheTyrIleProGlnValSerTyrAlaSerSerSerArgLeuLeuSerAsn

AAGAATCAATTCAAGTCTTTCCTCCGAACCATCCCCAATGATGAGCACCAGGCCACTGCCATGGCAGACATCATC   975
LysAsnGlnPheLysSerPheLeuArgThrIleProAsnAspGluHisGlnAlaThrAlaMetAlaAspIleIle

GAGTATTTCCGCTGGAACTGGGTGGGCACAATTGCAGCTGATGACGACTATGGGCGGCCGGGGATTGAGAAATTC  1050
GluTyrPheArgTrpAsnTrpValGlyThrIleAlaAlaAspAspAspTyrGlyArgProGlyIleGluLysPhe

CGAGAGGAAGCTGAGGAAAGGGATATCTGCATCGACTTCAGTGAACTCATCTCCCAGTACTCTGATGAGGAAGAG  1125
ArgGluGluAlaGluGluArgAspIleCysIleAspPheSerGluLeuIleSerGlnTyrSerAspGluGluGlu

ATCCAGCATGTGGTAGAGGTGATTCAAAATTCCACGGCCAAAGTCATCGTGGTTTTCTCCAGTGGCCCAGATCTT  1200
IleGlnHisValValGluValIleGlnAsnSerThrAlaLysValIleValValPheSerSerGlyProAspLeu

GAGCCCCTCATCAAGGAGATTGTCCGGCGCAATATCACGGGCAAGATCTGGCTGGCCAGCGAGGCCTGGGCCAGC  1275
GluProLeuIleLysGluIleValArgArgAsnIleThrGlyLysIleTrpLeuAlaSerGluAlaTrpAlaSer

TCCTCCCTGATCGCCATGCCTCAGTACTTCCACGTGGTTGGCGGCACCATTGGATTCGCTCTGAAGGCTGGGCAG  1350
SerSerLeuIleAlaMetProGlnTyrPheHisValValGlyGlyThrIleGlyPheAlaLeuLysAlaGlyGln
```

FIG. 49a.

```
         10        20        30        40        50        60        70
1234567890123456789012345678901234567890123456789012345678901234567890123456789012345
ATCCCAGGCTTCCGGGAATTCCTGAAGAAGGTCCATCCCAGGAAGTCTGTCCACAATGGTTTTGCCAAGGAGTTT   1425
IleProGlyPheArgGluPheLeuLysLysValHisProArgLysSerValHisAsnGlyPheAlaLysGluPhe

TGGGAAGAAACATTTAACTGCCACCTCCAAGAAGGTGCAAAAGGACCTTTACCTGTGGACACCTTTCTGAGAGGT   1500
TrpGluGluThrPheAsnCysHisLeuGlnGluGlyAlaLysGlyProLeuProValAspThrPheLeuArgGly

CACGAAGAAAGTGGCGACAGGTTTAGCAACAGCTCGACAGCCTTCCGACCCCTCTGTACAGGGGATGAGAACATC   1575
HisGluGluSerGlyAspArgPheSerAsnSerSerThrAlaPheArgProLeuCysThrGlyAspGluAsnIle

AGCAGTGTCGAGACCCCTTACATAGATTACACGCATTTACGGATATCCTACAATGTGTACTTAGCAGTCTACTCC   1650
SerSerValGluThrProTyrIleAspTyrThrHisLeuArgIleSerTyrAsnValTyrLeuAlaValTyrSer

ATTGCCCACGCCTTGCAAGATATATATACCTGCTTACCTGGGAGAGGGCTCTTCACCAATGGCTCCTGTGCAGAC   1725
IleAlaHisAlaLeuGlnAspIleTyrThrCysLeuProGlyArgGlyLeuPheThrAsnGlySerCysAlaAsp

ATCAAGAAAGTTGAGGCGTGGCAGGTCCTGAAGCACCTACGGCATCTAAACTTTACAAACAATATGGGGGAGCAG   1800
IleLysLysValGluAlaTrpGlnValLeuLysHisLeuArgHisLeuAsnPheThrAsnAsnMetGlyGluGln

GTGACCTTTGATGAGTGTGGTGACCTGGTGGGGAACTATTCCATCATCAACTGGCACCTCTCCCCAGAGGATGGC   1875
ValThrPheAspGluCysGlyAspLeuValGlyAsnTyrSerIleIleAsnTrpHisLeuSerProGluAspGly

TCCATCGTGTTTAAGGAAGTCGGGTATTACAACGTCTATGCCAAGAAGGGAGAAAGACTCTTCATCAACGAGGAG   1950
SerIleValPheLysGluValGlyTyrTyrAsnValTyrAlaLysLysGlyGluArgLeuPheIleAsnGluGlu

AAAATCCTGTGGAGTGGGTTCTCCAGGGAGGTGCCCTTCTCCAACTGCAGCCGAGACTGCCTGGCAGGGACCAGG   2025
LysIleLeuTrpSerGlyPheSerArgGluValProPheSerAsnCysSerArgAspCysLeuAlaGlyThrArg

AAAGGGATCATTGAGGGGGAGCCCACCTGCTGCTTTGAGTGTGTGGAGTGTCCTGATGGGGAGTATAGTGATGAG   2100
LysGlyIleIleGluGlyGluProThrCysCysPheGluCysValGluCysProAspGlyGluTyrSerAspGlu

ACAGATGCCAGTGCCTGTAACAAGTGCCCAGATGACTTCTGGTCCAATGAGAACCACACCTCCTGCATTGCCAAG   2175
ThrAspAlaSerAlaCysAsnLysCysProAspAspPheTrpSerAsnGluAsnHisThrSerCysIleAlaLys

GAGATCGAGTTTCTGTCGTGGACGGAGCCCTTTGGGATCGCACTCACCCTCTTTGCCGTGCTGGGCATTTTCCTG   2250
GluIleGluPheLeuSerTrpThrGluProPheGlyIleAlaLeuThrLeuPheAlaValLeuGlyIlePheLeu

ACAGCCTTTGTGCTGGGTGTGTTTATCAAGTTCCGCAACACACCCATTGTCAAGGCCACCAACCGAGAGCTCTCC   2325
ThrAlaPheValLeuGlyValPheIleLysPheArgAsnThrProIleValLysAlaThrAsnArgGluLeuSer

TACCTCCTCCTCTTCTCCCTGCTCTGCTGCTTCTCCAGCTCCCTGTTCTTCATCGGGGAGCCCCAGGACTGGACG   2400
TyrLeuLeuLeuPheSerLeuLeuCysCysPheSerSerSerLeuPhePheIleGlyGluProGlnAspTrpThr

TGCCGCCTGCGCCAGCCGGCCTTTGGCATCAGCTTCGTGCTCTGCATCTCATGCATCCTGGTGAAAACCAACCGT   2475
CysArgLeuArgGlnProAlaPheGlyIleSerPheValLeuCysIleSerCysIleLeuValLysThrAsnArg

GTCCTCCTGGTGTTTGAGGCCAAGATCCCCACCAGCTTCCACCGCAAGTGGTGGGGGCTCAACCTGCAGTTCCTG   2550
ValLeuLeuValPheGluAlaLysIleProThrSerPheHisArgLysTrpTrpGlyLeuAsnLeuGlnPheLeu

CTGGTTTTCCTCTGCACCTTCATGCAGATTGTCATCTGTGTGATCTGGCTCTACACCGCGCCCCCCTCAAGCTAC   2625
LeuValPheLeuCysThrPheMetGlnIleValIleCysValIleTrpLeuTyrThrAlaProProSerSerTyr

CGCAACCAGGAGCTGGAGGATGAGATCATCTTCATCACGTGCCACGAGGGCTCCCTCATGGCCCTGGGCTTCCTG   2700
ArgAsnGlnGluLeuGluAspGluIleIlePheIleThrCysHisGluGlySerLeuMetAlaLeuGlyPheLeu
```

FIG. 49b

```
         10        20        30        40        50        60        70
123456789012345678901234567890123456789012345678901234567890123456789012345
ATCGGCTACACCTGCCTGCTGGCTGCCATCTGCTTCTTCTTTGCCTTCAAGTCCCGGAAGCTGCCGGAGAACTTC   2775
IleGlyTyrThrCysLeuLeuAlaAlaIleCysPhePhePheAlaPheLysSerArgLysLeuProGluAsnPhe

AATGAAGCCAAGTTCATCACCTTCAGCATGCTCATCTTCTTCATCGTCTGGATCTCCTTCATTCCAGCCTATGCC   2850
AsnGluAlaLysPheIleThrPheSerMetLeuIlePhePheIleValTrpIleSerPheIleProAlaTyrAla

AGCACCTATGGCAAGTTTGTCTCTGCCGTAGAGGTGATTGCCATCCTGGCAGCCAGCTTTGGCTTGCTGGCGTGC   2925
SerThrTyrGlyLysPheValSerAlaValGluValIleAlaIleLeuAlaAlaSerPheGlyLeuLeuAlaCys

ATCTTCTTCAACAAGATCTACATCATTCTCTTCAAGCCATCCCGCAACACCATCGAGGAGGTGCGTTGCAGCACC   3000
IlePhePheAsnLysIleTyrIleIleLeuPheLysProSerArgAsnThrIleGluGluValArgCysSerThr

GCAGCTCACGCTTTCAAGGTGGCTGCCCGGGCCACGCTGCGCCGCAGCAACGTCTCCCGCAAGCGGTCCAGCAGC   3075
AlaAlaHisAlaPheLysValAlaAlaArgAlaThrLeuArgArgSerAsnValSerArgLysArgSerSerSer

CTTGGAGGCTCCACGGGATCCACCCCCTCCTCCTCCATCAGCAGCAAGAGCAACAGCGAAGACCCATTCCCACAG   3150
LeuGlyGlySerThrGlySerThrProSerSerSerIleSerSerLysSerAsnSerGluAspProPheProGln

CCCGAGAGGCAGAAGCAGCAGCAGCCGCTGGCCCTAACCCAGCAAGAGCAGCAGCAGCAGCCCCTGACCCTCCCA   3225
ProGluArgGlnLysGlnGlnGlnProLeuAlaLeuThrGlnGlnGluGlnGlnGlnGlnProLeuThrLeuPro

CAGCAGCAACGATCTCAGCAGCAGCCCAGATGCAAGCAGAAGGTCATCTTTGGCAGCGGCACGGTCACCTTCTCA   3300
GlnGlnGlnArgSerGlnGlnGlnProArgCysLysGlnLysValIlePheGlySerGlyThrValThrPheSer

CTGAGCTTTGATGAGCCTCAGAAGAACGCCATGGCCCACGGGAATTCTACGCACCAGAACTCCCTGGAGGCCCAG   3375
LeuSerPheAspGluProGlnLysAsnAlaMetAlaHisGlyAsnSerThrHisGlnAsnSerLeuGluAlaGln

AAAAGCAGCGATACGCTGACCCGACACCAGCCATTACTCCCGCTGCAGTGCGGGGAAACGGACTTAGATCTGACC   3450
LysSerSerAspThrLeuThrArgHisGlnProLeuLeuProLeuGlnCysGlyGluThrAspLeuAspLeuThr

GTCCAGGAAACAGGTCTGCAAGGACCTGTGGGTGGAGACCAGCGGCCAGAGGTGGAGGACCCTGAAGAGTTGTCC   3525
ValGlnGluThrGlyLeuGlnGlyProValGlyGlyAspGlnArgProGluValGluAspProGluGluLeuSer

CCAGCACTTGTAGTGTCCAGTTCACAGAGCTTTGTCATCAGTGGTGGAGGCAGCACTGTTACAGAAAACGTAGTG   3600
ProAlaLeuValValSerSerSerGlnSerPheValIleSerGlyGlyGlySerThrValThrGluAsnValVal

AATTCATAAAATGGAAGGAGAAGACTGGGCTAGGGAGAATGCAGAGAGGTTTCTTGGGGTCCCAGGGATGAGGAA   3675
AsnSer

TCGCCCCAGACTCCTTTCCTCTGAGGAAGAAGGGATAATAGACACATCAAATGCCCCGAATTTAGTCACACCATC   3750

TTAAATGACAGTGAATTGACCCATGTTCCCTTTAAAAAAAAAAAAAAAAAAAGCGGCCGC                  3809
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FIG. 49c.

```
         10        20        30        40        50        60        70
123456789012345678901234567890123456789012345678901234567890123456789012345
CGGGACTCTCCAGGCCGGCTCAGGCACCGGACTGTAGGTGTATTTGGAGGGATTTGGAGGCTGGAGACCCCAGGA    75

AGCACGCAGGCGGGAGCAGGCAAGGGGCGGAGCCCCGGGCCCGGCCAAGGTGGCCGTCAGAGGGTCTGCGGGGAG   150

GCAGTAGCTTGACCCAAGGCGACCAGGGAACTTCAGACGGTAGCACGCCACTCAAACAAATTAACTTGACATCGC   225

AAGCTGGGCGGGCTGGTACGACATCCTGACTTCAGCATCCAGCTGTTCCTGGGCAGACAGAGGGCCAACAGGTGT   300

TCCTGTGGAAGAAGCCAGGACAAGGACTCCAGAAAACATCTCGGGCAGCCTCTACATGATGTCACTTCTCAGGAC   375

TCGAGGACCAGCCACCCTACACCTCTACTACAGAGAAGGCAGAAATGGAGACCCAAAGGCCATCACTCCTGCTCT   450

GTCACTAACCACTCTGTAATCATGTCTCCCCACCAGAAGGTGTGAACCGCACCAGGGCCGTGGAGTTCTCGGGCT   525

CCCAATCCACTGACACCTTTACCTGTCCCCTGAAGAGAAGGCAACGCTATGGCATCGTACAGCTGCTGTTTGGCC   600
                                                MetAlaSerTyrSerCysCysLeuAla

CTATTGGCTCTTGCCTGGCACTCCTCTGCCTATGGGCCTGACCAGCGAGCCCAAAAGAAGGGGGACATTATCCTA   675
LeuLeuAlaLeuAlaTrpHisSerSerAlaTyrGlyProAspGlnArgAlaGlnLysLysGlyAspIleIleLeu

GGAGGTCTCTTTCCTATCCATTTTGGAGTAGCAGCCAAAGATCAAGATCTGAAGTCAAGACCAGAGTCTGTGGAG   750
GlyGlyLeuPheProIleHisPheGlyValAlaAlaLysAspGlnAspLeuLysSerArgProGluSerValGlu

TGCATTAGGTATAACTTCCGTGGATTCCGATGGTTACAAGCCATGATATTCGCCATAGAGGAGATAAACAGCAGC   825
CysIleArgTyrAsnPheArgGlyPheArgTrpLeuGlnAlaMetIlePheAlaIleGluGluIleAsnSerSer

CCCTCCCTTCTTCCCAACATGACACTGGGATATAGGATATTTGACACCTGTAACACCGTCTCCAAGGCGCTGGAA   900
ProSerLeuLeuProAsnMetThrLeuGlyTyrArgIlePheAspThrCysAsnThrValSerLysAlaLeuGlu

GCCACCTTGAGTTTTGTTGCCCAGAACAAAATCGATTCTTTGAACCTGGACGAGTTCTGCAACTGCTCTGAGCAC   975
AlaThrLeuSerPheValAlaGlnAsnLysIleAspSerLeuAsnLeuAspGluPheCysAsnCysSerGluHis

ATCCCTTCGACCATTGCCGTGGTGGGAGCCACCGGCTCCGGTGTCTCCACGGCGGTAGCCAACCTGCTGGGACTT  1050
IleProSerThrIleAlaValValGlyAlaThrGlySerGlyValSerThrAlaValAlaAsnLeuLeuGlyLeu

TTCTACATCCCCCAGGTGAGCTACGCCTCCTCCAGCAGGCTCCTCAGCAATAAGAACCAGTACAAATCCTTCCTC  1125
PheTyrIleProGlnValSerTyrAlaSerSerSerArgLeuLeuSerAsnLysAsnGlnTyrLysSerPheLeu

CGCACCATTCCCAATGACGAACACCAGGCAACCGCGATGGCCGACATCATCGAGTACTTCCGCTGGAACTGGGTG  1200
ArgThrIleProAsnAspGluHisGlnAlaThrAlaMetAlaAspIleIleGluTyrPheArgTrpAsnTrpVal

GGCACAATTGCAGCTGATGACGACTATGGCAGACCTGGCATTGAGAAGTTCCGAGAGGAAGCCGAAGAGAGGGAT  1275
GlyThrIleAlaAlaAspAspAspTyrGlyArgProGlyIleGluLysPheArgGluGluAlaGluGluArgAsp

ATCTGCATTGATTTTAGCGAGCTCATCTCCCAGTACTCTGACGAGGAAGAGATCCAGCAGGTGGTCGAAGTGATC  1350
IleCysIleAspPheSerGluLeuIleSerGlnTyrSerAspGluGluGluIleGlnGlnValValGluValIle
```

FIG. 50a.

```
         10        20        30        40        50        60        70
123456789012345678901234567890123456789012345678901234567890123456789012345
CAAAACTCTACGGCCAAGGTCATTGTCGTTTTCTCCAGCGGCCCGGACCTAGAACCTCTCATCAAGGAGATTGTG   1425
GlnAsnSerThrAlaLysValIleValValPheSerSerGlyProAspLeuGluProLeuIleLysGluIleVal

CGGCGTAACATCACAGGCAGGATCTGGCTGGCTAGCGAGGCCTGGGCCAGTTCCTCGCTGATTGCTATGCCTGAG   1500
ArgArgAsnIleThrGlyArgIleTrpLeuAlaSerGluAlaTrpAlaSerSerSerLeuIleAlaMetProGlu

TATTTCCATGTAGTCGGGGGCACCATTGGGTTCGGTCTGAAGGCTGGGCAGATTCCAGGCTTCAGAGAATTCCTA   1575
TyrPheHisValValGlyGlyThrIleGlyPheGlyLeuLysAlaGlyGlnIleProGlyPheArgGluPheLeu

CAGAAAGTTCATCCTAGGAAGTCTGTCCACAATGGTTTTGCCAAAGAGTTTTGGGAAGAAACTTTTAATTGCCAC   1650
GlnLysValHisProArgLysSerValHisAsnGlyPheAlaLysGluPheTrpGluGluThrPheAsnCysHis

CTCCAAGAAGGCGCAAAAGGACCTTTACCTGTGGACACCTTCGTGAGAAGTCACGAAGAAGGTGGCAACAGGTTA   1725
LeuGlnGluGlyAlaLysGlyProLeuProValAspThrPheValArgSerHisGluGluGlyGlyAsnArgLeu

CTCAATAGCTCTACTGCCTTCCGACCCCTCTGCACAGGGGATGAGAACATCAACAGTGTGGAGACCCCTTACATG   1800
LeuAsnSerSerThrAlaPheArgProLeuCysThrGlyAspGluAsnIleAsnSerValGluThrProTyrMet

GATTACGAACATTTACGGATATCCTACAATGTGTACTTAGCCGTCTACTCCATTGCGCATGCCCTACAAGATATA   1875
AspTyrGluHisLeuArgIleSerTyrAsnValTyrLeuAlaValTyrSerIleAlaHisAlaLeuGlnAspIle

TACACCTGCTTACCCGGAAGAGGGCTTTTCACCAACGGGTCCTGTGCAGACATCAAGAAGGTTGAGGCCTGGCAG   1950
TyrThrCysLeuProGlyArgGlyLeuPheThrAsnGlySerCysAlaAspIleLysLysValGluAlaTrpGln

GTCTTGAAGCACCTACGGCACCTGAACTTCACCAACAACATGGGGGAGCAGGTGACCTTCGATGAGTGTGGTGAT   2025
ValLeuLysHisLeuArgHisLeuAsnPheThrAsnAsnMetGlyGluGlnValThrPheAspGluCysGlyAsp

CTGGTGGGGAACTATTCTATCATCAACTGGCACCTCTCCCCAGAGGACGGCTCCATTGTGTTCAAGGAAGTTGGG   2100
LeuValGlyAsnTyrSerIleIleAsnTrpHisLeuSerProGluAspGlySerIleValPheLysGluValGly

TACTACAATGTGTATGCCAAGAAGGGAGAAAGACTCTTCATCAATGAGGAGAAGATCTTGTGGAGTGGGTTCTCC   2175
TyrTyrAsnValTyrAlaLysLysGlyGluArgLeuPheIleAsnGluGluLysIleLeuTrpSerGlyPheSer

AGAGAGGTGCCTTTCTCCAATTGCAGCCGGGACTGTCAGGCAGGGACCAGGAAGGGGATCATCGAGGGAGAGCCC   2250
ArgGluValProPheSerAsnCysSerArgAspCysGlnAlaGlyThrArgLysGlyIleIleGluGlyGluPro

ACCTGCTGCTTTGAGTGTGTGGAGTGTCCTGATGGAGAGTACAGTGGAGAGACAGATGCGAGTGCCTGTGACAAG   2325
ThrCysCysPheGluCysValGluCysProAspGlyGluTyrSerGlyGluThrAspAlaSerAlaCysAspLys

TGCCCGGATGACTTCTGGTCCAATGAGAACCACACTTCTTGCATTGCCAAGGAGATTGAGTTTCTGGCGTGGACC   2400
CysProAspAspPheTrpSerAsnGluAsnHisThrSerCysIleAlaLysGluIleGluPheLeuAlaTrpThr

GAGCCCTTTGGAATCGCTCTCACTCTCTTTGCGGTGCTGGGCATTTTCCTGACCGCCTTTGTGCTGGGTGTCTTC   2475
GluProPheGlyIleAlaLeuThrLeuPheAlaValLeuGlyIlePheLeuThrAlaPheValLeuGlyValPhe

ATCAAGTTCCGAAACACACCTATCGTCAAGGCCACCAACCGAGAACTGTCCTACCTCCTGCTCTTCTCCCTACTC   2550
IleLysPheArgAsnThrProIleValLysAlaThrAsnArgGluLeuSerTyrLeuLeuLeuPheSerLeuLeu

TGCTGCTTCTCCAGCTCCTTGTTCTTCATTGGGGAGCCCCAGGACTGGACGTGCCGCCTGCGACAGCCTGCTTTC   2625
CysCysPheSerSerSerLeuPhePheIleGlyGluProGlnAspTrpThrCysArgLeuArgGlnProAlaPhe

GGCATCAGCTTTGTGCTCTGTATCTCGTGCATCTTGGTGAAGACCAATCGCGTCCTCCTGGTATTTGAAGCCAAG   2700
GlyIleSerPheValLeuCysIleSerCysIleLeuValLysThrAsnArgValLeuLeuValPheGluAlaLys
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FIG. 50b.

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         10        20        30        40        50        60        70
123456789012345678901234567890123456789012345678901234567890123456789012345
ATACCCACCAGCTTCCACCGGAAGTGGTGGGGGCTCAACCTGCAGTTCCTGCTGGTTTTCCTCTGCACCTTCATG   2775
IleProThrSerPheHisArgLysTrpTrpGlyLeuAsnLeuGlnPheLeuLeuValPheLeuCysThrPheMet

CAGATCCTCATCTGCATCATCTGGCTCTACACGGCGCCCCCCTCTAGCTACCGCAACCATGAGCTGGAAGACGAA   2850
GlnIleLeuIleCysIleIleTrpLeuTyrThrAlaProProSerSerTyrArgAsnHisGluLeuGluAspGlu

ATCATCTTCATCACGTGCCATGAGGGCTCACTCATGGCACTTGGCTCCCTGATCGGCTATACCTGCCTGCTGGCT   2925
IleIlePheIleThrCysHisGluGlySerLeuMetAlaLeuGlySerLeuIleGlyTyrThrCysLeuLeuAla

GCCATCTGCTTCTTCTTTGCCTTCAAGTCCAGGAAGTTACCAGAGAACTTCAACGAAGCCAAGTTCATTACCTTC   3000
AlaIleCysPhePhePheAlaPheLysSerArgLysLeuProGluAsnPheAsnGluAlaLysPheIleThrPhe

AGCATGCTCATCTTCTTCATCGTCTGGATCTCCTTCATTCCAGCCTATGCCAGCACCTACGGCAAGTTTGTCTCT   3075
SerMetLeuIlePhePheIleValTrpIleSerPheIleProAlaTyrAlaSerThrTyrGlyLysPheValSer

GCCGTAGAGGTGATCGCCATTTTGGCAGCCAGCTTTGGCTTACTAGCCTGCATCTTCTTCAACAAGGTCTACATT   3150
AlaValGluValIleAlaIleLeuAlaAlaSerPheGlyLeuLeuAlaCysIlePhePheAsnLysValTyrIle

ATCCTCTTCAAGCCTTCCCGGAACACCATTGAGGAAGTCCGCTCCAGCACCGCAGCACATGCTTTCAAAGTAGCA   3225
IleLeuPheLysProSerArgAsnThrIleGluGluValArgSerSerThrAlaAlaHisAlaPheLysValAla

GCCCGCGCCACTCTACGCCGTCCCAACATCTCCCGGAAGCGGTCCAGCAGCCTTGGAGGCTCCACCGGCTCCATT   3300
AlaArgAlaThrLeuArgArgProAsnIleSerArgLysArgSerSerSerLeuGlyGlySerThrGlySerIle

CCCTCCTCCTCCATCAGCAGCAAAAGCAACAGCGAAGACCGGTTCCCGCAGCCAGAGAGGCAGAAGCAACAGCAA   3375
ProSerSerSerIleSerSerLysSerAsnSerGluAspArgPheProGlnProGluArgGlnLysGlnGlnGln

CCGCTGTCCCTGACCCAGCAAGAACAGCAGCAGCAGCCCCTGACCCTCCACCCACAGCAACAGCAGCAGCCACAG   3450
ProLeuSerLeuThrGlnGlnGluGlnGlnGlnGlnProLeuThrLeuHisProGlnGlnGlnGlnGlnProGln

CAGCCGAGATGCAAACAGAAGGTCATCTTCGGCAGTGGTACGGTCACCTTCTCTCTGAGTTTTGACGAGCCTCAG   3525
GlnProArgCysLysGlnLysValIlePheGlySerGlyThrValThrPheSerLeuSerPheAspGluProGln

AAGAATGCCATGGCCCACAGGAACTCCATGCGTCAGAACTCCCTGGAGGCCCAGAGGAGCAACGACACCTTGGGC   3600
LysAsnAlaMetAlaHisArgAsnSerMetArgGlnAsnSerLeuGluAlaGlnArgSerAsnAspThrLeuGly

AGACACCAGGCCCTGCTTCCCCTACAGTGTGCAGATGCGGACTCAGAAATGACCATTCAGGAAACGGGCCTGCAA   3675
ArgHisGlnAlaLeuLeuProLeuGlnCysAlaAspAlaAspSerGluMetThrIleGlnGluThrGlyLeuGln

GGGCCCATGGTGGGGGACCACCAGCCAGAAATGGAAAGCTCAGATGAAATGTCCCCAGCGCTGGTCATGTCCACC   3750
GlyProMetValGlyAspHisGlnProGluMetGluSerSerAspGluMetSerProAlaLeuValMetSerThr

TCTCGGAGCTTCGTCATTAGTGGTGGAGGTAGCTCTGTGACGGAAAACGTATTACACTCCTAATGGAGGGAAAGG   3825
SerArgSerPheValIleSerGlyGlyGlySerSerValThrGluAsnValLeuHisSer

CTATCCAGTTGAGAGGTTTTTCTTAGAGCCCTGAGCAAAAGGATGGGTCCTTCCTTTCTTCCCAGGAAGCCAGGG   3900

AGAGTAGGTACGTCAAAGCCTGTACTCAGTTGCACTGCTTTGAATGACAGTGAACTGACTGGTGTGCTCTTTAGA   3975

GTTAAAAGAAGAGCCATGTTTTGGGGTCGTTTTCCAGAGCTCAGTATCACACCTGGGTTTGCTGAAGTCTTTTCC   4050
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FIG. 50c.

```
         10        20        30        40        50        60        70
1234567890123456789012345678901234567890123456789012345678901234567890123456789012345
TCTGCTCTATCCACCATCAGTTCAGACGAAAGCAAGGCTCTAAGCTACCCATCTGCTTCCCTCAAAAAAAAAAAA   4125

AAAAAA                                                                         4131
```

FIG. 50d.

FIG. 51.

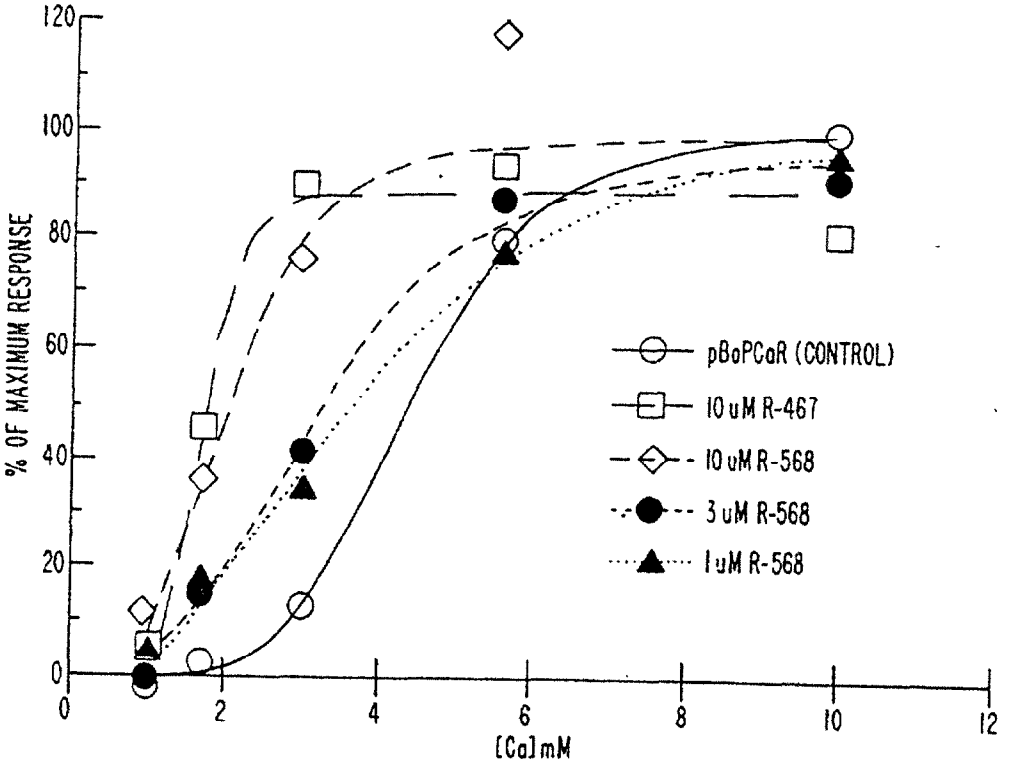
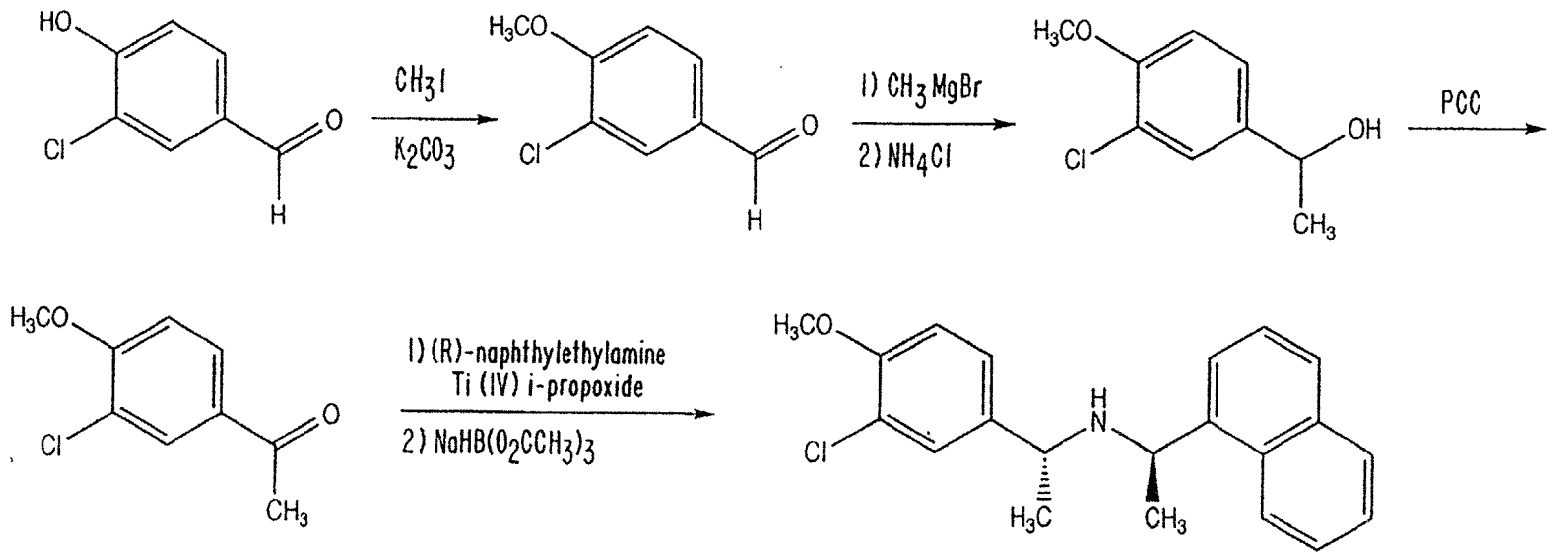

% OF MAXIMUM RESPONSE
120
100
80
60
40
20
0
0
2
4
6
8
10
12
[Ca] mM
pBoPCaR (CONTROL)
10 uM R-467
10 uM R-568
3 uM R-568
1 uM R-568

FIG. 52.


HO
Cl
O
H
CH_3I
K_2CO_3
H_3CO
Cl
O
H
1) CH_3MgBr
2) NH_4Cl
H_3CO
Cl
OH
CH_3
PCC
H_3CO
Cl
O
CH_3
1) (R)-naphthylethylamine
Ti (IV) i-propoxide
2) $NaHB(O_2CCH_3)_3$
H_3CO
Cl
H
N
H_3C
CH_3

17X

CALCIUM RECEPTOR-ACTIVE MOLECULES

RELATED APPLICATIONS

This is a continuation-in-part of Nemeth et al., PCT/US94/12117, filed Oct. 21, 1994, and a continuation-in-part of Nemeth et al., U.S. Ser. No. 08/292,827, filed Aug. 19, 1994, abandoned and a continuation-in-part of Nemeth et al., U.S. Ser. No. 08/141,248, filed Oct. 22, 1993, abandoned, and a continuation-in-part of Nemeth et al., U.S. Ser. No. 08/009,389, filed Feb. 23, 1993, abandoned, U.S. Ser. No. 08/009,389, is a continuation-in-part of Nemeth et al., U.S. Ser. No. 08/017,127, filed Feb. 12, 1993, abandoned, which is a continuation-in-part of Nemeth et al., U.S. Ser. No. 07/934,161, filed Aug. 21, 1992, abandoned, which is a continuation-in-part of Nemeth et al., U.S. Ser. No. 07/834,044, filed Feb. 11, 1992, abandoned, which is a continuation-in-part of Nemeth et al., U.S. Ser. No. 07/749,451, filed Aug. 23, 1991, abandoned, the whole of each of these applications including the drawings are hereby incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates to the design, development, composition and use of molecules able to modulate the activity of an inorganic ion receptor, preferably a calcium receptor. It also relates to a superfamily of receptors for inorganic ion (inorganic ion receptors) such as calcium receptors. The invention also relates to nucleic acids encoding such receptors, cells, tissues and animals containing such nucleic acids, antibodies to such receptors, assays utilizing such receptors, and methods relating to all of the foregoing.

BACKGROUND OF THE INVENTION

The following description provides a summary of information relevant to the present invention. It is not an admission that any of the information provided herein is prior art to the presently claimed invention, nor that any of the publications specifically or implicitly referenced are prior art to that invention.

Certain cells in the body respond not only to chemical signals, but also to ions such as extracellular calcium ions (Ca^{2+}). Changes in the concentration of extracellular Ca^{2+} (referred to herein as "$[Ca^{2+}]$") alter the functional responses of these cells. One such specialized cell is the parathyroid cell which secretes parathyroid hormone (PTH). PTH is the principal endocrine factor regulating Ca^{2+} homeostasis in the blood and extracellular fluids.

PTH, by acting on bone and kidney cells, increases the level of Ca^{2+} in the blood. This increase in $[Ca^{2+}]$ then acts as a negative feedback signal, depressing PTH secretion. The reciprocal relationship between $[Ca^{2+}]$ and PTH secretion forms the essential mechanism maintaining bodily Ca^{2+} homeostasis.

Extracellular Ca^{2+} acts directly on parathyroid cells to regulate PTH secretion. The existence of a parathyroid cell surface protein which detects changes in $[Ca^{2+}]$ has been suggested. This protein acts as a receptor for extracellular Ca^{2+} ("the calcium receptor"), and is suggested to detect changes in $[Ca^{2+}]$ and to initiate a functional cellular response, PTH secretion. For example, the role of calcium receptors and extracellular Ca^{2+} in the regulation of intracellular Ca^{2+} and cell function is reviewed in Nemeth et al., *Cell Calcium* 11: 319, 1990; the role of calcium receptors in parafollicular and parathyroid cells is discussed in Nemeth, *Cell Calcium* 11: 323, 1990; and the role of calcium receptors on bone osteoclasts is discussed by Zaidi, *Bioscience Reports* 10: 493, 1990.

Other cells in the body, specifically the osteoclast in bone, the juxtaglomerular, proximal tubule cells in the kidney, the keratinocyte in the epidermis, the parafollicular cell in the thyroid, intestinal cells, and the trophoblast in the placenta, have the capacity to sense changes in $[Ca^{2+}]$. It has been suggested that cell surface calcium receptors may also be present on these cells, imparting to them the ability to detect and to initiate or enable a response to changes in $[Ca^{2+}]$.

In parathyroid cells, osteoclasts, parafollicular cells (C-cells), keratinocytes, juxtaglomerular cells, trophoblasts, pancreatic beta cells and fat/adipose cells, an increase in $[Ca^{2+}]$ evokes an increase in intracellular free Ca^{2+} concentration ("$[Ca^{2+}]_i$"). Such an increase may be caused by influx of extracellular Ca^{2+} or by mobilization of Ca^{2+} from intracellular organelles. Changes in $[Ca^{2+}]_i$ are readily monitored and quantitated using fluorimetric indicators such as fura-2 or indo-1 (Molecular Probes, Eugene, Oreg.). Measurement of $[Ca^{2+}]_i$ provides an assay to assess the ability of molecules to act as agonists or antagonists at the calcium receptor.

In parathyroid cells, increases in the concentration of extracellular Ca^{2+} evoke rapid and transient increases in $[Ca^{2+}]_i$ which are followed by lower, yet sustained, increases in $[Ca^{2+}]_i$. The transient increases in $[Ca^{2+}]_i$ arise from the mobilization of intracellular Ca^{2+}, whereas the lower, sustained increases result from the influx of extracellular Ca^{2+}. The mobilization of intracellular Ca^{2+} is accompanied by increased formation of inositol-1,4,5-triphosphate (IP_3) and diacylglycerol, two biochemical indicators which are associated with receptor-dependent mobilization of intracellular Ca^{2+} in various other cells.

In addition to Ca^{2+}, various other di- and trivalent cations, such as Mg^{2+}, Sr^{2+}, Ba^{2+}, La^{3+} and Gd^{3+} also cause the mobilization of intracellular Ca^{2+} in parathyroid cells. Mg^{2+} and La^{3+} also increase the formation of IP_3. All of these inorganic cations depress the secretion of PTH. The postulated calcium receptor on the parathyroid cell is therefore promiscuous because it detects a variety of extracellular di- and trivalent cations.

The ability of various compounds to mimic extracellular Ca^{2+} in vitro is discussed by Nemeth et al., (spermine and spermidine) in "Calcium-Binding Proteins in Health and Disease," 1987, Academic Press, pp. 33–35; Brown et al., (e.g., neomycin) *Endocrinology* 128: 3047, 1991; Chen et al., (diltiazem and its analog, TA-3090) *J. Bone and Mineral Res.* 5: 581, 1990; and Zaidi et al., (verapamil) *Biochem. Biophys. Res. Commun.* 167: 807, 1990.

Brown et al., *J. Bone Mineral Res.* 6: 11, 1991 discuss theories regarding the effects of Ca^{2+} ions on parathyroid cells, and propose that the results may be explained by both a receptor-like mechanism and a receptor-independent mechanism as follows:

> Polyvalent cations [e.g. , divalent and trivalent cations] exert a variety of effects on parathyroid function, such as inhibition of parathyroid hormone (PTH) secretion and cAMP accumulation, stimulation of the accumulation of inositol phosphates, and elevation of the cytosolic calcium concentration. These actions are thought to be mediated through a "receptor-like" mechanism. The inhibition of agonist-stimulated cAMP accumulation by divalent and trivalent cations, for example, is blocked following preincubation with pertussis toxin. Thus, the putative polyvalent cation

receptor may be coupled to inhibition of adenylate cyclase by the inhibitory guanine nucleotide regulatory (G) protein, G_i.

We recently showed that the polycationic antibiotic, neomycin, mimics the actions of di- and trivalent cations in several aspects of parathyroid function. To determine whether these actions were specific to this agent or represented a more generalized action of polycations, we tested the effects of the highly basic peptides, polyarginine and polylysine, as well as protamine on the same parameters in dispersed bovine parathyroid cells. The results demonstrate that the parathyroid cell responds to a variety of polycations as well as to polyvalent cations, potentially via similar biochemical pathways. These results are discussed in terms of the recently postulated, "receptor-independent" modulation of G proteins by polycations in other systems.

The Ca^{2+} receptor has been presumed to be analogous to other G protein-coupled receptors [e.g., a glycoprotein], but recent studies with other cell types have raised the possibility that polycations can modulate cell function by alternative or additional mechanisms. In mast cells, for example, a variety of amphipathic cations, including mastoparan, a peptide from wasp venom, 48/80, a synthetic polycation, and polylysine, enhance secretion by a pertussis toxin-sensitive mechanism, suggesting the involvement of a G protein. No classic cell surface receptor has been identified that could mediate the actions of these diverse agents. Furthermore, these same compounds have been shown to activate directly purified G proteins in solution or in artificial phospholipid vesicles. On the basis of these observations, it has been proposed that amphipathic cations activate G proteins and, in turn, mast cell secretion by a "receptor-independent" mechanism.

Polycations have also been shown to interact strongly with acidic phospholipids. Polylysines of varying chain lengths (20–1000 amino acids) bind to artificial phospholipid vesicles with dissociation constants in the range of 0.5 nM to 1.5 μM. The binding affinity is directly related to the length of the polylysine chain, with polymers of 1000 amino acids having a K_d of 0.5 nM, shorter polymers having higher Kd values, and lysine not interacting to a significant extent. This relationship between potency and chain length is similar to that observed for the effects of polylysine 10,200, polylysine 3800, and lysine on parathyroid function.

It is possible that the binding of polycations to biomembranes produces some of their biologic actions. The permeabilization of the plasma membrane induced in some cell types by a variety of pore-forming agents, including polycations, has been postulated to be mediated by their interaction with a phosphatidylserine-like structure. In addition, the "receptor-independent" activation of purified G proteins by amphipathic cations is potentiated when these proteins are incorporated into phospholipid vesicles.

Calcium ions, in the millimolar concentration range, also produce marked changes in membrane structure. In some cases, calcium can either antagonize or potentiate the interaction of polycations with membrane lipids. These considerations raise the possibility that the actions of both polyvalent cations and polycations on parathyroid cells could involve a receptor-independent mechanism not requiring the presence of a classic, cell surface, G protein-coupled receptor. Further studies, however, are required to elucidate the molecular basis for Ca^{2+} sensing by this and other cell types. [Citations omitted.]

Shoback and Chen, *J. Bone Mineral Res.* 6 (Supplement 1) 1991, S135 and Racke et al., *J. Bone Mineral Res.* 6 (Supplement 1) 1991, S118) describe experiments which are said to indicate that a calcium receptor or Ca^{2+} sensor is present in parathyroid cells. Messenger RNA isolated from such cells can be expressed in oocytes and caused to provide those oocytes with a phenotype which might be explained by the presence of a calcium receptor protein.

SUMMARY OF THE INVENTION

The present invention relates to the different roles inorganic ion receptors have in cellular and body processes. The present invention features: (1) molecules which can modulate one or more inorganic ion receptor activities, preferably the molecule can mimic or block an effect of an extracellular ion on a cell having an inorganic ion receptor, more preferably the extracellular ion is Ca^{2+} and the effect is on a cell having a calcium receptor; (2) inorganic ion receptor proteins and fragments thereof, preferably calcium receptor proteins and fragments thereof; (3) nucleic acids encoding inorganic ion receptor proteins and fragments thereof, preferably calcium receptor proteins and fragments thereof; (4) antibodies and fragments thereof, targeted to inorganic ion receptor proteins, preferably calcium receptor protein; and (5) uses of such molecules, proteins, nucleic acids and antibodies.

The preferred use of the present invention is to treat diseases or disorders in a patient by modulating one or more inorganic ion receptor activities. Diseases or disorders which can be treated by modulating inorganic ion receptor activity include one or more of the following types: (1) those characterized by abnormal inorganic ion homeostasis; (2) those characterized by an abnormal amount of an extracellular or intracellular messenger whose production can be affected by inorganic ion receptor activity; (3) those characterized by an abnormal effect (e.g., a different effect in kind or magnitude) of an intracellular or extracellular messenger which can itself be ameliorated by inorganic ion receptor activity; and (4) other diseases or disorders in which modulation of inorganic ion receptor activity will exert a beneficial effect, for example, in diseases or disorders where the production of an intracellular or extracellular messenger stimulated by receptor activity compensates for an abnormal amount of a different messenger. Examples of extracellular messengers whose secretion and/or effect can be affected by modulating inorganic ion receptor activity include inorganic ions, hormones, neurotransmitters, growth factors, and chemokines. Examples of intracellular messengers include cAMP, cGMP, IP_3, and diacylglycerol.

Preferably, the compound modulates calcium receptor activity and is used in the treatment of diseases or disorders which can be affected by modulating one or more activities of a calcium receptor. Extracellular Ca^{2+} is under tight homeostatic control and controls various processes such as blood clotting, nerve and muscle excitability, and proper bone formation. Calcium receptor proteins enable certain specialized cells to respond to changes in extracellular Ca^{2+} concentration. For example, extracellular Ca^{2+} inhibits the secretion of parathyroid hormone from parathyroid cells, inhibits bone resorption by osteoclasts, and stimulates secretion of calcitonin from C-cells.

Preferably, the disease or disorder is characterized by abnormal bone and mineral homeostasis, more preferably

calcium homeostasis. Abnormal calcium homeostasis is characterized by one or more of the following activities: (1) an abnormal increase or decrease in serum calcium; (2) an abnormal increase or decrease in urinary excretion of calcium; (3) an abnormal increase or decrease in bone calcium levels, for example, as assessed by bone mineral density measurements; (4) an abnormal absorption of dietary calcium; (5) an abnormal increase or decrease in the production and/or release of messengers which affect serum calcium levels such as parathyroid hormone and calcitonin; and (6) an abnormal change in the response elicited by messengers which affect serum calcium levels. The abnormal increase or decrease in these different aspects of calcium homeostasis is relative to that occurring in the general population and is generally associated with a disease or disorder.

Diseases and disorders characterized by abnormal calcium homeostasis can be due to different cellular defects such as a defective calcium receptor activity or a defective intracellular protein acted on by a calcium receptor. For example, in parathyroid cells, the calcium receptor is coupled to the Gi protein which in turn inhibits cyclic AMP production. Defects in G protein can affect its ability to inhibit cyclic AMP production.

The inorganic ion receptor-modulating agents (e.g., molecules and compositions) can be used to treat patients. A "patient" refers to a mammal in which modulation of an inorganic ion receptor will have a beneficial effect. Patients in need of treatment involving modulation of inorganic ion receptors can be identified using standard techniques known to those in the medical profession. Preferably, a patient is a human having a disease or disorder characterized by one or more of the following: (1) abnormal inorganic ion homeostasis, more preferably abnormal calcium homeostasis; (2) an abnormal level of a messenger whose production or secretion is affected by inorganic ion receptor activity, more preferably affected by calcium receptor activity; and (3) an abnormal level or activity of a messenger whose function is affected by inorganic ion receptor activity, more preferably affected by calcium receptor activity.

Thus, a first aspect of the present invention features an inorganic ion receptor-modulating agent comprising a molecule which either evokes one or more inorganic ion receptor activities, or blocks one or more inorganic ion receptor activities. The agent has an EC_{50} of less than or equal to 5 μM at its respective receptor and is not protamine. Preferably, the inorganic ion receptor is a calcium receptor and the molecule has an EC_{50} of less than or equal to 5 μM at a calcium receptor and is not protamine.

Inorganic ion receptor activities are those processes brought about as a result of inorganic ion receptor activation. Such processes include the production of molecules which can act as intracellular or extracellular messengers.

Inorganic ion receptor-modulating agents include ionomimetics, ionolytics, calcimimetics, and calcilytics. Ionomimetics are molecules which bind to an inorganic ion receptor and mimics (i.e., evokes or potentiates) the effects of an inorganic ion at an inorganic ion receptor. Preferably, the molecule affects one or more calcium receptor activities. Calcimimetics are ionomimetics which affect one or more calcium receptor activities and bind to a calcium receptor.

Ionolytics are molecules which bind to an inorganic ion receptor and block (i.e., inhibits or diminishes) one or more activities caused by an inorganic ion on an inorganic ion receptor. Preferably, the molecule affects one or more calcium receptor activities. Calcilytics are ionolytics which inhibit one or more calcium receptor activities evoked by extracellular calcium and bind to a calcium receptor.

Ionomimetics and ionolytics may bind at the same receptor site as the native inorganic ion ligand binds or can bind at a different site (e.g., allosteric site). For example, NPS R-467 binding to a calcium receptor results in calcium receptor activity and, thus, NPS R-467 is classified as a calcimimetic. However, NPS R-467 binds to the calcium receptor at a different site (i.e., an allosteric site) than extracellular calcium.

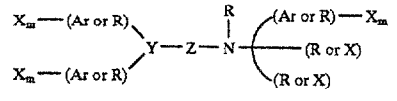
The EC_{50} is the concentration of agent which causes a half maximal mimicking effect. For example, the EC_{50} for calcium receptor activities can be determined by assaying one or more of the activities of extracellular calcium at a calcium receptor. Examples of suitable assays for measuring EC_{50} are described herein and include oocyte expression assays and measuring increases in intracellular calcium due to calcium receptor activity. Preferably, such assays measure the release or inhibition of a particular hormone associated with activity of a calcium receptor.

An inorganic ion receptor-modulating agent preferably selectively targets inorganic ion receptor activity in a particular cell. For example, selective targeting of a calcium receptor activity is achieved by an agent exerting a greater effect on a calcium receptor activity in one cell type than at another cell type for a given concentration of agent. Preferably, the differential effect is 10-fold or greater as measured in vivo or in vitro. More preferably, the differential effect is measured in vivo and the agent concentration is measured as the plasma concentration or extracellular fluid concentration and the measured effect is the production of extracellular messengers such as plasma calcitonin, parathyroid hormone, or plasma calcium. For example, in a preferred embodiment, the agent selectively targets PTH secretion over calcitonin secretion.

In one embodiment concerning the structure of the inorganic ion receptor-modulating agent, the molecule is positively charged at physiological pH, and is selected from the group consisting of branched or cyclic polyamines, positively charged polyamino acids, and arylalkylamines. Preferably, the branched polyamine has the formula $H_2N—(CH_2)_j—(NR_i—(CH_2)_j)_k—NH_2$ where k is an integer from 1 to 10, each j is the same or different and is an integer from 2 to 20, and each R_i is the same or different and is selected from the group consisting of hydrogen and $—(CH_2)_j—NH_2$, where j is as defined above, and at least one R_i is not hydrogen. Preferably, the inorganic ion receptor-modulating agent can modulate one or more calcium receptor activities.

In a preferred embodiment concerning the structure of inorganic ion receptor-modulating agents the arylalkylamine molecule has the formula:

STRUCTURE I



where each X independently is selected from the group consisting of H, CH_3, CH_3O, CH_3CH_2O, methylene dioxy, Br, Cl, F, I, CF_3, CHF_2, CH_2F, CF_3O, CF_3CH_2O, CH_3S, OH, CH_2OH, $CONH_2$, CN, NO_2, CH_3CH_2, propyl, isopropyl, butyl, isobutyl, t-butyl, and acetoxy;

Ar is a hydrophobic entity;

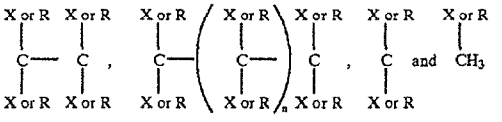
each R independently is selected from the group consisting of hydrogen, methyl, ethyl, propyl, isopropyl, allyl,

butyl, isobutyl, t-butyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, indenyl, indanyl, dihydroindolyl, thiodihydroindolyl, and 2-, 3-, or 4-piperid(in)yl;

Y is selected from the group consisting of CH, nitrogen and an unsaturated carbon; and

Z is selected from the group consisting of oxygen, nitrogen, sulfur,

STRUCTURE II



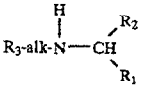
where each n is independently between 1 and 4 inclusive; and

each m is independently between 0 and 5 inclusive.

A hydrophobic entity refers to a non-polar group or moiety such as an aromatic or a cycloaliphatic ring or ring system. Preferably, the hydrophobic entity is selected from the group consisting of phenyl, cyclohexyl, 2-, 3-, or 4-pyridyl, 1- or 2-naphthyl, α- or β-tetrahydronaphthyl, 1- or 2-quinolinyl, 2- or 3-indolyl, benzyl, and phenoxy.

More preferably, the inorganic ion receptor-modulating agent is a substituted R-phenylpropyl-α-phenethylamine, substituted R-benzyl-α-1-napthylethylamine analogues, and derivatives having the formula:

STRUCTURE III



where alk is straight- or branched-chain alkylene of from 0 to 6 carbon atoms;

R_1 is lower alkyl of from 1 to 3 carbon atoms or lower haloalkyl of from 1 to 3 carbon atoms substituted with from 1 to 7 halogen atoms;

R_2 and R_3 are independently selected carbocyclic aryl or cycloalkyl groups, either monocyclic or bicyclic, having 5-to 7-membered rings optionally substituted with 1 to 5 substituents independently selected from lower alkyl of 1 to 3 carbon atoms, lower haloalkyl of 1 to 3 carbon atoms substituted with 1 to 7 halogen atoms, lower alkoxy of 1 to 3 carbon atoms, halogen, nitro, amino, alkylamino, amido, lower alkylamido of 1 to 3 carbon atoms, cyano, hydroxy, acyl of 2 to 4 carbon atoms, lower hydroxyalkyl of 1 to 3 carbon atoms or lower thioalkyl of 1 to 3 carbon atoms. Suitable carbocyclic aryl groups are groups having one or two rings, at least one of which has aromatic character and include carbocyclic aryl groups such as phenyl and bicyclic carbocyclic aryl groups such as naphthyl.

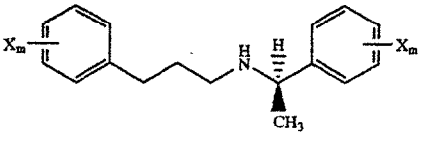
Preferred compounds include those where alk is n-propylene, methylene, or R-methyl methinyl. Also preferred are compounds where R_1 is R-methyl. Also preferred are those compounds where R_2 and R_3 are optionally substituted phenyl or naphthyl.

More preferred compounds are those where R_2 is monosubstituted phenyl, more preferably meta-substituted; or 1-naphthyl. More preferred R_3 groups are unsubstituted or monosubstituted phenyl, especially meta- or ortho-substituted, or 2-naphthyl. Preferred substituents for R_2 are halogen, haloalkyl, preferably trihalomethyl, alkoxy, preferably methoxy, and thioalkyl, preferably thiomethyl. Preferred substituents for R_3 are meta- or ortho-halogen, preferably chlorine, fluorine, or CF_3 and para- or ortho-alkoxy, preferably methoxy, and meta-lower alkyl, preferably methyl.

As is apparent from the above formula, preparation of the molecules may result in racemic mixtures containing individual stereoisomers. More preferred compounds are R-phenylpropyl-α-phenethylamine and R-benzyl-α-1-napthylethylamine derivatives which are believed to exhibit enhanced activity in lowering serum ionized calcium.

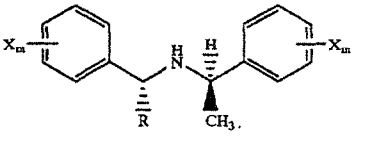
More preferably, the molecule is a substituted R-phenylpropyl-α-phenethylamine derivative, or a substituted R-benzyl-α-phenethylamine derivative, having the structure:

STRUCTURE IV



or

STRUCTURE V



where each X is preferably independently selected from the group consisting of Cl, F, I, CF_3, CH_3, isopropyl, CH_3O, CH_3S, CF_3O, CF_3CH_2O, an aliphatic ring and an attached or fused, preferably fused aromatic ring. Preferably, the aromatic and aliphatic rings have 5 to 7 members. More preferably, the aromatic and aliphatic rings contain only carbon atoms (i.e., the ring is not a heterocyclic ring); and

R is preferably H, CH_3, ethyl, or isopropyl.

In more preferred embodiments the molecule inhibits parathyroid hormone secretion from a parathyroid cell; inhibits bone resorption in vivo by an osteoclast; inhibits bone resorption in vitro by an osteoclast; stimulates calcitonin secretion in vitro or in vivo from a c-cell; or the molecule evokes the mobilization of intracellular Ca^{2+} to cause an increase in $[Ca^{2+}]_i$.

Preferably, the molecule is either a calcimimetic or calcilytic having an EC_{50} or IC_{50} at a calcium receptor of less than or equal to 5 μM, and even more preferably less than or equal to 1 μM, 100 nmolar, 10 nmolar, or 1 nmolar. Such lower EC_{50}'s or IC_{50}'s are advantageous since they allow lower concentrations of molecules to be used in vivo or in vitro for therapy or diagnosis. The discovery of molecules with such low EC_{50}'s and IC_{50}'s enables the design and synthesis of additional molecules having similar or improved potency, effectiveness, and/or selectivity.

In another preferred embodiment, the molecule has an EC_{50} or IC_{50} less than or equal to 5 μM at one or more, but not all cells chosen from the group consisting of: parathyroid cell, bone osteoclast, juxtaglomerular kidney cell, proximal tubule kidney cell, distal tubule kidney cell, central nervous

system cell, peripheral nervous system cell, cell of the thick ascending limb of Henle's loop and/or collecting duct, keratinocyte in the epidermis, parafollicular cell in the thyroid (C-cell), intestinal cell, trophoblast in the placenta, platelet, vascular smooth muscle cell, cardiac atrial cell, gastrin-secreting cell, glucagon-secreting cell, kidney mesangial cell, mammary cell, beta cell, fat/adipose cell, immune cell, GI tract cell, skin cell, adrenal cell, pituitary cell, hypothalamic cell and cell of the subfornical organ.

More preferably, the cells are chosen from the group consisting of parathyroid cell, central nervous system cell, peripheral nervous system cell, cell of the thick ascending limb of Henle's loop and/or collecting duct in the kidney, parafollicular cell in the thyroid (C-cell), intestinal cell, GI tract cell, pituitary cell, hypothalamic cell and cell of the subfornical organ. This presence of a calcium receptor in this group of cells has been confirmed by physical data such as in situ hybridization and antibody staining.

Another aspect of the present invention features a calcium receptor-modulating agent comprising a molecule selected from the group consisting of: NPS R-467, NPS R-568, compound 1D, compound 3U, compound 3V, compound 4A, compound 4B, compound 4C, compound 4D, compound 4G, compound 4H, compound 4J, compound 4M, compound 4N, compound 4P, compound 4R/6V, compound 4S, compound 4T/4U, compound 4V, compound 4W, compound 4Y, compound 4Z/5A, compound 5B/5C, compound 5W/5Y, compound 6E, compound 6F, compound 6R, compound 6T, compound 6X, compound 7W, compound 7X, compound 8D, compound 8J, compound 8K, compound 8R, compound 8S, compound 8T, compound 8U, compound 8X, compound 8Z, compound 9C, compound 9D, compound 9R, compound 9S, compound 10F, compound 11D, compound 11X, compound 11Y, compound 12L, compound 12U, compound 12V, compound 12W, compound 12Y, compound 13Q, compound 13R, compound 13S, compound 13U, compound 13X, compound 14L, compound 14Q, compound 14U, compound 14V, compound 14Y, compound 15G, compound 16Q, compound 16R, compound 16T, compound 16V, compound 16W, compound 16X, compound 17M, compound 170, compound 17P, compound 17R, compound 17W, compound 17X, compound 20F, compound 20I, compound 20J, compound 20R, compound 20S, compound 21D, compound 21F, compound 21G, compound 21O, compound 21P, compound 21Q, and compound 21R (see FIG. 36).

Another aspect of the present invention features a pharmaceutical composition made up of an inorganic ion receptor-modulating agent and a physiologically acceptable carrier. Such agents can be used to treat patients by modulating inorganic ion receptor activity.

Prior to this invention, applicant was unaware of any agent acting on the calcium receptor useful in the treatment of diseases caused by irregularity in operation or regulation of a calcium receptor or in diseases in an animal having normal calcium receptors, but which can be treated by modulating calcium activity.

A pharmacological agent or composition refers to an agent or composition in a form suitable for administration into a mammal, preferably a human. Considerations concerning forms suitable for administration are known in the art and include toxic effects, solubility, route of administration, and maintaining activity. For example, pharmacological agents or compositions injected into the blood stream should be soluble.

Pharmaceutical compositions can also be formulated as pharmaceutically acceptable salts (e.g., acid addition salts) and complexes thereof. The preparation of such salts can facilitate the pharmacological use of an agent by altering its physical characteristics without preventing it from exerting a physiological effect.

Another aspect of the present invention features a method for modulating inorganic ion receptor activity, preferably calcium receptor activity. The method involves the step of providing to a cell comprising an inorganic ion receptor an amount of an inorganic ion receptor-modulating molecule sufficient to either mimic one or more effects of an inorganic ion at the inorganic ion receptor, or block one or more effects of the inorganic ion at the inorganic ion receptor. The method can carried out in vitro or in vivo.

Preferably, the molecule is either a calcimimetic or a calcilytic which modulates one or more calcium receptor activity. Examples of calcium receptor-modulating molecules or agents are described herein. Additional calcium receptor-modulating agents can be obtained based on the present disclosure. More preferably, the method is carried out in vivo to treat a patient.

Another aspect the present invention features a method for treating a patient by modulating inorganic ion receptor activity. The method involves administering to the patient a therapeutically effective amount of an inorganic ion receptor-modulating agent.

In a preferred embodiment, the disease or disorder is treated by modulating calcium receptor activity by administering to the patient a therapeutically effective amount of a calcium receptor-modulating agent.

Preferably the disease or disorder is characterized by one or more of the following: (1) abnormal inorganic ion homeostasis, more preferably abnormal calcium homeostasis; (2) an abnormal level of a messenger whose production or secretion is affected by inorganic ion receptor activity, more preferably affected by calcium receptor activity; and (3) an abnormal level or activity of a messenger whose function is affected by inorganic ion receptor activity, more preferably affected by calcium receptor activity.

Diseases characterized by abnormal calcium homeostasis include hyperparathyroidism, osteoporosis and other bone and mineral-related disorders, and the like (as described, e.g., in standard medical text books, such as "Harrison's Principles of Internal Medicine"). Such diseases are treated using calcium receptor-modulating agents which mimic or block one or more of the effects of extracellular Ca^{2+} on a calcium receptor and, thereby, directly or indirectly affect the levels of proteins or other molecules in the body of the patient.

By "therapeutically effective amount" is meant an amount of an agent which relieves to some extent one or more symptoms of the disease or disorder in the patient; or returns to normal either partially or completely one or more physiological or biochemical parameters associated with or causative of the disease.

In a preferred embodiment, the patient has a disease or disorder characterized by an abnormal level of one or more calcium receptor-regulated components and the molecule is active on a calcium receptor of a cell selected from the group consisting of: parathyroid cell, bone osteoclast, juxtaglomerular kidney cell, proximal tubule kidney cell, distal tubule kidney cell, central nervous system cell, peripheral nervous system cell, cell of the thick ascending limb of Henle's loop and/or collecting duct, keratinocyte in the epidermis, parafollicular cell in the thyroid (C-cell), intestinal cell, trophoblast in the placenta, platelet, vascular smooth muscle cell, cardiac atrial cell, gastrin-secreting cell, glucagon-secreting cell, kidney mesangial cell, mammary cell, beta cell, fat/adipose cell, immune cell, GI tract cell, skin cell,

adrenal cell, pituitary cell, hypothalamic cell and cell of the subfornical organ.

More preferably, the cells are chosen from the group consisting of: parathyroid cell, central nervous system cell, peripheral nervous system cell, cell of the thick ascending limb of Henle's loop and/or collecting duct in the kidney, parafollicular cell in the thyroid (C-cell), intestinal cell, GI tract cell, pituitary cell, hypothalamic cell and cell of the subfornical organ.

In a preferred embodiment, the agent is a calcimimetic acting on a parathyroid cell calcium receptor and reduces the level of parathyroid hormone in the serum of the patient. More preferably, the level is reduced to a degree sufficient to cause a decrease in plasma Ca^{2+}. Most preferably, the parathyroid hormone level is reduced to that present in a normal individual.

In another preferred embodiment, the agent is a calcilytic acting on a parathyroid cell calcium receptor and increases the level of parathyroid hormone in the serum of the patient. More preferably, the level is increased to a degree sufficient to cause an increase in bone mineral density of a patient.

In another aspect, the invention features a method for diagnosing a disease or disorder in a patient characterized by an abnormal number of inorganic ion receptors, or an altered inorganic ion receptor. The method involves identifying the number and/or location and/or functional integrity of one or more inorganic ion receptor. The number and/or location and/or functional integrity is compared with that observed in patients characterized as normal or diseased as an indication of the presence of the disease or disorder.

Diagnoses can be carried out using inorganic ion receptor-binding agents. For example, calcium receptor-modulating agents binding to calcium receptors, and antibodies which bind to calcium receptors, can be used for diagnoses. Preferably, binding agents are labeled with a detectable moiety, such as a radioisotope or alkaline phosphatase.

An altered receptor has a different structure than the receptor has in normal individuals and is associated with a disease or disorder involving an inorganic ion receptor. Such alterations may affect receptor function, and can be detected by assaying for a structural difference between the altered and normal receptor. Binding agents which bind to an altered receptor, but not to a normal receptor, can be used to determine the presence of an altered receptor. Additionally, a binding agent which can bind to a normal receptor, but not to a particular altered receptor, can be used to determine the presence of the particular altered receptor.

Similarly, the number of receptors can be determined by using agents binding to the tested-for receptor. Such assays generally involve using a labeled binding agent and can be carried out using standard formats such as competitive, non-competitive, homogenous, and heterogenous assays.

In other preferred embodiments, the method is an immunoassay in which an antibody to a calcium receptor is used to identify the number and/or location and/or functional integrity of the calcium receptors; the assay involves providing a labeled calcimimetic or calcilytic molecule; the presence of a cancer, e.g., an ectopic tumor of the parathyroid, is tested for by measuring calcium receptor number or alteration; and conditions characterized by an above-normal number of osteoclasts in bone or an increased level of activity of osteoclasts in bone is tested for by measuring the number of calcium receptors.

In another aspect, the invention features a method for identifying a molecule useful as a therapeutic molecule to modulate inorganic ion receptor activity or as a diagnostic agent to diagnose patients suffering from a disease characterized by an abnormal inorganic ion activity. Preferably, the method is used to identify calcimimetics or calcilytics by screening potentially useful molecules for an ability to mimic or block an activity of extracellular Ca^{2+} on a cell having a calcium receptor and determining whether the molecule has an EC_{50} or IC_{50} of less than or equal to 5 μM. More preferably, the molecule is tested for its ability to mimic or block an increase in $[Ca^{2+}]_i$ elicited by extracellular Ca^{2+}.

Identification of inorganic ion receptor-modulating agents is facilitated by using a high-throughput screening system. High-throughput screening allows a large number of molecules to be tested. For example, a large number of molecules can be tested individually using rapid automated techniques or in combination using a combinational library. Individual compounds able to modulate inorganic ion receptor activity present in a combinational library can be obtained by purifying and retesting fractions of the combinational library. Thus, thousands to millions of molecules can be screened in a single day.

Active molecules can be used as models to design additional molecules having equivalent or increased activity. Preferably, the identification method uses a recombinant inorganic ion receptor, more preferably a recombinant calcium receptor. Recombinant receptors can be introduced into different cells using a vector encoding the receptor.

Preferably, the activity of molecules in different cells is tested to identify a calcimimetic or calcilytic molecule which mimics or blocks one or more activities of Ca^{2+} at a first type of calcium receptor, but not at a second type of calcium receptor.

Another aspect of the present invention features a purified nucleic acid containing at least 12 contiguous nucleotides of a nucleic acid sequence provided in SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3 or SEQ. ID. NO. 4. By "purified" in reference to nucleic acid is meant the nucleic acid is present in a form (i.e., its association with other molecules) other than found in nature. For example, purified receptor nucleic acid is separated from one or more nucleic acids which are present on the same chromosome. Preferably, the purified nucleic acid is separated from at least 90% of the other nucleic acids present on the same chromosome.

Another example of purified nucleic acid is recombinant nucleic acid. Preferably, recombinant nucleic acid contains nucleic acid encoding an inorganic ion receptor or receptor fragment cloned in an expression vector. An expression vector contains the necessary elements for expressing a cloned nucleic acid sequence to produce a polypeptides. An expression vector contains a promoter region (which directs the initiation of RNA transcription) as well as the DNA sequences which, when transcribed into RNA, will signal synthesis initiation.

Recombinant nucleic acid may contain nucleic acid encoding for an inorganic ion receptor, receptor fragment, or inorganic ion receptor derivative, under the control of its genomic regulatory elements, or under the control of exogenous regulatory elements including an exogenous promoter. By "exogenous" is meant a promoter that is not normally coupled in vivo transcriptionally to the coding sequence for the inorganic ion receptor. Preferably, the nucleic acid is provided as a substantially purified preparation representing at least 75%, more preferably 85%, most preferably 950 of the total nucleic acids present in the preparation.

Nucleic acid sequences provided in SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3, and SEQ. ID. NO. 4 each encode for a calcium receptor. Nucleic acid sequences encoding

both full length calcium receptors, calcium receptor fragments, derivatives of full length calcium receptors, and derivatives of calcium receptor fragments are useful in the present invention.

Uses of nucleic acids encoding cloned receptors or receptor fragments include one or more the following: (1) producing receptor proteins which can be used, for example, for structure determination, to assay a molecule's activity on a receptor, and to obtain antibodies binding to the receptor; (2) being sequenced to determine a receptor's nucleotide sequence which can be used, for example, as a basis for comparison with other receptors to determine conserved regions, determine unique nucleotide sequences for normal and altered receptors, and to determine nucleotide sequences to be used as target sites for antisense nucleic acids, ribozymes, hybridization detection probes, or PCR amplification primers; (3) as hybridization detection probes to detect the presence of a native receptor and/or a related receptor in a sample; and (4) as PCR primers to generate particular nucleic acid sequence regions, for example to generate regions to be probed by hybridization detection probes.

Preferably, the nucleic acid contains at least 14, more preferably at least 20, more preferably at least 27, and most preferably at least 45, contiguous nucleic acids of a sequence provided in SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3, or SEQ. ID. NO. 4. Advantages of longer-length nucleic acid include producing longer-length protein fragments having the sequence of a calcium receptor which can be used, for example, to produce antibodies; increased nucleic acid probe specificity under higher stringent hybridization assay conditions; and more specificity for related inorganic ion receptor nucleic acid under lower stringency hybridization assay conditions.

Another aspect of the present invention features a purified nucleic acid encoding an inorganic ion receptor or fragment thereof. The nucleic acid encodes at least 6 contiguous amino acids provided in SEQ. ID. NO. 5, SEQ. ID. NO. 6, SEQ. ID. NO. 7 or SEQ. ID. NO. 8. Due to the degeneracy of the genetic code, different combinations of nucleotides can code for the same polypeptide. Thus, numerous inorganic ion receptors and receptor fragments having the same amino acid sequences can be encoded for by different nucleic acid sequences. In preferred embodiments, the nucleic acid encodes at least 12, at least 18, or at least 54 contiguous amino acids of SEQ. ID. NO. 5, SEQ. ID. NO. 6, SEQ. ID. NO. 7 or SEQ. ID. NO. 8.

Another aspect of the present invention features a purified nucleic acid having a nucleic acid sequence region of at least 12 contiguous nucleotides substantially complementary to a sequence region in SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3 or SEQ. ID. NO. 4. By "substantially complementary" is meant that the purified nucleic acid can hybridize to the complementary sequence region in nucleic acid encoded by SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3 or SEQ. ID. NO. 4 under stringent hybridizing conditions. Such nucleic acid sequences are particularly useful as hybridization detection probes to detect the presence of nucleic acid encoding a particular receptor. Under stringent hybridization conditions, only highly complementary nucleic acid sequences hybridize. Preferably, such conditions prevent hybridization of nucleic acids having 4 mismatches out of 20 contiguous nucleotides, more preferably 2 mismatches out of 20 contiguous nucleotides, most preferably one mismatch out of 20 contiguous nucleotides. In preferred embodiments, the nucleic acid is substantially complementary to at least 20, at least 27, or at least 45, contiguous nucleotides provided in SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3, or SEQ. ID. NO. 4.

Another aspect of the present invention features a purified polypeptide having at least 6 contiguous amino acids of an amino acid sequence provided in SEQ. ID. NO. 5, SEQ. ID. NO. 6, SEQ. ID. NO. 7 or SEQ. ID. NO. 8. By "purified" in reference to a polypeptide is meant that the polypeptide is in a form (i.e., its association with other molecules) distinct from naturally occurring polypeptide. Preferably, the polypeptide is provided as a substantially purified preparation representing at least 75%, more preferably 85%, most preferably 95% of the total protein in the preparation. In preferred embodiments, the purified polypeptide has at least 12, 18, or 54 contiguous amino acids of SEQ. ID. NO. 5, SEQ. ID. NO. 6, SEQ. ID. NO. 7 or SEQ. ID. NO. 8.

Preferred receptor fragments include those having functional receptor activity, a binding site, epitope for antibody recognition (typically at six amino acids), and/or a site which binds a calcimimetic or calcilytic. Other preferred receptor fragments include those having only an extracellular portion, a transmembrane portion, an intracellular portion, and/or a multiple transmembrane portion (e.g., seven transmembrane portion). Such receptor fragments have various uses such as being used to obtain antibodies to a particular region and being used to form chimeric receptors with fragments of other receptors to create a new receptor having unique properties.

The invention also features derivatives of full-length inorganic ion receptors and fragments thereof having the same, or substantially the same, activity as the full-length parent inorganic ion receptor or fragment. Such derivatives include amino acid addition(s), substitution(s), and deletion (s) to the receptor which do not prevent the derivative receptor from carrying out one or more of the activities of the parent receptor.

Another aspect of the present invention features a recombinant cell or tissue. The recombinant cell or tissue is made up of a recombined nucleic acid sequence encoding at least 6 contiguous amino acids provided in SEQ. ID. NO. 5, SEQ. ID. NO. 6, SEQ. ID. NO. 7 or SEQ. ID. NO. 8 and a cell able to express the nucleic acid. Recombinant cells have various uses including acting as biological factories to produce polypeptides encoded for by the recombinant nucleic acid, and Eor producing cells containing a functioning calcium receptor. Cells containing a functioning calcium receptor can be used, for example, to screen for calcimimetics or calcilytics.

In preferred embodiments, the recombinant nucleic acid encodes a functioning calcium receptor, more preferably a human calcium receptor; the cell or tissue is selected from the group consisting of: parathyroid cell, bone osteoclast, juxtaglomerular kidney cell, proximal tubule kidney cell, distal tubule kidney cell, central nervous system cell, peripheral nervous system cell, cell of the thick ascending limb of Henle's loop and/or collecting duct, keratinocyte in the epidermis, parafollicular cell in the thyroid (C-cell), intestinal cell, trophoblast in the placenta, platelet, vascular smooth muscle cell, cardiac atrial cell, gastrin-secreting cell, glucagon-secreting cell, kidney mesangial cell, mammary cell, beta cell, fat/adipose cell, immune cell, GI tract cell, skin cell, adrenal cell, pituitary cell, hypothalamic cell and cell of the subfornical organ; and the recombinant nucleic acid encodes at least 12, 18 or 54 contiguous amino acids of SEQ. ID. NO. 5, SEQ. ID. NO. 6, SEQ. ID. NO. 7 or SEQ. ID. NO. 8.

Another aspect of the present invention features a calcium receptor-binding agent able to bind a polypeptide having an

amino acid sequence of SEQ. ID. NO. 5, SEQ. ID. NO. 6, SEQ. ID. NO. 7 or SEQ. ID. NO. 8. The binding agent is preferably a purified antibody which recognizes an epitope present on a polypeptide having an amino acid sequence of SEQ. ID. NO. 5, SEQ. ID. NO. 6, SEQ. ID. NO. 7 or SEQ. ID. NO. 8. Other binding agents include molecules which bind to the receptor, for example, calcimimetics and calcilytics binding to the calcium receptor.

By "purified" in reference to an antibody is meant that the antibody is in a form (i.e., its association with other molecules) distinct from naturally occurring antibody, such as in a purified form. Preferably, the antibody is provided as a purified preparation representing at least 1%, more preferably at least 50%, more preferably at least 85%, most preferably at least 95% of the total protein in the preparation.

Antibodies able to bind inorganic ion receptors have various uses such as being used as therapeutic agents to modulate calcium receptor activity; as diagnostic tools for determining calcium receptor number and/or location and/or functional integrity to diagnose a Ca^{2+}-related disease; and as research tools for studying receptor synthesis, structure, and function. For example, antibodies targeted to the calcium receptor are useful to elucidate which portion of the receptor a particular molecule such as the natural ligand, a calcimimetic, or calcilytic, binds.

In preferred embodiments, the binding agent binds to an extracellular region of a calcium receptor and the binding agent binds to a calcium receptor expressed in tissue or cells selected from the group consisting of: parathyroid cell, bone osteoclast, juxtaglomerular kidney cell, proximal tubule kidney cell, distal tubule kidney cell, central nervous system cell, peripheral nervous system cell, cell of the thick ascending limb of Henle's loop and/or collecting duct, keratinocyte in the epidermis, parafollicular cell in the thyroid (C-cell), intestinal cell, trophoblast in the placenta, platelet, vascular smooth muscle cell, cardiac atrial cell, gastrin-secreting cell, glucagon-secreting cell, kidney mesangial cell, mammary cell, beta cell, fat/adipose cell, immune cell, GI tract cell, skin cell, adrenal cell, pituitary cell, hypothalamic cell and cell of the subfornical organ. More preferably, the cells are chosen from the group consisting of parathyroid cell, central nervous system cell, peripheral nervous system cell, cell of the thick ascending limb of Henle's loop and/or collecting duct in the kidney, parafollicular cell in the thyroid (C-cell), intestinal cell, GI tract cell, pituitary cell, hypothalamic cell and cell of the subfornical organ.

In other preferred embodiments, the binding agent is coupled to a toxin. Binding agents coupled to a toxin can be used to deliver the toxin to a cell containing a particular receptor. For example, an antibody coupled to a toxin directed to a cancer cell characterized by an abnormal receptor can selectively kill the cancer cell.

In other aspects, the invention provides transgenic, nonhuman mammals containing a transgene encoding an inorganic ion receptor or a gene affecting the expression of an inorganic ion receptor and methods of creating a transgenic nonhuman mammal containing a transgene encoding an inorganic ion receptor. Preferably, these aspects use a calcium receptor.

Transgenic nonhuman mammals are particularly useful as an in vivo test system for studying the effects of introducing an inorganic ion receptor, preferably a calcium receptor; regulating the expression of an inorganic ion receptor, preferably a calcium receptor (i.e., through the introduction of additional genes, antisense nucleic acids, or ribozymes); and studying the effect of molecules which mimic or block the effect of inorganic ions on an inorganic ion receptor, preferably mimic or block the effect of calcium on a calcium receptor. In preferred embodiments, the transgene encodes a calcium receptor; alters the expression of a calcium receptor; inactivates the expression of the inorganic ion receptor, preferably a calcium receptor; and up-regulates or down-regulates the expression of the inorganic ion receptor, preferably a calcium receptor.

Another aspect of the present invention features a method for treating a patient by administering a therapeutically effective amount of nucleic acid encoding a functioning inorganic ion receptor. Preferably, nucleic acid encoding a functioning calcium receptor is administered to a patient having a disease or disorder characterized by one or more of the following: (1) abnormal calcium homeostasis; (2) an abnormal level of a messenger whose production or secretion is affected by calcium receptor activity; and (3) an abnormal level or activity of a messenger whose function is affected by calcium receptor activity. The nucleic acid can be administered using standard techniques such as through the use of retroviral vectors and liposomes.

Another aspect of the present invention features a method for treating a patient by administering a therapeutically effective amount of a nucleic acid which inhibits expression of an inorganic ion receptor. Preferably, the administered nucleic acid inhibits expression of a calcium receptor and the disease or disorder is characterized by one or more of the following: (1) abnormal calcium homeostasis; (2) an abnormal level of a messenger whose production or secretion is affected by calcium receptor activity; and (3) an abnormal level or activity of a messenger whose function is affected by calcium receptor activity.

Nucleic acids able to inhibit expression of an inorganic ion receptor include anti-sense oligonucleotides, ribozymes and nucleic acid able to combine through homologous recombination with an endogenous gene encoding the receptor. Target sites of inhibitory nucleic acid include promoters, other regulatory agents acting on promoters, mRNA; pre-processed mRNA, and genomic DNA. Administration can be carried out by providing a transgene encoding the agent or by any other suitable method depending upon the use to which the particular method is directed.

Another aspect of the present invention features a method for identifying an inorganic ion receptor-modulating agent. The method involves contacting a cell containing a recombinant nucleic acid encoding an inorganic ion receptor with the agent and detecting a change in inorganic ion receptor activity. Preferably, the method is used to identify a calcium receptor-modulating agent.

Thus, the present invention features agents and methods useful in the diagnosis and treatment of a variety diseases and disorders by targeting inorganic ion receptor activity. For example, molecules mimicking external calcium may be used to selectively depress secretion of parathyroid hormone from parathyroid cells, or depress bone resorption by osteoclasts, or stimulate secretion of calcitonin from C-cells. Such molecules can be used to treat diseases characterized by abnormal calcium homeostasis such as hyperparathyroidism and osteoporosis.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1*a*–1*f* depict representative molecules useful in the invention.

FIG. 2 is a graphical representation showing increases in $[Ca^{2+}]_i$ induced by extracellular Ca^{2+} in quin-2- or fura-2-