CHAPTER 3

SMOOTH MUSCLE RELAXANTS

Malaysia is gifted with beautiful, luxurious forests which are known to contain plants with biologically active compounds, and these can provide invaluable cures for various diseases. Local scientists should initiate interest in discovering the medicinal potentials of our local forests since there is an abundance of information yet to be documented. The fact that scientists from other countries are tirelessly collecting and doing laborious studies on our local plants has made most local scientists realise the need to retain as much information as possible and take steps to prevent the loss of our own plant species for the sake of the future generations. One of the promising fields currently under active study is the search for plant derived compounds with smooth muscle relaxant activities. These compounds can be used for treatment of hypertension, angina, asthma and other forms of generalised muscle spasms.

3.1 THE CONTRACTILE PROCESS AND THE REGULATION OF CALCIUM IN SMOOTH MUSCLES

Muscles are defined as tissue or organ of the animal body characterized by the ability to contract, usually in response to a stimulus from the nervous system by way of converting potential energy to kinetic energy[73,74]. There are three types of muscles;

i. *Smooth or unstriated muscle.*

This is composed of spindle-shaped cells, usually 2 to 5 microns in diameter and 50 to 200 microns in length. The cells have no cross striations, although they do exhibit faint longitudinal striations. Smooth muscle can be divided into two types; the multiunit and the visceral

smooth muscle. Examples of multiunit smooth muscle are the iris of the eye and the smooth muscle of major blood vessels, whereas the visceral smooth muscle is found in most organs of the body especially in the walls of the uterus and the gut.

ii. *Skeletal or striated muscle.*

This muscle is composed of long fibres surrounded by a membranous sheath, the sarcolemma. The fibres are elongated, sausage-shaped cells containing many nuclei, and clearly display longitudinal and cross striations. Most skeletal muscles are attached to portions of the skeleton by connective-tissue attachments called tendons. Contractions of skeletal muscle serve to move the various bones and cartilages of the skeleton. This muscle is supplied with nerves from the central nervous system, and because it is partly under conscious control it is also called voluntary muscle.

iii. *Cardiac muscle.*

This muscle tissue composes most of the vertebrate heart. The cells show both longitudinal and imperfect cross striations. This muscle is not under voluntary control; it is supplied with nerves from the autonomic nervous system, but autonomic impulses merely speed or slow its action and are not responsible for the continuous rhythmic contraction characteristic of living cardiac muscle.

There are three distinct types of filaments in various smooth muscle cell; thin filaments (6-8 nm in diameter) composed mainly of actin monomers, polymerized into a double-helical strand, and associated tropomyosin arranged

along the length of the actin filament; intermediate filaments (10 nm in diameter) composed of desmin and vimentin; and thick filaments (12-18 nm in diameter) composed of aggregated myosin molecules[75]. The contractile filaments (actin and myosin) of smooth muscle may function in a similar way to striated muscle filaments, that is contributing to muscle shortening by sliding mechanism. Proteins in smooth muscle are divided into contractile, regulatory and cytoskeletal. The activities of most of them are, to some extent, regulated by the level or concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$).

Two integrated membrane systems that play key roles in the regulation of smooth muscle $[Ca^{2+}]_i$ are; the plasmalemma, under the control of membrane depolarization and agonists; and the sarcoplasmic reticulum (SR), controlled by second messengers[75]. Sustained contraction of vascular smooth muscle is initiated by the increase in $[Ca^{2+}]_i$ resulting from the increased Ca^{2+} influx into the cell via two types of Ca^{2+} channels, i.e. receptor-linked Ca^{2+} channel and voltage-dependent Ca^{2+} channel. The former involves binding of stimulatory transmitters (e.g. noradrenaline) or other substances to specific receptors, while the latter involves directly changing membrane electrical properties[76,77]. However, a more transient contraction can be obtained by release of Ca^{2+} from the cellular store, i.e. the sarcoplasmic reticulum (SR)[78,79].

Figure 3.1 illustrates the mechanism of vascular smooth muscle contraction as reported by Karaki[76]. As shown in the figure, receptor activation (1) by stimulant or agonist may stimulate turnover of membrane phosphoinositides (3)[80] and, in this

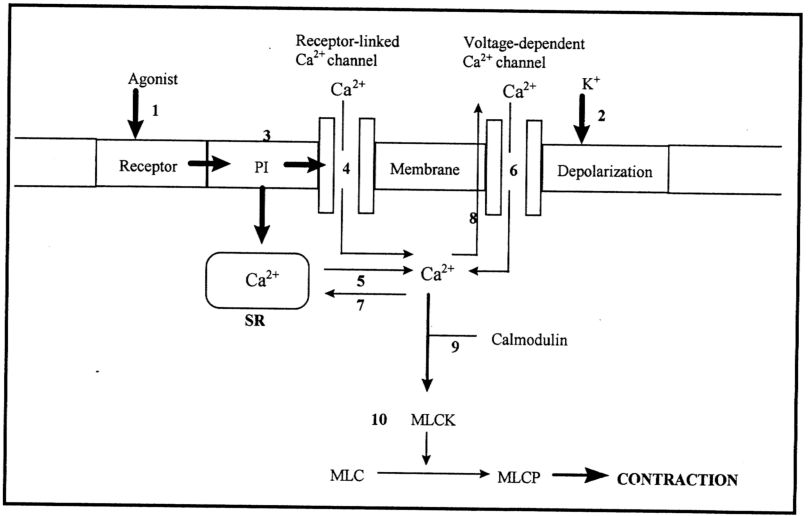


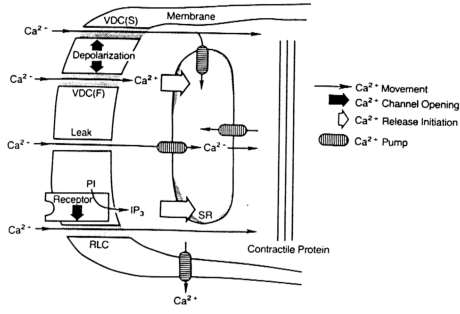
FIGURE 3.1 MECHANISM OF CONTRACTION OF VASCULAR SMOOTH MUSCLE

Note : Receptor-linked Ca^{2+} channel is also referred to as non-selective cation channel.

manner, open receptor-linked Ca^{2+} channels (or non-selective cation channels[81]) (4) located in the membrane to permit influx of external Ca^{2+} and/or release cellular bound Ca^{2-} from the sarcoplasmic reticulum (5)[82,83]. (N.B. Non-selective cation channel is permeable not only to monovalent cations but also to Ca^{2+}; opening of this channel results in an increase in Ca^{2+} influx)[81]. Membrane depolarization (2), which can be induced by high K^+ concentration (high $[K^+]$) *in vitro*, opens the voltage-dependent Ca^{2+} channel (6) allowing, again, the influx of external Ca^{2+} into the cytoplasm. The resulting increase in concentration of cytoplasmic free Ca^{2+} together with the protein calmodulin (9) (i.e. Ca^{2+} binds to calmodulin)[84] activate myosin light-chain kinase (MLCK) (10), phosphorylate myosin light-chain (MLC) to MLC phosphate (MLCP) and induce muscle contraction. Cytoplasmic free Ca^{2+} is either sequestered at cellular binding sites, presumably sarcoplasmic reticulum (7), or extruded from the cell by an active pumping mechanism (8)[85]. ATP is supplied by both aerobic and anaerobic metabolism to support these processes. Increase in cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) contents inhibit the contraction mainly by inhibiting the receptor-linked Ca^{2+} channels (4) and by inhibiting Ca^{2+} release (5)[86,87].

In another report, Karaki *et.al.* illustrated a model of Ca^{2+} release in smooth muscle as depicted in figure 3.2[78].

FIGURE 3.2 CALCIUM MOVEMENTS IN SMOOTH MUSCLE[78]



Four different Ca^{2+} influx pathways are illustrated:

i. the resting influx Ca^{2+} channel (leak),
ii. the slow-inactivating, voltage-dependent Ca^{2+} channel (VDC(S)),
iii. the fast-inactivating, voltage-dependent Ca^{2+} channel (VDC(F)),and,
iv. the receptor-linked Ca^{2+} channel (RLC).

Also indicated is storage site (SR) Ca^{2+} accumulation (Ca^{2+} pump) or release (Ca^{2+} release initiation). SR Ca^{2+} is released by a Ca^{2+}-induced Ca^{2+} release (CCR) (after VDC(F)) entry) or by a receptor-activated conversion of phosphatidylinositol (PI) to IP_3. Released SR Ca^{2+} or directly entering Ca^{2+} (RLC or some VDC(S) Ca^{2+}) activates contractile filaments and is then either extruded from the cell or reaccumulated in the SR.

Figure 3.2 summarizes at least four different pathways for Ca^{2+} influx. Firstly, Ca^{2+} entering the cell through resting influx or leak mechanisms is taken up by the storage sites (SR) and does not directly increase the $[Ca^{2+}]_i$. Secondly, Ca^{2+} entering the cell through voltage-dependent Ca^{2+} channels (of the slow-inactivating type) is also accumulated in the storage sites and, when entering Ca^{2+} reaches the cytoplasm, directly activates contractile filaments. Thirdly, Ca^{2+} entering through another type of voltage-sensitive channel (of the fast-inactivating type) may release rather than fill the Ca^{2+} store. And finally, Ca^{2+} entering through receptor-linked Ca^{2+} channels does not fill the store (SR) because the storage site cannot accumulate or retain Ca^{2+} during receptor activation. Hence, all of the Ca^{2+} entering through receptor-linked Ca^{2+} channels is available to directly activate contractile filaments.

As mentioned above, intracellular Ca^{2+}, $(Ca^{2+})_i$, is tightly regulated in vascular smooth muscle. $(Ca^{2+})_i$ is required for many cellular processes and changes in $[Ca^{2+}]_i$ play an important role in excitation-contraction coupling smooth muscle[88]. The resting cell membrane potential in vascular smooth muscle is usually around -60 mV and extracellular diffusible Ca^{2+} is about 1.6 mM. Despite of this steep electrochemical gradient for Ca^{2+} entry into the cell, measurements of $[Ca^{2+}]_i$ in vascular smooth muscle have yielded estimates of around 100 - 300 nM. The low $[Ca^{2+}]_i$ means that the opening of calcium channels (unlike Na^+ and K^+ channels) admits sufficient Ca^{2+} ions to significantly raise $[Ca^{2+}]_i$, even when the powerful Ca^{2+} buffering systems of the cell are taken into account and these channels represent the major route by which Ca^{2+} enters the vascular smooth muscle cell.

Drugs affecting vascular smooth muscle contraction may modify any of the above mentioned processes directly by acting on smooth muscle cells or indirectly to release substances modifying muscle contractility by acting either on nerve terminals located in the vascular wall[89] or on the endothelium[90].

3.2 MECHANISM OF ACTIONS OF VASCULAR SMOOTH MUSCLE RELAXANTS

Hypertension is more specifically defined as an elevated blood pressure and can result when total peripheral vascular resistance is increased[91]. Various mechanisms have been postulated for the causes and genesis of hypertension and one thing is apparent, that is the pathways involved at the smooth muscle cells of the resistance vessels elicit a directly related increase in the contractile tone of the muscle cells.

Vasodilators are drugs that relax the vascular smooth muscles and can be classified into six major groups based on their mode of actions.

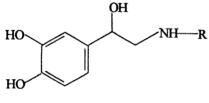
3.2.1 α_1-Adrenoceptor antagonists

Many systems of the body (e.g. digestion and circulation) are controlled automatically by the autonomic nervous system[92]. There are many sensory fibres carrying information to centres in the hypothalamus and medulla; these centres control the outflow of the autonomic nervous system. The autonomic nervous system is divided into two major parts: the

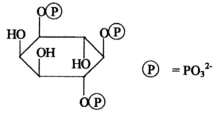
sympathetic system and the parasympathetic system. In the sympathetic system, the transmitter substance released at the sympathetic nerve endings is noradrenaline **50** (adrenaline **51** and noradrenaline **50** are released from the adrenal medulla). Noradrenaline **50** and adrenaline **51** produce their actions on effector organs by acting on adrenoceptors. Adrenoceptors are divided into two main types: α-adrenoceptors and β-adrenoceptors. α-Adrenoceptors, in addition, are divided into α_1 (postsynaptic) and α_2 (presynaptic). Receptors mainly found in vascular smooth muscle are α_1-adrenoceptors[92].

The α_1-adrenoceptors in rat aorta and rabbit mesenteric artery are coupled to inositol-1,4,5-triphosphate (IP_3) **52** / Ca^{2+} release system[81,93,94] which then lead to muscle contraction. Activation of the α_1-adrenoceptors by noradrenaline or other agonists, such as phenylephrine **53**, releases Ca^{2+} in rat aorta and portal vein[95]. This receptor may also be directly coupled to the L-type Ca^{2+} channel and to Ca^{2+} sensitizing mechanism in some types of smooth muscle.

α_1-Blockers or α_1-adrenoceptor antagonists, such as prazosin **54** and doxazosin **55**, cause vasodilation by selectively blocking vascular α_1-adrenoceptors[96].



50 R = H
51 R = Me



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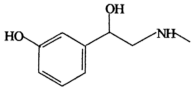

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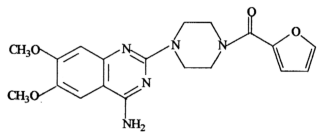

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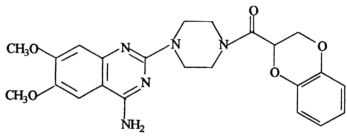

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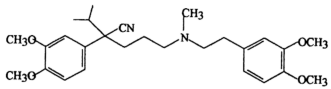
3.2.2 Calcium antagonists

This group of compounds are usually referred to as calcium antagonists, calcium channel antagonists, or calcium channel blockers[97], and are known to be a chemically heterogenous group of agents with the common property of selective antagonism of Ca^{2+} movements through Ca^{2+} channel. Calcium antagonists were first developed by Fleckenstein in the early 1960s in Freiburg, West Germany[84]. Karaki reported that calcium antagonists inhibit voltage-dependent Ca^{2+} channel[98]. This group of drugs do not exhibit strong tissue specificity (although its main effects are registered in cardiac and smooth muscle tissue) and only interfere with cross-membrane flow of Ca^{2+}, and not with other cellular processes involving calcium. The compounds vary markedly in chemical structure, and their inhibitory effects cannot be completely offset by increases in extracellular Ca^{2+} concentration. By reducing Ca^{2+} influx into the smooth muscle cells through a blockade of some of the slow channels, the tension is decreased through reduced activated calmodulin (bound to Ca^{2+}), and there is less interaction between actin and myosin, hence, there is less contraction and results in decreased vascular resistance, permitting an increased blood flow.

In general calcium antagonists are amphiphiles with a large hydrophobic region, usually one or more ring structures, and a hydrophilic portions such as an amine or other nitrogen-containing group[84]. Examples of calcium antagonists include the clinically available verapamil **56**, nifedipine **57** and diltiazem **58**[97-99].



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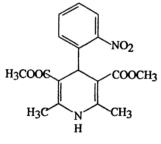

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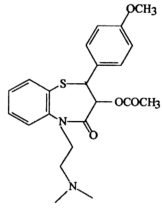

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3.2.3 Angiotensin converting-enzyme inhibitors and angiotensin antagonists

Angiotensin was first discovered in the late 1930s[100]. The inhibition of the angiotensin-converting enzyme is established as one modern therapeutic principle in the treatment of hypertension [101]. Angiotensin is involved in the so-called renin-angiotensin-aldosterone system which regulate the blood pressure[102]. This system comprises a proteolytic enzyme, renin, that is synthesized by many organs. However, most of the circulating renin that acts in the vascular system is synthesized by the kidney. Within the plasma, renin cleaves a decapeptide known as angiotensinogen **59** into

angiotensin I **60**, a prohormone, which is then converted to the active hormone angiotensin II **61** by an enzyme called angiotensin-converting enzyme. This enzyme is found in association with the luminal surface of the plasma membrane of endothelial cells in both pulmonary and systemic circulations. Finally, angiotensin II has a direct stimulatory effect on aldosterone secretion, and then is further degraded into smaller fragments. Angiotensin II is a powerful constrictor of vascular smooth muscle and this vasopressor effect has been shown to be a major factor contributing to the development and maintenance of high blood pressure in certain forms of arterial hypertension[102].

Examples of angiotensin-converting enzyme inhibitors are captopril **62** and enalapril **63**[96]. The blockade of the angiotensin-converting enzyme results in the accumulation of bradykinin **64**, a potent vasodilator[102]. There are also drugs that lower the blood pressure by blocking angiotensin receptors, eg. saralasin **65**[96].

3.2.4 Drugs that act by increasing cyclic nucleotides concentration

Increase in levels of cyclic guanosine monophosphate (cGMP) **66** and cyclic adenosine monophosphate (cAMP) **67** will inactivate the myosin light chain kinase (MLCK) and may facilitate calcium efflux which leads to smooth muscle relaxation[74]. The nucleotide, cGMP has emerged as a potent physiological second messenger involved in both vasodilator action and failure of vasoconstrictor activity[103]. The vasodilators related to cGMP

H_3N^+-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser-R

59 R = the remainder of the amino acid sequence

H_3N^+-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-COO^-

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H_3N^+-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-COO^-

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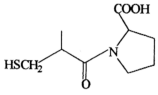

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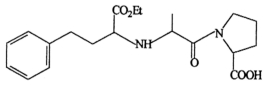

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H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH

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Ser-Arg-Val-Tyr-Val-His-Pro-Ala

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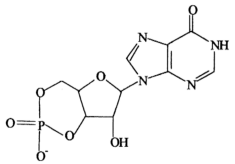

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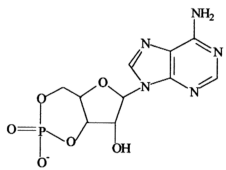

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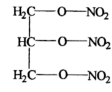
include nitrocompounds such as nitroglycerine **68** and sodium nitroprusside **69** (these compounds produce nitric oxide (NO) in cells which has a direct activating effect on guanylate cyclase)[104,105], cholinergic agonists such as acetylcholine **70** and carbachol **71**, and atrial natriuretic peptides[76]. These compounds activate guanylate cyclase, increase cGMP content, activate cGMP-dependent protein kinase, and phosphorylate target proteins[87]. Resulting effects include membrane hyperpolarization, which somehow uncouples receptor activation from Ca^{2+} movements, inhibition of phosphoinositides turnover, inhibition of receptor-linked Ca^{2+} channels[106], inhibition of Ca^{2+} release from SR, increase in Ca^{2+} extrusion and inhibition of contractile filaments[76]. Eventhough cholinergic agonists such as acetylcholine is known to induce contraction on smooth muscle, they, however, act on vascular endothelium to release endothelium-derived relaxing factors (EDRF)[90,107], which then stimulate guanylate cyclase and increase cGMP content in cells[87].

Examples of vasodilators related to cAMP are β-adrenoceptor agonists such as isoproterenol **72**, forskolin **73**, adenosine **74**, papaverine **75**, xanthines **76** and amrinone **77**. Similarly, these drugs activate adenylate cyclase, increases cAMP content, activates cAMP-dependent protein kinase and phophorylates target proteins. These sequence of events then result in membrane hyperpolarization, inhibition of Ca^{2+} channels[108], increase in Ca^{2+} extrusion and inhibition of contractile filaments.



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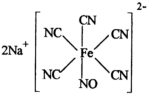

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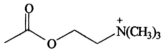

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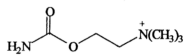

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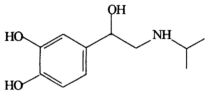

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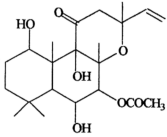

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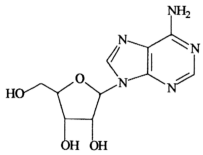

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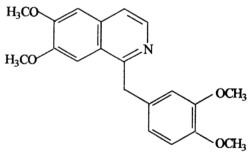

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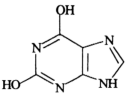
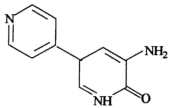


76 (enol form)



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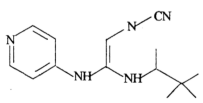
3.2.5 Potassium channel openers

Potassium channel openers comprise a diverse group of molecules. These compounds open K^+ channels, hyperpolarize the membrane, inhibit the opening of the L-type (long-lasting type) Ca^{2+} channel, inhibit Ca^{2+} influx, decrease $[Ca^{2+}]_i$, and inhibit contraction[95]. In addition, hyperpolarization of the plasma membrane caused by K^+ channel openers also inhibits the production of inositol-1,4,5- triphosphate (IP_3) **52** and Ca^{2+} release from the SR. Examples of K^+ channel openers are pinacidil **78**, nicorandil **79**, chromakalim **80** and minoxidil **81**[95,96]. Chromakalim is a more specific K^+ channel opener than pinacidil and nicorandil.



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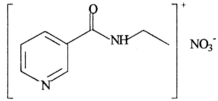

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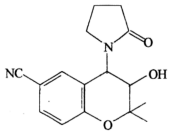

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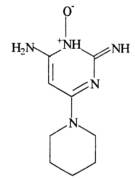

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3.2.6 Contractile protein modulators

According to Weiss *et. al.*, inhibition of vascular contractile protein interactions remains as potential sites of action for the discovery and development of novel antihypertensive agents[91]. Direct pharmacological regulation of vascular contractile protein interactions can theoretically occur at several sites, among which include the sites of regulation on the thin filament (caldesmon) or on the thick filament (phosphorylation of the MLC). Much light has been put upon the direct alteration of MLC phosphorylation which can conceivably occur by four distinct modes:

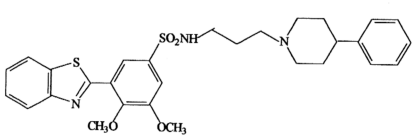
i. inhibition of Ca^{2+} binding to calmodulin,

ii. inhibition of Ca^{2+}-calmodulin activation of MLCK,

iii. direct inhibition of MLCK catalytic activity, or

iv. direct stimulation of MLC phosphatase activity.

Calmodulin antagonism is currently the most popular method for inhibiting smooth muscle MLC phosphorylation. By far, the largest group of calmodulin antagonists are those agents that compete with the regulated enzyme for the Ca^{2+}-calmodulin complex. An example of agents that directly modify Ca^{2+} binding to calmodulin is a drug known as HT-74 **82**[109].



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