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Review

Natural products with calmodulin inhibitor properties

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Abstract

This review summarizes properties of various naturally occurring compounds with reported calmodulin (CaM)-inhibitory properties, which include about 159 natural products belonging to different structural classes. Most inhibitors are alkaloid and peptide type of compounds and have been isolated from a wide variety of natural sources, including many plant species. Among the most potent natural anti-CaM substances, however, are several animal venoms and the antibiotic polymixin. The largest number of compounds described were discovered by means of enzymatic functional assays.

Keywords: Natural products; Calmodulin; *c*AMP phosphodiesterase; PDE1; Alkaloids; Peptides; Coumarins; Stilbenoids; Flavonoids; Polyketides; Terpenoids

Contents

1. Introduction

Calmodulin (CaM) is an ubiquitous Ca^{2+}-binding protein which regulates many Ca^{2+}-dependent cellular events by interacting with a heterogeneous group of target proteins in both plant and animal cells. Calmodulin is a small protein (16–18 kD) composed by ~148 amino acids organized in two distinct N- and C-terminal globular domains connected by a flexible central linker. Both domains possess a pair of intimately linked EF hands, each binding a single Ca^{2+} ion (Chin and Means, 2000; Bouché et al.,

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2005). Calcium binding to CaM causes conformational changes in each domain; in consequence, a series of hydrophobic regions are exposed on the surface of the protein. The hydrophobic areas, rich in Met residues, are the primary surface for hydrophobic interactions with 12–30 contiguous positively charged amino acids in target proteins. The stability of the CaM-target complex is also achieved by electrostatic interactions. According to X-ray studies, the two globular domains of CaM wrap around the target enzyme forming an almost globular structure.

CaM is a multi-functional receptor for intra- and extracellular Ca^{2+} stimulus in animal and plant cells regulating many important physiological functions (Daye et al., 2001) alike in all eukaryotes. In wheat, potato, *Arabidopsis* and soybean there are multiple *CaM* genes which code for numerous CaM isoforms (Lee et al., 2000; Bouché et al.,

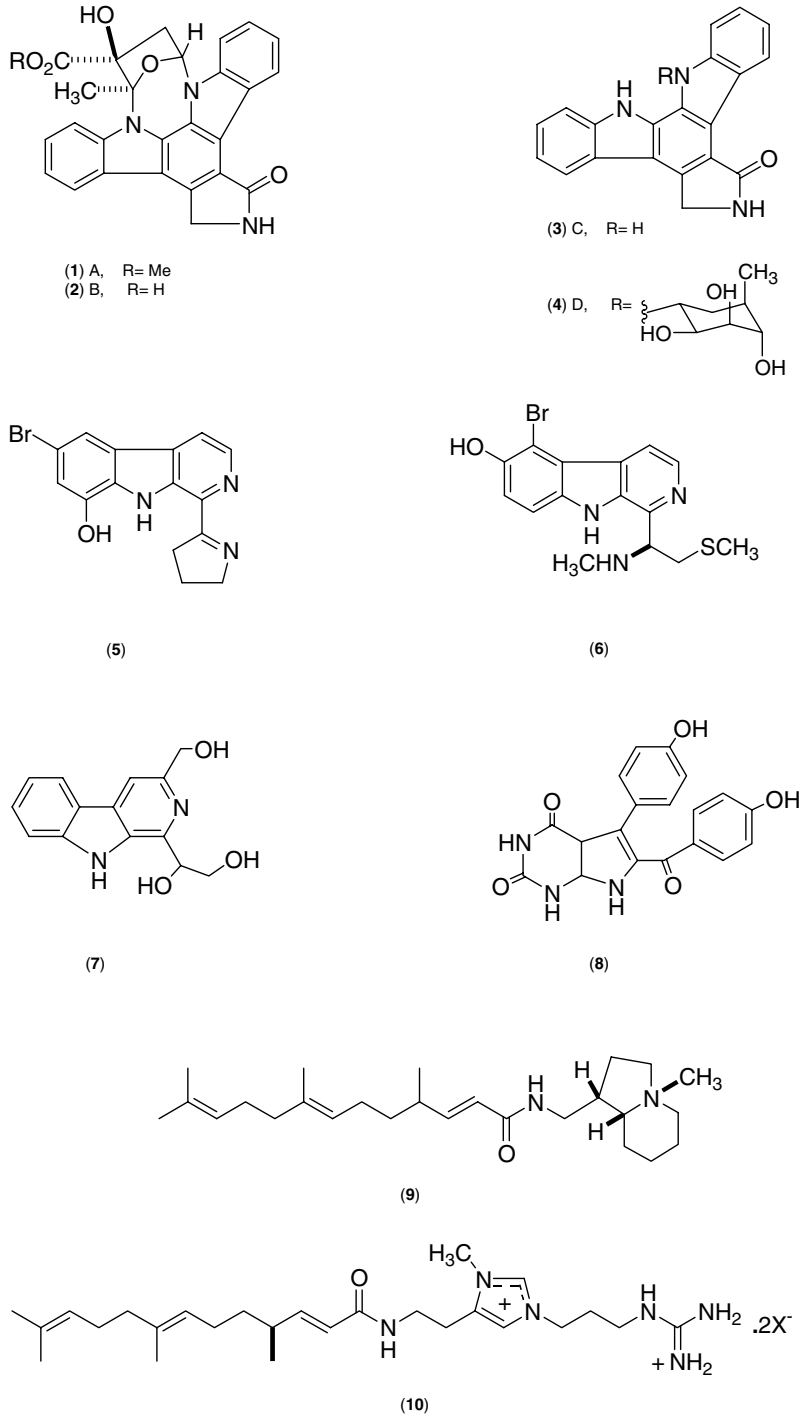
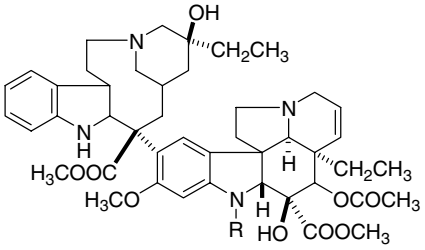


Fig. 1. Alkaloids.

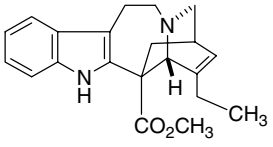
2005; Yamniuk and Vogel, 2005) some of which (SCaM-1 to SCaM-3) are more than 90% identical with mammalian CaM. However, the isoforms SCaM-4 and SCaM-5 exhibit only 78% identity and are the most divergent isoforms reported so far in the plant or animal kingdoms.

The existence of multiple divergent CaM isoforms in plants raise the question whether or not they allow differential regulation of target enzymes and can confer different Ca^{2+} sensitivity to CaM-binding enzymes or proteins (CaMBPs). However, in mammalian cells at least three differentially regulated *CaM* genes code for the same protein. Recently, subtractive hybridization analyses have identified a human CaM-like protein that is 85% identical with CaM (Lee et al., 2000; Yamniuk and Vogel, 2005).

More than 50 enzymes and ion channels are regulated by CaM in plants and vertebrates, and the number of CaM-modulated proteins being reported is ever increasing. Such proteins include several kinases, (i.e CaM kinase II), the protein phosphatase calcineurin, phosphodiesterase, the nitric oxide synthases, adenylate cyclases 1 and 8, several ion channels, notably voltage-gated Ca^{2+} channels and both ryanodine and IP3 receptors, as well as a number of cytoskeleton proteins, including caldesmon, spectrin, MARCKS and adducin (Toutenhoofd and Strehler, 2000;



(11) R=CH_3
(12) R=CHO



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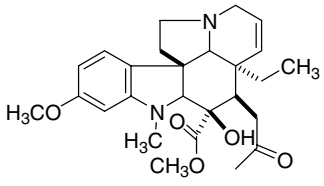

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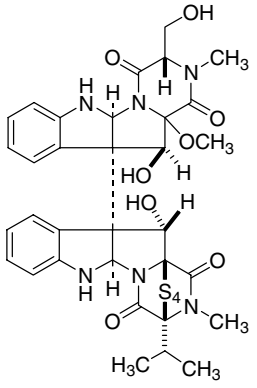

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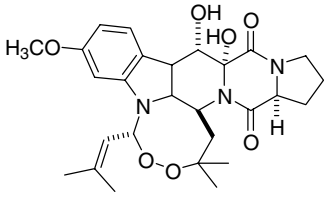

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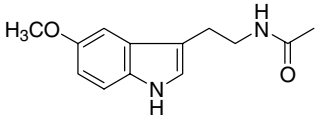

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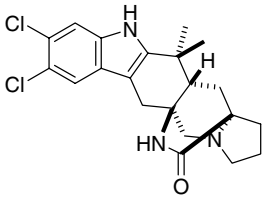

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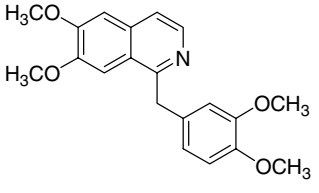

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Fig. 1 (*continued*)

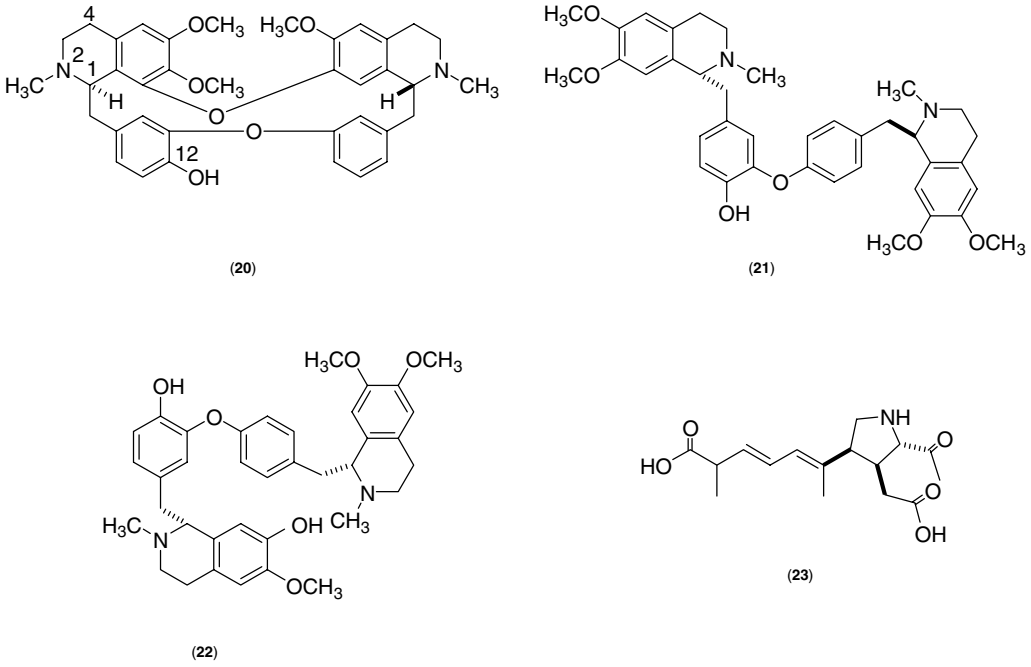


Fig. 1 (*continued*)

Black et al., 2004; Bouché et al., 2005). The stoichiometry of CaM-target enzyme varies with the target since recent studies revealed different ratios in several complexes. For example, with the Ca^{2+}-pump, the ratio CaM-target enzyme is 1:1; with potassium channel is 2:2, and with petunia glutamate decarboxylase (GAD) is 1:2. Some targets interact with both the C- and N-terminal lobes of CaM, while others interrelate with one lobe (Chin and Means, 2000; Bouché et al., 2005).

Altogether, CaM and its targets represent a complex network which participate in most cellular processes including regulation of metabolism, cytoskeleton, ion transport, protein folding, cell proliferation, cell division, transcription, protein phosphorylation and dephosphorylation, phospholipid metabolism, among others (Black et al., 2004; Kortvely and Gulya, 2004). Calmodulin shows contradictory behavior in target enzyme activation, activating many enzymes that catalyze counter reactions, such as protein phosphorylation and dephosphorylation, cyclic nucleotide synthesis and breakdown, and Ca^{2+} channels and Ca^{2+} pumps. The reasons which permit CaM to order these reciprocal events remain an open question.

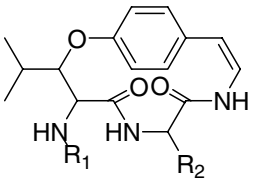
Some of the target enzymes of CaM are only found in plants; some examples are the chimeric Ca^{2+}-responsive CaM-binding protein kinases; the Ca^{2+}-ATPase (ACA9), of particular importance for normal pollen tube growth and fertilization; and PP7 ser/thr phosphatase, the pollen-specific protein NPG1 which is also important for plant development. Other enzymes, although not exclusive to plants, are regulated by CaM only in plants; for example, glutamate decarboxylase, apyrase, and NAD kinase. A third group of proteins such as CaM-regulated plasma membrane Ca^{2+}-ATPases, protein kinases, and kinesin are regulated by CaM in all type of organisms (Bouché et al., 2005).

Anti-CaM compounds might inhibit CaM in different ways i.e. binding to the CaM molecule or to the CaM-enzyme complex. Calmodulin inhibitors have been detected by means of several methods including affinity chromatography or labeling (Molnar et al., 1995; Ovadi, 1989), limited proteolysis (Ovadi, 1989), UV-CD spectroscopy (Molnar et al., 1995; Harmat et al., 2000; Vertessy et al., 1998), gel electrophoresis (Leung et al., 1985), fluorescence-based technologies, including fluorescence resonance energy (Au et al., 2000; Molnar et al., 1995; Sharma et al., 2005), NMR (Zhou et al., 1994; Craven et al., 1996), X-ray diffraction (Vertessy et al., 1998; Harmat et al., 2000), site-directed mutagenesis (Odom et al., 1997) and functional enzymatic assays using CaM-sensitive enzymes as reporters (Orosz et al., 1988, 1990; Ovadi, 1989; Molnar et al., 1995; Sharma et al., 1997). The functional enzymatic assays measure the inhibition of the enzymatic activity of an enzyme modulated by CaM being CaM-sensitive *c*AMP phosphodiesterase (PDE1), isolated from bovine brain or heart, the most widely used; this enzyme catalyzes the hydrolysis of cyclic nucleotides to nucleotides monophosphates. The activity of PDE1 can be correlated with the amount *c*AMP released during the enzymatic reaction using radioimmunoassay measurement. Alternatively, the amount of inorganic phosphate (Pi) released by the hydrolysis of *c*AMP in the presence of CaM and a nucleotidase can be quantify spectrophotometrically or radiometrically (Sharma and Wang, 1979). Myosin light chain kinase (MLCK), Ca^{2+}-ATPase and calcineurin are other CaM-regulated enzymes broadly used to detect CaM antagonists; recently the use of

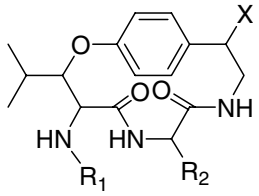
CaM-dependent NADK was reported (Mata et al., 2003a). Since in the functional studies employed to detect CaM-inhibitors, a limited number of CaM-dependent enzymes have been used, caution must be used in extrapolating data to all CaM-dependent processes.

For most assays the phenothiazines trifluoperazine (TFP) and chlorpromazine, the naphthalenesulfonamide W-7 [*N*-(6-aminohexyl)-5-chloro-1-napththalenesulfonamide] as well as calmidazolium have been extensively used as positive controls.

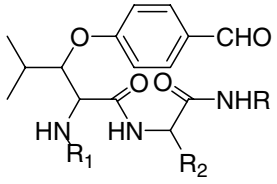
During the 1980s and early 1990s a few natural products with anti-CaM properties were already discovered using functional enzymatic assays; then, the interest in the topic dwindled. However, more recent research in several laboratories world-wide revealed a renewed interest for discovering new CaM inhibitors. This concern is probably due to the significant progress in the knowledge of this regulatory protein including structural features, the discovery of new CaM target enzymes and isoforms, its role in the regulation of several physiological processes such as plant growth and defense (Bouché et al., 2005), muscle contraction and relaxation (Somlyo and Somlyo, 2003), learning and memory, immune responses (Horikawa et al., 2005), osteoclastogenesis (Seales et al., 2006), inflammation (Horikawa et al., 2005), mood and anxiety physiology (Du et al., 2004), to mention a few. Furthermore, potential modulation of physiological targets of CaM by natural or synthetic compounds offers great possibilities for the discovery of new drugs or pesticide agents and valuable research tools to understand the complex CaM messenger



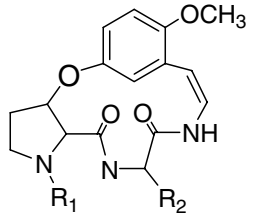
	R1	R2
(**24**)	$N(CH_3)_2$Phe(S)-	$(CH_3)_2CHCH_2$-
(**25**)	$N(CH_3)_2$Phe(R)-	$(CH_3)_2CHCH_2$-
(**26**)	$N(CH_3)$Phe-	$(CH_3)_2CHCH_2$-
(**27**)	$N(CH_3)_2$Phe-	$(CH_3)_2CHCH(OH)$
(**28**)	$N(CH_3)_2$Leu-	$(CH_3)_2CHCH_2$-
(**29**)	t-cinnamoyl-	$(CH_3)_2CHCH_2$-



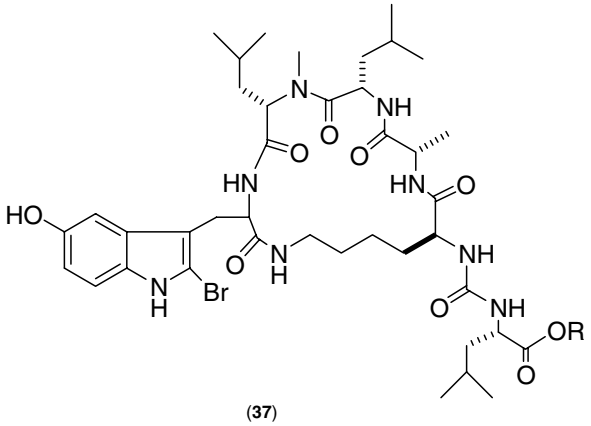
	R1	R2	X
(**30**)	$N(CH_3)_2$Phe(S)-	$(CH_3)_2CHCH_2$-	H
(**31**)	$N(CH_3)_2$Phe(R)-	$(CH_3)_2CHCH_2$-	OCH_3
(**32**)	$N(CH_3)$Phe-	$(CH_3)_2CHCH_2$-	OH



	R1	R2	X
(**33**)	$N(CH_3)_2$Phe(S)-	$(CH_3)_2CHCH_2$-	H
(**34**)	$N(CH_3)_2$Phe(R)-	$(CH_3)_2CHCH_2$-	CHO

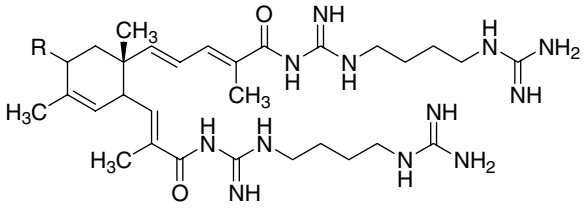


	R1	R2
(**35**)	$N(CH_3)_2$Try-	$CH_3CH_2CH(CH_3)$-
(**36**)	$NH(CH_3)$Ala-Val-	Benzyl-



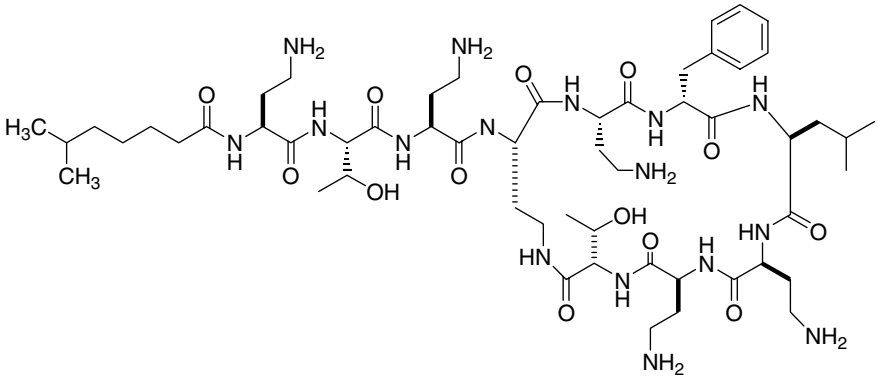
(**37**)

Fig. 2. Peptides.



(**39**) R= H

(**40**) R=



(**38**)

GIGAVLKVLTTGLPALISWILRLRQQ-NH_2

(**41**)

CNCKAPETALCARRCQQ-NH_2

(**42**)

GLLDILKKVGKVA-NH_2

(**43**)

GLFDII KK I AES I-NH_2

(**44**)

GLFDVIKKVASVIGGL-NH_2

(**45**)

GLFDIVKKVVGALGSL-NH_2

(**46**)

GLFDIVKKVVGIAGSL-NH_2

(**47**)

GLFDIVKKVVGTLAGL-NH_2

(**48**)

GLLGSIGNAIGAFIANKLKP-OH

(**49**)

GLMSVLGHAVGNVLGGLFKPKS-OH

(**50**)

GLVSSIGKALGGLLADVVKSKGQPA-OH

(**51**)

GLLGVLGSIAKHVLPHVVPVIAEHL-NH_2

(**52**)

GLLSVLGSVAKHVLPHVVPVIAEKL-NH_2

(**53**)

GLFSVLGAVAKHVLPHVVPVIAEKL-NH_2

(**54**)

GLFKVLGSVAKHLLPHVVPVIAEKL-NH_2

(**55**)

GLFGVLGSI AKHVLPHVVPVIAEKL-NH_2

(**56**)

Fig 2. (*continued*)

system in plants and animals. In this article, the structures, natural sources and biological activities of natural anti-CaM compounds reported to date are systematically reviewed.

2. Alkaloids and peptides

The alkaloid family contains the largest number of Ca^{2+}–CaM inhibitors (Fig. 1); most of these are indole alkaloids of different structural classes. The unusual indole-carbazole alkaloids from a culture broth of a *Nocardiopsis* sp, namely K-252a-K-252d (**1**–**4**), inhibited specifically the activity of both bovine brain and heart Ca^{2+}–CaM-stimulated PDE1's (Kase et al., 1986; Nakanishi et al., 1986); the most potent inhibitors were **1** ($IC_{50} = 2.9$ and 1.3 μM, respectively) and **2** ($IC_{50} = 10.9$ and 12.5 μM, respectively). The brominated β-carbolines eudistomidins A (**5**) and C (**6**), obtained from the Okinawan tunicate *Eudistoma glaucus*, were the first CaM antagonists isolated from a marine source (Kobayashi et al., 1986; Kobayashi et al., 1990a); both compounds were more potent than W-7 ($IC_{50} = 300$ μM) as inhibitors of the complex Ca^{2+}–CaM–PDE1, with IC_{50} values of 20 μM and 30 μM, correspondingly. Pyridindolol (**7**) from *Streptoverticillium album* K-251 is another β-carboline alkaloid that inhibited the activity of bovine brain and heart PDE1's with IC_{50} values of 320 μM and 251 μM, respectively (Matsuda et al., 1988). Among the pyrrolopyrimidine-type of alkaloids from the Okinawan marine tunicate *Eudistoma* cf. *rigida*, rigidin (**8**) is the only CaM inhibitor, affecting bovine-brain PDE1 activity with an IC_{50} of 50 μM (Kobayashi et al., 1990b). Stellettamide A (**9**), an octahydroindolizine containing a farnesyl moiety, from a sponge species of the genus *Stelletta* sp, inhibited CaM activity assessed by two Ca^{2+}–CaM-dependent enzymes, namely the (Ca^{2+}–Mg^{2+})-ATPase of erythrocyte membrane ($IC_{50} = 100$ μM) and PDE1 prepared from bovine cardiac muscle with an ($IC_{50} = 52$ μM). The inhibitory effect on PDE1 activity was antagonized by increasing CaM concentration. The interaction between **9** and CaM was demonstrated by instantaneous quenching of the intrinsic tyrosine fluorescence of CaM by **9** (3–300 μM). Similar results were obtained in the presence or absence of Ca^{2+} suggesting that **9** binds to CaM, and that Ca^{2+} is not essential for this binding. Furthermore, **9** inhibited myosin light chain (MLC) phosphorylation with an IC_{50} of 152 μM in a smooth muscle contractile system containing CaM, MLC and MLCK. Once more, the effect of the alkaloid was antagonized by increasing the concentration of CaM (Abe et al., 1997). Stellettazole A (**10**), obtained from the same sponge, also inhibited by 45% the activity of PDE1 at a concentration of 100 μM (Tsukamoto et al., 1999b). The antitumoral bis-indol alkaloids vinblastine (**11**) and vincristine (**12**) bind to CaM in a Ca^{2+}-dependent manner. The interaction was quantified by fluorescence energy transfer and CD measurements. The calculated dissociation constant (3 μM) of the CaM–vinblastine complex revealed that the binding affinity of the alkaloid to CaM and tubulin is comparable (Molnar et al., 1995). The monomers, catharantine (**13**) and vindoline (**14**), were practically ineffective in these tests revealing the dramatic differences between the CaM-antagonistic effect of dimeric and monomeric indole alkaloids. In recent years, it has been suggested that the anti-CaM effect of vinca alkaloid account for some of their side effects (Roufogalis et al., 1999).

The cytotoxic dimeric alkaloid leptosin M (**15**), biosynthesized by a strain of *Leptosphaeria* sp OUPS-4 and the algae *Sargassum tortil*, inhibited Ca^{2+}–CaM dependent kinase III (CaMKIII) as well as protein tyrosine kinase (PTK) at a concentration of 10 μg/ml by 40–70%. It is important to point out that there is no direct evidence that compound **15** directly inhibits CaM or the basal activity of CaMKIII. On the other hand, alkaloid **15** specifically inhibited topoisomerase II with an IC_{50} value of 59.1 μM without affecting topoisomerase I (Yamada et al., 2002).

Verruculogen (**16**) is a very potent mycotoxin produced by several *Penicillium* species. This toxin inhibited PDE1 ($IC_{50} = 42$ μM) activity in a concentration dependent manner without affecting the basal activity of PDE1. Interestingly, verruculogen (**16**) showed little effect on *N*-pheny-1-naphthylamine (NPN) fluorescence and the Ca^{2+}-dependent complex formation of dansyl-CaM and PDE (Pala et al., 1999).

Melatonin (**17**), a hormone found in all living creatures from algae to humans acts as a CaM antagonist inducing conformational changes in the estrogen receptor α–CaM (ERα–CaM) complex, thus impairing the binding of E2–ERα–CaM complex to DNA and, therefore, preventing ERα-dependent transcription. Moreover the mutant ERα (K302G, K303G), unable to bind CaM, becomes insensitive to melatonin (**17**). The effect of melatonin (**17**) is specific since other related indol alkaloids neither interacts with CaM nor inhibits ERα-mediated transactivation (Del Rio et al., 2004).

Bioassay-directed fractionation of an ethyl acetate extract (mycelia and broth) of the coprophilous fungus *Malbranchea aurantiaca* led to the isolation of the unusual prenylated indole alkaloid malbrancheamide (**18**). The absolute configuration of its chiral centers was established by X-ray analysis (Martínez-Luis et al., 2006). Compound **18** possesses one preferred conformation according to the conformational studies using molecular mechanics studies. Alkaloid **18** inhibited the activation of PDE1 ($IC_{50} = 3.65$ μM) in a concentration-dependent manner; the effect was comparable to that of chlorpromazine ($IC_{50} = 2.75$ μM). The results of a kinetic analysis ($K_i = 47.4$ μM) suggested that **18** acts as competitive antagonist of CaM competing with the formation of the CaM–PDE1 active complex. This compound caused also moderate inhibition of radicle growth of *Amaranthus hypochondriacus* ($IC_{50} = 0.37$ μM) with a similar potency

to 2,2-dichlorophenoxyacetic acid [2,4-D; $IC_{50} = 0.18$ μM]. The CaM antagonistic effect of **18** might be related with its phytotoxic action and other pharmacological properties yet to be discovered. From a structural point of view, it is important to point out that malbrancheamidely is the first chlorinated indole alkaloid possessing a bicyclo [2.2.2] ring (Martínez-Luis et al., 2006).

The isoquinoline papaverine (**19**) inhibits PDE1 (Ronca-Testoni et al., 1985) and strongly reduced the activity of the isoform PDE10 which is insensitive to CaM (Boswell-Smith et al., 2006; Siuciak et al., 2006). This natural product **19** also exerts a weak inhibitory effect on the basal activity of PDE1 (~8%). The bisbenzylisoquinolines alkaloids berbamine (**20**), dauricine (**21**) and daurisoline (**22**) isolated from *Menispermun dauricum* and *Berberis poiretii*, inhibited the ability of CaM to activate PDE1 (Hu et al., 1988). The most active was berbamine ($IC_{50} = 22$ μM) which displayed a similar activity to trifluoperazine ($IC_{50} = 7.5$ μM). Structure–activity relationship studies revealed that an increment of the hydrophobicity in the alkaloids provoked an augmentation of the anti-CaM activity; for example, a berbamine analog possessing a *n*-propionyl moiety at C-12 was two times more potent than the parent compound; the C-12 mono acetyl derivative of daurisoline (**22**) also showed stronger anti-CaM activity ($IC_{50} = 1.2$ μM). It is important to point out that the nature of the polar terminus of the bisbenzylisoquinoline (tertiary amine vs. a quaternary amine) did not modify the anti-CaM activity (Hu et al., 1988; Chen et al., 1990).

Domoic acid (**23**), a tricarboxylic amino acid, is a shellfish toxin which produces neurodegeneration and CNS dysfunction, notably a loss of short-term memory. This toxin is localized in the stomach of blue mussels (*Mytilus edulis*) overfed with the algae *Nitzschia pungens*. The toxin was found to inhibit Ca^{2+} and CaM-stimulated adenylate cyclase activity in brain membranes, resulting in reduced level of *c*AMP; this effect in turn reduce the feedback control of *c*AMP on Ca^{2+} influx via Ca^{2+} channels, thus, allowing continuing enhanced Ca^{2+} influx; this Ca^{2+} overload adversely affects many intracellular processes to provoke toxicity (Nijjar and Nijjar, 2000).

From the Korean medicinal herbs *Zizyphus vulgaris* var. *spinosus* and *Z. jujube* var *inermis* (Rhamnaceae), there were isolated several cyclopeptides (**24**–**32**), two linear peptides (**33** and **34**) and the peptide alkaloids **35** and **36** (Fig. 2). The plants, as well as the isolates, have sedative properties. Their effects on the CaM dependent enzymes PDE1, Ca^{2+}-ATPase and protein kinase II (PKII) were tested and the results are summarized in Table 1. In general, the peptides were good CaM inhibitors, more potent than the positive controls chlorpromazine and pimozide. The effects on Ca^{2+}-ATPase showed good correlation with the sedative properties of the compounds (Han et al., 1993, 2005; Hwang et al., 2001). Sanjoinine A (**24**) binds to CaM in a Ca^{2+}-dependent manner at two sets of binding sites; the binding ratio was calculated as 1:2 (CaM to **24**) at the high affinity (K_d, 1.1 μM) sites and 1:4 (CaM to **24**) at the low affinity (K_d, 3.1 μM) sites (Han et al., 1993).

Table 1
Effect of peptides **24**–**36** on the enzymes PDE1, Ca^{2+}-ATPase, and PKII in the presence of CaM

Compounds	IC_{50} (μM)		
	PDE1	Ca^{2+}-ATPase	PKII
Sanjoinine A (**24**)	82	4.6	24.1
Sanjoinine Ahl (**25**)	55	4.0	27.2
Sanjoinine B (**26**)	[a]	[a]	23.5
Sanjoinine F (**27**)	94	7.9	18.2
Daechuine S4 (**28**)	21	19.6	86.0
Sanjoinenine (**29**)	25.5	11.0	35.0
Dihydrosanjoinine A (**30**)	21	23.7	35.0
Sanjoinine D (**31**)	9	23	33
Sanjoinine G1 (**32**)	140	14.6	28.5
Sanjoinine G2 (**33**)	125	7.2	19.0
Sanjoinine A dialdehyde (**34**)	34	2.3	40.5
Daechuine S10 (**35**)	4.9	29.0	57.0
Daechuine S27 (**36**)	16.8	27.0	2.95
Chlorpromazine	4.6	27.0	[a]

[a] Data noncalculated.

Konbamide (**37**), a unique peptidic alkaloid (Fig. 2) produced by the Okinawan marine sponge *Theonella* sp, exhibited moderate inhibition of PDE1 with an IC_{50} of 100 μM (Kobayashi et al., 1991). Polymyxin B (**38**), a cyclic peptide antibiotic (Fig. 2) produced by *Bacillus polymyxa*, is widely used as a pharmacological tool to investigate the involvement of protein kinase C (PKC) on physiological processes. The peptide inhibited bovine heart-PDE1 with a remarkably low IC_{50} value (80.5 nM) in the presence of 500 μM Ca^{2+}. The inhibition was competitive with respect to CaM. The interaction of polymyxin B with CaM was further supported by affinity chromatography of the protein on polymixin β-agarose and polyacrylamide gel electrophoresis under non-denaturing conditions (Hegemann et al., 1991). Polymyxin B inhibited also other CaM-dependent enzymes such as MLCK and PKII with K_i values of 17 μM and 950 μM, respectively (Hegemann et al., 1991). The bistellettadines A (**39**) and B (**40**) (Fig. 2) moderately inhibited PDE1 (40% inhibition at 100 μM) (Tsukamoto et al., 1999a).

Several peptides found in insect venoms (Fig. 2), including melittin (**41**), apamin (**42**) and mastoparans inhibited the activity of PDE1 at concentrations that had no appreciable effect on basal PDE1 activity; the K_i value of melittin (**41**) for inhibiting CaM activity was 30 nM. Acetylation of the peptides reduced their inhibitory effect on CaM, suggesting that a net positive charge was an important structural feature for anti-CaM activity (Barnette et al., 1983). Melittin (**41**) appears to competitively inhibit the binding of PDE1 to CaM suggesting that the binding site may be the same. Small-angle X-ray scattering has revealed that the dumbbell shape of CaM is changed to a globular shape upon binding melittin (**41**) in the presence of Ca^{2+} (Kataoka et al., 1989). Peptide **41** inhibited also PKC, Ca^{2+}–CaMKII, MLCK, Na^{+}–K^{+}-ATPase (synaptosomal

Table 2
Effect of peptides **43**–**56** on CaM-dependent *n*NOS

Peptides	IC_{50} (μM)
Lesueurin (**43**)	16.2
Aurein 1.1 (**44**)	33.9
Citropin 1.1 (**45**)	8.2
Aurein 2.2 (**46**)	4.3
Aurein 2.3 (**47**)	1.8
Aurein 2.4 (**48**)	2.1
Not named (**49**)	3.2
Frenatin 3 (**50**)	6.8
Splendipherin (**51**)	8.5
Caerin 1.1 (**52**)	36.6
Caerin 1.10 (**53**)	41.0
Caerin 1.6 (**54**)	8.5
Caerin 1.8 (**55**)	1.7
Caerin 1.9 (**56**)	6.2

membrane) and the H^+–K^+-ATPase. Apamin (**42**) is a very potent and highly selective antagonist of the Ca^{2+}-activated K^+-channel, which is regulated by CaM. The inhibition of CaM activity by these insect venom peptides may explain some of their biochemical or toxicological effects.

Fourteen (**43**–**56**) peptides (Fig. 2) from the glandular skin secretions of Australian anurans of the *Litoria* genus were found to inhibit (Table 2) CaM-dependent neuronal nitric oxide synthase (*n*NOS). It has been proposed that the amphibian peptides inhibit *n*NOS by blocking the attachment of the enzyme to Ca^{2+}–CaM. Several experiments support this proposal: First, the inhibition of the enzyme by selected peptides (**43**, **45**, **50** and **56**) was reduced by the addition of Ca^{2+}–CaM to the assay buffer. The maximum reduction of inhibition under the experimental conditions used was 50%. Second, these peptides inhibited the activity of calcineurin, another enzyme that requires activation by CaM; lesueurin (**43**) at 74 μM inhibited calcineurin by 33%; citropin 1.1 (**45**) reduced calcineurin activity by 96% at 62 μM; frenatin 3 (**50**) inhibited calcineurin by 38% at 46 μM; finally, caerin 1.9 (**56**) inhibited calcineurin by 48.1% at 19.3 μM. A kinetic analysis revealed that mechanism of inhibition is noncompetitive with respect to arginine, the *n*NOS substrate (Doyle et al., 2002).

3. Polyamines

The natural polyamines (Fig. 3) spermine (**57**) and 1,12-diaminododecane (**58**) inhibited CaM-sensitive bovine heart MLCK. According to a kinetic analysis, the inhibition of **57** was noncompetitive with respect to Ca^{2+} ($K_i = 1.82$ mM) and CaM ($K_i = 2.73$ mM); whereas **58** inhibited the enzyme competitively with regard to CaM ($K_i = 0.63$ mM) and non-competitively respect to Ca^{2+} ($K_i = 1.5$ mM). The latter result suggested that the amine interacts only with the enzyme-binding site of CaM. The analog amines spermidine (**59**), cadaverine (**60**), putrescine (**61**) and 1,10-diaminodecane (**62**) were less active suggesting that the spacing between the two positively charged terminal nitrogen atoms is crucial to interfere with MLCK activation by CaM (Qi et al., 1983).

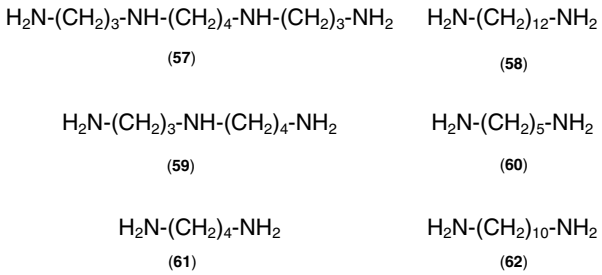


Fig. 3. Polyamines.

4. Terpenoids

Ophiobolin A (**63**) (Fig. 4) is the natural CaM inhibitor most investigated so far; it is a potent phytotoxin isolated from the pathogenic fungi *Bipolaris oryzae* and inhibited CaM-dependent PDE1 activity with an IC_{50} of 9 μM. Its effect was accredited to inhibition of CaM in the assay system because the basal activity of PDE1 without CaM was not affected (Leung et al., 1984). In maize roots, the phytotoxic effect of ophiobolin A (**63**) correlated very well with CaM inhibition. The effects were time-dependent and irreversible. When ophiobolin A (**63**) was adminstered to maize roots, a smaller amount of active CaM was found in the root extract, thereby indicating that the sesterpenoid possibly inhibited CaM in vivo. The direct interaction between **63** and CaM was shown by quenching the intrinsic tyrosine fluorescence of bovine brain CaM upon the addition of this sesterterpenoid. In UV spectroscopic studies, ophiobolin A (**63**) reacted with CaM to give a new chromophore with an absorption maximum at 272 nm; this type of study revealed also that one CaM reacted with two molecules of ophiobolin A (**63**). More recently, Au and Leung (1998), using site-directed mutagenesis to define the ophiobolin A (**63**) inhibitory site in the bovine-brain CaM, found that lysine 75 is the inhibitory site and that lysine 148 is another binding site, but is not an inhibitory site for **63** (Au and Leung, 1998; Au et al., 2000). Recently, Evidente et al. (2006) demonstrated that ophiobolin A (**1**) and some analogs, assayed by means on punctured detached leaves of several grass and dicotyledon weeds, had the potential for the development of herbicidal agents against grass weeds. Ophiobolin A (**63**) caused the appearance of large necrosis on the grasses tested, even at the lowest concentration assayed. Some structural features are important for the noted phytoxicity of **63**, in particular the hydroxy group at C-3, the stereochemistry at C-6, and the aldehyde group at C-7 (Evidente et al., 2006).

Fasciculic acids A–C (**64**–**66**) and fasciculols C–E (**67**–**69**) (Fig. 4), isolated from the mushroom *Naematoloma fasciculare*, are inhibitors of CaM-sensitive and -insensitive

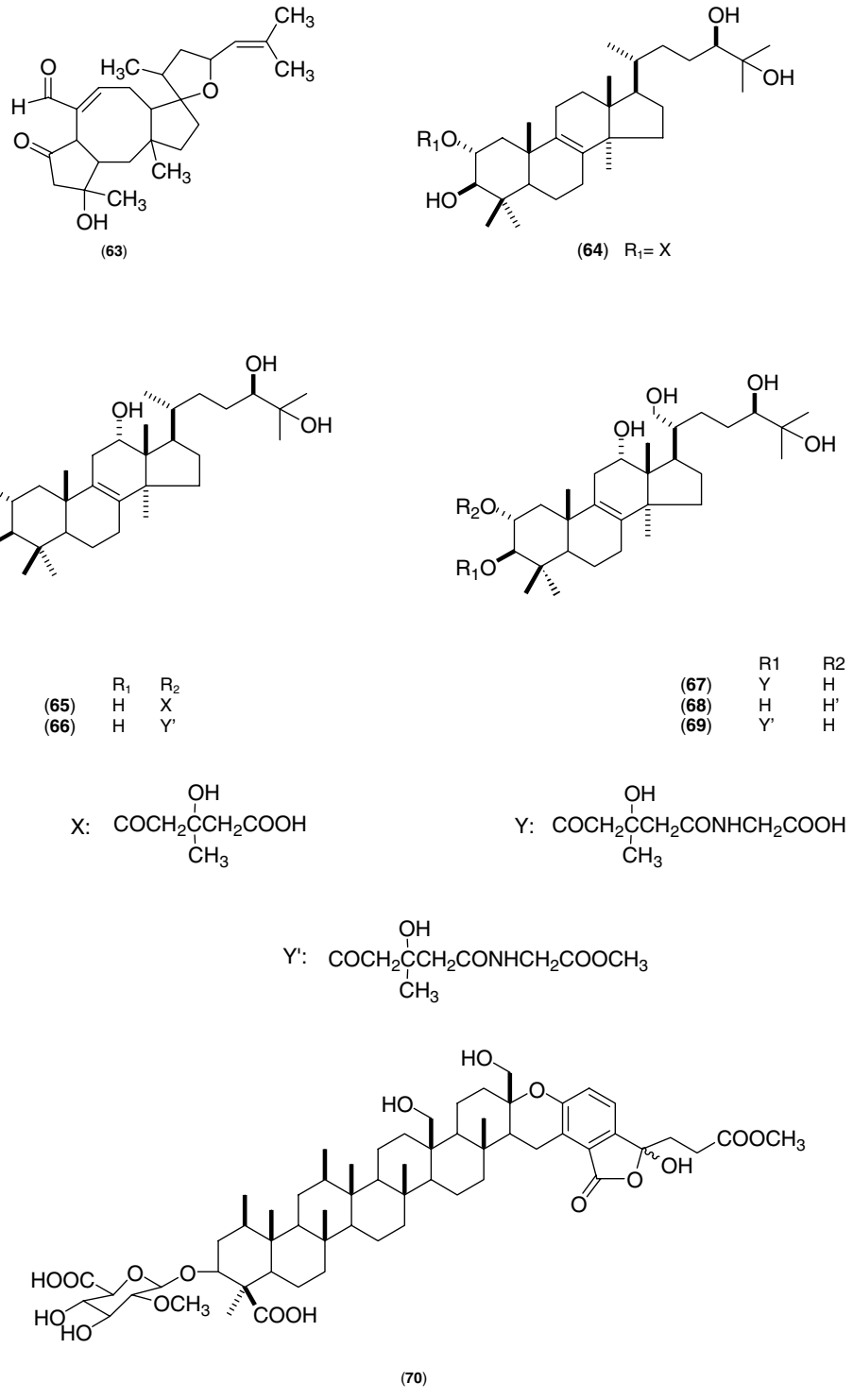


Fig. 4. Terpenoids.

PDE with different selectivity; the IC_{50} values for both enzymes ranged from 6 to >100 μM. In general, they were more active than W-7 ($IC_{50} = 65$ μM), a well known CaM inhibitor. Compound **64** was the only fasciculic acid that selectively inhibited PDE1 activity ($IC_{50} = 10$ μM) without affecting CaM-insensitive PDE (Takahashi et al., 1989; Kubo et al., 1985).

KS-505a (**70**), from *Streptomyces argenteolus* A-2, is a terpenoid (Fig. 4) possessing a unique structure with a 2-*O*-methylglucuronic acid moiety, a decacyclic isoprenoid skeleton and a tautomeric aromatic γ-hydroxy-γ-lactone. KS-505a potently inhibited PDE1 from bovine brain ($IC_{50} = 0.065$ μM) and required much higher doses to inhibit PDE1 from bovine heart ($IC_{50} = 1.4$ μM); the activity

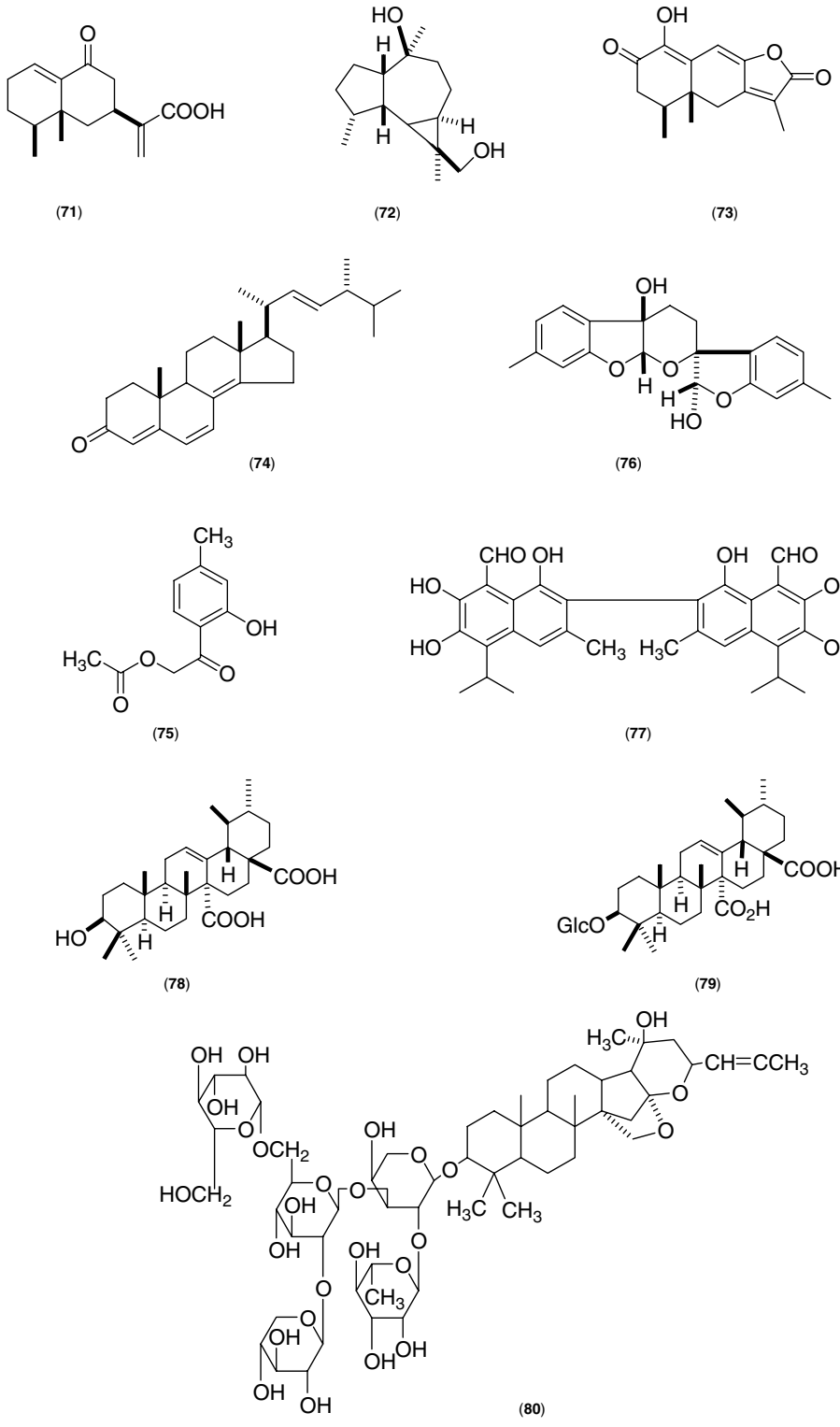


Fig. 4 (*continued*)

was compared to calmodazolium ($IC_{50} = 0.12$ μM). Kinetic analysis indicated that the inhibitory mode of KS-505a for the bovine brain isoenzyme was competitive with respect to Ca^{2+}–CaM with a K_i of 0.089 μM. KS-505a did not interfere with the interaction between Ca^{2+}–CaM and NPN, a hydrophobic fluorescent probe, nor was it adsorbed to CaM-conjugated gels in the presence of Ca^{2+}; then, KS-505a bound to a site in the Ca^{2+}–CaM-binding domain of the 61 kDa isoenzyme and selectively inhibited its activity (Nakanishi et al., 1992; Ichimura et al., 1996).

Dehydroflourensic acid (**71**) and flourensadiol (**72**) (Fig. 4) are the major phytotoxic principles of *Flourensia cernua* (Asteraceae). According to an SDS–PAGE electrophoresis experiment these compounds affected the electrophoretic mobility of CaM. In addition, the activation of the CaM-sensitive PDE1 was inhibited in the presence of both sesquiterpenoids with IC_{50} values of 23.2 and 5.2 μM, respectively; compound **72** (Fig. 4) was more active than chlorpromazine ($IC_{50} = 10.2$ μM) (Mata et al., 2003b). Another phytotoxic eremophyllane that inhibits CaM is **73** (Fig. 4); it was isolated from the coprophilous fungus *Malbranchea aurantiaca*; this sesquiterpenoid inhibited activation of PDE1 ($IC_{50} = 10.2$ μM) with a higher potency than chlorpromazine ($IC_{50} = 18.4$ μM) (Martínez-Luis et al., 2005). The fungal sterol **74** (Fig. 4) isolated from *Guanomyces polytrix* also inhibited activation of CaM sensitive enzymes PDE1 (bovine-brain) and NADK isolated from peas with IC_{50} values of 5.2 and 90.0 μM, respectively (Mata et al., 2003a).

The unusual phytotoxic thymol derivatives **75** and **76** (Fig. 4) from *Hofmeisteria schaffneri* (Asteraceae) inhibited radicle growth of *A. hypochondriacus* and the activation of PDE1 in the presence of CaM with IC_{50} values of 4.4 and 4.2 μM, respectively. Their effect was comparable

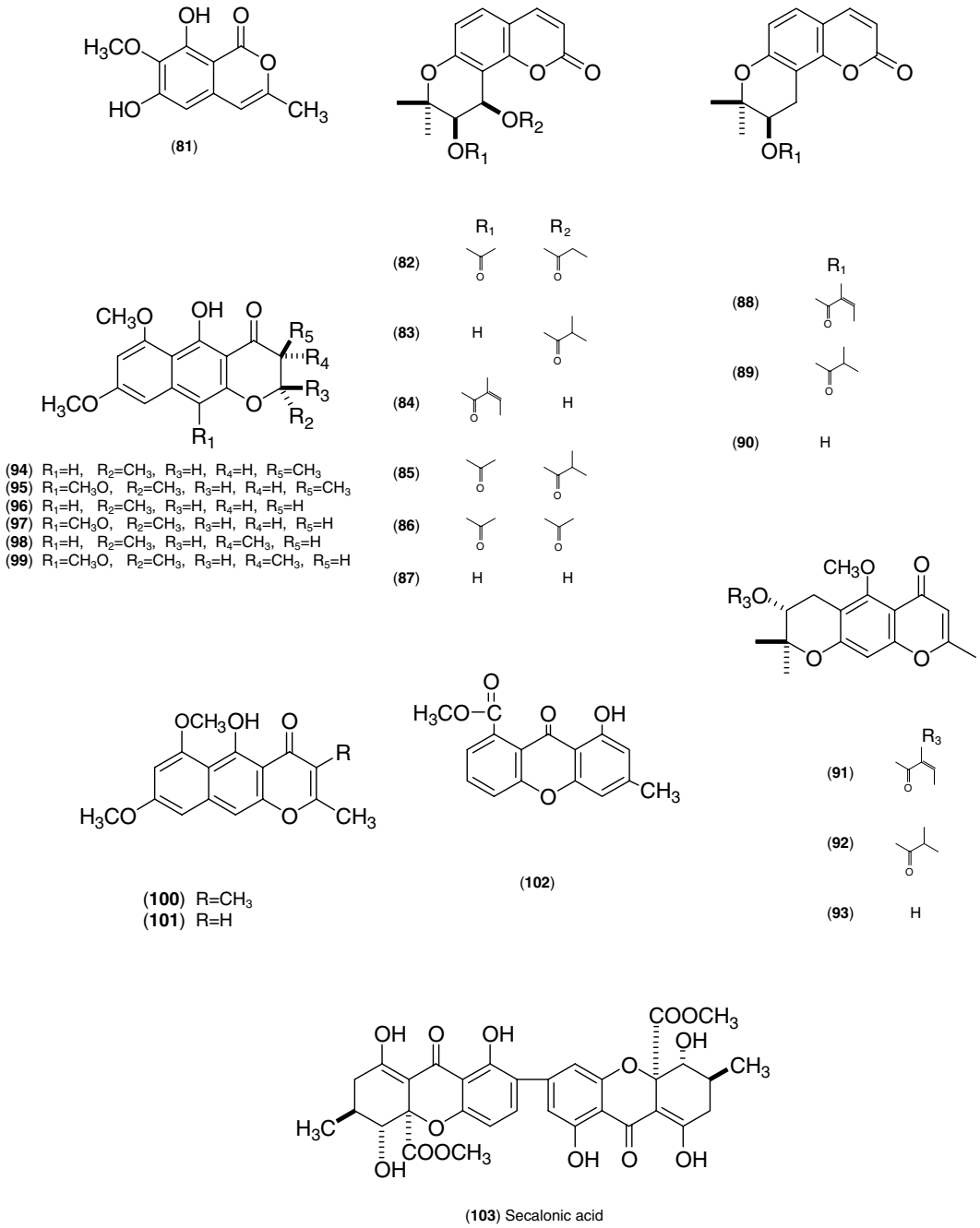


Fig. 5. Coumarins, γ-pyrones and xanthones.

to chlorpromazine ($IC_{50} = 6.8$ μM) (Pérez-Vasquez et al., 2005).

Gossypol (**77**), a sesquiterpenoid dimer (Fig. 4) produced by cotton plants, inhibited noncompetitively Ca^{2+}-ATPase in isolated plasma membrane of human spermatozoa with an IC_{50} value of 13 μM (Roufogalis et al., 1999). It also inhibited in a reversible way the Ca^{2+}–CaM-dependent protein Ser/Thr phosphatase, which is a key enzyme for most cyclosporin A (CsA)- and tacrolimus (FK506)-mediated biological effects. Inhibition of calcineurin by gossypol (**77**) was found to be independent of calcineurin activation by CaM, although the terpenoid binds to CaM at an independent site (Baumgrass et al., 2001).

Quinovic acid (**78**) and quinovic glycoside C (**79**) (Fig. 4) from *Mitragyna stipulosa* and other Rubiaceae, inhibited PDE1 activity with IC_{50}'s of 0.166 mM and 0.374 mM, respectively. Cysteine was used as a standard inhibitor ($IC_{50} = 0.748$ mM) (Fatima et al., 2002). Jujuboside A (**80**), a triterpenoid (Fig. 4) from the Chinese herbal medicine sanzaoren, is a noncompetitive inhibitor of CaM. The interaction of this natural product with CaM was investigated using both ^{1}H NMR and spin labeled EPR spectroscopies. The NMR experiment revealed that jujuboside A (**80**) has two binding sites on CaM, one located in the N-terminus and the second at the C-terminal region of the protein; jujuboside A (**80**) probably binds CaM through hydrophobic interactions (Zhou et al., 1994).

5. Coumarins, γ-pyrones and xanthones

Reticulol (**81**) (Fig. 5) from *Streptoverticillium album* K-251 inhibited PDE1 from bovine brain in a concentration-related manner; the IC_{50} value was 89.6 μM (Matsuda et al., 1988). The pyranocumarins **82**–**90** and the γ-pyrones **91**–**93** (Fig. 5) from the Umbelliferae *Prionosciadium watsoni* showed significant phytotoxicity against *A. hypochondriacus*, *Echinochloa crus-galli* and *Lemna pausicostata* (duckweed) and modified the electrophoretic mobility of bovine brain and spinach CaMs (Valencia-Islas et al., 2002).

From the mycelium and culture broth of the fungus *Guanomyces polythrix* several phytotoxic naphthopyrones **94**–**101** and the xanthone **102** (Fig. 5) affected the electrophoretic mobility of both spinach and bovine brain CaMs (Macías et al., 2000, 2001). In addition, these compounds inhibited the activity of CaM dependent-NADK and PDE1 (bovine brain) in a concentration dependent manner (Table 3). Affinity chromatography on CaM-agarose beads indicated that the interaction of these compounds with CaM through covalent linkages.

Secalonic acid (**103**) is a dimeric xanthone (Fig. 5) produced in large amounts by *Penicillium oxalicum* cultured on corn. Secalonic acid inhibited PDE1 activity ($IC_{50} =$ 45 μM) in a concentration-dependent manner. The interaction of **103** with CaM was confirmed by fluorescence spectroscopy (Pala et al., 1999).

Table 3
Effect of compounds **94**–**101** on PDE1 and NADK

Compound	IC_{50} (μM)	
	PDE1[b]	NADK[c]
94	8.1	40.1
95	7.2	22.0
96	6.1	ND
97	5.8	ND
98	7.6	42.2
99	6.6	24.3
100	4.8	17.1
101	4.7	13.3
Chlorpromazine[a]	10.6	ND
Quercetin[a]	20.1	ND

ND = not determined.
[a] Controls.
[b] In the presence of 0.2 μg of bovine-brain CaM.
[c] In the presence of 2.0 μg of spinach CaM.

6. Anthracyclins and anthraquinones

Antraquinone K-259-2 (**104**) (Fig. 6), isolated from the cultures of the bacteria *Micromonospora olivasterospora* K-259, inhibited PDE1 from bovine brain and heart with IC_{50} values of 6.6 and 2.9 μM, respectively. However, the effect was not specific since bovine brain and heart CaM-independent cyclic nucleotide PDEs were inhibited by this compound to a lesser extent with IC_{50} values of 27.4 and 40.7 μM, respectively (Matsuda et al., 1987, 1990). KS-619-1 (**105**) (Fig. 6), from the cultures of the *Streptomyces californicus*, inhibited the bovine brain and heart CaM-dependent PDE1s in a concentration-dependent manner. The IC_{50} values were 2.0 and 1.5 μM, respectively. This compound also inhibited CaM-independent PDE from bovine heart with less potency than the CaM-dependent enzyme with IC_{50} values of 12.3 and 25.9 μM, respectively (Matsuda and Kase, 1987; Yasuzawa et al., 1987). Adriamycin (**106**), an anthracycline-aminoglycoside (Fig. 6) anticancer compound produced by several *Streptomyces* species, weakly inhibited CaMK ($IC_{50} \sim 50$–85 μM) and other CaM functions (30–300 μM) by binding to a hydrophobic region of the protein. However, the effect on PDE1 activity was very weak ($IC_{50} = 700$ μM) (Matsuda and Kase, 1987). Emodin (**107**) (Fig. 6), isolated from several plant and fungal sources also affected the electrophoretic mobility of plant and animal CaMs (Macias et al., 2001).

7. Lignans

Four furofurane type of lignans (**108**–**111**, Fig. 7) from *Leucophyllum ambiguum* (Scrophulariaceae) interact with bovine brain-CaM as distinguished in a SDS–PAGE electrophoresis method. CaM treated with the lignans had lower electrophoretic mobility than untreated CaM. In addition, the activation of the CaM-dependent enzyme PDE1 was inhibited in the presence of **108**–**111** and CaM

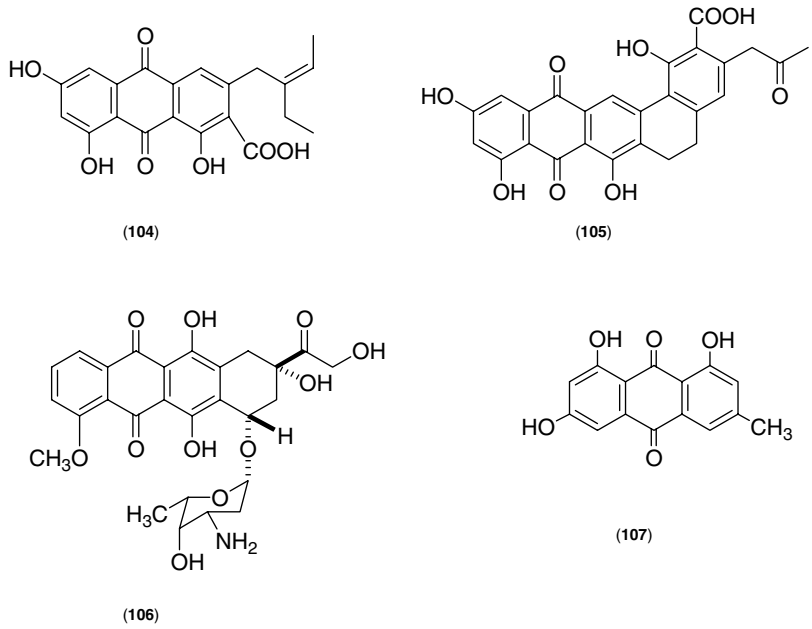


Fig. 6. Anthracyclins and anthraquinones.

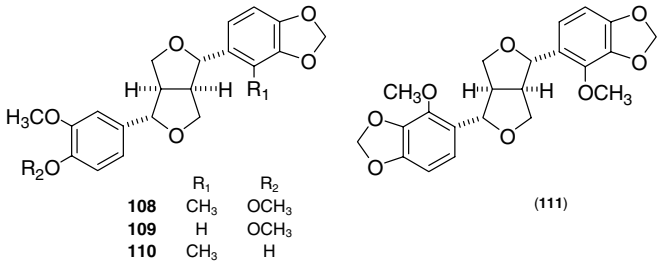


Fig. 7. Lignans.

(IC_{50} = 14.4, 26.4, 7.4, and 5.6 μM, respectively). The inhibitory activity of compounds **110** and **111** was higher than that of chlorpromazine (IC_{50} = 10.2 μM) (Rojas et al., 2003).

8. Stilbenoids

Asakawa et al. (1991) reported that the prenyl bibenzyls **112** and **113** (Fig. 8), isolated from the liverwort *Radula kojana*, showed CaM inhibitory activity (IC_{50} = 4.9 μg/ml and 4.0 μg/ml, respectively). However, throughout that paper it is not described how the activity was measured.

The stilbenoids **114**–**121** (Fig. 8) isolated from several Mexican orchids showed CaM inhibitor properties. These aromatic compounds inhibited PDE1 (Table 4) without affecting the basal activity of the enzyme. Since most of these compounds are smooth muscle relaxants, it was proposed that CaM is involved in their pharmacological effect; this suggestion was supported by means of a functional experiment, since the concentration–response curves of **116** and **117** were shifted to the left when the spasmolytic effect was determined in the presence of chlorpromazine (0.1 μM). These results are consistent with chlorpromazine, **116** and **117** being agonists. Furthermore, the effect of L-NAME, an inhibitor of CaM regulated-*n*NOS, on *c*GMP production was antagonized by these stilbenoids (Hernández-Romero et al., 2004). The carbocyclic skeleton of nidemone (**114**) is unusual and it can be envisaged that it arises by an oxidative contraction (Bayer–Villiger type) of an aromatic ring of a suitable dihydrophenanthrene (Hernández-Romero et al., 2004). It is interesting to point out that compounds **116**, **117** and **119** showed also significant phytotoxic activity against *A. hypochondriacus* and *L. pausicostata* (Hernandez-Romero et al., 2005). Piceatanol (**122**, Fig. 8), a widespread stilbenoid that interacts with CaM, inhibits the enzyme activity of MLCK and Ca^{2+}-dependant protein kinase (CDPK) with IC_{50}'s of 12 and 19 μM, respectively (Wang et al., 1998).

9. Miscellaneous polyketides

The alkyl phenolic antibiotics KS-501 (**123**) and KS-502 (**124**) (Fig. 9), isolated from the fungus Sporothrix sp. KAC-1985, inhibited both bovine brain and heart PDE's (IC_{50} = 1.8 and 4.3 μM for bovine brain, and 15 and 5.9 μM for bovine heart, respectively) (Nakanishi et al., 1990a; Yasuzawa et al., 1990). Methyl orsellinate (**125**) (Fig. 9), a phytotoxin isolated from Flourensia cernua and several lichens (Mata et al., 2003), inhibited activation of PDE1 with an IC_{50} value of 8.1 μM, being more active than chlorpromazine (IC_{50} = 10.2 μM). The butyrolactones (**126**–**134**) (Fig. 9), and the related compounds (**135**–**137**) (Fig. 9), isolated from the culture of Streptomyces viridochromogenes, showed weak inhibitory activity of

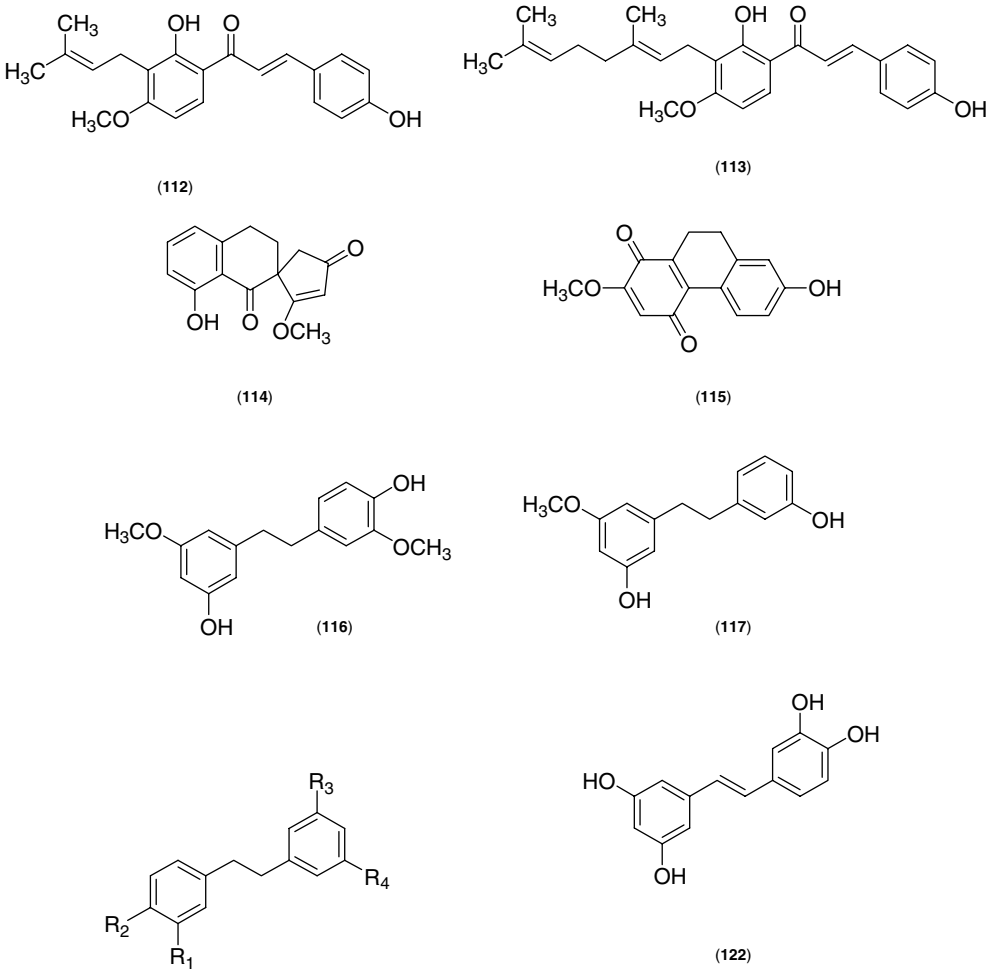


Fig. 8. Stilbenoids.

Table 4
Effect of stilbenoids **114**–**121** on PDE1

Compound	IC_{50} (μM)
114	3.2
115	10.9
116	7.0
117	13.3
118	9.0
119	10.1
120	36.6
121	21.2
Chlorpromazine	10.3

PDE1; the IC_{50}'s ranged from 2 to 15 mM (Hoff et al., 1992; Ritzau et al., 1993). The unusual diester antibiotics MS-282a (138) and MS-282b (139) (Fig. 9) were isolated from the culture broth of Streptomyces tauricus ATCC 27470 as inhibitors of MLCK. These compounds inhibited Ca^{2+}–CaM-dependent activity of chicken gizzard MLCK in a concentration-dependent manner with IC_{50} values of 3.8 μM and 5.2 μM, respectively. CaM-dependent cyclic nucleotide phosphodiesterase ($IC_{50} = 4.2$ μM) was also inhibited by 138 (Nakanishi et al., 1994).

A series of unusual polychlorinated natural products, KS-504's (**140**–**143**) (Fig. 9), were isolated from the fungus *Mollisia ventosa* kac-1148. These compounds suppress in vivo cell responses, such as contraction of isolated rabbit aortic strips, rabbit platelet aggregation and serotonin secretion, as well as histamine release from rat peritoneal mast cells. These compounds also inhibited several CaM dependent enzymes (Table 5). CaM-independent activities of the enzymes were not affected by the compounds at the same concentration ranges. Ca^{2+}-dependent interaction of the compounds with CaM was shown using hydrophobic fluorescence probes. These data indicated that the compounds exerted its effects on CaM-PDE1 by interacting with CaM. In the case of KS-504a (**140**), kinetic analysis using MLCK as a probe revealed that the inhibition mechanism was competitive with respect to CaM (Nakanishi et al., 1990b).

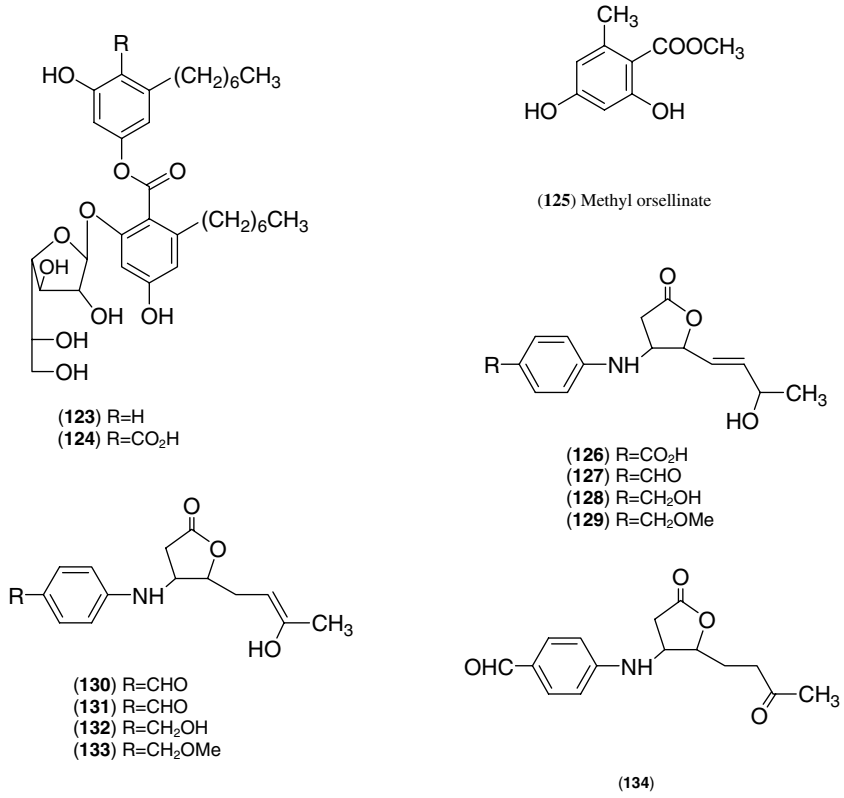


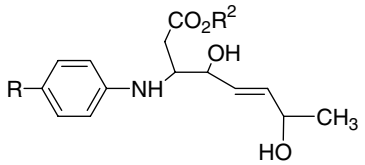
Fig. 9. Miscellaneous polyketides.

The nonenolides herbarumins I–III (**144**–**146**) (Fig. 9), isolated from the mycelium and culture broth of the fungus *Phoma herbarum* (Sphaeropsidaceae), caused relevant inhibition of radicle growth of seedlings of *A. hypochondriacus* when tested by the Petri dish bioassay. Compounds **144**–**146** interacted with bovine brain-CaM as detected in a SDS–PAGE electrophoresis. CaM treated with the lactones had lower electrophoretic mobility than untreated CaM. The effect was comparable to that of chlorpromazine. In addition, different concentrations of compounds **144** and **145** inhibited PDE1. The inhibitory activity of **144** ($IC_{50} = 14.2\ \mu M$) and **145** ($IC_{50} = 6.6\ \mu M$) was not higher than that of chlorpromazine ($IC_{50} = 9.8\ \mu M$). It is important to indicate that neither the basal activity of the enzyme nor the CaM-independent form of the enzyme was inhibited by the fungal metabolites (Rivero-Cruz et al., 2003).

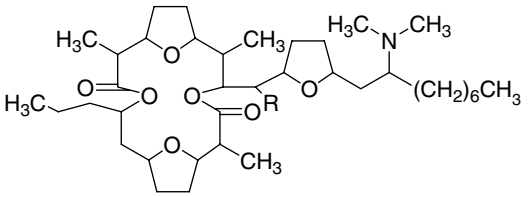
Citreoviridin (**147**) (Fig. 9) is a toxic secondary metabolite isolated from several *Penicillium* species. Its anti-CaM activity was proved using the CaM-deficient bovine brain PDE1 assay, binding of fluorescent probe NPN to CaM and alterations in the fluorescence of dansyl-CaM. Citreoviridin (**147**) inhibited CaM-stimulated PDE activity in a concentration-dependent manner with IC_{50} value of 44 μM without affecting the basal activity of CaM-deficient PDE1. Citreoviridin also inhibited NPN fluorescence and Ca^{2+}-dependent complex formation of dansyl-CaM and PDE with IC_{50} value of 13 μM (Pala et al., 1999). The authors speculated that the poisonous effect of **147** could be related to its anti-CaM activity.

10. Flavonoids and other phenolic compounds

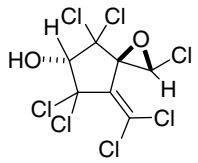
Quercetin (**148**, Fig. 10), one of the flavonoids most common in plants, at a concentration of 50 μM completely depressed CaM-induced NPN fluorescence; the interaction of this flavonol with CaM was confirmed by examining the binding activity of ^{14}C-quercetin to Ca^{2+}–CaM by the equilibrium binding technique of Hummel and Dryer (Nishino et al., 1984). The isoflavones genistein (**149**) and biochanin A (**150**) as well as the flavanone naringenin (**151**) and the flavonol glycoside rutine (**152**) (Fig. 10) inhibited Ca^{2+}–CaM-dependent activity of PDE1 from bovine brain in a concentration-related manner without appreciably affecting its basal activity; the IC_{50}'s calculated were 220, 320, 529 and 317 μM, respectively. The effect was compared with that of quercetin ($IC_{50} = 52.8\ \mu M$), however the latter compound as well as the flavan-3-ols, catechin (**153**) and epicatechin (**154**) (Fig. 10), were not CaM specific inhibitors; they inhibited both the basal and CaM-dependent activity of the phosphodiesterase (Paliyath and Poovaiah, 1985; Goto et al., 1987). On the other hand, the isoflavones tectorigenin (**155**) and daidzein (**156**) (Fig. 10), isolated from the culture broth of *Streptoverticillium album* K-251, inhibited PDE1 with IC_{50} values



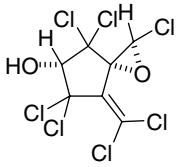
(**135**) R_1 = CHO, R_2 = H
(**136**) R_1 = CHO, R_2 = H
(**137**) R_1 = CH_2OH, R_2 = CH_3



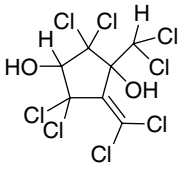
(**138**) R=CH_3, n=4
(**139**) R=C_2H_5, n=3



(**140**)



(**141**)



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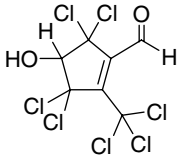

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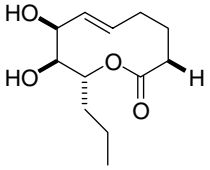

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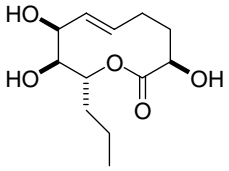

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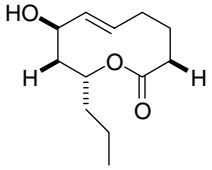

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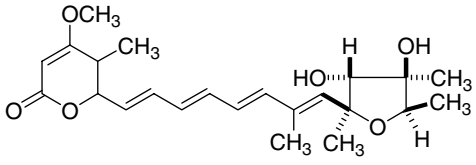


(**147**)

Fig. 9 (*continued*)

Table 5
Effect of compounds **140**–**143** on selected CaM-dependent enzymes

Enzyme	IC_{50} (μM)					
	140	**141**	**142**	**143**	TFP*	W-7*
Brain CaM-PDE	122	109	>500	139	8.6	42
Heart CaM-PDE	226	207	NT	169	14	44
MLCK	6.3	35	40	4.6	6.9	32
Ca^{2+}-ATPase	41	11	34	9.8	11	34
CAM-independent PDE	>270	>270	NT	57	270	>1000
Protein kinase C	>270	>270	>270	171	91	250

NT, not tested.
* Positive controls.

of 39.6, and 119 μM, respectively, at CaM concentration of 4 units/ml. Both flavonoids inhibited also *c*AMP-dependent PKC with values of 252 and 262 μM, respectively (Matsuda et al., 1988). The antitumor-promoting chalcones 4-hydroxyderricin (**157**) and xanthoangelol (**158**) (Fig. 10), isolated from *Angelica keiskei* (Umbelliferae), depressed Ca^{2+}–CaM-enhanced fluorescence of NPN at the concentrations of 1 and 0.5 μg/ml, respectively (Okuyama et al., 1991). Among other tested phenolic compounds, the flavanone naringenin (**151**) and cafeic acid (**159**) (Fig. 10) inhibited significantly CaM-stimulated PDE1 (IC_{50} values of 67, and 8 μM, respectively) while a series of aromatic acids including chlorogenic, vainillic, homovanillic, ferulic, gentisic, *trans*-cinnamic, phenylacetic and *o*-coumaric acids were inactive displaying IC_{50}'s higher than 500 μM (Paliyath and Poovaiah, 1985).

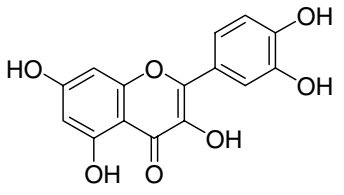
11. Concluding remarks

Research in recent years has made significant progress in understanding the structure and physiological role of CaM in plants and animals. Additional research is still needed to better understand the many aspects of this protein and its target enzymes, including their interaction

with biodynamic naturally occurring compounds. A broad range of plant and microorganism natural products covering a wide diversity of chemical structures have been reported to be CaM inhibitors. Among the most potent active compounds were several toxic peptides (**41** and **42**) and the antibiotic polymyxin ($IC_{50} = 80.5$ nM). Since CaM is involved in the modulation of a number of cell processes, these CaM inhibitors might exhibit a wide spectrum of biological activities, including the toxic effects provoked by several natural products isolated from insects (**41** and **42**), anurans (**43**–**56**), fungi (**16**, **103** and **147**) and marine microorganisms (**23**) with anti-CaM effect. These compounds would be useful for a better understanding of the CaM cellular systems and the mode of action of several toxins.

Natural CaM inhibitors might play an important role in the development of new drugs for neurodegenerative diseases; in this regard, it is important to point out that some synthetic pharmacological inhibitors of CaM, several of which are already used clinically as anti-cancer and anti-psychotic agents, inhibit osteoclastic acid transport, suggesting their potential as bone-sparing drugs.

Finally, CaM could be also a specific target for natural phytotoxins with potential as herbicide agents due to the following reasons: (i) In higher plants, CaM is a fundamental component of the Ca^{2+} signal transduction pathway during plant growth. Indeed, more than 50 enzymes and ion channels are regulated by CaM in plants, some of which are important for plant development. A few



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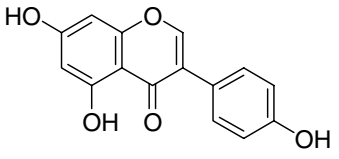

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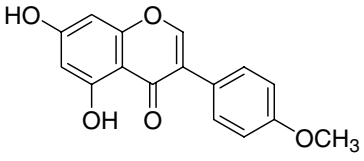

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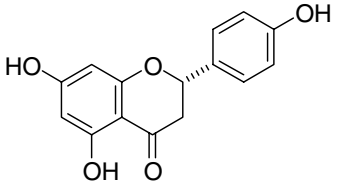

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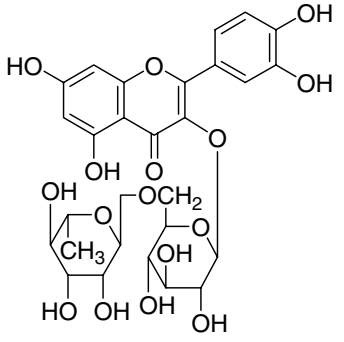

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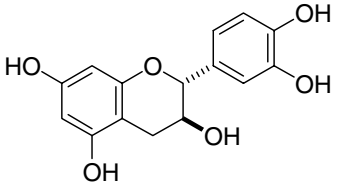

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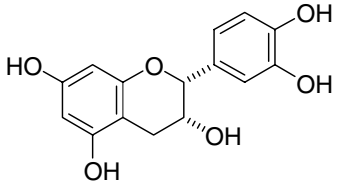
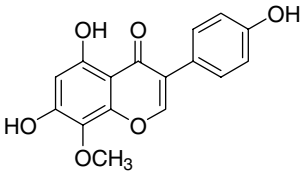
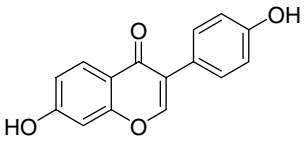


(**154**)

Fig. 10. Flavonoids and other phenolic compounds.



(155)



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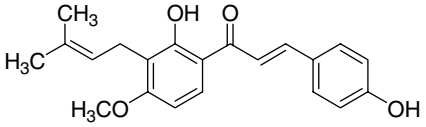
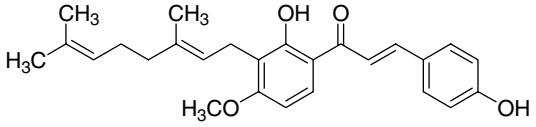
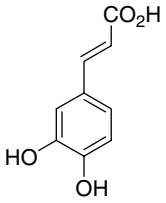


(157)



(158)



(159)

Fig. 10 (*continued*)

of these proteins are found only in plants. Other proteins, although not exclusive to plants, are regulated by CaM only in plants. (ii) Last but not least, unlike animals, higher plants express multiple divergent CaM isoforms, some of which (SCaM-4 and SCaM-5) share 78% of structural identity with vertebrate CaM. These isoforms might differentially regulate CaM-binding enzymes therefore they could be specific targets for plant-growth inhibitors. It has been proposed that the phytoxicity of compounds **18**, **63**, **71**–**76**, **94**–**102**, **114**–**121**, **125** is related with its anti-CaM activity.

According to the scarce kinetic data available, a few metabolites (**9**, **18**, **38**, **41**, **58**, **70**, **140**–**143**) compete with CaM for the binding site of the target enzymes, accordingly acting as competitive antagonists, while others (**43**, **45**, **50**, **56**, **77** and **80**) do not compete with the activation site, thus behaving as noncompetitive or noncompetitive inhibitors of CaM. The various target sites for inhibiting CaM function by natural products remains to be discovered.

Unfortunately, in vivo studies for most of CaM-inhibitors are scarce. In addition, more data on the in vivo concentrations of the putative inhibitory compounds is needed.

The analysis of the sensitivity of CaM-like proteins to natural CaM-inhibitors could be useful in understanding the role of these understudied proteins in plants. These CaM-like proteins are composed of EF-hand Ca^{2+}-binding motifs, with no other particular functional domains. According to McCormack and Braam (2003), these proteins are at least 16% identical to CaM.

Acknowledgements

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