CALCIUM CHANNEL BLOCKERS

This application is a continuation-in-part of U.S. Serial No. 09/107,037 filed 30 June 1998 and now allowed, and the contents of which are incorporated herein by reference.

Technical Field

The invention relates to compounds useful in treating conditions associated with calcium channel function. More specifically, the invention concerns compounds containing benzhydril and 6-membered heterocyclic moieties that are useful in treatment of conditions such as stroke and pain.

Background Art

Native calcium channels have been classified by their electrophysiological and pharmacological properties as T, L, N, P and Q types (for reviews see McCleskey, E.W. *et al. Curr Topics Membr* (1991) 39:295-326, and Dunlap, K. *et al. Trends Neurosci* (1995) 18:89-98). T-type (or low voltage-activated) channels describe a broad class of molecules that transiently activate at negative potentials and are highly sensitive to changes in resting potential. The L, N, P and Q-type channels activate at more positive potentials (high voltage activated) and display diverse kinetics and voltage-dependent properties. There is some overlap in biophysical properties of the high voltage-activated channels, consequently pharmacological profiles are useful to further distinguish them. L-type channels are sensitive to dihydropyridine agonists and antagonists, N-type channels are blocked by the *Conus geographus* peptide toxin, ω-conotoxin GVIA, and P-type channels are blocked by the peptide ω-agatoxin IVA from the venom of the funnel web spider, *Agelenopsis aperta*. A fourth type of high voltage-activated calcium channel (Q-type) has been described, although whether the Q- and P-type channels are distinct molecular entities is controversial (Sather, W.A. *et al. Neuron* (1995) 11:291-303; Stea, A. *et al. Proc Natl Acad Sci USA* (1994) 91:10576-10580; Bourinet, E. *et al. Nature Neuroscience* (1999) 2:407-415). Several types of calcium conductances do not fall neatly into any of the above categories and there is variability of properties even within a category suggesting that additional calcium channels subtypes remain to be classified.

Biochemical analyses show that neuronal high voltage activated calcium channels are heterooligomeric complexes consisting of three distinct subunits (α_1, $\alpha_2\delta$ and β) (reviewed by De Waard, M. *et al. Ion Channels* (1997) vol. 4, Narahashi, T. ed. Plenum Press, NY). The α_1 subunit is the major pore-forming subunit and contains the voltage sensor and binding sites for calcium channel antagonists. The mainly extracellular α_2 is disulfide-linked to the transmembrane δ subunit and both are derived from the same gene and are proteolytically cleaved *in vivo*. The β subunit is a nonglycosylated, hydrophilic protein with a high affinity of binding to a cytoplasmic region of the α_1 subunit. A fourth subunit, γ, is unique to L-type calcium channels expressed in skeletal muscle T-tubules. The isolation and characterization of γ-subunit-encoding cDNAs is described in U.S. Patent No. 5,386,025 which is incorporated herein by reference.

Recently, each of these α_1 subtypes has been cloned and expressed, thus permitting more extensive pharmacological studies. These channels have been designated α_{1A}-α_{1I} and α_{1S} and correlated with the subtypes set forth above. α_{1A} channels are of the P/Q type; α_{1B} represents N; α_{1C}, α'_{1D}, α_{1F} and α_{1S} represent L; α_{1E} represents a novel type of calcium conductance, and α_{1G}-α_{1I} represent members of the T-type family, reviewed in Stea, A. *et al.* in Handbook of Receptors and Channels (1994), North, R.A. ed. CRC Press; Perez-Reyes, *et al. Nature* (1998) 391:896-900; Cribbs, L.L. *et al. Circulation Research* (1998) 83:103-109; Lee, J.H. *et al. Journal of Neuroscience* (1999) 19:1912-1921.

U.S. Patent No. 5,646,149 describes calcium antagonists of the formula A-Y-B wherein B contains a piperazine or piperidine ring directly linked to Y. An essential component of these molecules is represented by A, which must be an antioxidant; the piperazine or piperidine itself is said to be important. The exemplified compounds contain a benzhydril substituent, based on known calcium channel blockers (see below). U.S. Patent No. 5,703,071 discloses compounds said to be useful in treating ischemic diseases. A mandatory portion of the molecule is a tropolone residue; among the substituents permitted are piperazine derivatives, including their benzhydril derivatives. U.S. Patent No. 5,428,038 discloses compounds which are said to exert a neural protective and antiallergic effect. These compounds are coumarin derivatives which may include derivatives of piperazine and other six-membered heterocycles. A permitted substituent on the heterocycle is diphenylhydroxymethyl. Thus, approaches in the art for various indications which may involve calcium channel blocking activity have employed

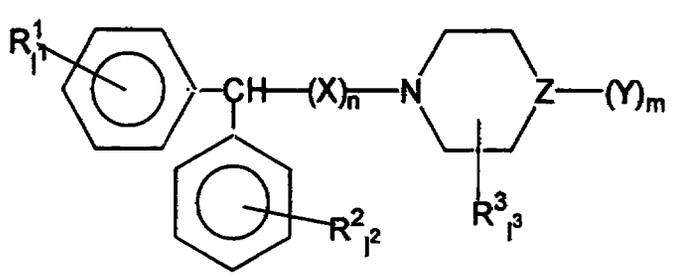
compounds which incidentally contain piperidine or piperazine moieties substituted with benzhydril but mandate additional substituents to maintain functionality.

Certain compounds containing both benzhydril moieties and piperidine or piperazine are known to be calcium channel antagonists and neuroleptic drugs. For example, Gould, R.J. *et al. Proc Natl Acad Sci USA* (1983) 80:5122-5125 describes antischizophrenic neuroleptic drugs such as lidoflazine, fluspirilene, pimozide, clopimozide, and penfluridol. It has also been that fluspirilene binds to sites on L-type calcium channels (King, V.K. *et al. J Biol Chem* (1989) 264:5633-5641) as well as blocking N-type calcium current (Grantham, C.J. *et al. Brit J Pharmacol* (1944) 111:483-488). In addition, lomerizine, as marketed by Kenebo KK, is a known calcium channel blocker. A review of publications concerning lomerizine is found in Dooley, D., *Current Opinion in CPNS Investigational Drugs* (1999) 1:116-125.

The present invention is based on the recognition that the combination of a six-membered heterocyclic ring containing at least one nitrogen coupled optionally through a linker to a benzhydril moiety not only results in calcium channel blocking activity, but also enhanced specificity for N-type channels, thus making these compounds particularly useful for treating stroke and pain. By focusing on these moieties, compounds useful in treating indications associated with excessive calcium channel activity and combinatorial libraries that contain these compounds can be prepared.

Disclosure of the Invention

The invention relates to compounds useful in treating conditions such as stroke, chronic and acute pain, epilepsy, hypertension, cardiac arrhythmias, and other indications associated with calcium metabolism. The compounds of the invention are benzhydril derivatives of piperidine, piperazine, or morpholine with substituents which enhance the calcium channel blocking activity of the compounds but do not contain substituents that are antioxidants, tropholones or coumarins. Thus, in one aspect, the invention is directed to therapeutic methods that employ compounds of the formula



(1)

wherein m is 0, 1 or 2;

wherein when m is 0, Z is O, when m is 1, Z is N, and when m is 2, Z is C;

Y is H, OH, NH_2, or an organic moiety of 1-20C, optionally additionally containing 1-8 heteroatoms selected from the group consisting of N, P, O, S and halo;

each l^1 and l^2 is independently 0-5;

l^3 is 0 or 1;

each of R^1, R^2 and R^3 is independently alkyl (1-6C), aryl (6-10C) or arylalkyl (7-16C) optionally containing 1-4 heteroatoms selected from the group consisting of halo, N, P, O, and S or each of R^1 and R^2 may independently be halo, COOR, $CONR_2$, CF_3, CN or NO_2, wherein R is H or lower alkyl (1-4C) or alkyl (1-6C);

n is 0 or 1;

X is a linker;

with the proviso that Y is not a tropolone, a coumarin, or an antioxidant containing an aromatic group and with the further proviso that if l^3 is 0, neither R^1 nor R^2 represents F in the para position.

The invention is directed to methods to antagonize calcium channel activity using the compounds of formula (1) and thus to treat associated conditions. It will be noted that the conditions may be associated with abnormal calcium channel activity, or the subject may have normal calcium channel function which nevertheless results in an undesirable physical or metabolic state. In another aspect, the invention is directed to pharmaceutical compositions containing these compounds.

The invention is also directed to combinatorial libraries containing the compounds of formula (1) and to methods to screen these libraries for members containing particularly potent calcium channel blocking activity or for members that antagonize other receptors. The libraries may also contain compounds of formula (1) where the provisos do not apply.

Brief Description of the Drawings

Figure 1 shows the structure of several known compounds which have been shown to exhibit calcium channel antagonistic activity.

Figure 2 shows the structure of several known compounds which have been demonstrated to lack calcium channel blocking activity at acceptable concentrations.

Modes of Carrying out the Invention

The compounds of formula (1), useful in the methods of the invention, exert their desirable effects through their ability to antagonize the activity of calcium channels. While the compounds of formula (1) generally have this activity, the availability of a multiplicity of calcium channel blockers permits a nuanced selection of compounds for particular disorders. Thus, the availability of this class of compounds provides not only a genus of general utility in indications that are affected by excessive calcium channel activity, but also provides a large number of compounds which can be mined and manipulated for specific interaction with particular forms of calcium channels. The availability of recombinantly produced calcium channels of the α_{1A}-α_{1I} and α_{1S} types set forth above, facilitates this selection process. Dubel, S.J. *et al. Proc Natl Acad Sci USA* (1992) 89:5058-5062; Fujita, Y. *et al. Neuron* (1993) 10:585-598; Mikami, A. *et al. Nature* (1989) 340:230-233; Mori, Y. *et al. Nature* (1991) 350:398-402; Snutch, T.P. *et al. Neuron* (1991) 7:45-57; Soong, T.W. *et al. Science* (1993) 260:1133-1136; Tomlinson, W.J. *et al. Neuropharmacology* (1993) 32:1117-1126; Williams, M.E. *et al. Neuron* (1992) 8:71-84; Williams, M.E. *et al. Science* (1992) 257:389-395; Perez-Reyes, *et al. Nature* (1998) 391:896-900; Cribbs, L.L. *et al. Circulation Research* (1998) 83:103-109; Lee, J.H. *et al. Journal of Neuroscience* (1999) 19:1912-1921.

Thus, while it is known that calcium channel activity is involved in a multiplicity of disorders, the types of channels associated with particular conditions is the subject of ongoing data collection. The association of, for example, N-type channels, as opposed to other types, in a specific condition would indicate that compounds of the invention which specifically target N-type receptors are most useful in this condition. Many of the members of the genus of compounds of formula (1) are likely to specifically target N-type channels. Other members of the genus may target other channels

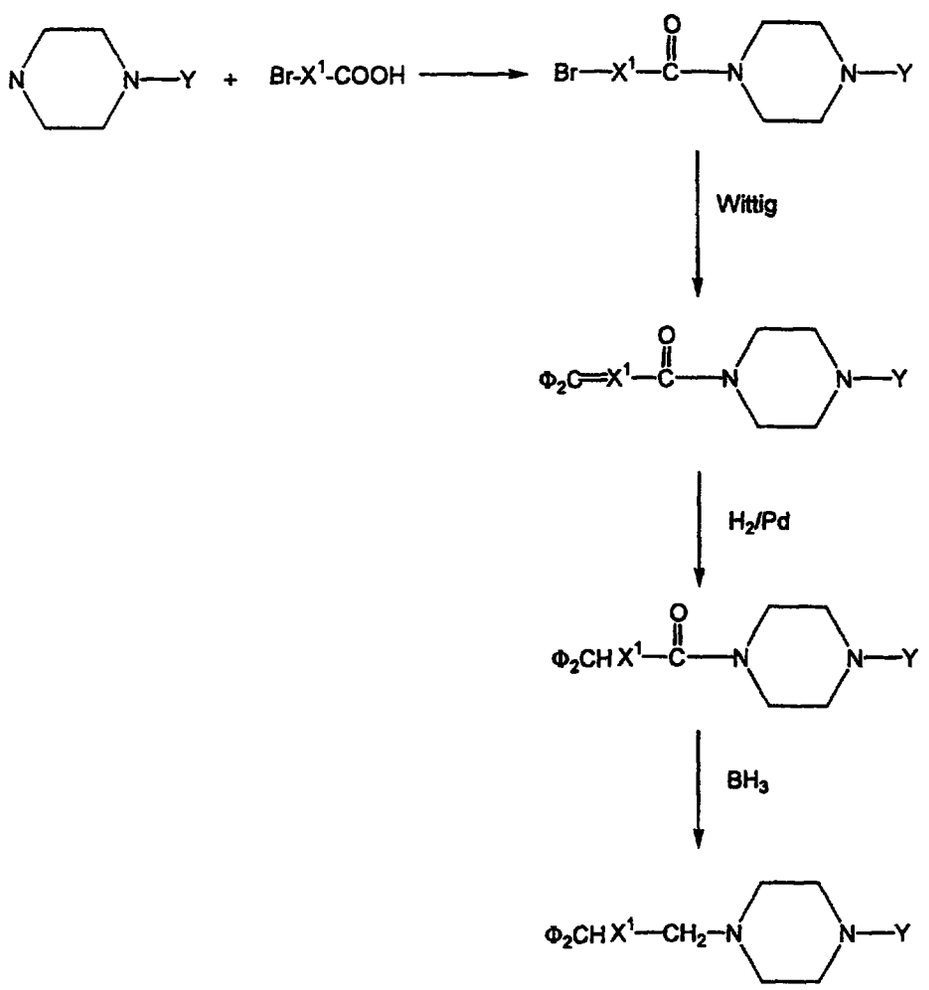
Among the conditions associated in which blocking excessive calcium would be of therapeutic value are stroke, epilepsy, and chronic and acute pain. Other cardiovascular conditions include hypertension and cardiac arrhythmias. Calcium is also implicated in other neurological disorders such as migraine, epilepsy and certain degenerative disorders.

The availability of the libraries containing the compounds of formula (1) (including those to which the provisos do not apply) also provides a source of compounds which may be screened for activity with regard to additional ion channels and receptors.

These channels and receptors are also associated with conditions that are susceptible to treatment. Blockers of sodium channels, for example, are useful as local anesthetics, and in treating cardiac arrhythmias, as anticonvulsants, and in treating hyperkalemic periodic paralysis. Potassium channel blockers are useful in treating hypertension and cardiac arrhythmias; various other receptors are associated with psychoses, schizophrenia, depression, and apnea. Thus, the library of compounds of the invention is useful in standard screening techniques as a source of effective pharmaceutical compounds.

The compounds of the invention may be synthesized using conventional methods. Illustrative of such methods are Schemes 1 and 2:

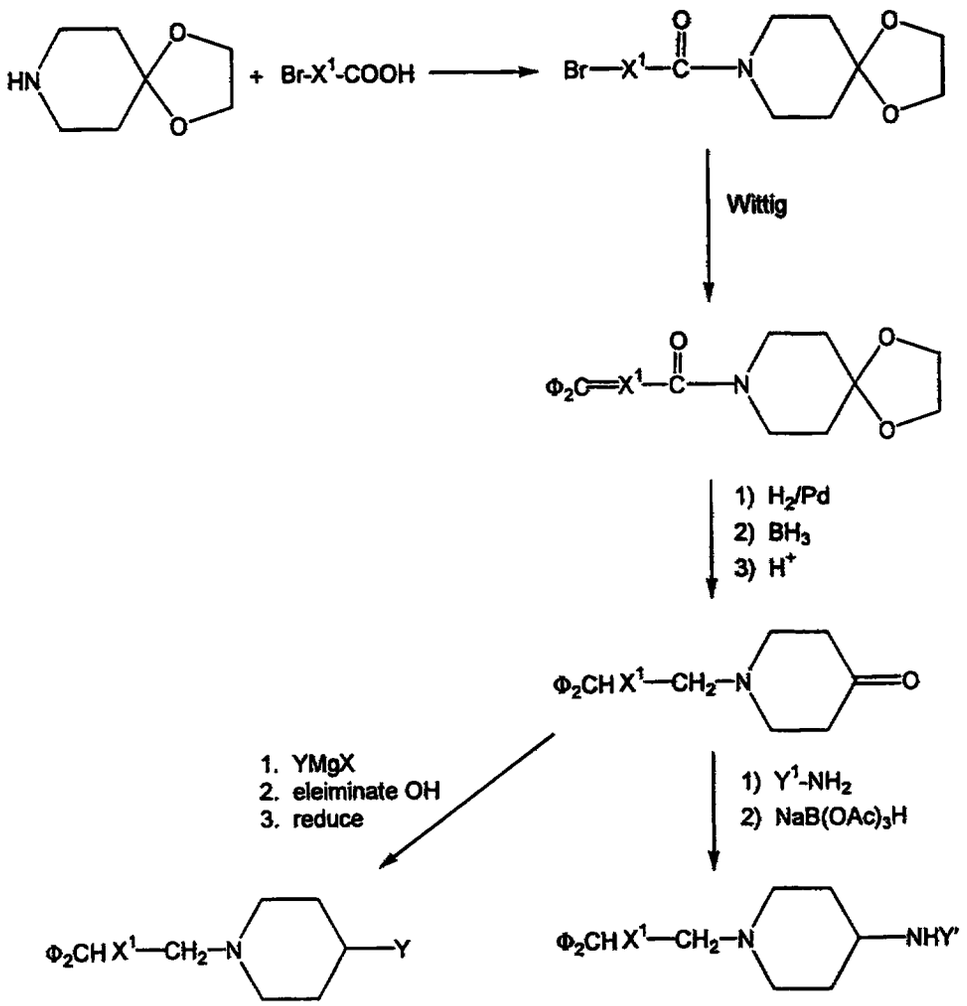
Scheme 1 (Z is N)



Alternatively, a carboxylic acid containing the benzhydril moiety can be synthesized and then reacted with the piperazine (or piperidine) moiety and subsequently reduced. Under those circumstances, an ω-bromo carboxylic acid is refluxed with triphenylphosphine in the presence of methyl nitrile and then treated with lithium hexamethyldisilazide in a solvent such as THF. The resulting unsaturated carboxylic acid

containing the two phenyl substituents is then reduced as shown in Scheme 1 with hydrogen on a palladium catalyst and then reacted with derivatized piperazine (or piperidine) to form the amide. The amide can then be reduced as shown above.

Scheme 2 (Z is CH)



The compounds of formula (1) are defined as shown in terms of the embodiments of their various substituents:

Z may be O, N or C where m has the appropriate value, i.e., O when m is 0, N when m is 1 or C when m is 2. When m is 2, one of the Y substituents is preferably H, OR, NR_2, wherein R is H alkyl (1-6C) or one Y may be itself alkyl (1-6C). Preferred forms of Z are N, and C where one Y is H or OH.

Y is H, OH or NH_2, or an organic moiety of 1-25C, optionally additionally containing 1-8 heteroatoms selected from the group consisting of N, P, O, S and halo. Preferred forms of at least one Y include those that comprise an aromatic ring system, including fused ring systems and rings containing one or more heteroatoms. Particularly

preferred forms of at least one Y are those which include phenyl moieties. The aromatic moieties included within Y may be substituted or unsubstituted; the "substituents" may include alkyl (1-6C), halo, OR, SR, NR_2, COOR, or $CONR_2$ wherein each R is independently H or alkyl (1-6C) or the "substituents" may be CN, CF_3, or NO_2. This set of moieties will be referred to herein as "substituents." Of course, if Z is O, Y is not present (m=0).

Additional preferred embodiments of Y include: aminoindane, azulene, cyclohexane, cyclohexanol, hexahydroazepin, indane, indene, indazole, indole, indolazine, morpholine, phenothiazine, phenoxazine, piperidine, pyrrole, pyridine, pyrimidine, thionaphthene, thiomorpholine, thiazine, and thiazole or these systems linked through an additional linker. When m is 2, the two Y groups may be the same or different and preferred forms are those set forth above. Particularly preferred, however, are embodiments where, when m is 2 and Z is C, one Y is selected from the foregoing list and the other Y is H or OH.

R^3 may be alkyl (1-6C) aryl (6-10C), or arylalkyl (7-16C) optionally containing 1-4 heteroatoms selected from the group consisting of N, P, O, S, and halo; preferred embodiments of R^3 include methyl. Typically, l^3 is 0 or 1.

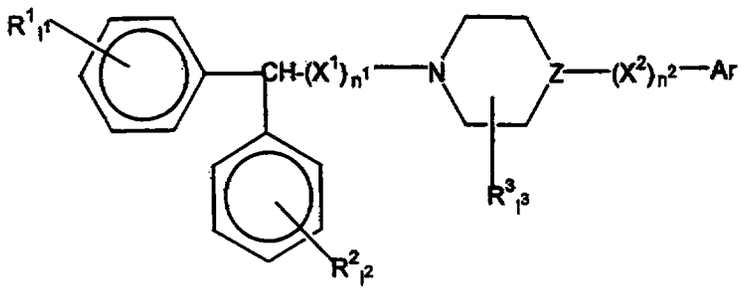
As n may be 0 or 1, X may be present or not. X is a suitable linker containing 1-10C which may be saturated or unsaturated and may contain a ring. The linker may also contain one or two heteroatoms selected from N, O and S and may be substituted with the "substituents" listed above. Preferred embodiments of X include $-(CH_2)_n-$ wherein n is 1-10, preferably 1-6.

R^1 and R^2 may independently be alkyl (1-6C) aryl (6-10C), or arylalkyl (7-16C) optionally containing 1-4 heteroatoms and optionally containing any of the "substituents" set forth above or R^1 and R^2 may themselves independently be said substituents; l^1 and l^2 are each independently 0-5, but preferably 0-3. Preferred embodiments of l^1 and l^2 include 1, where the substituent is in the para position (1p) or 3 where the substituents are in the two ortho positions and the para position (3o,p) or 2 where the substituents are in the meta positions (2m). Preferred forms of R^1 and R^2 include phenyl, phenylalkyl, Cl, Br, I, CF_3, amino and alkyl.

In the methods of treatment using the compounds of formula (1), Y must be other than a tropolone, a coumarin, or an oxidant containing an aromatic group. In addition, in these methods the compounds of formula (1) cannot include those wherein l^3 is 0 and

either R^1 or R^2 represents F in the para position. In the libraries containing compounds of formula (1), these provisos do not apply.

Preferred compounds for use in the method of the invention include those of the formulas (1a)



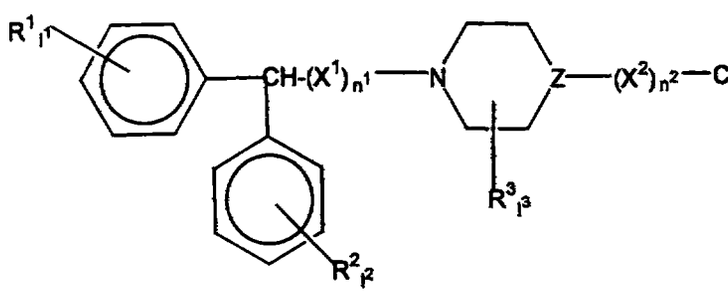
(1a)

wherein Z is N or CH;

wherein each of n^1 and n^2 is independently 0 or 1;

X^1 and X^2 are linkers; and

Ar represents one or two substituted or unsubstituted aromatic or heteroaromatic rings, and of (1b)



(1b)

wherein Z is N or CH;

wherein each of n^1 and n^2 is independently 0 or 1;

X^1 and X^2 are linkers; and

Cy represents one or two substituted or unsubstituted aliphatic cyclic or heterocyclic moieties, or consists of one substituted or unsubstituted aliphatic cyclic or heterocyclic moiety and one substituted or unsubstituted aromatic or heteroaromatic moiety.

Thus, formulas (1a) and (1b) are similar, except that compounds of formula (1a) contain aromatic substituents linked to the heterocyclic 6-membered ring and those of (1b) contain aliphatic cyclic or heterocyclic moieties. In each case, preferably when X^2 is present, X^2 represents a linker which spaces the Ar or Cy moiety from Z at a distance of 3-20Å, and may contain at least one heteroatom which is nitrogen or oxygen. Included in such linkers are amines and carbonyl functionalities, including amides. The linker may

also be unsaturated or may be an alkylene group. Typically, X_2 is $(CH_2)_{1-8}$ or $(CH_2)_{1-5}$-CH=CH-$(CH_2)_{0-3}$-. Similarly, X^1, when present, spaces the benzhydril moiety from the nitrogen of the heterocyclic ring at a distance of 3-20Å and may contain a heteroatom. Preferred embodiments are similar to those for X^2.

In both cases, when there are two aromatic or heterocyclic moieties, X^2 must accommodate this and a typical embodiment is -$(CH_2)_{0-6}$-CH, which may also contain a π-bond.

Thus, in preferred forms of formulas (1a) and (1b), n^1 is 1 and X^1 is $(CH_2)_{1-5}CO(CH_2)_{0-3}$, $(CH_2)_{1-5}NH(CH_2)_{0-3}$, $(CH_2)_{1-5}CONH(CH_2)_{0-3}$, and $(CH_2)_{1-5}NHCO(CH_2)_{0-3}$.

The preferred embodiments for X^2 are similar except that in instances where Ar or Cy represent two rings, the two rings are coupled to CH as the terminal portion of the linker X^2. When X^1 and X^2 are selected from these preferred embodiments, although it is preferred that l^1 and l^2 are both 0, substitution by R^1 and R^2 in the benzhydril system is permitted as set forth in the description of the invention above, and may also include, in these instances, a para-fluoro substituent.

It is believed that halogenation of the compounds of the invention is helpful in modulating the *in vivo* half-life, and it may be advantageous to include halogen substituents as R^1 and R^2. In formulas (1a) and (1b), such substituents may also be included on Ar and Cy.

The invention compounds may also be supplied as pharmaceutically acceptable salts. Pharmaceutically acceptable salts include the acid addition salts which can be formed from inorganic acids such as hydrochloric, sulfuric, and phosphoric acid or from organic acids such as acetic, propionic, glutamic, glutaric, as well as acid ion-exchange resins.

Utility and Administration

For use as treatment of animal subjects, the compounds of the invention can be formulated as pharmaceutical or veterinary compositions. Depending on the subject to be treated, the mode of administration, and the type of treatment desired -- e.g., prevention, prophylaxis, therapy; the compounds are formulated in ways consonant with these parameters. A summary of such techniques is found in Remington's Pharmaceutical Sciences, latest edition, Mack Publishing Co., Easton, PA.

In general, for use in treatment, the compounds of formula (1) may be used alone, as mixtures of two or more compounds of formula (1) or in combination with other pharmaceuticals. Depending on the mode of administration, the compounds will be formulated into suitable compositions to permit facile delivery.

Formulations may be prepared in a manner suitable for systemic administration or topical or local administration. Systemic formulations include those designed for injection (e.g., intramuscular, intravenous or subcutaneous injection) or may be prepared for transdermal, transmucosal, or oral administration. The formulation will generally include a diluent as well as, in some cases, adjuvants, buffers, preservatives and the like. The compounds can be administered also in liposomal compositions or as microemulsions.

For injection, formulations can be prepared in conventional forms as liquid solutions or suspensions or as solid forms suitable for solution or suspension in liquid prior to injection or as emulsions. Suitable excipients include, for example, water, saline, dextrose, glycerol and the like. Such compositions may also contain amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as, for example, sodium acetate, sorbitan monolaurate, and so forth.

Various sustained release systems for drugs have also been devised. See, for example, U.S. Patent No. 5,624,677.

Systemic administration may also include relatively noninvasive methods such as the use of suppositories, transdermal patches, transmucosal delivery and intranasal administration. Oral administration is also suitable for compounds of the invention. Suitable forms include syrups, capsules, tablets, as in understood in the art.

For administration to animal or human subjects, the dosage of the compounds of the invention is typically 0.1-100 μg/kg. However, dosage levels are highly dependent on the nature of the condition, the condition of the patient, the judgment of the practitioner, and the frequency and mode of administration.

Screening Methods

The compounds of the invention can be synthesized individually using methods known in the art *per se*, or as members of a combinatorial library. In general, the benzhydril portion of the molecule, typically containing any R^1 and R^2 substituents is coupled, along with any linking moiety, to the nitrogen of the morpholine, piperazine or

piperidine ring. This ring itself is generally appropriately substituted prior to this coupling. Typically, the benzhydril-linker portion is supplied containing a suitable electron-withdrawing leaving group, thus effecting the coupling to the ring nitrogen.

In addition to condensing a halogenated derivative of a benzhydril moiety to the nitrogen-containing heterocycle, additional conventional ways of condensing the relevant portions of the molecule can be used. For example, a brominated form of appropriately substituted benzhydril may be converted to a Grignard reagent which can then be condensed with, for example, the morpholine, piperidine, or piperazine ring extended at the nitrogen through the moiety $(CH_2)_nCHO$ wherein n is an integer from 1-4. Alternatively, a bromoalkylated form of the nitrogen-containing heterocycle may be converted to a Grignard reagent and condensed with the appropriately substituted diphenylketone. In addition, an aminoalkylated form of the nitrogen-containing heterocycle may be condensed with appropriately substituted diphenylketone to obtain the imine which can then be reduced, if desired. Finally, the two phenyl moieties associated with the benzhydril group can be prepared separately and condensed to obtain benzhydril alcohol using a Grignard reagent prepared from one phenyl group and the appropriately substituted benzaldehyde. The benzhydril alcohol can then be brominated or further extended by alkylation and condensed with the morpholine, piperidine or piperazine derivative.

Synthesis of combinatorial libraries is now commonplace in the art. Suitable descriptions of such syntheses are found, for example, in Wentworth, Jr., P. *et al. Current Opinion in Biol* (1993) 9:109-115; Salemme, F.R. *et al. Structure* (1997) 5:319-324. The libraries contain compounds with various embodiments of R^1, R^2, R^3, X, Y and Z. These libraries, which contain, as few as 10, but typically several hundred members to several thousand members, may then be screened for compounds which are particularly effective against a specific subtype of calcium channel. In addition, using standard screening protocols, the libraries may be screened for compounds which block additional channels or receptors such as sodium channels, potassium channels and the like.

Methods of performing these screening functions are well known in the art. Typically, the receptor to be targeted is expressed at the surface of a recombinant host cell such as human embryonic kidney cells. The ability of the members of the library to bind the receptor or channel is measured, for example, by the ability of the compound in the library to displace a labeled binding ligand such as the ligand normally associated with

the receptor or an antibody to the receptor. More typically, ability to antagonize the receptor is measured in the presence of the appropriate agonist and the ability of the compound to interfere with the signal generated is measured using standard techniques.

In more detail, one method involves the binding of radiolabeled agents that interact with the calcium channel and subsequent analysis of equilibrium binding measurements including, but not limited to, on rates, off rates, K_d values and competitive binding by other molecules. Another method involves the screening for the effects of compounds by electrophysiological assay whereby individual cells are impaled with a microelectrode and currents through the calcium channel are recorded before and after application of the compound of interest. Another method, high-throughput spectrophotometric assay, utilizes loading of the cell lines with a fluorescent dye sensitive to intracellular calcium concentration and subsequent examination of the effects of compounds on the ability of depolarization by potassium chloride or other means to alter intracellular calcium levels.

The following examples are intended to illustrate but not to limit the invention.

Example 1

Correlation of Calcium Channel Blocking with the Presence of a Piperidine/Piperazine Ring

Antagonist activity was measured using nystatin patch recordings on human embryonic kidney cells either stably or transiently expressing rat α_{1B}+α_{2b}+β_{1b} channels with 5 mM barium as a charge carrier.

For transient expression, host cells, such as human embryonic kidney cells, HEK 293 (ATCC# CRL 1573) are grown in standard DMEM medium supplemented with 2 mM glutamine and 10% fetal bovine serum. HEK 293 cells are transfected by a standard calcium-phosphate-DNA coprecipitation method using the rat $\alpha_{1B} + \beta_{1b} + \alpha_2\delta$ N-type calcium channel subunits in a vertebrate expression vector (for example, see *Current Protocols in Molecular Biology*).

After an incubation period of from 24 to 72 hrs the culture medium is removed and replaced with external recording solution (see below). Whole cell patch clamp experiments are performed using an Axopatch 200B amplifier (Axon Instruments, Burlingame, CA) linked to an IBM compatible personal computer equipped with

pCLAMP software. The external recording solution is 5-20 mM $BaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 40 mM TEACl, 10 mM Glucose, 65 mM CsCl (pH 7.2). The internal pipette solution is 105 mM CsCl, 25 mM TEACl, 1 mM $CaCl_2$, 11 mM EGTA, 10 mM HEPES (pH 7.2). Currents are typically elicited from a holding potential of -100 mV to various test potentials. Data are filtered at 1 kHz and recorded directly on the hard drive of a personal computer. Leak subtraction is carried out on-line using a standard P/5 protocol. Currents are analyzed using pCLAMP versions 5.5 and 6.0. Macroscopic current-voltage relations are fitted with the equation $I = \{1/(1+\exp(-(V_m-V_h)/S)\} \times G - (V_m-E_{rev})$, where V_m is the test potential, V_h is the voltage at which half of the channels are activated, and S reflects the steepness of the activation curve and is an indication of the effective gating charge movement. Inactivation curves are normalized to 1 and fitted with $I = (1/1+\exp((V_m-V_h)/S)$ with V_m being the holding potential.

The results of three experiments were averaged. The structures of most of the compounds tested are shown in Figures 1 and 2. The compounds of Figure 1 showed effective blocking activity:

Penfluridol has an IC_{50} of 5 μM; the block develops over 60-90 sec at 10 μM concentrations and is poorly reversible (pKa=9.0).

Pimozide shows an IC_{50} of about 2-3 μM; the block develops in 90 sec at 10 μM and is completely reversible (pKa=7.32). More than 80% of the activity is blocked at 10 μM.

Haloperidol has an IC_{50} of 90 μM and the block develops in less than 16 sec. It is reversible within 15 sec (pKa=8.3). At 10 μM concentrations, the block is about 10%.

Flunarizine has an IC_{50} of <1 μM; the block develops over about 120 sec at 10 μM concentration and reverses over about 5 min. The block is 90-95% effective at 10 μM.

On the other hand, less activity was shown by prenylamine (IC_{50} >40 μM); pridinol (IC_{50} >400 μM); primidone (IC_{50} >500 μM); and piperidolate (IC_{50} >300 μM). Additional compounds which showed high values for IC_{50} include bupivacaine, tolylpiperazine, piperine, trifluoromethylphenothiazine, morpholineacetophenone, morpholinebenzophenone and chloroethylpiperazine. As shown, the compounds of formula (1) which show activity comprise those wherein the CH attached to X is in turn bound to two phenyl rings and Y contains one phenyl ring, optionally substituted by halo.

Example 2

Synthesis of Illustrative Compounds of Formula (1)

A. Synthesis of 6,6-Diphenyl Hexanoic Acid.

6-Bromohexanoic acid (7.08g, 36.3 mmole) and triphenylphosphine (10g, 38.2 mmole) were mixed in dry CH_3CN (40 ml), heated to reflux overnight and allowed to cool to RT. The solution was concentrated under reduced pressure to give a viscous gel. Approximately 75 ml of THF was added to the reaction mixture and the walls of the flask were scratched with a spatula to start crystallization. The resulting solid was filtered under vacuum, washed with THF and dried under reduced pressure and used without further purification.

This product (1.5g) was suspended in dry THF (10ml) and the flask purged with N_2 and cooled to -78°C. To the stirred reaction was added lithium hexamethyldisilazide (LiHMDS) (10ml, 1M in THF). The yellow solution was stirred at -78°C for 1h over which time the reaction darkened slightly. The cooling bath was removed and the reaction allowed to warm to RT. The reaction was kept at RT for 1h during which time the solution turned a dark red color and most of the solids went into solution. Benzophenone (0.54g in 3ml THF) was added to the reaction and allowed to react overnight. The yellow solution was concentrated under reduced pressure to give a yellow solid. The resulting solid was partitioned between ether and 10% HCl. The organic layer was washed with water (2x) and extracted with 10% NaOH (3x). The combined aqueous base fraction was acidified with conc. HCl to a pH of 4. The water layer was extracted with ether (3x) and the combined organic fractions dried over Na_2SO_4.

The ether was evaporated to dryness under reduced pressure to give a colorless oil which crystallized on standing to give a waxy solid, 6,6-diphenyl hex-5-enoic acid, which was dissolved in 30ml MeOH and mixed with 5% Pd-C and placed in a Parr hydrogenator. The reaction vessel was purged with hydrogen and pressurized to 60 PSIG and reacted at RT for 4h. The reaction mixture was sampled and analyzed by TLC. If the TLC when stained with $KMnO_4$ showed a positive test for alkenes the reaction mixture was resubjected to the reaction conditions. The solution was then filtered through a plug of celite and the methanol filtrate containing 6,6-diphenyl hexanoic acid was concentrated under vacuum.

B. Reaction with Substituted Piperazine.

6,6-Diphenylhexanoic acid (0.4 mmoles) was mixed with the desired N-alkylated piperazine (0.35 mmoles) in dry THF (7ml). EDC (0.5 mmoles) and DMAP (cat) were added and the mixture heated to 40°C with shaking overnight. The reaction was diluted with ethyl acetate and washed with water (4x) and 10% NaOH (3x) and dried over sodium sulfate and evaporated to dryness. The resulting residue was purified by column chromatography (silica gel, 1:1 hexane:EtOAc), and the products were characterized by HPLC-MS.

Piperazines used in the foregoing procedure include phenylpiperazine, benzylpiperazine, benzhydrilpiperazine, and piperazine substituted at the 1-position with BOC or Φ-CH=CH_2-.

The resulting compounds contain a carbonyl adjacent to the ring nitrogen of piperazine. These compounds are of formula (1) and exhibit calcium ion channel blocking activity.

C. Reduction of CO in X^1.

The compounds prepared in paragraph B were dissolved in dry THF (5ml) and reacted with $LiAlH_4$ (1M in THF) and allowed to react for 6h. The reactions were quenched with EtOAc (15ml) and extracted with water (5x) 10% NaOH (10x), brine (1x), dried over sodium sulfate and concentrated under reduced pressure. Most of the products at this stage were >80% pure. Those <80% were purified for running a short column (silica gel, 1:1 hex:EtOAc).

D. Preparation of Compounds of Formula (1) from Benzhydrylpiperazine Derivatives.

N-(Diphenylmethyl)piperazine (0.5 mmole) was dissolved in dry THF (10ml). To each reaction flask was added powdered K_2CO_3 and acid chloride of the formula Y'-CO-Cl (0.7 mmole). The reaction was stirred at RT for 2h and quenched with 105 NaOH (10ml) and extracted with EtOAc (10ml). The organic layer was washed with 10% NaOH (4x) and dried over sodium sulfate, concentrated, and purified by column chromatography (silica gel, 1:1 hex:EtOAc) to give the desired amide. Acyl halides used in this procedure included cyclohexyl COCl, ΦCOCl and ΦCH=CHCOCl.

To reduce the resulting amide, the above product was dissolved in dry THF (5ml) and reacted with $LiAlH_4$ (1M in THF) and allowed to react for 6h. The reaction was quenched with EtOAc (15ml) and extracted with water (5x) 10% NaOH (10x), brine (1x),

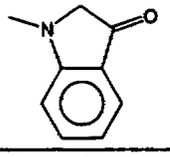
dried over sodium sulfate and concentrated under reduced pressure. Most of the products at this stage were >80% pure. Those <80% were purified for running a short column (silica gel, 1:1 hex:EtOAc).

Example 3

Synthesis of Additional Compounds of Formula (1)

Following the general procedure described above in Reaction Schemes 1 and 2, the following compounds of formula (1) are synthesized as shown in Table A.

Table A

R^1	L^1	R^2	l^2	X	n	Y	Y	R^3	l^3	Z	m
CH_3	1p	CH_3	1p	-	0	Φ	-	-	0	N	1
-	0	Cl	2m	$-CH_2-$	1	$-CH_2\varphi$	H	CH_3	1	CH	2
F	1p	F	3o,p	$-CH_2CH_2-$	1	$-CH_2CH\varphi$	OH	CH_3	1	COH	2
-	0	CF_3	1p	-	0		H	C_2H_5	1	CH	2
Φ	1p	-	0	$-(CH_2)_3-$	1	Amino-indane	-	-	0	O	-
CH_3	3o,p	COOMe	2m	$-CH_2-$	1	Azulene	Azulene	C_2H_5	1	C	2
Cl	3o,p	Cl	2m	-CONH-	1	Cyclohexane	-	CH_3	1	N	1
CN	2m	CF_3	2m	-CH=CH-	1	Pyrimidine	-	-	0	N	1
$N(CH_3)_2$	1p	C_2H_5	2m	$-CH_2CH_2-$	1	Indole	Pyrrole	-	0	C	2

Example 4

Channel Blocking Activities of Various Invention Compounds

Using the procedure set forth in Example 1, various compounds of the invention were tested for their ability to block N-type calcium ion channels. The results are shown in Tables 1-3, where IC_{50} is given in μM (micromolar). Table 1 represents results for compounds of formula (1a) where Z is CH; Table 2 represents results for compounds of formula (1a) where Z is N; and Table 3 represents the results the results for compounds of formula (1b) where Z is N. In all cases, l^1, l^2 and l^3 are 0.

Table 1
Formula (1a), Z is CH

n^1	X^1	n^2	X^2	Ar	IC_{50}	% reversibility
1	CH_2	1	$NHCH_2$	Φ	3	80
1	$(CH_2)_2$	1	$NHCH_2$	Φ	2	67

Table 2
Formula (1a), Z is N

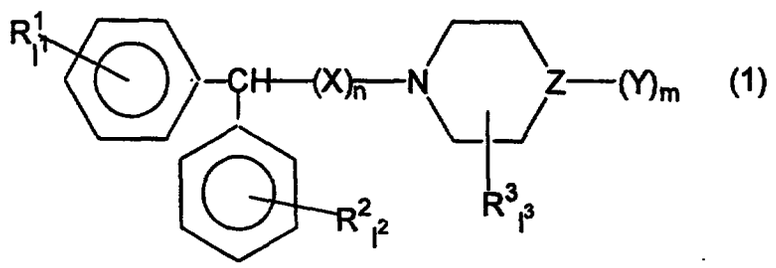
n^1	X^1	n^2	X^2	Ar	IC_{50}	% reversibility
1	$(CH_2)_6$	1	CH_2	Φ	2-5	52
1	$(CH_2)_6$	1	$-CH_2-CH=CH-$	Φ	1-3	44
1	$(CH_2)_5$	0	-	Φ	±10	83
1	$(CH_2)_5$	1	CH_2	Φ	±5	71
1	$(CH_2)_5$	1	$(CH_2)_2$	Φ	5-10	72
1	$(CH_2)_4CO$	1	CH_2	Φ	±5	66
1	$(CH_2)_4CO$	1	$-CH_2CH=CH-$	Φ	2-3	58
1	$(CH_2)_4CO$	1	$-COCH_2-$	Φ	5	78
1	$(CH_2)_5CO$	1	CH_2	Φ	2-5	84
1	$(CH_2)_3CO$	1	$-CH_2CH=CH-$	Φ	2-5	39
1	$(CH_2)_4$	0	-	Φ	3-5	13
1	$(CH_2)_4$	1	$-CH_2CH=CH-$	Φ	±5	48
0	-	1	$(CH_2)_2CH$	2Φ	2-5	40
0	-	1	$COCH_2CH$	2Φ	2-5	40
1	CH_2CO	1	COCH	2Φ	±5	60
1	CO	1	COCH	2Φ	>20	90
1	CH_2CO	1	CH	2Φ	2-5	40
1	$(CH_2)_2$	1	CH	2Φ	2-5	40
1	CO	1	CH	2Φ	>50	-
0	-	1	CH_2	Φ	±15	70
1	CH_2	1	CH_2	Φ	±50	0
1	CO	1	CH_2	Φ	±50	85
1	$(CH_2)_2$	1	CH_2	Φ	±20	70
1	CO	0	-	Φ	±50	85
1	CO	1	CH_2	Φ	±50	85
1	CO	1	CH	2Φ	>50	-
1	CO	1	$-CH_2CH=CH-$	Φ	±50	90
1	CH_2CO	0	-	Φ	>50	90
1	CH_2CO	1	CH_2	Φ	±15	80
1	CH_2CO	1	$-CH_2CH=CH-$	Φ	±20	80
1	CH_2	0	-	Φ	>50	0
1	CH_2	1	CH_2	Φ	±50	0
1	CH_2	1	CH	2Φ	>50	90
1	CH_2	1	$-CH_2CH=CH-$	Φ	35	60
1	$(CH_2)_2CO$	0	-	Φ	±9	11
1	$(CH_2)_2CO$	1	CH_2	Φ	±10	13
1	$(CH_2)_2CO$	1	CH	2Φ	±23	28

Table 2 Formula (1a), Z is N						
n^1	X^1	n^2	X^2	Ar	IC_{50}	% reversibility
1	$(CH_2)_2CO$	1	$-CH_2CH=CH-$	Φ	±5	20

Table 3 Formula (1b), Z is N						
n^1	X^1	n^2	X^2	Cy	IC_{50}	% reversibility
1	CH_2	1	NHCH	cyclohexyl	3-4	62
1	$(CH_2)_2$	1	NHCH	cyclohexyl	2-3	68
1	$(CH_2)_6$	1	CH_2	cyclohexyl	±1	5
1	$(CH_2)_5$	1	CH_2	cyclohexyl	5-10	66

Claims

1. A method to treat conditions associated with calcium channel activity in a subject which method comprises administering to a subject in need of such treatment a compound of the formula



(1)

wherein m is 0, 1 or 2;

wherein when m is 0, Z is O, when m is 1, Z is N, and when m is 2, Z is C;

Y is H, OH, NH_2, or an organic moiety of 1-20C, optionally additionally containing 1-8 heteroatoms selected from the group consisting of N, P, O, S and halo;

each l^1 and l^2 is independently 0-5;

l^3 is 0 or 1;

each of R^1, R^2 and R^3 is independently alkyl (1-6C), aryl (6-10C) or arylalkyl (7-16C) optionally containing 1-4 heteroatoms selected from the group consisting of halo, N, P, O, and S or each of R^1 and R^2 may independently be halo, COOR, $CONR_2$, CF_3, CN or NO_2, wherein R is H or lower alkyl (1-4C) or alkyl (1-6C);

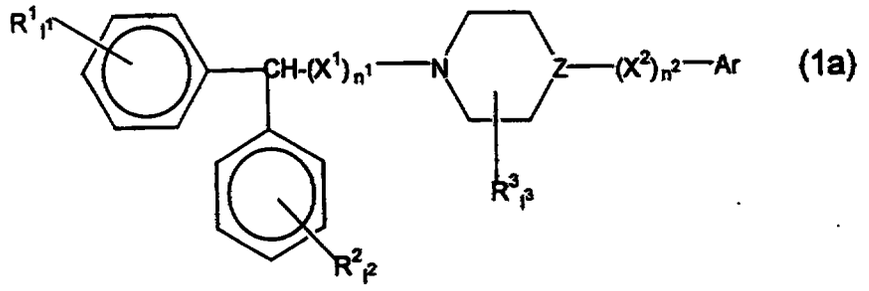
n is 0 or 1;

X is a linker;

with the proviso that Y is not a tropolone, a coumarin, or an antioxidant containing an aromatic group and with the further proviso that if l^3 is 0, neither R^1 nor R^2 can be F in the para position.

2. The method of claim 1 wherein at least one of R^1, R^2 and R^3 is a halo substituent.

3. The method of claim 1 wherein the compound of formula (1) is of the formula (1a)



(1a)

wherein Z is N or CH;

wherein each of n^1 and n^2 is independently 0 or 1;

X^1 and X^2 are linkers; and

Ar represents one or two substituted or unsubstituted aromatic or heteroaromatic rings.

4. The method of claim 3 wherein Ar represents one or two unsubstituted phenyl moieties.

5. The method of claim 3 wherein n^2 is 1 and X^2 represents a linker which spaces Ar from Z at a distance of 3-20Å.

6. The method of claim 5 wherein n^2 is 1 and X^2 contains at least one heteroatom selected from N and O.

7. The method of claim 5 wherein n^2 is 1 and X^2 represents $-(CH_2)_{1-8}-$ or $-(CH_2)_{1-5}-CH=CH-(CH_2)_{0-3}-$.

8. The method of claim 5 wherein Ar represents two phenyl moieties and n^2 is 1 and X^2 is of the formula $-(CH_2)_{0-6}-CH$.

9. The method of claim 3 wherein l^3 is 0.

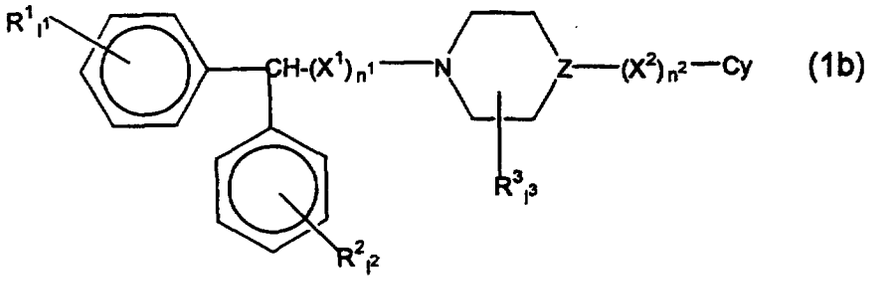
10. The method of claim 3 wherein l^1 and l^2 are 0.

11. The method of claim 3 wherein n^1 is 1 and X^1 represents a linker which spaces the benzhydril moiety from N at a distance of 3-20Å.

12. The method of claim 11 wherein n^1 is 1 and X^1 contains at least one heteroatom selected from O and N.

13. The method of claim 11 wherein n^1 is 1 and X^1 represents $-(CH_2)_{1-8}-$ or $-(CH_2)_{1-5}-CH=CH-(CH_2)_{0-3}-$.

14. The method of claim 1 wherein the compound of formula (1) is of the formula (1b)



(1b)

wherein Z is N or CH;

wherein each of n^1 and n^2 is independently 0 or 1;

X^1 and X^2 are linkers; and

Cy represents one or two substituted or unsubstituted aliphatic cyclic or heterocyclic moieties or consists of one substituted or unsubstituted aliphatic cyclic or heterocyclic moiety and one substituted or unsubstituted aromatic or heteroaromatic moiety.

15. The method of claim 14 wherein n^2 is 1 and X^2 represents a linker which spaces Cy from Z at a distance of 3-20Å.

16. The method of claim 15 wherein n^2 is 1 and X^2 contains at least one heteroatom selected from N and O.

17. The method of claim 15 wherein n^2 is 1 and X^2 represents $-(CH_2)_{1-8}-$ or $-(CH_2)_{1-5}-CH=CH-(CH_2)_{0-3}-$.

18. The method of claim 14 wherein l^3 is 0.

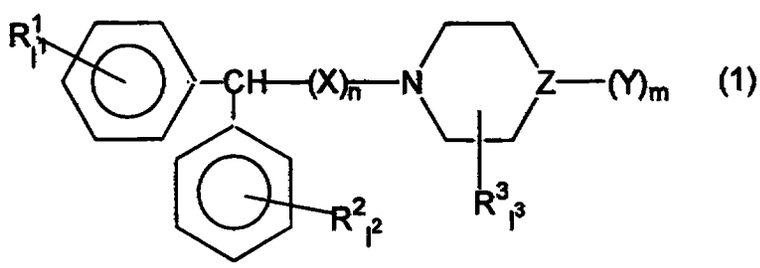
19. The method of claim 14 wherein l^1 and l^2 are 0.

20. The method of claim 14 wherein n^1 is 1 and X^1 represents a linker which spaces the benzhydril moiety from N at a distance of 3-20Å.

21. The method of claim 20 wherein n^1 is 1 and X^1 contains at least one heteroatom selected from O and N.

22. The method of claim 20 wherein n^1 is 1 and X^1 represents $-(CH_2)_{1-8}-$ or $-(CH_2)_{1-5}-CH=CH-(CH_2)_{0-3}-$.

23. A pharmaceutical composition for use in treating conditions characterized by calcium channel activity which composition comprises, in admixture with a pharmaceutically acceptable excipient, a dosage amount of a compound of the formula



(1)

wherein m is 0, 1 or 2;

wherein when m is 0, Z is O, when m is 1, Z is N, and when m is 2, Z is C;

Y is H, OH, NH_2, or an organic moiety of 1-20C, optionally additionally containing 1-8 heteroatoms selected from the group consisting of N, P, O, S and halo;

each l^1 and l^2 is independently 0-5;

l^3 is 0 or 1;

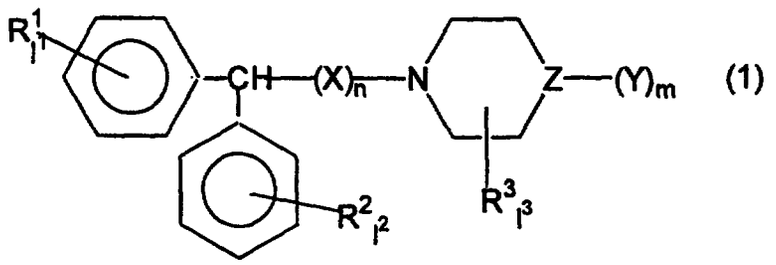
each of R^1, R^2 and R^3 is independently alkyl (1-6C), aryl (6-10C) or arylalkyl (7-16C) optionally containing 1-4 heteroatoms selected from the group consisting of halo, N, P, O, and S or each of R^1 and R^2 may independently be halo, COOR, $CONR_2$, CF_3, CN or NO_2, wherein R is H or lower alkyl (1-4C) or alkyl (1-6C);

n is 0 or 1;

X is a linker;

with the proviso that Y is not a tropolone, a coumarin, or an antioxidant containing an aromatic group and with the further proviso that if l^3 is 0, neither R^1 nor R^2 can be F in the para position.

24. A library comprising at least ten different compounds of the formula



(1)

wherein m is 0, 1 or 2;

wherein when m is 0, Z is O, when m is 1, Z is N, and when m is 2, Z is C;

Y is H, OH, NH_2, or an organic moiety of 1-20C, optionally additionally containing 1-8 heteroatoms selected from the group consisting of N, P, O, S and halo;

each l^1 and l^2 is independently 0-5;

l^3 is 0 or 1;

each of R^1, R^2 and R^3 is independently alkyl (1-6C), aryl (6-10C) or arylalkyl (7-16C) optionally containing 1-4 heteroatoms selected from the group consisting of halo, N, P, O, and S or each of R^1 and R^2 may independently be halo, COOR, $CONR_2$, CF_3, CN or NO_2, wherein R is H or lower alkyl (1-4C) or alkyl (1-6C);

n is 0 or 1; and

X is a linker.

25. A method to identify a compound which antagonizes a target receptor which method comprises contacting host cells displaying said target receptor in the presence of an agonist for said receptor and with the members of the library of claim 14;

assessing the ability of the members of the library to affect the response of the receptor to its agonist; and

identifying as an antagonist any member of the library which diminishes the response of the receptor to its agonist.

26. The method of claim 25 wherein the receptor is an ion channel.