Hypotensive Natural Products: Current Status

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Abstract: Hypertension is a common and progressive disorder that possesses a major risk for cardiovascular and renal disease. Recent data have revealed that the global burden of hypertension is an important and increasing public health problem worldwide and that the level of awareness, treatment and control of hypertension varies considerably among countries. Hypertension is often called a 'silent killer' because persons with this pathological condition can be asymptomatic for years and then have a fatal heart attack or stroke. The field of naturally occurring antihypertensive agent is a research area rapidly expanding due to the high potential of such molecules as new antihypertensive drugs. Recently, a great number of plant-derived substances have been evaluated as possible antihypertensive agents through different mechanisms of action such as alkaloids, flavonoids and terpenoids. In this mini-review we will discuss the medicinal chemistry of these compounds.

Key Words: Hypertension, alkaloids, terpenoids, poliphenols, miscellaneous.

INTRODUCTION

Hypertension remains the most common risk factor for cardiovascular morbidity and mortality. Despite massive and costly efforts to identify and treat hypertension, the availability of over seventy five different antihypertensive agents organised in nine different classes, less than a third of individuals with a usual blood pressure (BP) exceeding 140/90 mmHg are adequately treated [1]. Even in individuals whose hypertension is thereby presumed to be well controlled, less than a third are protected from subsequent strokes and heart attacks. The inadequacy of current practice is obvious: too few individuals at risk, because of raised BP are being diagnosed and treated effectively. Another reason is the complexity of the origin of hypertension, a multi-factorial disease [2]. In fact, from the pathophysiological view point, hypertensive disease involves changes in at least one of three hemodynamic variables (cardiac output, arterial stiffness, or peripheral resistance) that determine the measurable BP. Moreover, there are still some doubts regarding the long-term safety of antihypertensive therapy [3]. A modern therapeutic approach should not target only BP but also normalize vascular structure and function. Therefore, much improved population-wide and individual approaches to the prevention and control of hypertension are needed. Since more than a quarter of the world adult population is already hypertensive and this number is projected to increase to 29%, 1·56 billion, by 2025. Even today, in the century of combinatorial chemistry, plants secondary metabolites are still an important source for the development of new drugs [4]. About fifty percent of the drugs introduced to the market during the last two decades are derived from naturally occurring compounds. The potential use of natural products has also been successfully demonstrated in the field of hypertension in several articles in which hypotension induced by plants extracts were evaluated on different mechanism of actions including: K^+ and Ca^{2+} channel function, the eicosanoid system, the nitric oxide (NO) system, the adrenergic blockade, inhibition of Angiotensin Converting Enzyme (ACE), angiotensin receptors blocker and diuretic action. Since today, several works reported just a collection of data regard plants extracts with anti-hypertensive activity [5, 6]. The intent of this article is to address the *in vitro* and *in vivo* studies on natural products with anti-hypertensive activity. The mechanisms of action and the Structure Activity Relationships (SAR) were also discussed, were it is possible. The knowledge offer from this review should help to provide leads to the ultimate goal of developing new therapeutic drugs more efficacy and safety for the treatment of hypertension.

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2. ALKALOIDS

2.1. Aporphine Sheleton

Dicentrine (**1**), isolated from *Lindera megaphylla* (Lauraceae), was found to be a selective potent α_1-adrenoceptor (-AR) antagonist in vascular smooth muscle cells (VSMCs). This compound acts as competitive antagonist of noradrenaline (NA) or phenylephrine (PE) -induced vasoconstriction. These effects still persisted in denuded aorta. Moreover, dicentrine (**1**) suppressed the inositol-5'-monophosphate (IMP) formation induced by NA in rat thoracic aorta [7]. Roemerine (**2**), anonaine (**3**) and dehydroroemerine (**4**), isolated from *Annona cherimolia* (Annonaceae), exhibited a relaxant effects on isolated strips of rat thoracic aorta. Anonaine (**5**) was the most promising alkaloid inhibiting the KCl-induced contraction (IC_{50} 1.2 x 10^{-5} M), whereas the contraction elicited by NA was more potent by roemerine (**2**) (IC_{50} 5.8 x 10^{-6} M). The experiments, carried out in Ca^{2+}-free medium using two different agonists (NA and caffeine), which mobilize intracellular Ca^{2+} ($[Ca^{2+}]_i$) by different mechanisms of action, showed that the alkaloids made no contribution to $[Ca^{2+}]_i$ processes. So the relaxant effects pro-

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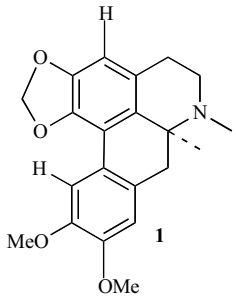
duced by aporphine alkaloids may be due to the blockade of Ca^{2+} movements across the cell membrane, mainly through voltage-operated channels (VOCs) and to the disruption of α_1-AR connected to receptor-operated channels (ROCs). The presence of a methyl group at N6 in **2** could allow this compound to acquire a greater activity in inhibiting the NA induced contraction. Dehydroroemerine (**4**) showed a loss of potency probably due to the less lipophilic character and the absence of the chiral carbon atom [8]. Valiente *et al.* [9] demonstrated that the roemerine (**2**), anonaine (**3**) and pukateine (**5**) showed affinity for three human cloned α_1-AR subtypes and exhibited slightly but significantly lower affinities for the α_{1B}- with regard to the α_{1A}- or α_{1D}-AR subtypes. Affinities for different subtypes were in the order $\alpha_{1A} > \alpha_{1D} > \alpha_{1B}$ for **2** and **3** and $\alpha_{1A} = \alpha_{1D} > \alpha_{1B}$ for pukateine (**5**). The presence of an hydroxyl group at C11 (**5** *vs* **2**) is associated with a significant decrease in the affinity for the $\alpha_{1A} > \alpha_{1B}$ subtypes without changing the affinity for the α_{1D}-AR. Earlier studies indicated that hydroxyl substituent on one or the other of the aporphine aromatic rings as determinants of the affinity of these alkaloids for each subtypes. In a series of 1,2,9,10-tetraoxygenated (*S*)-aporphine alkaloids a free hydroxyl group at C2 is associated with increased affinity for α_{1A}-AR and to a lesser extent for the α_{1B}-subtype, while it does not affect the affinity of the alkaloid for α_{1D}-AR. Conversely, in a set of (*R*)-10,11-dihydroxyaporphines, a free hydroxyl group at C10 increased the affinity for α_{1D}-AR without changing the affinity for the α_{1A}- or α_{1B}-AR subtypes. Introduction of a methyl group on the nitrogen atom (**2** *vs* **3**) significantly increased affinity for all three subtypes. All compounds exhibited a greater affinity for [^{3}H]-prazosin than of [^{3}H]-(+)-c*is*-diltiazem binding sites. SAR analysis demonstrated that the presence of a methyl group on the nitrogen atom (**2** *vs* **3**) was associated with increased affinity as Ca^{2+}-channel blocker, while the presence of an hydroxyl group at C11 as in **7** led to decreased affinity. Similar effects of hydroxyl substitution at other positions of the aporphine structure suggest that the presence of hydroxyl groups may impede the interaction between the compound and the benzothiazepine site of the Ca^{2+}-channel. Several alkaloids, isolated from *Hernandia nymphaeifolia* (Hernandiaceae), exhibited an effective inhibitory activities on the contraction of VSMCs induced by high K^+ and NE. In particular, noraporphines ovigerine (**6**), laurotetanine (**7**) and hernangerine (**8**) showed marked inhibition of aortic contractions caused by high K^+ and norepinephrine (NE), but the corresponding oxoaporphines hernandonine (**9**), atheroline (**10**) and oxohernangerine (**11**) were reduced. Ovingerine (**6**) showed significant inhibition on aortic contractions but its dimer, ovigeridimerine (**12**), lost vasorelaxing effects. Hernangerine (**8**) exhibited marked inhibitory activity on aortic concentrations, but hernovine (**13**) showed reduced inhibitoy activity due to the effect of 1,2-methylendioxy substitution. Among the oxoaporphines, oxohernangine (**14**) and oxohernangerine (**11**), both with 10-hydroxy substitution, showed marked inhibitory activity on aortic concentrations induced by high K^+ and NE. *N*-Methyllaurotetanine (**15**) showed marked inhibitory activation aortic contractions induced by NE, while laurotetanine (**7**) did not show any inhibitory activity due to the lack of *N*-methyl group. Moreover, hernandaline (**16**) exhibited marked inhibitory activity on aortic concentrations induced by high K^+ and NE, but dehydrohernandaline (**17**) did not show any inhibitory activity due to the effect of the aromatic ring C. Although the vasorelaxing activites of these natural compounds are much weaker than compared with the known α_1-AR blocker, and Ca^{2+} blocker [10].

(+)-Nantenine (**18**), isolated from *Platycapnos spicata* (Papaveraceae), totally relaxed the contractions induced by NA or by a high KCl concentration in intact rat aortic rings. Mechanical removal of endothelium and/or pretreatment of aorta rings with glinbenclamide (GB) or tetraethylammonium chloride (TEA) did not significantly modify the vasorelaxant effects of this alkaloid. In experiments with $^{45}Ca^{2+}$, **30** did not modify the basal uptake of $^{45}Ca^{2+}$ but decreased, in a concentration-dependent fashion, the influx of $^{45}Ca^{2+}$ induced by NA and KCl in rat aortic rings with/without endothelium [11]. Hypotension and bradycardia elicited by intravenous (i.v.) administration of this aporphine alkaloid in anaesthetized normotensive rats (NTR) seem to be due, at least in part, to a combined α_1-AR and 5-HT_{2A} receptor blockade but not to the release of nitric oxide (NO) from vascular endothelium, to an α_2-AR antagonism or to a Ca^{2+} antagonist activity [12]. (±)-Domesticine (**19**), a (±)-nantenine derivatives, inhibited the concentration-response curve induced by PE in the rat aorta more than (±)-nantenine (**18**). The experiment involving IMP accumulation was done to confirm the α_1-AR blocking action of these compounds in A10 cells. (±)-Domesticine (**19**), (±)-nordomesticine (**20**) and (±)-nantenine (**18**) suppressed the NE-induced accumulation of [^{3}H]IMP in A10 cells through α_1-AR blockage. SAR analysis revealed that an hydroxyl group at C1 and a methyl group at N6 position of the aporphine skeleton of **18** are important for increasing affinity for the α_1-AR. It has been reported that a small change in aporphine structure may lead to dramatic changes in the pharmacological profile. For example, the hydroxyl group at the C2 position of (+)-boldine **(21)** has been reported to be a critical factor for discrimination between α_1AR subtypes [13]. Martinez *et al.* [14] reported that halogen substitution at the C3 position of **34** increased selectivity for the α_{1A}-AR rather than for the α_{1B}- AR while **20** was selective for the α_{1D}- AR rather than for the α_{1A}- and α_{1B}-AR and it was more selective than **18**. In conclusion, the relative order of affinity on α_1-AR was **19** > **20** > **18** > **21**. *N*-Allylsecoboldine (**22**) exerted vasorelaxing effect on the rat aorta blocking Ca^{2+} channels and it also has an antagonistic effect at α_1-AR [15]. Successively, Teng *et al.* [16] demonstrated that another secoboldine derivatives, *N*-benzylsecoboldine (**23**), exhibited vasodilatating property on the vasoconstriction induced by high K^+ medium and Ca^{2+}, with a mechanism of action involving both suppressing the Ca^{2+} influx and also antagonistic effect on α_1-ARs. The alkaloids harmine (**24**) and harmaline (**25**) induced the relaxation in the aorta pre-contracted with NA or KCl. However, the removal of endothelium or pretreatment of intact aortic ring with Nω-nitro-L-arginine methyl esters (L-NAME) or with indomethacin (INDO) reduced significantly the vasorelaxant response of harmaline (**25**) but not of harmine (**24**). According to their IC_{50} values, prazosin reduced the vasorelaxant effect only of **25**, whereas pre-treatment with 3-isobutyl-1-methylxanthine K affects both the **25** and **24**-responses. Inhibitions of L-type VOCs in endothelium-intact aortic rings with diltiazem depressed the relaxa-



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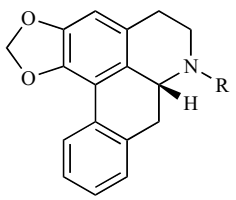
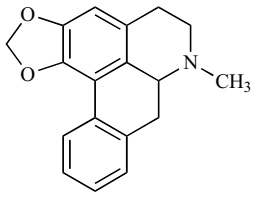


2 R= CH_3
3 R= H



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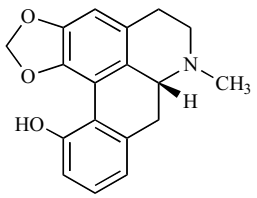

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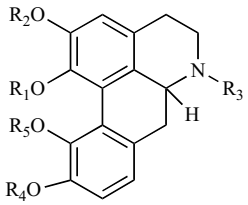
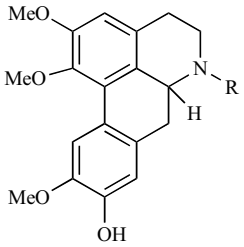
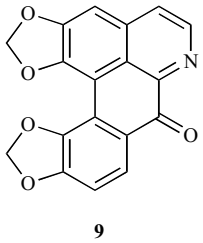


6 R_1+R_2=R_4+R_5= CH_2, R_3= H
8 R_1+R_2= CH_2, R_3=R_4= H, R_5= CH_3
13 R_1=R_5= CH_3, R_2=R_3=R_4= H



7 R= H
15 R= CH_3



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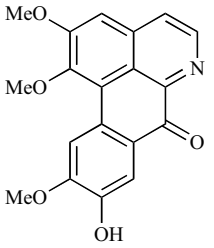

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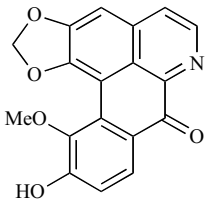

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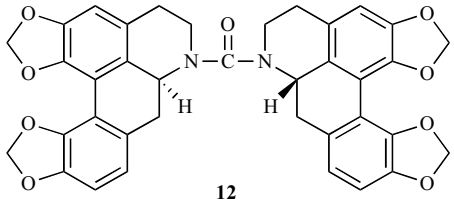

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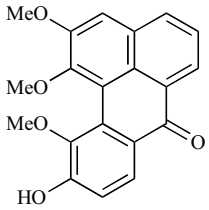

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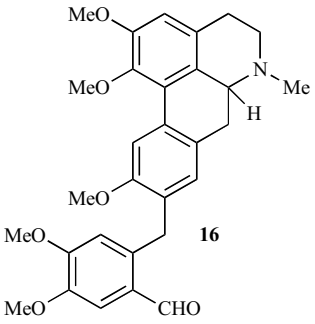

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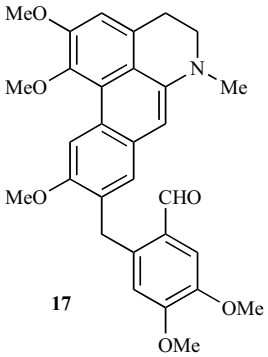

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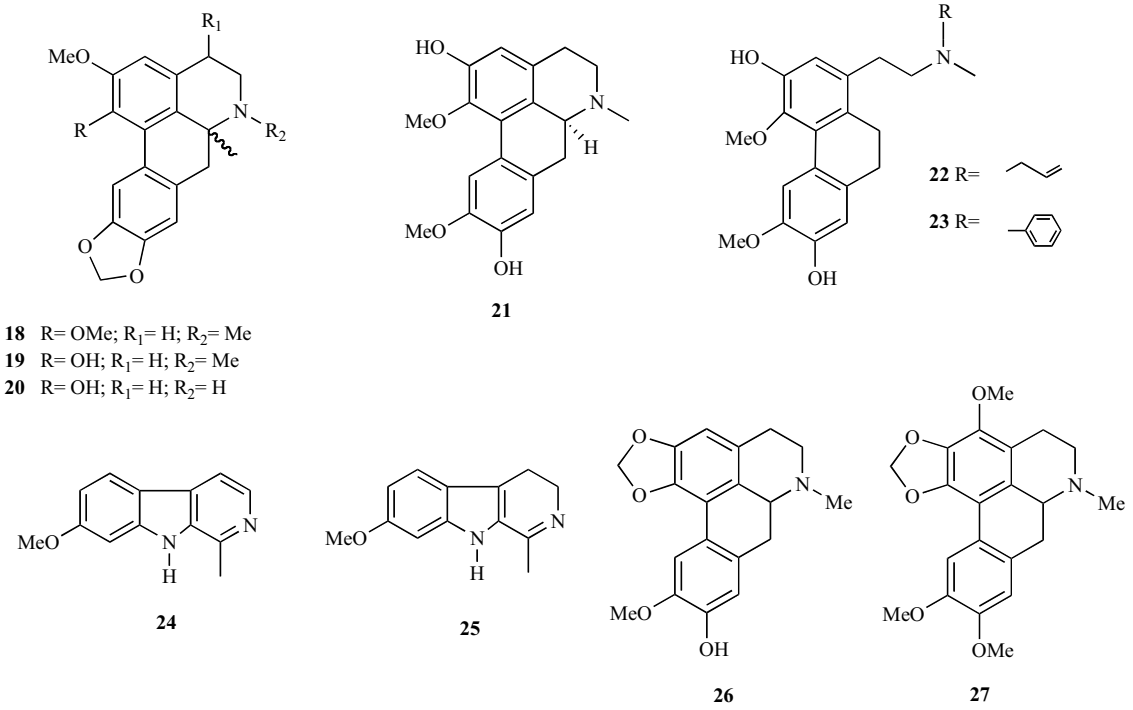
Scheme 1.

tion evoked by harmaline (**25**) as well as by harmine (**24**). Pretreatment with **24** or **25** shifted the PE-induced dose response curves to the right and the maximum response was attenuated indicating that the antagonist effect of both alkaloids on α_1-AR was non-competitive. Related to their SAR, the difference in the relaxant potency between **24** and **25** might be related to the change of carboline to dihydro-β-carboline. Therefore, the results suggest that the vasorelaxant effect of harmaline (**25**) but not harmine (**24**) is related to its action on the prostacyclin pathway and on the endothelial cells (ECs) to release nitric oxide (NO) [17]. *N*-methylactinodaphnine (**26**), isolated from *Illigera luzonensis* (Her-

nandiaceae), analysed by functional and binding experiments exhibited a simple competitive antagonist of contractions elicited by PE in rat thoracic aorta and it also competitively antagonised the clonidine-induced inhibition of the twitch response of rat vas deferens. These results indicated that **26** is a selective α_1-AR antagonist. Furthermore, it is more selective for the α_{1B}- than for the α_{1A}-AR subtype [18]. Ocoteine (**27**), isolated from *Cassytha filiformis* (Lauraceae), was found to be an α_1-AR blocking agent in rat thoracic aorta as revealed by its competitive antagonism of PE-induced vasoconstriction. Removal of endothelium from the aorta did not affect its antagonistic potency. [^{3}H]-IMP formation caused by NA was suppressed by **27**. Ocoteine (**27**) did not affect the contraction induced by a tromboxane A2 agonist U-46619, $PGF_{2\alpha}$ or angiotensin II, but inhibited slightly those by high K^+ and endothelin I. Neither the cAMP nor cGMP content of rat thoracic aorta was changed by **27**. Moreover, **27** also slightly antagonized the clonidine-induced inhibition of the twitch response evoked by field stimulation in rat vas deferens [19].

2.2. Indole Skeleton

Kopsingine (**28**) from *Kopsia teoi* (Apocynaceae), produced dose-related decreases in the mean arterial blood pressure (MABP) and heart rate (HR) in anesthetized spontaneously hypertensive rats (SHR), which were similar to those seen in normotensive controls. Minor modifications in the molecular structure of kopsingine (**28**), as in kopsaporine (**29**) and 14,15-dihydrokopsingine (**30**) did not significantly alter the hypotensive responses, whereas a more drastic change in the structure, as in the heptacyclic kopsidine A (**31**) and the 3-to-17 oxo-bridged compound **32**, resulted in an increase in BP. The antihypertensive effects of kopsingine (**28**) and its congeners **29** and **30** along with the pressor effects produced by the heptacyclic oxo-bridged compounds **31** and **32** could be ascribed to central as well as peripheral actions. A drastic modification of the molecular skeleton, such as an intramolecular closure to form the heptacyclic oxo-bridged derivatives **31** and **32** produced a pronounced alteration in the cardiovascular effect of kopsingine (**28**) [20]. Rhynchophylline (**33**) and isorhynchophylline (**34**), isolated from *Uncaria rhynchphylla* (Rubiaceae), relaxed aortic rings pre-contracted with PE. Removal of endothelium and pre-incubation with L-NAME slightly inhibited but did not prevented this relaxant response. These results indicated that **33** and **34** act largely in an endothelium-independent manner and may act *via* L-type VOCs [21]. Evidence of the antihypertensive and vasodilative effects of *Uncariae ramulus* et *Uncus* has been accumulated by several studies [22-25]. Its major alkaloid geissoschizine methyl ether (**35**) have been demonstrated to possess a vasodilative effect that is composed of two different mechanisms: endothelial dependency with NO release and endothelial independency with VOCs blocking activity [26]. Caracasandiamide (**36**), isolated from *Verbesina caracasana* (Amaranthaceae) at higher doses stimulated cardiac inotropism and induced by reducing peripheral vascular resistance arterial hypotension with reduction of both aortic flow and stroke volume [27]. These cardiovascular effects appear to involve complex interactions at the level of the peripheral β_1-, β_2- and α_2-AR-dependent as well as muscarinic M_2- and M_4-cholinergic receptor-dependent transductional pathways both in cardiovascular myocells and at the level of the postganglionic sympathetic endings. Notably, the dimer **36** was more potent as a hypotensive and positive inotropic agent than the monomer (*Z*)-caracasanamide (**37**). Like (*Z*)-caracasanamide (**37**), **36** did not decrease cardiac inotropism in contrast with all the tested hypotensive drugs. Furthermore, the hypotensive effect of **36**



Scheme 2.

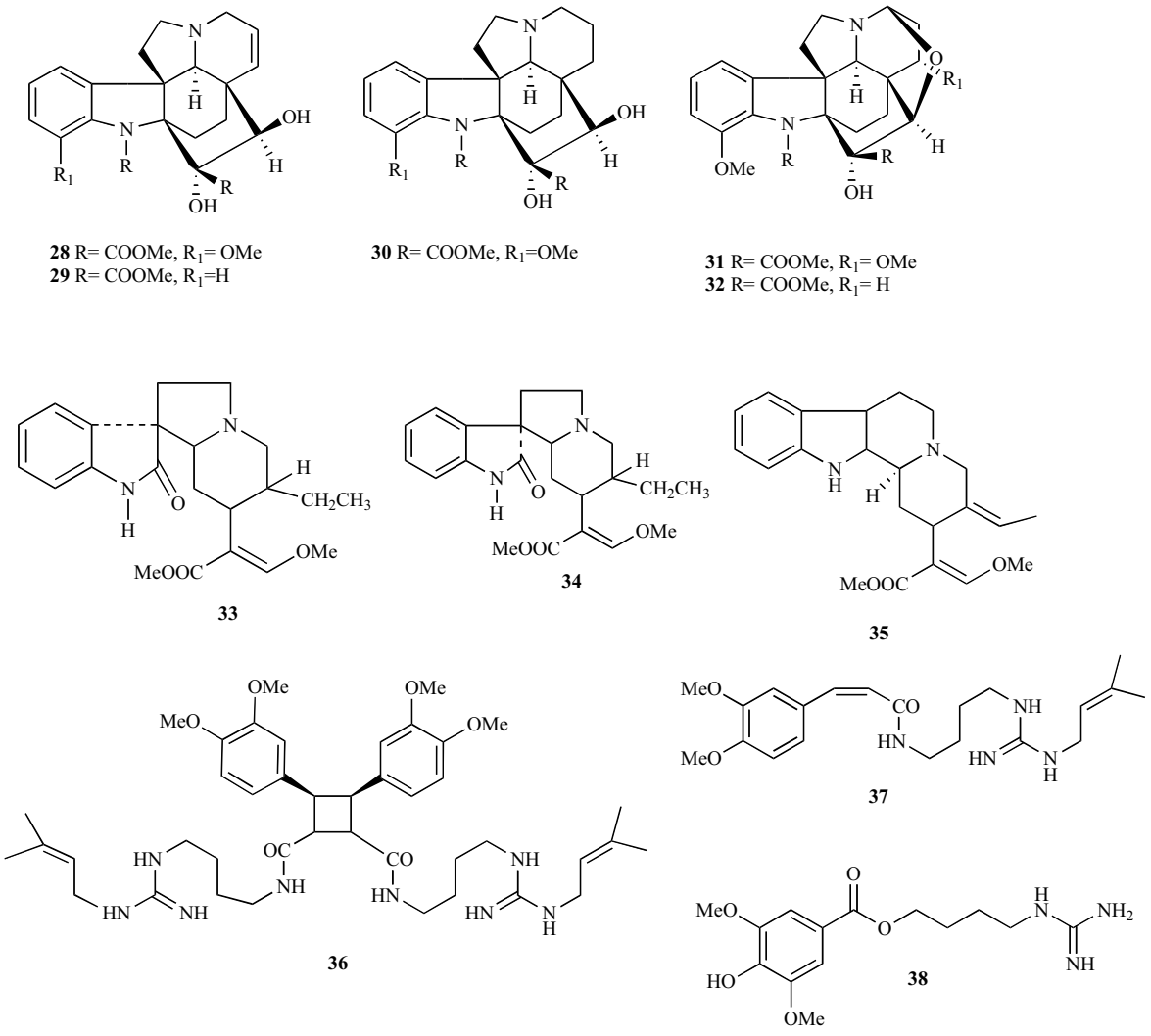
was longer than that of **37**, which was already found to last more than those of drugs acting by peripheral vasodilatatory mechanisms. Leonurine (**38**) induced concentration-dependent and endothelium-independent relaxation of PE-pretreated rat aortic arterial rings. It inhibited the responses of aortic smooth muscle to PE acting on the release of $[Ca^{2+}]_i$. Leonurine (**38**) is not a specific α-AR blocker, since it also caused a concentration-dependent inhibition of vascular contractile responses to KCl with an IC_{50} value of 96.4 μM, suggesting that **38** also blocks the L-type VOCs. In addition, **38** relaxed the aortic contraction induced by $PGF_{2\alpha}$. The inhibitory effects of leonurine (**38**) were reversible and did not affect the resting tension. The inhibitory effects of **38** on vascular stimulating agents were reversible, indicating that it does not cause tissue damage [28].

2.3. Steroidal Skeleton

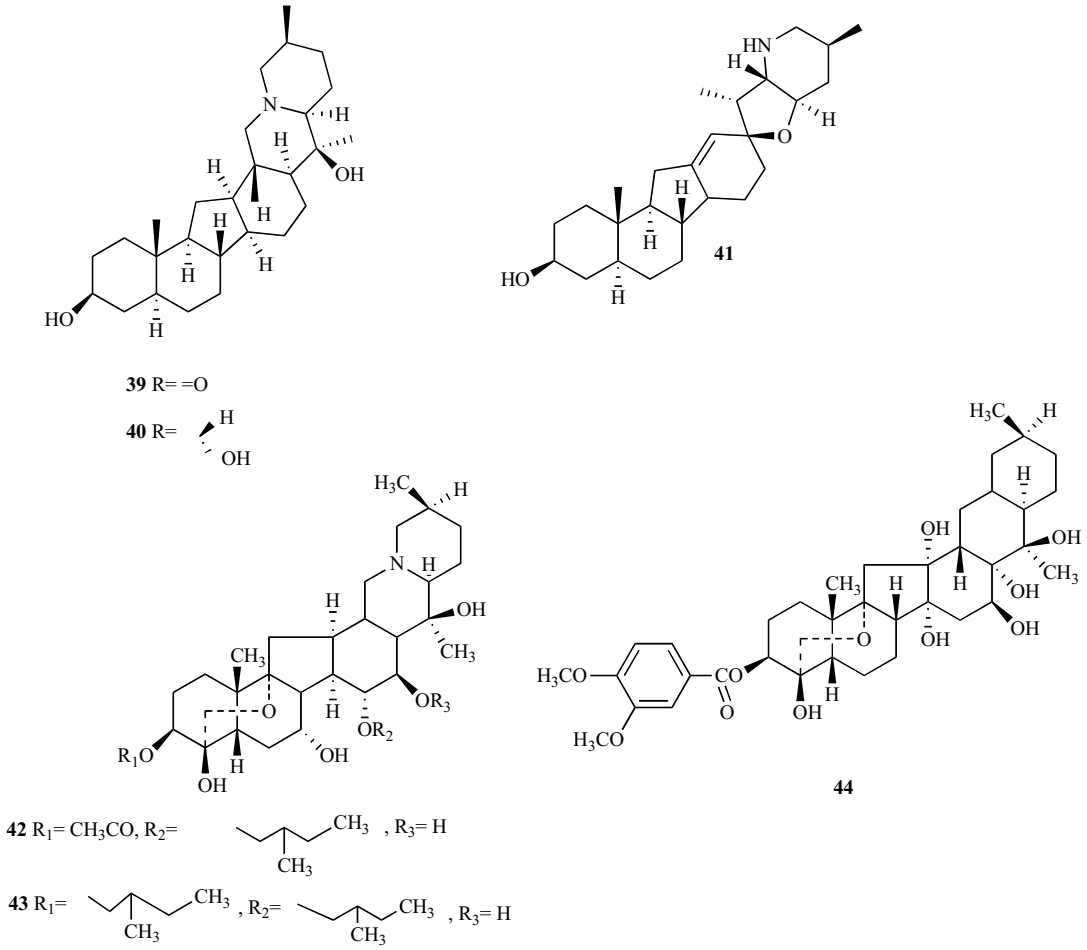
Verticinone (**39**), verticine (**40**) and peimisine (**41**), isolated from *Fritilaria ussuriensis* (Liliaceae), exhibited an interesting activity against ACE. The most active compound **39** showed an IC_{50} value of 165 μM, while values of 312.8 and 526.5 μM were found for **40** and **41**, respectively [29]. The *Veratrum* (Liliaceae) alkaloids germidine (**42**) and germerine (**43**) were able to lowered BP accompanied with positive chronotropy and inotropy in mice. In particular, **43** was more potent than **42** in both BP lowering and positive inotropy, whereas veratridine (**44**) produced negative chronotropy and positive inotropy. An acyl group at 3-O-R_1 position and a 2-methylbutyroyl group at 15-O-R_2 position in germine type skeleton were important to produce the positive inotropy and chronotropy. The presence of a veratroyl group at 3-O-R_1 position and a free hydroxyl group at 15-O-R_2 position may be essential to produce the negative chronotropy by **44** [30].

2.4. Isoquinoline Skeleton

Berbamine (**45**) and oxyacanthine (**46**), isolated from *Mahonia aquifolium* (Berberidaceae), relaxed K^+-precontracted rat aortal rings with IC_{50} values of 20 μM. Both alkaloids inhibited contractions induced by NA, PE and serotonine (5-HT) in a non-competitive manner. They seemed to inhibit both the influx of Ca^{2+} into the cell, preferentially



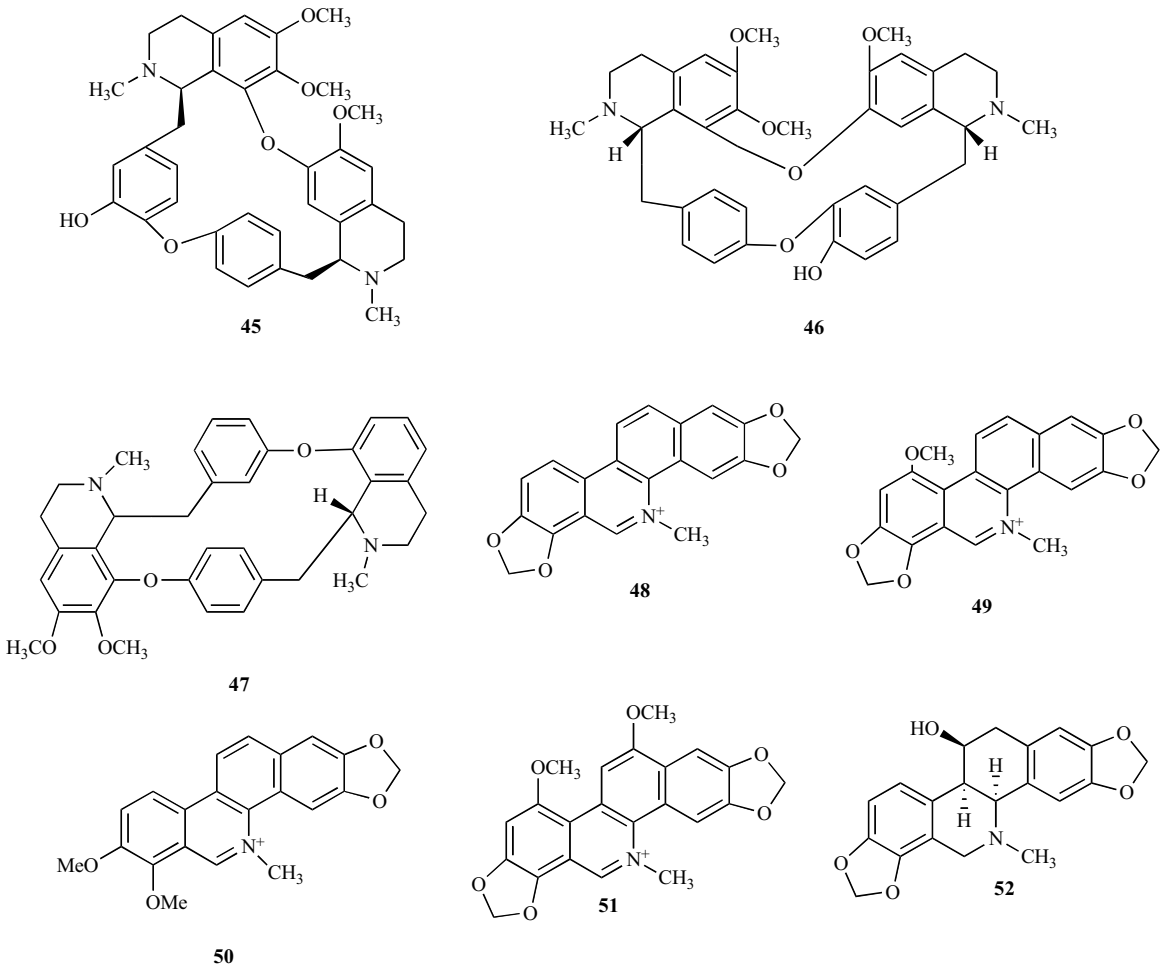
Scheme 3.



Scheme 4.

through ROCs and the release of $[Ca^{2+}]_i$ from stores. Moreover, they appear to be antagonists of α_1-ARs [31]. With a Ca^{2+}-antagonist property acts cycleanine (**47**), extracted from *Stephania glabra* (Menispermaceae). Compound **47** inhibited the KCl-induced contraction of rabbit aortic rings with higher potency than nifedipine. Action potential duration of rat right ventricular strips was decreased by **47**. L-type Ca-current (I_{CaL}) of single rat ventricular cardiomyocytes was inhibited by **47** in a voltage- and frequency-dependent manner [32]. Sanguinarine (**48**), chelirubine (**49**) and chelerythrine (**50**), isolated from *Bocconia frutescens* (Papaveraceae), blocked the human angiotensin II AT1 and endothelin ET_{A1} receptors. Substitutions at C10 and C12 of the benzonucleus like macarpine (**51**) reduced drastically the activity. Moreover, chelidonine (**52**) showed low activity suggesting the need for a fully aromatic nucleus. Apparently at physiological pH the iminium ion form at position C5 of the quaternary benzoskeleton become an alkanolamine form, which increases lipopilicity. The planarity of **48** molecule probably is responsible of the founded more affinity of sanguinarine (**48**) for AT_1 receptor than chelidonine (**52**) [33]. Compound **48** inhibited the binding of specific radioligands to the angiotensin AT_1 receptor in the μM range. Although, **48** inhibited cell viability at a similar concentration range as the inhibition of [^{3}H]candesartan binding, [^{3}H]candesartan binding was not restricted to intact cells, but also found on membranes indicating that there is no strict requirement to have apoptosis or cytotoxicity to perceive the inhibition of angiotensin AT_1 receptor binding [34].

Tetrandrine (**53**), purified from *Stephania tetrandrae* (Menispermaceae), exerted antihypertensive action due to its vasodilatory properties. Compound **53** prevented or inhibited vascular contraction induced by membrane depolarization with KCl or α-AR activation with PE and apparently inhibited multiple Ca^{2+} entry pathways as demonstrated in cell types lacking the L-type Ca^{2+} channels. In cardiac muscle cells, tetrandrine (**53**) inhibited both L- and T-type VOCs. In addition to its actions on cardiovascular tissues, **53** exerted its anti-hypertensive action *via* a Ca^{2+}-dependent manner on other tissues intimately involved in the modulation of BP control, such as adrenal glands. Other than the Ca^{2+} antagonistic effects, **53** also interacted with the α-AR and muscarinic receptors based on functional as well as radioligand binding studies [35]. Comparative studies of the effects of tetrandrine (**53**) and fangchinoline (**54**) on hypotensive effects on stroke-prone spontaneously hypertensive rats (SHRSP) were performed [36]. Both compounds inhibited high K^+ and induced sustained contraction in the rat aorta smooth muscle strips with IC_{50} values of 0.27 and 9.53 μM

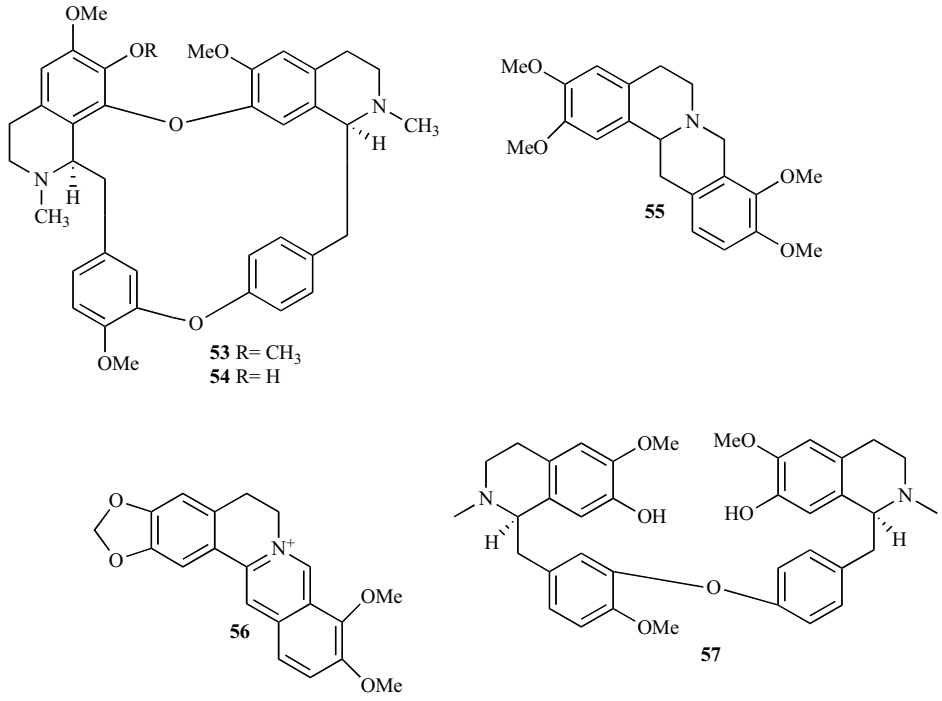


Scheme 5.

for **53** and **54,** respectively. For NE-induced contraction **53** and **54** showed an IC_{50} value of 3.08 and 14.20 μM, respectively. At the molecular level, $^{45}Ca^{2+}$ uptake tests revealed that **53** also inhibited high K^+ and NE-stimulated Ca^{2+} influx in rat aorta strips. An *in vivo* study demonstrated that **53** and **54** administered by i.v. bolus injection lowered the MABP significantly during the period of observation in conscious SHRSP. Fangchinoline (**54**) generally showed less potent inhibitory effects than **53**, on high K^+ and NE-induced sustained contractions and on Ca^{2+} influx in the rat aorta and showed a hypotensive effect on SHRSP [37]. dl-Tetrahydropalmatine (**55**), isolated from *Corydalis racemosa* (Fumariaceae), has been found to have antihypertensive effects trough a mechanism of action involving the Ca^{2+} influx. In fact, in an isolated perfused rat heart model, **55** was found to have a negative effect on left ventricular pressure. In isolated cardiomyocytes, **55** inhibited in concentration-dependent manner the $^{45}Ca^{2+}$ influx [38]. Administration of **55** i.v. elicited a proportional decrease in either the MABP or HR, which could be mimicked by i.v. injection of 5-HT_2- receptor antagonists [39]. Berberine (**56**), isolated from *Hydrastis canadensis* (Ranunculaceae), exerted long-term benefit in the vascular system trought vasorelaxant and antiproliferative effects. In particular, NO from endothelium may account primarily for the berberine-induced endothelium-dependent relaxation, while activation of tetrapentylammonium-, 4-aminopyridine- and Ba^{2+}-sensitive K^+ channels, inhibition of $[Ca^{2+}]_i$ release from caffeine-sensitive pools, or a direct relaxant effect, is likely responsible for the **56**-induced endothelium-independent relaxation [40]. Thaligrisine (**57**), isolated from *Pseudoxandra esclerocarpa* (Annonaceae), inhibited the contractile response induced by depolarization in rat aorta (IC_{50} 8.9 μM) and in tail artery (IC_{50} 3.04 μM) or NA induced contraction in rat aorta (IC_{50} 23.0 μM) and in tail artery (IC_{50} 3.8 μM). In rat aorta, **57** concentration-dependently inhibited NA-induced contraction in Ca^{2+}-free solution (IC_{50} 13.3 μM). This alkaloid also relaxed the spontaneous contractile response elicited by extracellular Ca^{2+} after depletion of NA-sensitive intracellular stores IC_{50} 7.7 μM) [41].

2.4. Diterpene Skeleton

Taxine B (**58**), isolated from *Taxus baccata* (Taxaceae), inhibited concentration-dependently the Ca^{2+}-induced contractions of the aorta depolarized by K^+ in Ca^{2+} free media. Compound **58** relaxed the aorta which was precontracted by K^+ with IC_{50} of 4.78 x 10^{-6} g/ml. Similar effects of **58** were observed in the endothelium-denuded aortic strips. Moreo-

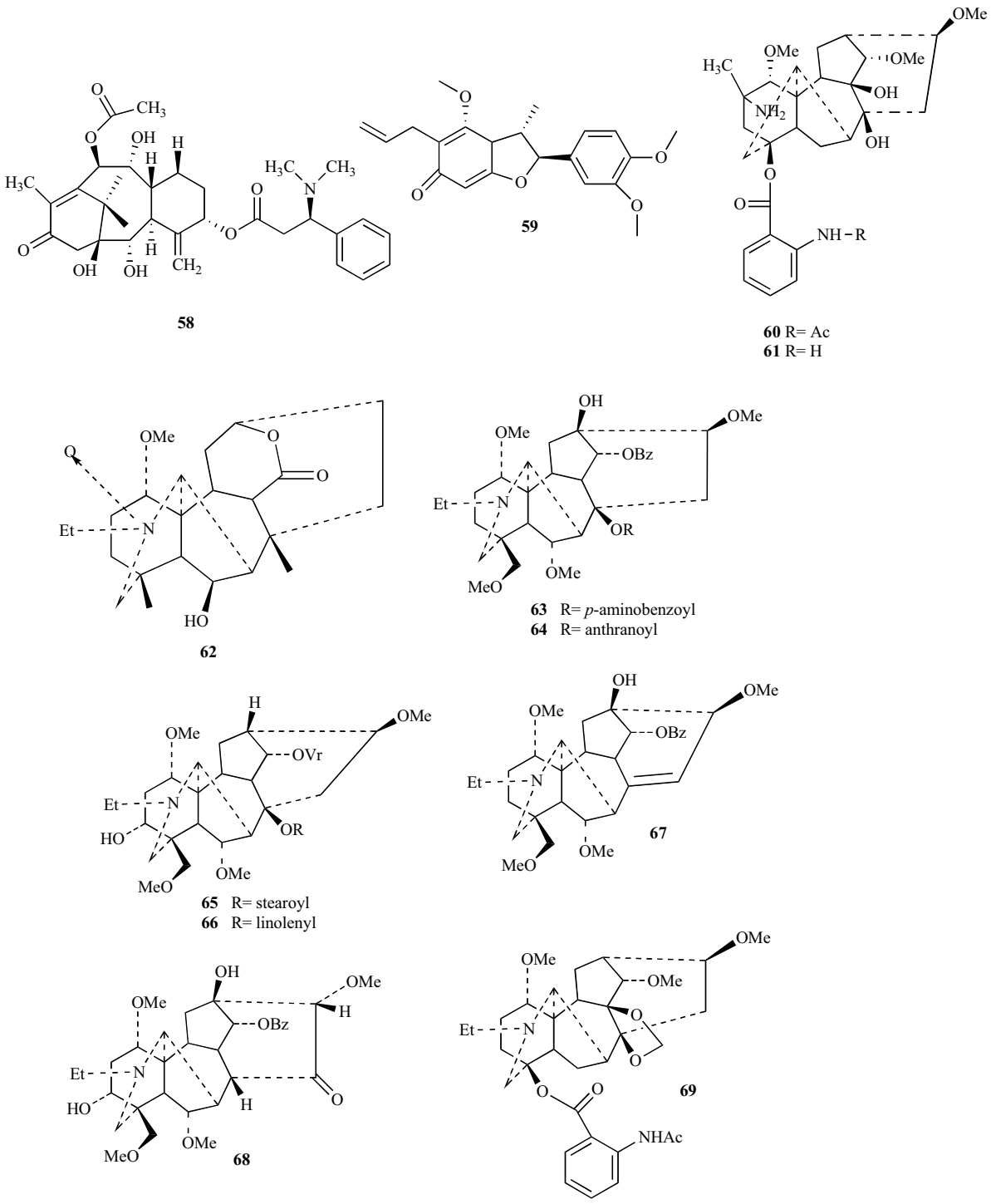


Scheme 6.

ver, **58** produced concentration-dependent negative inotropic and chronotropic effects on isolated atrium preparations. The IC_{50} values of **58** were 3.63 x 10^{-7} and 5.75 x 10^{-7} g/ml for contraction and rate, respectively [42]. Denudatin B (**59**), isolated from *Magnolia fargesii* (Magnoliaceae), inhibited the vasoconstriction of rat thoracic aorta induced by high K^+ solution, NE and caffeine. The contraction of rat aorta caused by high K^+ and cumulative concentrations of $CaCl_2$ was inhibited by **59** with an IC_{50} of 21.2 μg/ml. Moreover, NE-induced contractions of rat aorta were inhibited by pretreatment with denudatin B (**59**). The relaxing action persisted in denuded aorta. In quin-2/AM-loaded cultured rat VSMCs, **59** inhibited the increase of intracellular calcium caused by NE in the presence or absence of extracellular calcium ($[Ca^{2+}]_e$). Neverthless, **59** did not affect the caffeine-induced contraction and the increase in $[Ca^{2+}]_i$. These results indicate that **59** relaxed VSMCs by inhibiting the Ca^{2+} influx through bothVOCs and ROCs. Denudatin B (**59**) increased the cGMP level and this effect may enhance the vasorelaxation [43]. Lappaconitine (**60**), at a dose of 150 μg/kg (i.v.), increased cardiac vagal afferent nerve activity (16.2%) and reduced cardiac sympathetic efferent nerve activity (12.5%). A polar analog, *N*-deacetyllappaconitine (**61**), at the same dose, increased cardiac vagal afferent nerve activity (40%) and reduced cardiac sympathetic efferent nerve activity (23.5%). Both of these agents also reduced arterial BP and HR [44]. Several derivatives of norditerpenoid alkaloids including *N*-oxide (**62**), 8-deacetyl-8-*p*-aminobenzoyldelphinine (**63**), 8-deacetyl-8-anthranoyldelphinine (**64**), 8-stearoylfalconerine (**65**), 8-linolenylfalconerine (**66**), pyrodelphinine (**67**), 16-epipyroaconitine (**68**) and 8,9-(methylenedioxy) lappaconitine (**69**), tested *in vivo* for their cardiovascular action (hypotensive, bradycardic and ventricular arrhythmias) in male Sprague-Dawley rats, exhibited prominent hypotensive and bradycardic activity without prominent arrhythmias [45].

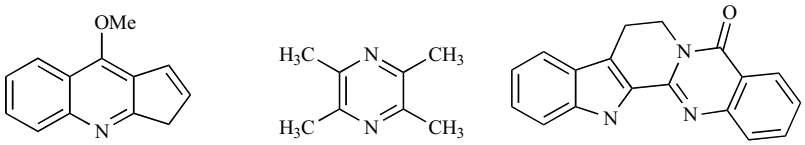
2.5. Other Skeleton Types

The component of *Dictamnus dasycarpus* (Rutaceae) dictamine (**70**) was able to inhibit K^+ medium, Ca^{2+}-induced vasoconstriction in dose-dependent manner. The tonic contraction elicited by NA was also relaxed by **70** in the nifedipine-treated aorta. The relaxing effect of **70** persisted in endothelium-denuded aorta [46]. Tetramethylpirazine (**71**), isolated from *Ligusticum wallichii* (Apiaceae), induced dose-dependent reductions of portal venous pressure and MABP after i.v. administration. Also, total peripheral resistance was significantly reduced by **71** and cardiac index was slightly increased [47]. Successively, Tsai *et al.* [48] demonstrated that only the inhibitors specific to small conductance Ca^{2+}-activated K^+ channel or adenosine triphosphate (ATP)-sensitive K^+ channel inhibited the relaxant action of **71**. Moreover, the **75**-induced relaxation was reversed by the inhibitor of soluble guanylyl cyclase in a way similar to that of ATP-sensitive K^+ channel blockade. From *Evodia rutaecarpa* (Rutaceae) Chiou *et al*. [49] isolated rutaecarpine (**72**), that relaxed PE-precontracted aorta in concentration and endothelium-dependent manners. Studies with appropriate antagonists indicated that this was coupled to NO and guanylyl cyclase. $[Ca^{2+}]_e$ removal and treatment with the $[Ca^{2+}]_i$ antagonist suggested that influx of $[Ca^{2+}]_e$ was the major factor contributing to the action of **72**. The actions of **72** in ECs and VSMCs were also evaluated [50]. With K^+ and Na^+ channels blocked, whole-cell patchclamp studies in isolated cultured ECs indicated that **72** elicited macroscopic ionic currents. In fact, **72** modulated Ca^{2+} fluxes directly through some **72**-sensitive mechanisms in the Ca^{2+} channels. However, there was a conspicuous temporal discrepancy between **72**-



Scheme 7.

induced membrane activation, as reflected by the rise in Ca^{2+} currents and increase in $[Ca^{2+}]_i$ flux. Several possibilities might help to explain such a temporal lapse. First, patch-clamp measurements represent only changes in the Ca^{2+} currents *per se*. The rapid development of the ruteacarpine-induced inward Ca^{2+} currents might reflect the opening of the Ca^{2+} channel, whereas the subsequent decay might reflect the kinetics of drug and action site interactions and inner kinetics of Ca^{2+} channels themselves. Second, Ca^{2+} channel opening may just trigger off a cascade of intracellular events leading eventually to increase in $[Ca^{2+}]_i$, a process that may take much longer to complete. Finally, a finite time is needed



70 **71** **72**

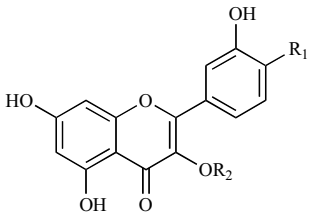
Scheme 8.

for the penetration of **72** from the edge of the confluent ECs to those cells in the optical recording field used in the fura-2/AM studies. The fact that the NO inhibitors and hydroquinone readily blocked **72**-induced endothelium-dependent relaxation in the rat aorta suggested that NO was likely the mediator responsible. Together with a concomitant elevation in cGMP production these observations were consistent with the notion that a rise in $[Ca^{2+}]_i$ led to enhancement of the NO-cGMP vasorelaxing axis. Patch-clamping studies in isolated VSMCs indicated again **72** directly inhibited Ca^{2+}-generated currents in the L-type VOCs, the predominant Ca^{2+} channels in VSMCs. However, **72** also inhibited NE-induced contraction, suggesting that it also attenuated Ca^{2+} influx through ROCs. Thus, it seems that suppression of both VOCs and ROCs in VSMCs are involved. Therefore, in addition to inhibition of $[Ca^{2+}]_i$ release from stores previously reported, it seems that **72** can also suppress membrane VOCs and ROCs in VSMCs [51].

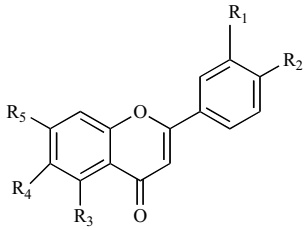
3. POLIPHENOLS

3.1. Flavonoids

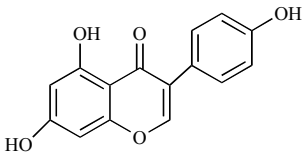
Chronic oral administration of quercetin (**73**) exerted antihypertensive effects in spontaneously hypertensive rats (SHR). Starting from this observation the vasodilatator effect of quercetin (**73**) and isorhamnetin (**74**) was investigated in isolated thoracic aorta, iliac artery and on the isolated perfused mesenteric resistance vascular bed from SHR and normotensive Wistar Kyoto (NWKY) rats. In NA-precontracted vessels from SHR there was an inverse correlation between the relaxant potency of quercetin (**73**) and isorhamnetin (**74**). Moreover, both flavonoids were more potent in endothelium-denuded aortae and iliac arteries from SHR than from NWKY rats. In addition, in aorta from SHR both flavonoids restored the endothelial-dependent vasodilation. Compound **74**, but not **73**, also reduced the endothelium-dependent contractile responses induced by acethylcholine (ACh). These direct vasodilator effects, together with the improvement of endothelial function, are good candidates to explain the BP reduction and vascular protective effects of quercetin (**74**) in animal models of hypertension and possibly in human cardiovascular diseases [52]. Galisteo *et al.* [53] demonstrated that quercetin (**74**) and verapamil showed similar antihypertensive effects in mineralocorticoid hypertension, but **74** was superior to verapamil in improving endothelial-dependent aortic dilatation, suggesting a better vascular protection in volume expansion deoxycorticosterone acetate (DOCA)-salt rats hypertension model. Quercetin (**74**) was able also to inhibit ACE activity through the cardiovascular response to bradykinin and angiotensin I [54]. With a multiple mechanism of action operated apigenin (**75**). It relaxed the PE-precontracted endothelium aortic rings. Pretreatment with apigenin (**75**) significantly potentiated the relaxant effect of ACh on PE-induced contraction. Pretreatment with L-NAME or methylene blue (MB) reduced the relaxant effect of this flavonoid at the same time **75** increased the cGMP content of endothelium-intact tissues. These findings suggest that besides influx and release of Ca^{2+}, NO and cGMP may account for the apigenin-induced endothelium-dependent relaxation and hypotensive activity. The isoflavonoid genistein (**76**) inhibited membrane Na^+-K^+-Cl^- co-transporters and induced a maximal salidiuretic action similar to that of furosemide (natriuresis EC_{50} 237 and 560 μM for **76** and furosemide, respectively). Genistein (**76**) had no significant effect on glomerular filtration rate but was able to significantly reduce renal vascular resistance with respect to vehicle isolated perfused kidney [55]. Quercetin (**73**), hesperidin (**77**), quercitrin (**78**) and rhoifolin (**79**) exhibited only a partial vasorelaxing effect. On the contrary, acacetin (**80**), apigenin (**75**), chrysin (**81**), hesperetin (**82**), luteolin (**83**), pinocembrin (**84**), 4'-hydroxyflavanone (**85**), 5-hydroxyflavone (**86**), 5-methoxyflavone (**87**), 6-hydroxyflavanone (**88**) and 7-hydroxyflavone (**89**) showed full vasorelaxing effects. The vasodilatory activity of **82**, **83**, **87** and **90** were antagonised by TEA, indicating the possible involvement of Ca^{2+}-activated K^+ (BK(Ca)) channels. Finally, GB inhibited the vasorelaxing action of luteolin (**83**) and 5-hydroxyflavone (**87**), suggesting that adenosine 5'-triphosphate (ATP)-sensitive K^+ channels may also be involved in their mechanism of action [56]. Naringenin (**90**) induced concentration-dependent relaxation in endothelium-denuded rat aortic rings pre-contracted with either KCl or NA. TEA, iberiotoxin, 4-aminopyridine and KCl antagonised **90**-induced vasorelaxation, while GB did not produce any significant antagonism. In rat tail artery myocytes, **90** increased large conductance (BK(Ca)) currents in a concentration-dependent manner. Moreover, **90** accelerated the activation kinetics of BK(Ca) current, shifted, by 22 mV, the voltage dependence of the activation curve to more negative potentials and decreased the slope of activation [57]. Bioguided fractionation of *Selaginella tamariscina* (Selaginellaceae) afforded an active biflavonoid, amentoflavone (**91**). This compound induced concentration-dependent relaxation of the PE-precontracted aorta, which disappeared by removal of functional endothelium. Pretreatment of the aortic tissues with L-NAME, MB, or ^{1}H-[1,2,4]-oxadiazole-[4,3- a]-quinoxalin-1-one (ODQ) inhibited the relaxation induced by **91**. Incubation of endothelium-intact aortic rings with amentoflavone (**91**) increased the production of cGMP, but this effect was blocked by endothelium-denudation or pretreatment with L-NAME or ODQ. These results suggested that **91**-iduced relaxation *via* endothelium-dependent NO-cGMP signaling with possible involvement of non-specific K^+ and Ca^{2+} channels [58]. Apigenin (**75**), luteolin (**83**), kaempferol-3-*O*-α-arabinopyrano-



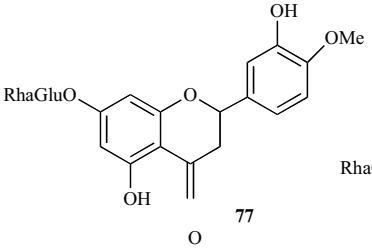
73 R_1= OH, R_2= H
74 R_1= OMe, R_2= H
78 R_1= OH, R_2= Rhamnose
RUTIN R_1= OMe, R_2= Rutinose



75 R_1=R_4= H, R_2=R_3=R_5= OH
81 R_1=R_2=R_4=H, R_3=R_5= OH
83 R_1=R_2=R_3=R_5= OH, R_4=H
84 R_1=R_2=R_4= H, R_3=R_5= OH
95 R_1=R_2=R_3= OH, R_5= OGlc, R_4=H



76



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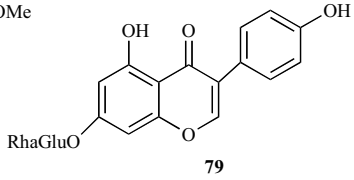

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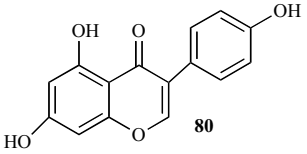

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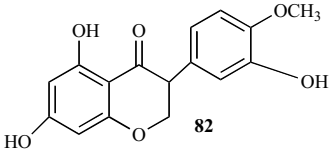
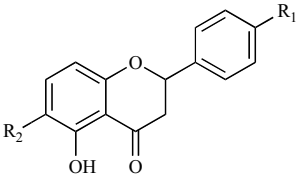
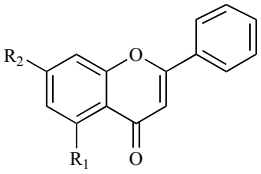


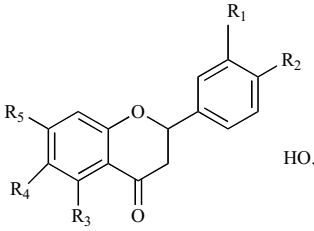
82



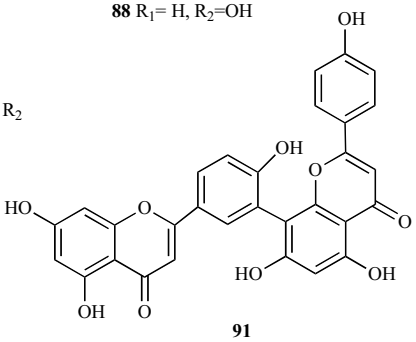
85 R_1= OH, R_2=H
88 R_1= H, R_2=OH



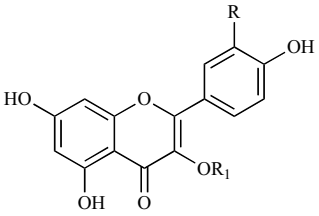
86 R_1= OH, R_2=H
87 R_1=OCH_3, R_2=H
89 R_1=H, R_2=OH



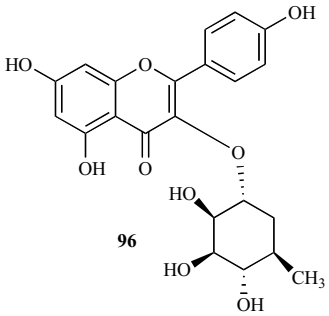
90 R_1=R_4= H, R_2=R_3=R_5= OH



91



92 R= H, R_1= Ara
93 R= H, R_1= Gal
94 R=OH, R_1=Ara



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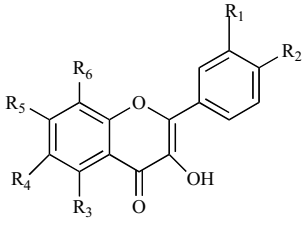
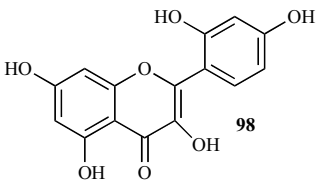


97 R_1=R_2=R_5= OH, R_3=R_4=R_6=H
101 R_1=OCH_3, R_2=R_3=R_5=R_6= OH, R_4= H



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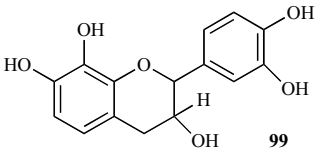

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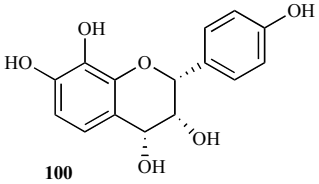

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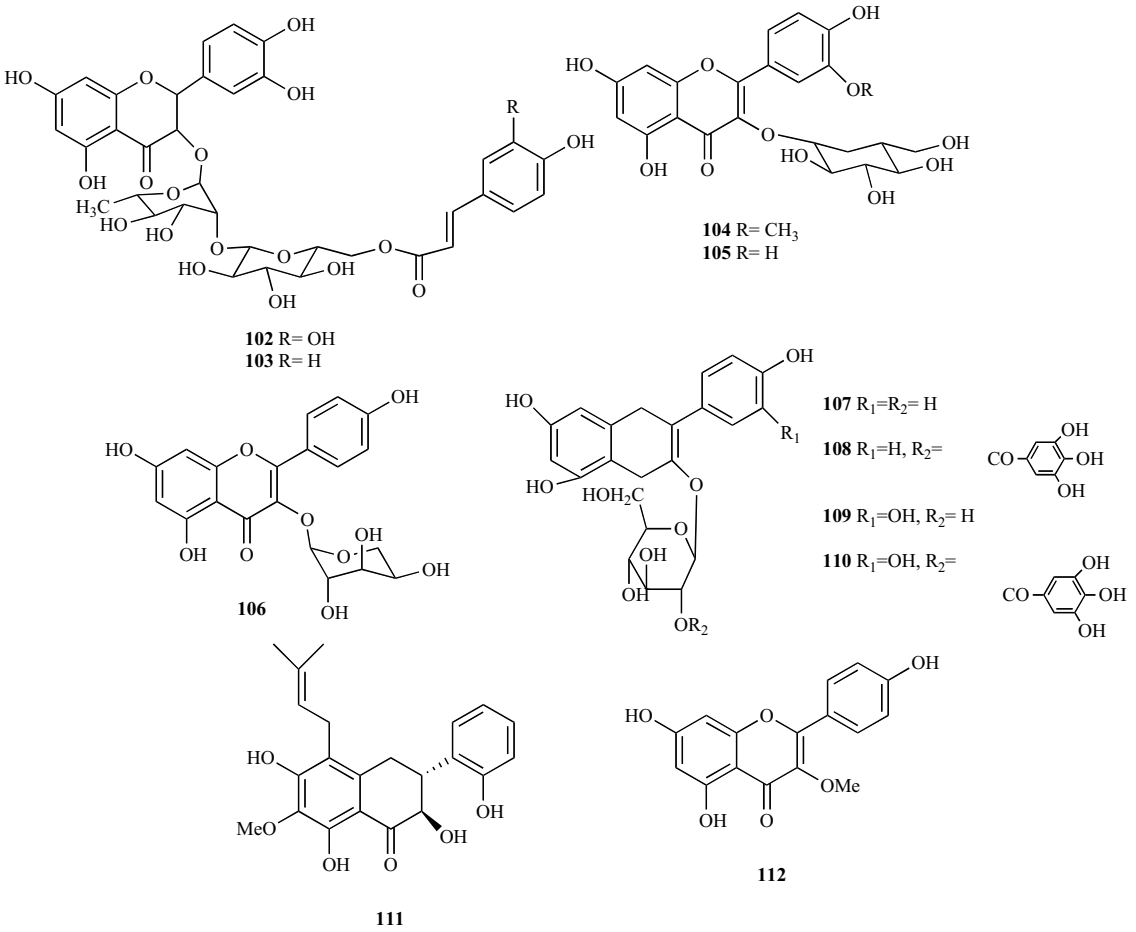
Scheme 9



Scheme 10.

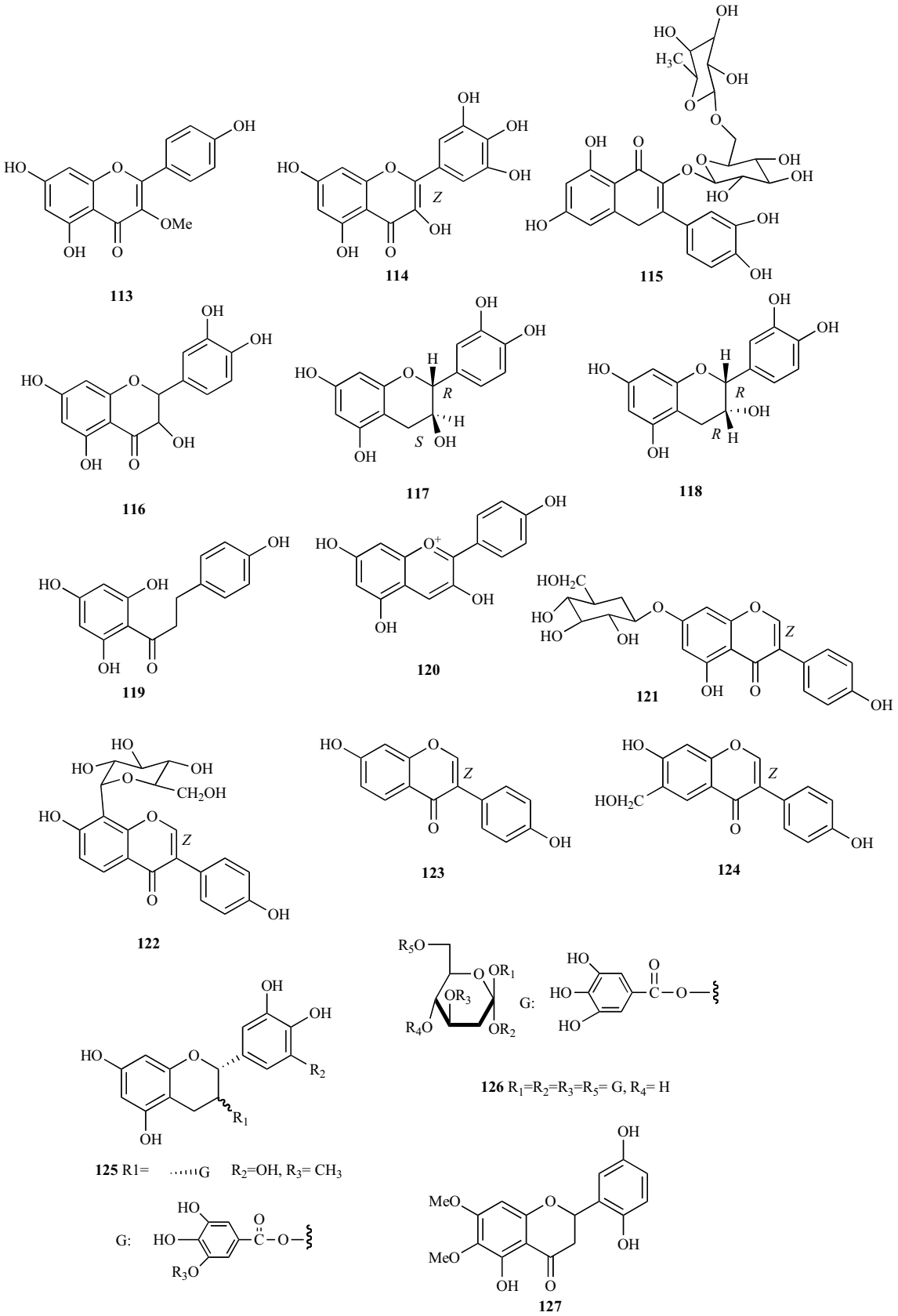
side (**92**), kaempferol-3-*O*-β-galactopyranoside (**93**), quercetin-3-*O*-α-arabinopyranoside (**94**) and luteolin-7-*O*-β-glucopyranoside (**95**) isolated from *Ailanthus excelsa* (Simaroubaceae) exhibited ACE inhibitory activity in a dose-dependent manner. Among them **92** showed the most promising activity (IC_{50} 260 μM). The arabinosyl moiety seems to have negative influence on the ACE inhibition, in fact quercetin-3-*O*-α-arabinopyranoside (**94**) showed a value of inhibition less high then others compounds (IC_{50} 310 μM). Luteolin (**83**) exhibited an IC_{50} of 290 μM; the introduction of glucopyranoside moiety in C7 weakly reduce the flavonoid activity as in **95** (IC_{50} 280 μM) while **75** showed an IC_{50} value of 280 μM on ACE activity. Free hydroxyl groups of phenolic compounds are also suggested to be important structural moieties to chelate the zinc ions, thus inactivate the ACE activity [59]. Previously Hansen *et al*. [60] demonstrated the ability of quercitrin (**78**) and afzelin (**96**) to inhibit ACE with IC_{50} of 0.67 and 2.8 mM, respectively. The removal of the 3'-hydroxyl group in quercitrin decreases the ACE-inhibitory activity approximately by a factor four. The rhamnosyl moiety also seems to be influence the ACE inhibitory activity. In fact, Eble *et al*. [61] founded that the aglicone of quercitrin (**78**), quercetin (**73**) did not show ACE-inhibitory activity. The removal of the 5-hydroxy group in **78** resulted in fisetin (**97**) which exhibit an ACE inhibitory activity with IC_{50} of 0.15 mM [62]. Among flavonoids group interesting activity against ACE were also founded with morin (**98**), mesquitol (**99**), teracacidin (**100**), amentoflavon (**91**) and gossipetin (**101**). It is remarkable that all tested flavonoids are characterized by a hydroxyl group in the vicinity of a heterocyclic oxygen atom. This group may create a chelate complexe with the zinc atom within the active centre of ACE as previously reported [63].

Bioassay-guided fractionation of *Sedum sarmentosum* (Crassulaceae) afforded quercetin-3-*O*-α-(6""-caffeoylglucosyl-β-1,2-rhamnoside) (**102**), quercetin 3-*O*-α-(6""-*p*-coumaroylglucosyl-β-1,2-rhamnoside) (**103**), isorhamnetin-3-β-glucopyranoside (**104**), quercetin-3-β-glucopyranoside (**105**), and kaempferol-3-α-arabinopyranoside (**106**). Compounds **102**-**106** inhibited ACE activity with IC_{50} ranging from 158.9 to708.8 μM. In particular **102** was two-fold more active than its closely related **103**. This higher activity could be linked by hydroxyl group at the C-6"" [64]. Astragalin (**107**), kaempferol-3-*O*-(2"-*O*-galloyl)-glucoside (**108**), isoquercitrin (**109**), and quercetin-3-*O*-(2"-*O*-galloyl)-glucoside (**110**), isolated from *Diospyros kaki* (Ebenaceae), inhibited the ACE activity in a dose-dependent manner. In particular, the IC_{50} of **107** and **108** were 180 and 280 μg/ml, respectively [65]. Floranol (**111**), isolated from *Dioclea grandiflora* (Leguminosae), induced a concentration-dependent vasodilator effect in PE-precontracted vessels with an IC_{50} value of 19.9 μM.

The removal of endothelium or pretreatment of vessels with L-NAME did not change the IC_{50} and the maximal values for the relaxant effect (E_{max}) for **111**-induced vasorelaxation it is clear that endothelium-derived vasorelaxant factors such as NO do not account for the vasorelaxant effect of floranol (**111**) in the rat aorta and that direct vasorelaxant mechanism could be supposed in the rat-aorta smooth muscle cells [66]. Isokaempferide (**112**), isolated from *Amburana cearensis* (Fabaceae), induced relaxation of contracted guinea-pig isolated trachea with EC_{50} of 77.4 μM in intact tissue and 15.0 μM in denuded epithelium [67].

Flavones apigenin (**75**) and luteolin (**83**), flavonols kaempferol (**113**), quercetin (**73**), myricetin (**114**) and rutin (**115**), flavononoles naringenin (**90**) and taxifolin (**116**), flavanoles (+)-catechin (**117**) and (-)-epicatechin (**118**), chalcone pholoretin (**119**), anthocyanidins pelargonidin (**120**) and isoflavones genistein (**76**), genistin (**121**), puerarin (**122**), daidzein (**123**) and glycetein (**124**) were investigated for their vasorelaxant activities in porcine coronary arteries with/without an intact endotelium. In endothelium-intact porcine coronary artery all flavonoids except genistin (**121**) showed a significant vasorelaxant activity. According to their ability to induce vasorelaxation, it is possible to classify these flavonoids into three categories taken into account of their potency and efficacy. The flavonoids that induce almost 100% relaxation at 30 μM are considered to be good relaxing agents and this group includes apigenin (**75**), luteolin (**83**), kaempferol (**113**) and genistein (**76**). Flavonoids inducing 50% relaxation at concentration around 30 μM and good relaxation (from 85% to >100%) at the maximum concentration tested, i.e. 100 μM, are moderate relaxing agents such as quercetin (**73**), naringenin (**90**), daidzein (**123**), whereas those with little or no relaxation at 30 μM but some effect (45% to 80%) at 100 μM are weak vasodilators such as myricetin (**114**), rutin (**115**) etc. On comparison of the relaxing effect in the artery with/without endothelium, it appears that flavonoids induced relaxation mainly through direct action on the VSMCs. The release of endothelium-derived relaxing factors such as NO and endothelium-derived hyperpolarizing factor are largely responsible for the vasorelaxing effect of flavonoids together with antagonism on thromboxane A2 receptor or through hyperpolarization with activation of K^+ channels. Both **73** and **114** reduced relaxation activity compared with **113**. The glycosylation at the 3-OH position of **77** as in rutin (**115**) that causing steric hindrance reduced the relaxation effect. Compounds **75**, **83**, **113** and **76** (good relaxing compounds) possessed a 5-OH, 7-OH, 4'-OH, C(2)=C(3) double bond and C(4)=O functionalities. In the isoflavone class, the B-ring is connected at the 3-position to the C-ring. Genistein (**76**) had that possess a 5-OH, 7-OH, 4'-OH and a C(2)=C(3) double bond had the strongest vascular effect. Daidzein (**123**) lacks the 5- OH group and this may account for the smaller vascular relaxation effect than genistein (**76**). Genistin (**121**), which is glycosylated at the 7-OH position, had no relaxation effect compared with **76**. Puerarin (**122**) had a C-glycosyl group at C8 and strongly decreased vascular relaxation as compared with **123**. (+)-Catechin (**117**) and (-)-epicatechin (**118**), which have no C(4)=O and C(2)=C(3) double bonds, exhibited a very weak vascular relaxation effect. Both phloretin (**119**) and pelargonidin (**120**) showed a moderate relaxation effect. For the former, it had a broken C-ring and this attenuates coplanarity of the A- and B-rings. The latter compound had positive charges at the 1-position in the C-ring. Both compounds also lack a C(2)=C(3) double bond [68]. An *in vivo* study demonstrated that epigallocatechin-3-*O*-methylgallate (**125**) and 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**126**) reduced the vasopressor activity of exogenous angiotensin I 10 min after their administration, which was consistent with the effect of captopril. Both compounds, reduced BP to a significantly greater extent than captopril. Puerarin (**122**) elicited endothelium-independent vasodilation while endothelium-dependent vasodilations were unaffected. Due to the structural similarity, it is likely that **122** may exert its action *via* a similar mechanism to the estrogen trought the cAMP cascade. Indeed, the modulatory effects of puerarin (**122**) on endothelium-independent vasorelaxation were abolished by a cAMP antagonist. Puerarin (**122**) enhanced sodium nitroprusside-induced relaxation, possibly *via* the cyclic AMP-dependent pathway in U46619-contracted porcine coronary artery rings [69]. Hesperetin (**82**), isolated from *Citrus* fruits (Rutaceae), concentration-dependently relaxed the isometric contractions induced by NA or by a high extracellular KCl concentration in intact rat isolated thoracic aorta rings. However, **82** did not affect the contractile response induced by okadaic acid. Mechanical removal of endothelium and/or pretreatment of aorta rings with GB, TEA or nifedipine did not significantly modify the vasorelaxant effects of this flavonoid **82**. Hesperitin (**82**) significantly reversed the inhibitory effects of NA and high KCl on cAMP and cGMP production in cultured rat aortic myocytes. This flavonoid preferentially inhibited calmodulin-activated PDE_1 and PDE_4 isolated from bovine aorta with IC_{50} values of about 74 μM and 70 μM respectively [70]. Dioclein (**127**), isolated from *Dioclea grandiflora* (Campanulaceae), induced a concentration-dependent relaxation of aortic rings trought the inhibition of contractions dependent on activation of protein kinase C, voltage-dependent Ca^{2+} influx and on the release of $[Ca^{2+}]_i$ stores sensitive to NA [71]. The hypotensive and vasorelaxant effect of dioclein (**127**) in resistance mesenteric arteries was studied in intact animals and isolated vessels, respectively. In intact animals, initial bolus administration of **127** produced transient hypotension accompanied by an increase in HR. In endothelium-containing or -denuded vessels precontracted with PE, **127** produced a concentration-dependent vasorelaxation. In conclusion, **127** lowered arterial pressure probably through a decrease in peripheral vascular resistance. The underling mechanism implicated in the vasorelaxant effect of dioclein (**127**) appears to be the opening of Ca^{2+} activated channel and voltage activated K^+ channel and subsequent membrane hyperpolarization [72].

Galangin (**128**) caused NO release from aortic rings and abolished the increase in $[Ca^{2+}]_i$ triggered by PE or KCl in aortic smooth muscle cells, either in presence and in absence of $[Ca^{2+}]_e$ [73]. Gentiacaulein (**129**) and gentiakochianin (**130**), isolated from *Gentiana kochiana* (Gentianaceae), exhibited vasorelaxing activity in rat aortic preparations, precontracted by NE and KCl [74]. Kang *et al*., [75] evidenced that the administration of the chalcone butein (**131**) lowered BP in a dose-dependent manner. Moreover, **131** had a significantly inhibitory activity against ACE. Xanthoangelol (**132**), 4-hydroxyderricin (**133**), xanthoangelols B (**134**), E


113
114
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123
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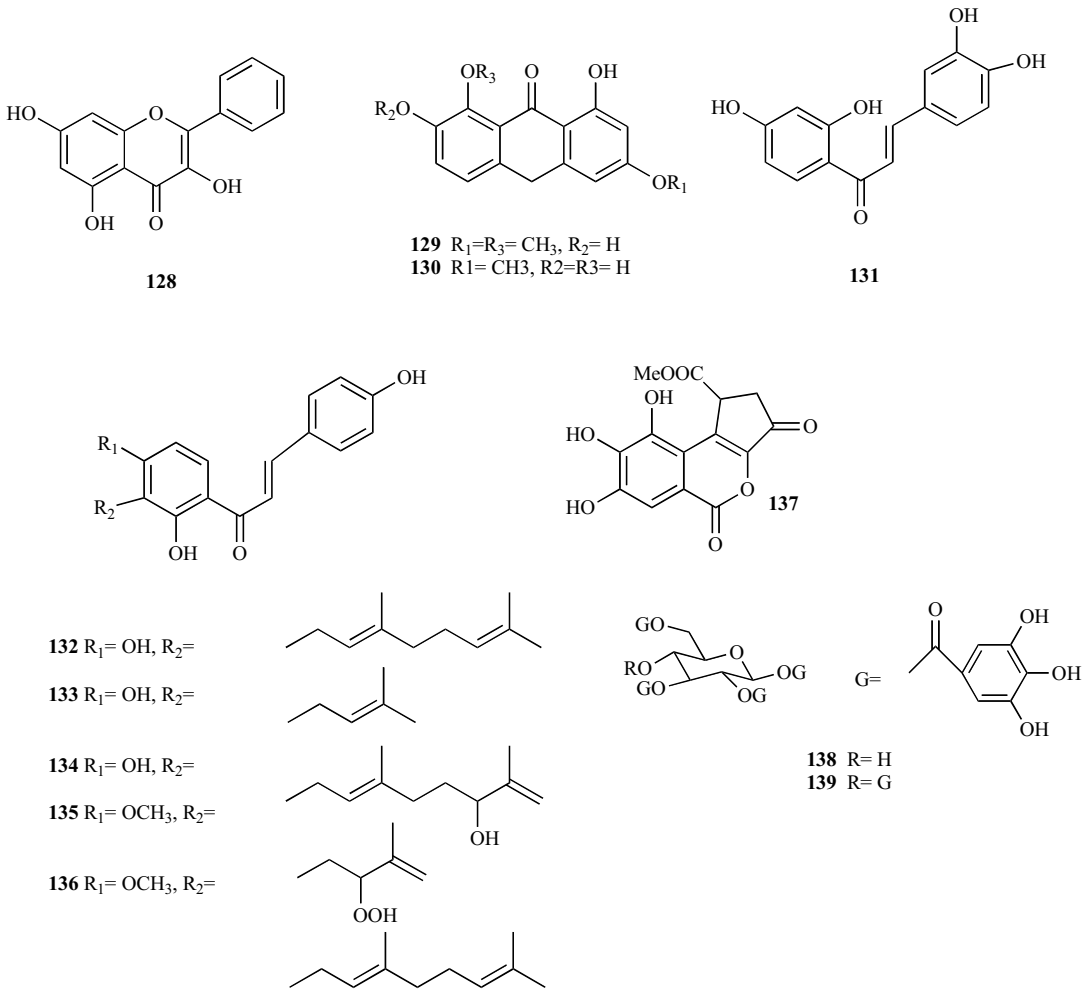
125 R1= G R2=OH, R3= CH3
126 R1=R2=R3=R5= G, R4= H
127
G:
G:

Scheme 11.

(**135**) and F (**136**), isolated from *Angelica keiskei* (Umbelliferae), inhibited the PE-induced vasoconstriction through endothelium-derived relaxing factor (EDRF) production and/or NO production. Among them, xanthoangelol B (**134**) inhibited the PE-induced vasoconstriction most strongly and it inhibited the PE-induced vasoconstriction in the presence or absence of endothelium and in the presence or absence of NG-Monomethyl-L-Arginina (L-NMMA). Furthermore, **133** and **134** concentration-dependently inhibited the elevation of free $[Ca^{2+}]_i$ induced by PE. These results demonstrated that compounds **132**, **133**, **135** and **136** inhibit PE-induced vasoconstriction through endothelium-dependent production of EDRF/NO and/or through the reduction of the $[Ca^{2+}]_i$ elevation induced by PE. The inhibitory mechanism of **133** on PE-induced vasoconstriction might involve the direct inhibition of smooth muscle functions through the reduction of $[Ca^{2+}]_i$ elevation without affecting EDRF/NO production. The inhibitory action of **135**, which has a γ,γ-dimethylallyl group at the C3' position was stronger than that of **132** or **136** which have a geranyl group at this position. Furthermore, the inhibitory action of **135** in which a 2-hydroxyperoxy-3-methyl-3-butenyl group is substituted for γ,γ-dimethylallyl (3-methyl-2-butanyl) group of **133** at C3', was attenuated as compared to that of **133**. The activity of **133** and **135** were also shown to be mediated through endothelim-dependent NO/EDRF production. These findings suggest that the difference of the side chain at C3' of these chalcones may be closely related to their inhibitory strength. It was of great interest that **134** with an (*E*)-6-hydroxy-3,7-dimethyl-2,7-octadenyl moiety at C3' inhibited PE-induced vasoconstriction without affecting endothelium-dependent NO/EDRF production [76]. Ogawa *et al*. [77] demonstrated the ability of 4-hydroxyderricin (**133**) to suppress the elevation of systolic blood pressure (SBP) in stroke-prone SHR after 7 weeks of diets containing 0.07% of **133**. Methyl brevifolincarboxylate (**137**), isolated from *Phyllanthus niruri* (Euphorbiaceae), showed a vasorelaxant effect on rat aortic rings. The inhibition of NE-induced vasocontraction by **137** is in part attributable to a decrease in $[Ca^{2+}]_i$ through receptor-operated Ca^{2+} channels [78]. Tetragalloylglucose (**138**) and pentagalloylglucose (**139**), isolated from *Paeonia moutan* (Paeoniaceae), potently relaxed PE-induced contraction of rat aortic preparations in a concentration-dependent manner though increases in the release of NO from ECs with IC_{50} of 5.1 and 3.6 μM, respectively [79].



Scheme 12.

3.2. Coumarins

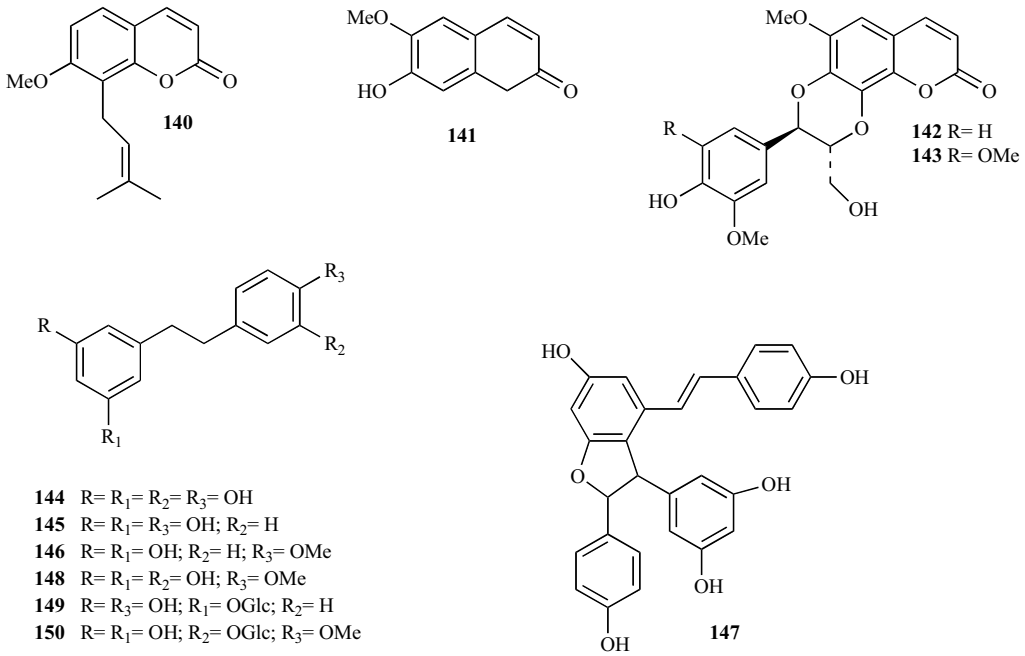
The pharmacological effects of osthole (**140**), isolated from *Angelica pubescens* (Apiaceae), revealed that it act blocking the Ca^{2+}-channel and elevating cGMP levels in VSMCs [80]. Scopoletin (**141**), cleomiscosin A (**142**) and aquillochin (**143**), isolated from *Acer nikoense* (Aceraceae), exhibited slow vasorelaxant effects on aortic rings with/ without endothelium. When NE was added to the organ bath in Ca^{2+}-free Krebs–Henseleit solution, the aortic rings showed transient phasic contractions. The transient phasic contractions were significant and markedly reduced after treatment with **141**-**143**. Thus **141**-**143** may inhibit NE-induced Ca^{2+} release and **141** showed vasorelaxant effects by inhibiting $[Ca^{2+}]_i$ mobilization. Therefore it is presumable that **142** and **143** might also have the same action as **141**. Compounds **142** and **143** have moderate inhibitory effects on the contractions induced by cumulatively applied Ca^{2+} in aortic rings preincubated with NE and nicardipine in the Ca^{2+}-free medium, although the aortic rings were not significantly affected by treatment with **141**. Furthermore, the Ca^{2+}-induced vasoconstriction of high K^+-depolarized rat aorta was also attenuated by treatment with **142** and **143**, but not by **141**. The results indicated that the vasorelaxant effects of **142** and **143** may be attributed to the inhibition of both the nicardipine-sensitive and -insensitive Ca^{2+} channels [81].

3.3. Stilbenes

The activity-guided fractionation of the *Rheum undulatum* (Polygonaceae) extract led to the isolation of several hydroxystilbene components as active principles. Four stilbenes, such as piceatannol (**144**), resveratrol (**145**), desoxyrhapontigenin (**146**) and ε-viniferin (**147**), relaxed the PE induced contraction of rat aorta (EC_{50} 2.4, 28.6, 18.5 and 8.4 μM, respectively). Piceid (**148**), rhaponticin (**149**) and rhapontigenin (**150**) showed poor activity [82]. These results suggest the following structural requirements of stilbenes for the vascular relaxant effect: the number and position of the hydroxyl substituents on the stilbene scaffold may play an essential role in the activity; the glucosyl group attached to the stilbene skeleton may reduce the activity, suggesting that a glucosyl group may be ascribed to the steric hindrance which would not allow them to reach the target site. In particular, piceatannol (**144**) with four hydroxyl groups exhibited a more potent vascular relaxant effect than the other stilbene components. The relaxant effect of **144** in aortic tissue was completely abolished by denudation of the endothelial layer, suggesting that the vasorelaxation caused by **144** was endothelium-dependent NO signaling.

3.4. Phenylethanoid and Phenylpropanoid Glycosides

Inhibitory effects of the major constituents of *Cistanche tubulosa* (Orobanchaceae), kankanoside F (**151**), kankanose (**152**), echinacoside (**153**), acteoside (**154**), isoacteoside (**155**), cistanoside F (**156**) and salidroside (**157**) on NA-induced contractions in isolated rat thoracic aorta were recently examined [83]. Compounds **152**-**154** and **152** having a caffeoyl group in C4' significantly inhibited the contractions, time- and/or concentration dependently, while **155** having a 6'-*O*-caffeoyl moiety showed weak activity. Compound **151** with a catechol group showed significant inhibition, while **157** with a phenol group did not show such effect. Compounds **151**, **152**-**154** and **156** inhibited the contractions *via* ROCs, but not *via* VOCs. In another study, acteoside (**154**) isolated from *Ligustrum purpurascen* (Oleaceae) induced relaxation of precontracted rings (IC_{50} 0.22 mg/ml), but it caused an increase in K^+-induced tone. Removal of endothelium enhanced the relaxing effect of acteoside (**154**). Besides, pretreatment with **154** inhibited endothelium/NO-mediated relaxation. The inhibitory effect on endothelial NO-mediated relaxation suggests that acteoside (**154**) could

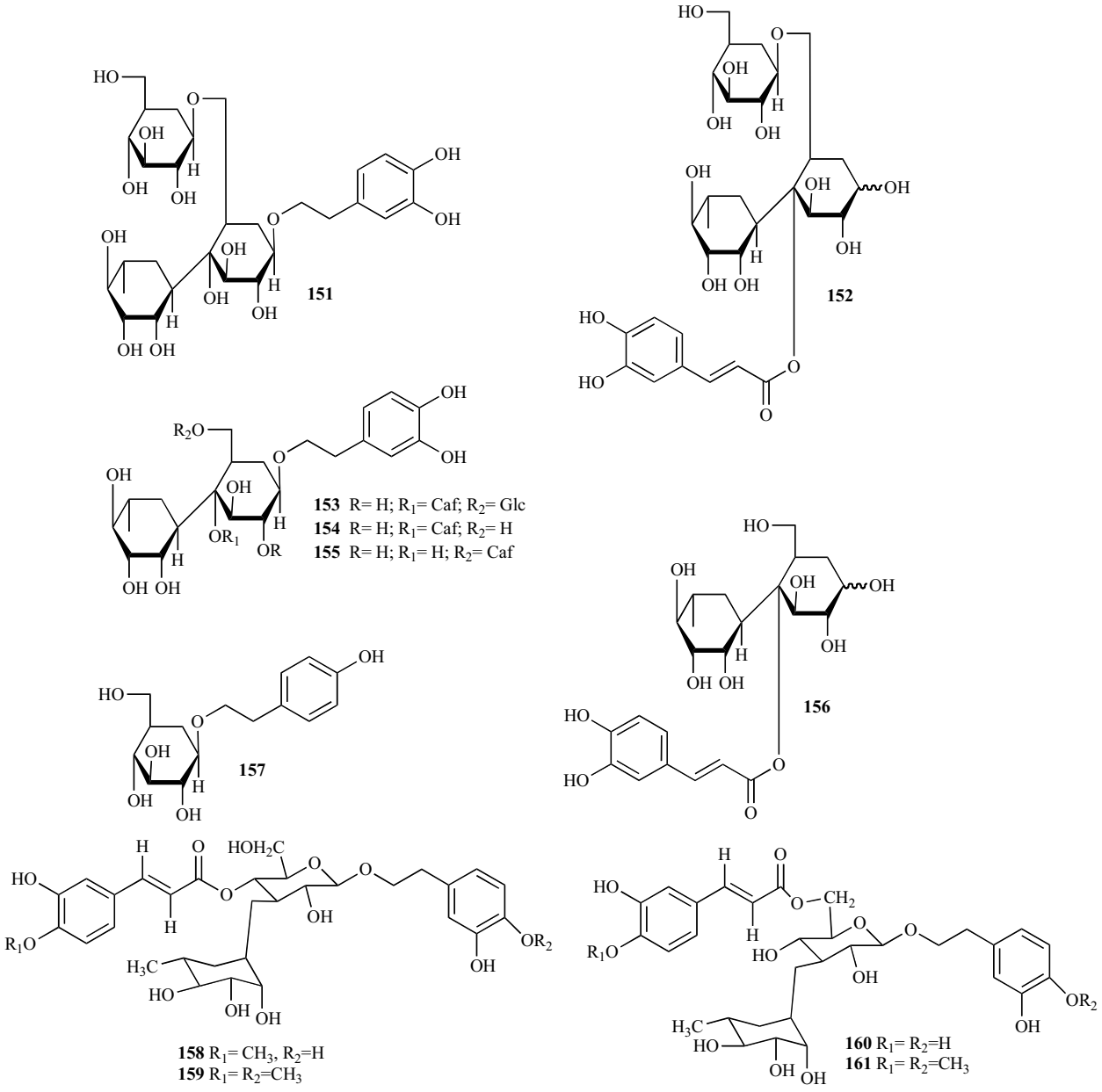


Scheme 13.

also act on the ECs to reduce NO release [84]. Lau *et al.* [85] demonstrated that **154** impairs endothelial NO-mediated aortic relaxation partially through inhibition of agonist-induced endothelial Ca^{2+} mobilization and Ca^{2+}-dependent NO production and subsequent suppression of cGMP formation without affect the expression of endothelial NOS mRNA in endothelium-intact rings. Moreover, the ability of acteoside (**154**), isoacteside (**155**), leucosceptoside A (**158**), martynoside (**159**), acteoside isomer (**160**) and isomartynoside (**161**) to inhibit ACE was demonstrated. In general, the inhibitory activities of phenylpropanoid glycosides showed a tendency to increase with increasing hydroxyl group on aromatic rings [86, 87].

3.5. Cinnamic Acids

3,5-di-*O*-Caffeoylquinic acid (**162**), methyl 3,5-di-*O*-caffeoylquinate (**163**), 3,4-di-*O*-caffeoylquinic acid (**164**), and methyl 3,4-di-*O*-caffeoylquinate (**165**), isolated from *Cuscuta japonica* (Cuscutaceae), exhibited the ACE in dose-dependent manner with IC_{50} values ranging from 460 to 596 μM. Although it is not significantly varied, the enhanced activities of methyl esters as compared to the respective acid counterparts were observed. In addition, slightly enhanced inhibitory activities were observed for the acid (**164**) and methyl ester (**165**) with caffeoyl groups at C3 and C4 positions compared to those of respective acid (**162**) or methyl



Scheme 14.

ester (**163**) with caffeoyl groups at C3 and C5 positions [88]. Mishima *et al*. [89] reported the hypotensive activity of **162**, **163** and 3,4,5-tri-*O*-caffeoylquinic acid (**166**) *in vivo*. Single oral administration of 10 mg/kg of each **162**, **163** and **166** significant decreased of BP beginning approximately 1 h after administration. The SBP decreased 9.3 mmHg with **163**, 8.4 mmHg with **162** and 13.0 mmHg with **166** compared with starting SBP. As regards the HR, no differences were observed within or between groups with all three components before and after administration after oral administration in SHR. Liu *et al*. [90] evaluated through SAR analysis the ACE inhibitory activity of caffeoylquinates in comparison with flavan-3-ols and gallotannins. Among caffeoylquinates **165** exhibited a 2-fold more potent activity than **166** with an IC_{50} of 82.9 μM. The IC_{50} values of flavano-3-ols ranging from 18.3 to 195.9 μM for epicatechin-3-*O*-gallate (**167**) and gallocatechin (**168**), respectively. Gallotannins showed IC_{50} ranging from 73.1 to 101.4 μM. Comparison of the structures of caffeoylquinates and ACE inhibitory activity suggested that both two caffeoyl groups (R_1, R_2, or R_1, R_3) and methylation at the R_4 position are the prerequisites of stronger ACE inhibition. Other tannins, for example, with one caffeoyl (R_1) and methylation at the R_4 position, such as methylchlorogenate (**169**), or with two caffeoyl groups (R_1, R_2, or R_1, R_3), but no methylation at the R_4 position, such as 3,4 di-*O*-caffeoylquinate (**164**), present less ACE inhibitor activity. The ACE inhibitory activity of the three prototype flavan-3-ols, gallocatechin, epicatechin and catechin were quite low. This data suggested that the R_5, hydroxyl group might be important in determining ACE inhibitory activity. The inclusion of a hydroxyl group at R_3 position and gallate into the flavan-3-ol structure increased the inhibitory activity of the flavan-3-ols. Each of the tannin classes appeared to exhibit a non-competitive mode of inhibition, indicating that the substrate and inhibitor bind to the ACE simultaneously in a reversible manner. Tannin-induced ACE inhibition resulting from protein precipitation was only observed with 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**170**), moreover, **170** is to be a non-specific inhibitor of ACE activity by similarly reducing the activity of both chymotrypsin and trypsin. Likewise, two flavan-3-ols, epigallocatechin-3-*O*-gallate (**171**) and epigallocatechin-3-*O*-methylgallate (**125**) were also found to have reduced the activity of these enzymes, suggesting they are also non-specific inhibitors of ACE activity. The vasorelaxant effects of forsythiaside (**172**), isolated from *Forsythia suspensa* (Oleaceae), on isolated rat aortic rings demonstrated that it induce a slow relaxation activity against NE-induced contractions of rat aorta with/without endothelium. Compound **172** did not affect contractions induced by a high concentration of K^+, while inhibited NE-induced vasocontraction in the presence of nicardipine. These results suggest that **172** induce vasorelaxant effect due to a decrease in Ca^{2+} influx from the extracellular space caused by NE [91]. Oral administration of ferulic acid (**173**) to SHR, SBP significantly decreased in a dose-dependent manner. Furthermore, the depressor effect of i.v. administration of **173** was significantly attenuated by pretreatment with L-NAME [96]. Compound **173** increased NO bioavailability and decreased NADPH-dependent superoxide anion levels in SHR aortas. Moreover, **173** improved ACh-induced endothelium-dependent vasodilation in SHR, but not in NWKY rats [92,93].

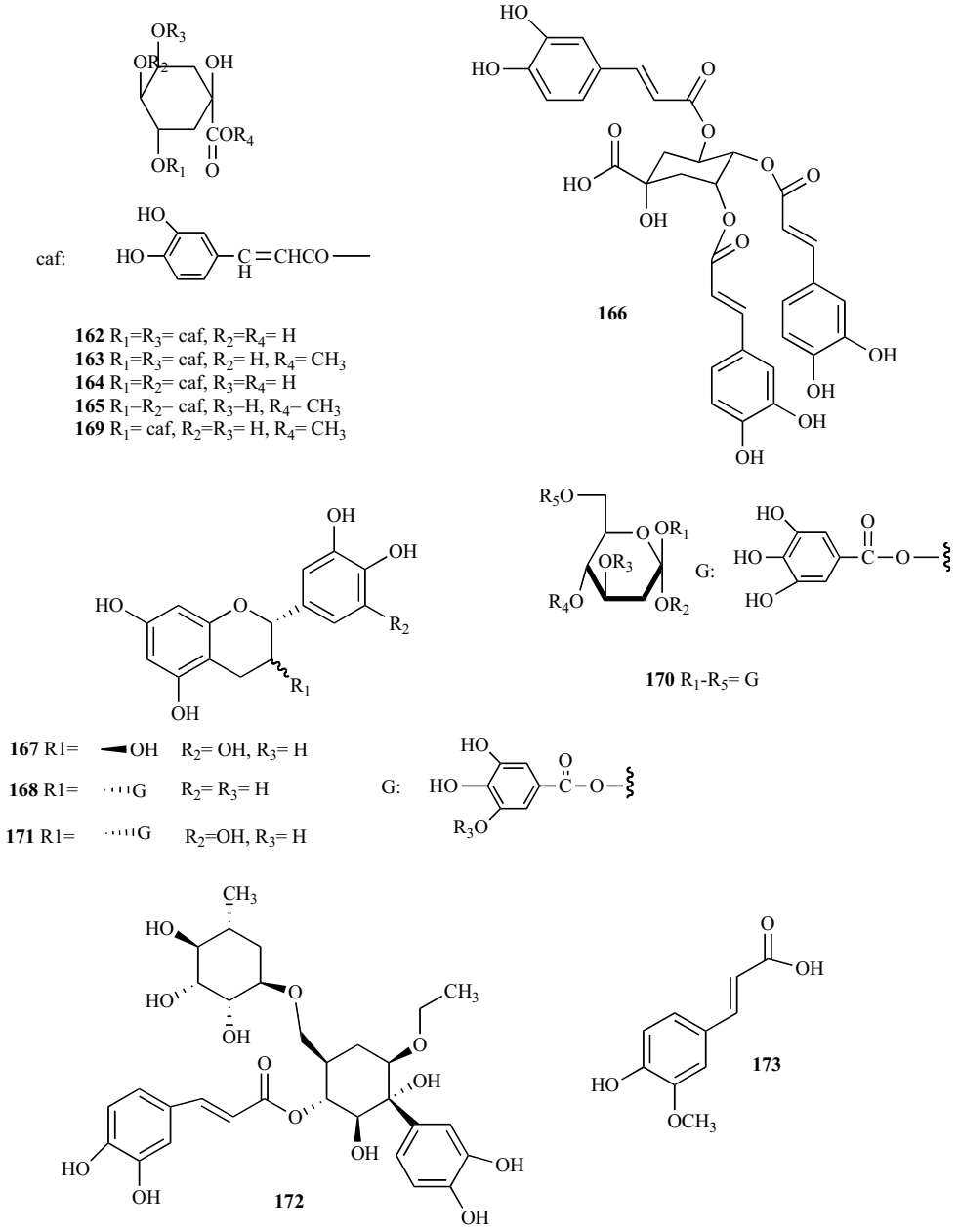
3.6. Phenolic Acids

Salvianolic acid B (**174**), one of the main constituents of *S. miltiorrhiza* (Lamiaceae) or danshen, exerted vasorelaxant effects by inhibition of Ca^{2+} influx in the VSMCs [94, 95]. Recently, Gao *et al.* [96] reported the ability of salvianolic acid B (**174**) to inhibt ACE with an IC_{50} of 0.02 g/l. Another lipid-soluble constituent of danshen, lithospermic acid B (**175**) exhibited Ca^{2+} channel antagonism. Previously, Nagai *et al*. [97] demonstrated the potent vasodilator effects of 8-epiblechnic acid (**176**), a **175** derivative. In fact pretreatment with **176** significantly attenuated the NE-induced concentration-dependent contraction of aortic strips while concentration-response curves for Ca^{2+}-induced contracture of depolarized aortic strips with isotonic high K^+ were not affected. Moreover, pretreatment of aortic strips with **176** slightly inhibited the phorbol ester-induced contraction. With antagonistic effect against VOCs work, the main water-soluble principle of *S. miltiorrhizae,* magnesium lithospermate B (**177**). In fact, **177** reversibly inhibited L-type Ca^{2+} current ($I_{Ca,L}$). The inhibition was use-dependent and voltage-dependent. In the presence of 100 μM of **177**, both the activation and steady-state inactivation curves of $I_{Ca,L}$ were markedly shifted to hyperpolarizing membrane potentials, whereas the time course of recovery of $I_{Ca,L}$ from inactivation was not altered. Compound **177** up to 300 μM had no significant effect on the fast-inactivating Na^+ current, delayed rectifier K^+ current and inward rectifier K^+ current [98]. Previously, Kamata *et al*. [99], demonstrated that **175** acts with a multiple mechanism of action. Firstly, i.v. of **175** decreased the BP in a dose-dependent manner. Secondly, in perfused mesenteric arterial bed precontracted with methoxamine, **175** produced a concentration-dependent relaxation. Thirdly, vasodilator response of the mesentery in response to **175** was reversed by L-NMMA while the inhibition of vasodilation by L-NMMA was reversed by L-arginine. Moreover, lithospermic acid B (**175**) demonstrated ACE inhibitory activity with an IC_{50} of 120 μM [100]. Danshensu (**178**), isolated from *S. miltiorrhiza*, relaxed coronary artery rings with an IC_{50} of 71.5 μg/ml. Removal of the endothelium did not significantly affect its vasodilator potency (IC_{50} 84.8 μg/ml). The possible involvement of Ca^{2+} channels was investigated in artery rings incubated with Ca^{2+}-free buffer and primed with KCl for 5 min prior to addition of $CaCl_2$ to elicit contraction. In 5-HT-primed preparations, the $CaCl_2$-induced vasoconstriction was abolished by 200 μg/ml of **178**, whereas, in KCl-primed preparations, 600 μg/ml of danshensu (**178**) were required to abrogate the vasoconstriction. These findings suggest that the vasorelaxant action of **178** was produced by inhibition of Ca^{2+} influx in the VSMCs. The opening of K^+ channels had a minor contribution to the response, while endothelium-dependent mechanisms were not involved [101].

4. TERPENES

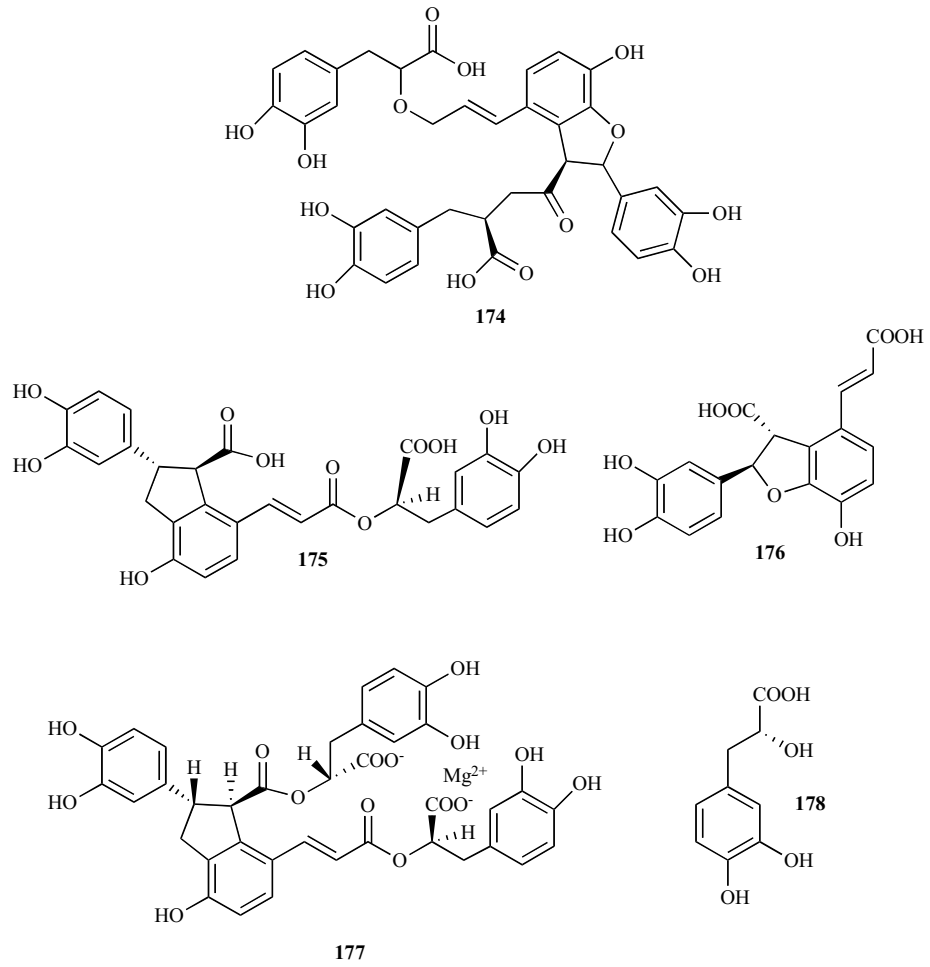
4.1. Monoterpenes

Carvacrol (**179**), at the dose of 100 μg/kg, decreased HR, MABP, SBP and diastolic blood pressure (DBP) in anesthe-



Scheme 15.

tized rats. It was able to induce hypotension and to inhibit L-NAME-induced hypertension. The lack of inhibitory action of carvacrol (**179**) (10^{-4} M) on the $CaCl_2$- and PE-induced contractions of isolated rat aorta showed that neither ARs nor L-type VOCs were involved. This finding is in contrast with founded blocking activity of **179** on cardiac L-type VOCs [102]. Eugenol (**180**), the main constituent of the essential oil of *Ocimum gratissimum* (Lamiaceae), induced dose-dependent hypotension and bradycardia in DOCA-salt-hypertensive rats [103]. Its isomer isoeugenolol (**181**) produced a dose-dependent bradycardia and a decrease in BP in anesthetized Wistar rats. It inhibited the tachycardia effects induced by (-)isoproterenol, but had no blocking effect on the arterial pressor responses induced by PE. Compound **181** produced a mild direct cardiac depression at high concentration and was without intrinsic sympathomimetic activity. In isolated rat thoracic aorta, this terpenoid relaxed more potently the contractions induced by PE than those by high K^+. From the comparison of the -log IC_{50} values of **181** for predominate β_1-, β_2- and β_3-AR sites emerging that it was found to be a highly selective β_1-AR antagonist with a -log IC_{50} of 5.82 [104]. Also anethole (**182**) was evaluated for its effect on rat isolated aorta and compared with those of eugenol (**180**) and their respectively isomeric forms, estragole (**183**) and isoeugenol (**181**). In aortic rings precontracted with PE, anethole (**182**, 10^{-6}-10^{-4} M) induced contraction in prepara-

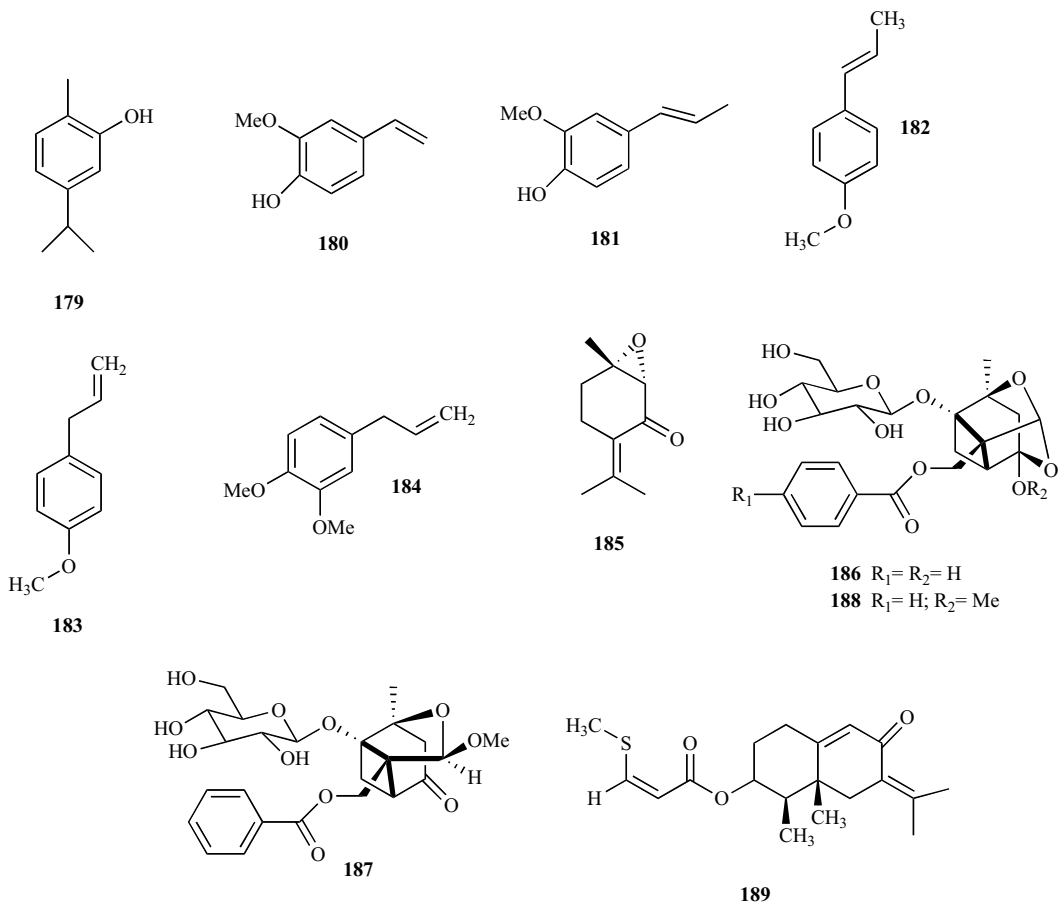


Scheme 16.

tions possessing an intact endothelium, but not in endothelium-denuded tissues. At higher concentrations (10^{-3}-10^{-2} M), **191**-induced concentration-dependent and complete relaxation of all precontracted preparations, irrespective of whether the endothelium was intact or not, an action shared by eugenol (**180**), estragole (**183**) and isoeugenol (**181**). The contractile and relaxant effects of **182** in PE-precontracted preparations were not altered by L-NAME or INDO, indicating that neither NO nor prostaglandins were involved in these actions. The mixed profile of effects was not confined to PE-mediated contraction, since similar responses were obtained to **182** when tissues were precontracted with KCl. Anethole (**182**) and estragole (**183**), but not eugenol (**180**) or isoeugenol (**181**), increased the basal tonus of endothelium-denuded aortic rings, an action that was abolished by VOCs blockers or by withdrawal of $[Ca^{2+}]_e$. These data suggest complex effects of **182** on isolated blood vessels, inducing contraction at lower doses, mediated *via* opening of VOCs, and relaxant effects at higher concentrations that are shared by structural analogues [105]. In both pentobarbital-anesthetized and conscious rats, i.v. bolus injections of methyleugenol (**184**) elicited similar and dose-dependent decreased in mean aortic pressure. In rat isolated thoracic aorta preparations, **184** induced a concentration-dependent reduction of K^+-induced contraction [106]. Rotundifolone (**185**), isolated from *Mentha x villosa* (Lamiaceae), induced vasorelaxation in the rat aorta, at least in part by inhibiting both VOCs and $[Ca^{2+}]_i$ release selectively due to inositol 1,4,5-triphosphate activation [107]. Activity-guided fractionation of *Paeonia moutan* (Paeoniaceae) led to the isolation of paeoniflorin (**186**), paeonidanin (**187**) and methylpaeoniflorin (**188**), that demonstrated a strong relaxed activity in rat aortic preparations precontracted with PE *via* NO release with IC_{50} ranging from 7.9 to 19.4 μM [79].

4.2. Sesquiterpenes

From *Petasites formosanus* (Asteraceae) *iso*-S-petasin (**189**) was isolated. Acute application of **189** (10^{-7} to 10^{-4} M) elicited a concentration-dependent inhibition in peak shortening (PS) and Ca^{2+}-induced Ca^{2+} release, with maximal inhibitions of 51.0% and 31.0%, respectively. Compound **189** also induced a concentration-dependent inhibition of maximal velocity of shortening/re-lengthening (+/-dL/dt) without affecting time to PS, time to 90% re-lengthening, baseline $[Ca^{2+}]_i$ level or $[Ca^{2+}]_i$ decay. Elevation of $[Ca^{2+}]_e$ from 1.0 mM to 2.7 mM significantly antagonized the **189**-induced depression in PS and Ca^{2+}-induced Ca^{2+} release. These results demonstrated a direct depressant action of **189**



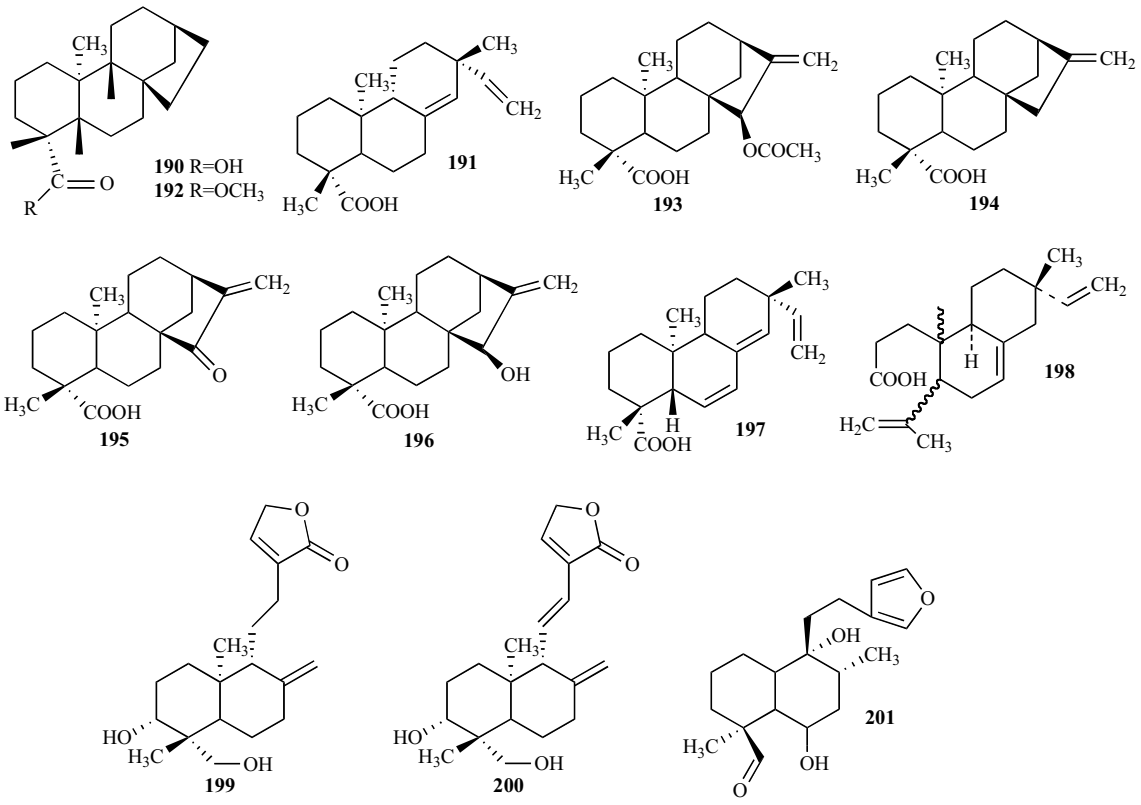
Scheme 17.

on ventricular contraction, which may work in concert with its antihypertensive action to reduce the cardiac load [108]. Further observations revealed that *iso-S*-petasin (**189**) inhibited VOCs activities and KCl-induced increase of $[Ca^{2+}]_i$ in cultured VSMCs, suggesting that the decrease in the agonist-stimulated increase in $[Ca^{2+}]_i$ in VSMCs was likely attributable to reduced $[Ca^{2+}]_e$ influx through L-type VOCs which may in turn account, at least in part, for the hypotensive action of **189**. Moreover, the vasorelaxing response of *iso-S*-petasin (**189**) was due to a direct effect on the arterial smooth muscle.

4.3. Diterpenes

Kaurenoic acid (**190**) vasorelaxant action involved $[Ca^{2+}]_e$ influx blockage. Its effects are also partly mediated by the activation of NO-cGMP pathway and the opening of K^+ channels sensitive to charybdotoxin and 4-amynopiridine. Additionally, **190** induce the activation of the endothelial and neuronal nitric oxide synthase (NOS) isoforms [109]. The vascular effects of **190** were compared with those of pimaradienoic acid (**191**) [110]. Both compounds inhibited PE and KCl-induced contraction in intact and denuded- endothelium of rat aortic rings. These compounds also reduced $CaCl_2$-induced contraction in Ca^{2+}-free solution containing KCl. Both **190** and **191** concentration dependently relaxed endothelium-denuded aortic rings pre-contracted with KCl. Similarly, the relaxation induced by **190** on aortic rings pre-contracted with PE was less pronounced than that found for **191**. Incubation of endothelium-denuded rings for different periods showed that **190** and **191** achieved maximum inhibitory activity on KCl-induced contraction and that this action is time dependent. The results provide evidence that structural differences between diterpenes, independent of the C19 carboxylic acid site, influenced selectivity for VOCs and rate of equilibrium with the target site for their vasorelaxant action in rat aortic rings. Ambrosio *et al.* [111] investigated the influence of the methylation of the C19 carboxyl group site of kaurenoic acid (**190**). Vascular reactivity experiments showed that **190** concentration-dependently inhibited KCl-induced contraction in both endothelium-intact and denuded rat aortic rings. The methyl-kaur-16-en-19-oate (**192**) attenuated KCl-induced contraction at 100 μM. Compound **190** also reduced $CaCl_2$-induced contraction in Ca^{2+}-free solution containing KCl. Again, **192** produced a less accentuated reduction in $CaCl_2$-induced contraction than that induced by the acid **190**. Methyl-kaur-16-en-19-oate (**192**) concentration-dependently relaxed KCl-precontracted rings (82.57 and 70.55%, respectively) with denuded endothelium. Similarly, the relaxation induced by **190** on PE-precontracted rings (73.06 %) was more pronounced than that found for **192** (53.68 %). Preincubation of denuded rings for different periods with **190** and **192** showed that the equilibrium periods

required by each compound to achieve its maximal inhibitory response on KCl-induced contraction are different. This result provide functional evidence that methylation of the C19 carboxyl group of **190** reduces but does not abolish the vasorelaxant activity displayed by acid. Several kaurene derivatives isolated from *Xylopia aethiopica* (Annonaceae) were screened for their potential cardiovascular and diuretic activity. Xylopic acid (**193**) produced pronounced and significant hypotensive effect on both SBP and DBP after intraperitoneal application, with significant gradual decrease of HR. I.v. application of *ent*-kaur-16-en-19-oic acid (**194**) and *ent*-kaur-16-en-15-one-19-oic acid (**195**) showed immediate decrease of SBP, no change of DBP and significant decrease of HR. Moreover, **194**, **195** and 15α-hydroxy- *ent*-kaur-16-en-19-oic acid (**196**) increased coronary flow. A comparable natriuretic activity and half saluretic activity to that of hydrochlorthiazide were found with **193**, **194** and **195** [112]. On the contrary, *ent*-pimara-8(14),15-dien-19-oic acid (**197**) isolated from *Viguiera arenaria* (Asteraceae) inhibited rat carotid rings contraction trought α_1-AR blockade. In addition, the effect elicited by **197** could be related to a reduction in the $[Ca^{2+}]_e$ influx since it inhibited KCl-induced contraction, which is mainly dependent on $[Ca^{2+}]_e$ mobilization [113]. Tirapelli *et al*. [114] demonstrated that **197** relaxed PE-precontracted rings with/without endothelium through a mechanism endothelium-independent that blocked $[Ca^{2+}]_e$ influx. Moreover, **197** induce the release of NO from the VSMCs through an activation of guanylyl cyclase-dependent mechanism and are related to the release of metabolites derived from the arachidonic acid pathway. I.v. administration of 3,4-seicosopimar-4(18),7,15-triene-3-oic acid (**198**), isolated from *Salvia cinnabarina* (Lamiaceae), led to a fall in MABP, that was not modified by treatment of the rat with chlorisondamine nor with L-NAME. These results demonstrate the hypotensive effect of **198** due to a peripheral mechanism but independent of endothelial NO release [115]. The 14-deoxy-11,12-didehydroandrographolide (**199**), isolated from *Andrographis paniculata* (Acanthaceae), produced significant falls in MABP and HR in anaesthetised rats in a dose-dependent manner. Compound **199** seems to work *via* AR, autonomic ganglia receptor and ACE. In the isolated right atria, **199** caused negative chronotropic action and antagonised isoproterenol-induced positive chronotropic actions in a non-competitive and dose-dependent manner. These results further supported the bradycardia-inducing and β-AR antagonistic properties of **199** *in vivo* [116]. Successively it was demonstrated that **199** vasorelaxant activity was mediated through the activation of NOS and guanylyl cyclase [117]. With the same mechanism of action but a less potency operate 14-deoxyandrographolide (**200**). A potent L-type VOCs blocker, marrubenol (**201**) was isolated from *Marrubium vulgare* (Lamiaceae). In fact, in fura-2-loaded aorta, **201** simultaneously inhibited the Ca^{2+} signal and the contraction evoked by KCl, and decreased the quenching rate of fura-2 fluorescence by Mn^{2+}. Moreover, patch-clamp data obtained in aortic smooth muscle cells indicated that it inhibited Ba^{2+} inward current in a voltage-dependent manner [118].



Scheme 18.

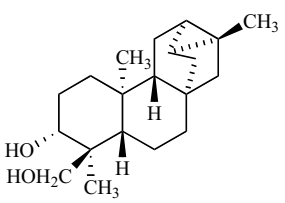
From *Croton zambesicus* (Euphorbiaceae) ent-18-hydroxytrachyloban-3β-ol (**202**) and ent-18-hydroxyisopimara-7,15-diene-3β-ol (**203**) were isolated. The mixture of the two compounds inhibited the KCl-induced contraction of male Wistar rat aorta with an IC_{50} of 1 μg/ml, while the purified **202** and **203** showed a lower activity than the mixture [119]. SAR analysis revealed that the presence of a carbonyl group at C3 could play an important role in the vasorelaxant activity of this type of natural diterpenes [120]. In fact, diterpene **204**, had a carbonyl group at C3 and a hydroxymethyl group at C4, exhibited a much more potent activity than **205**, which has a hydroxyl group at C3 and a hydroxymethyl group at C4. However, this is not the only important functional characteristic for high activity since *ent*-trachyloban-3-one (**206**), which also had a carbonyl group at C3, showed a lower vasorelaxant activity. As deduced from the comparison of the activity of **206** and **204**, the presence of a hydroxymethyl group at C4 in addition to a carbonyl group at C3, seems to greatly increase the inhibitory activity of this type of diterpenes on KCl-induced contractions. Among the active compounds, diterpenes **207**, **208** and **209** showed IC_{50} values of 3.5, 4.6 and 5.8 μM, respectively, while compounds **210** and **211** were inactive in the same range. Comparison of the results for the synthetic compounds highlights the importance of the location of some substituents at strategic positions of the trachylobane framework for the vasorelaxant activity. The presence of a C3 β-halogen atom in the 19-*nor*-trachylobane system markedly reduced the vasorelaxant effect. Thus, **211** and **212**, with chlorine and bromine atoms at C3, respectively were significantly less active than the parent non-halogenated analog **207**. Compound **209**, with carbonyl groups at C14 and C15 and **208** with a carbonyl group at C14 and a hydroxy group at C15, were more active than **213**, which have a hydroxy group at C14 and C15. The strong vasorelaxant activities of **208** and **209** support the idea that a carbonyl group at C14 may be important for the activity. However, the positive effect associated with the presence of the carbonyl group at C14 seems to be suppressed by the presence of another carbonyl at C3, as inferred from comparison of the data for **208** and **214**, or **209** and **215**. The replacement of the oxygenated function at C15 by a less polar group, produced a decrease of activity (**210** *vs* **216**). Authors observed a small difference in the vasorelaxant activity of the enantiomeric trachylobanes **217** and **215** or **207** and **210**. In addition neither **214**, from the *ent*-series, nor **218**, from the normal series, exhibited significant vasorelaxing activity. Cleavage of the C13-C16 bond did not result in a modification of the activity (**219** *vs* **217**). However, cleavage of the C12-C13 bond led to an important loss of activity: **220** is about 50% less active than trachylobane **210**. The cleavage of the C13-C16 cyclopropyl bond did not produced an important modification of the geometry of the C ring, which in both the initial trachylobane system and the resulting atisane framework adopts a boat conformation, while cleavage of the C12-C13 bond led to a beyerane skeleton in which the C ring adopts a chair conformation. However, this is not observed in the *ent*-series. In this case, the cleavage of the C12-C13 bond induced a small positive effect on the vasorelaxant activity, as evidenced by comparison of the activity of **221**. It is important to note that **221** has a hydroxy group at C14, while **220** has a carbonyl group at this position. Therefore, cleavage of the C12-C13 bond had a positive or a negative effect depending on the presence of a hydroxy or a carbonyl group at C14 and/or the absolute configuration. *trans*-Dehydrocrotonin (**222**), isolated from *Croton cajucara* (Euphorbiaceae), in anesthetized NTR reduced the MABP and HR in a dose-dependent manner. The hypotensive effect of **222** appears not mediated through effects on the muscarinic receptor, β-AR, or ganglionic blockade. Compound **222** showed no significant influence on inotropism. In isolated rat aortic rings with/without endothelium, **222** relaxed the tonic contraction induced by PE. The hypotensive and bradycardia effects of **222**, possibly is related in part to the release of NO and in part to direct effects on VSMCs and cardiac pacemaker activity [121]. Forskolin (**223**), isolated from *Coleus forskohii* (Lamiaceae), displayed BP lowering properties in normotensive and hypertensive models. It also had potent, positive inotropic action and a vasodilatory effect. Its mode of action as an antihypertensive agent was related essentially to its peripheral vasodilatory properties. Four analogues **224**-**227** were investigated for their ability to reduce BP in anesthetized cat. 7-Deacetylforskolin (**224**) and 6-acetyl-7-deacetylforskolin (**227**), produced falls in BP on the contrary 9-deoxyforskolin (**225**) and 1,9-dideoxyforskolin (**226**), which lack the hydroxyl group of the respective position in **224**, were inactive. Compound **221** that lack of acetyl group in C7 produce fall in BP comparable to forskolin (**223**) together with the 6-acetyl-7-deacetyl derivative (**227**) [122].

4.4. Triterpenes

Two pentacyclic triterpenoids oleanolic acid (**228**) and erythrodiol (**229**) were isolated from 'Orujo' olive oil. Both **228** and **229**, accumulatively added, showed vasorelaxant activities in aortic rings with endothelium pre-contracted by PE. The relaxation was significantly attenuated by pretreatment with L-NAME demonstrating that it was mediated by the endothelial production of NO [123]. Previously, **228** demonstrated a significantly increased sodium urinary excretion (13.41 mEq/3h/kg) and a low K^+ excretion (1.99 mEq/3h/kg) [124].

Interferring with the receptor binding site of aldosterone act the saikosaponin H (**230**) that exhibited an IC_{50} of 22 μM. Moreover, **230** inihibited the decrease in urine volume in aldosterone-loaded mice [125]. α-Hederin (**231**), β-escine (**232**) and kryptoescine (**233**), members of olean-12-ene type saponins, gave a stronger inhibition of binding to the AT_1 than the ET_A receptors [126].

Asiatic acid (**234**) inhibited selectively the [^{3}H]-BQ-123 binding to the ET_A receptor, while oleanolic acid (**228**) and β-glycyrrhetinic acid (**235**) strongly inhibited the [^{3}H]-angiotensin II binding to the AT_1 receptor. Ursolic acid (**236**) showed moderate inhibition of both radioligands. In the lupane series, betulinic acid (**237**) showed some inhibition of the [^{3}H]-BQ-123 binding, while betulin (**238**) remained inactive. Cardiovascular and diuretic/saluretic activity of oleanolic (**228**) and ursolic (**236**) acid were studied in Dahl salt-sensitive rats (DSS). Although both triterpenoids did not have direct hypotensive effect, intraperitoneal application in a daily dose of 60 mg/kg b.w., prevented the development of severe hypertension. This antihypertensive effect was attrib-



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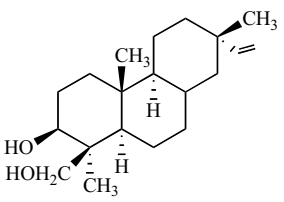

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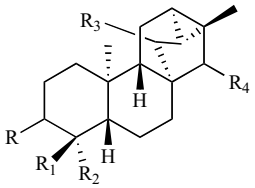
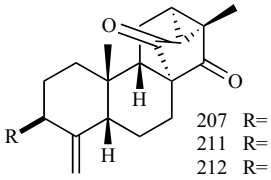
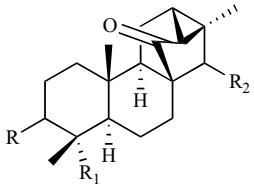
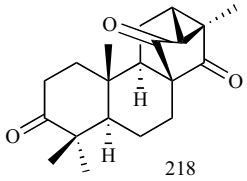


204 R= =O; R1= CH2OH; R2= Me; R3= R4= H
205 R= b-OH; R1= CH2OH; R2= Me; R3= R4= H
206 R= =O; R1= R2= Me; R3= R4= H
208 R= H; R1= R2= Me; R3= =O; R4= b-OH
209 R= H; R1= R2= Me; R3= R4= =O
213 R= =O; R1= R2= Me; R3= =O; R4= b-OH
214 R= =O; R1= R2= Me; R3= R4= =O
221 R= H; R1= R2= Me; R3= a-OH; R4= b-OH

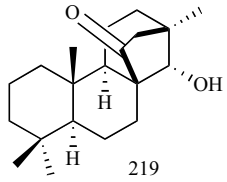


207 R= H
211 R= Cl
212 R= Br



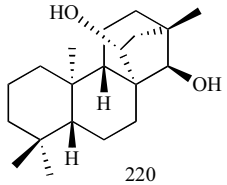


218



219

210 R= H; R1= Me; R2= a-OH
215 R= H; R1= Me; R2= b-I
216 R= =O; R1= Me; R2= =O
217 R= =O; R1= Me; R2= a-OH



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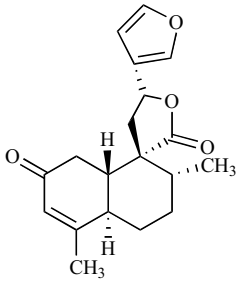

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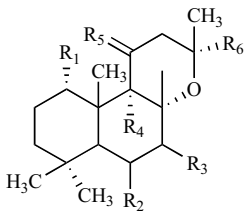


223 R1=R2=R4= OH, R3= OCOCH3, R5= O, R6= CH=CH2
224 R1=R2=R3=R4= OH, R5= O, R6= CH=CH2
225 R1=R2= OH, R3= OCOCH3, R4= H, R5= O, R6= CH=CH2
226 R1=R4= H, R2= OH, R3= OCOCH3, R5= O, R6= CH=CH2
227 R1=R3=R4= OH, R2= OCOCH3, R5= O, R6= CH=CH2

Scheme 19.

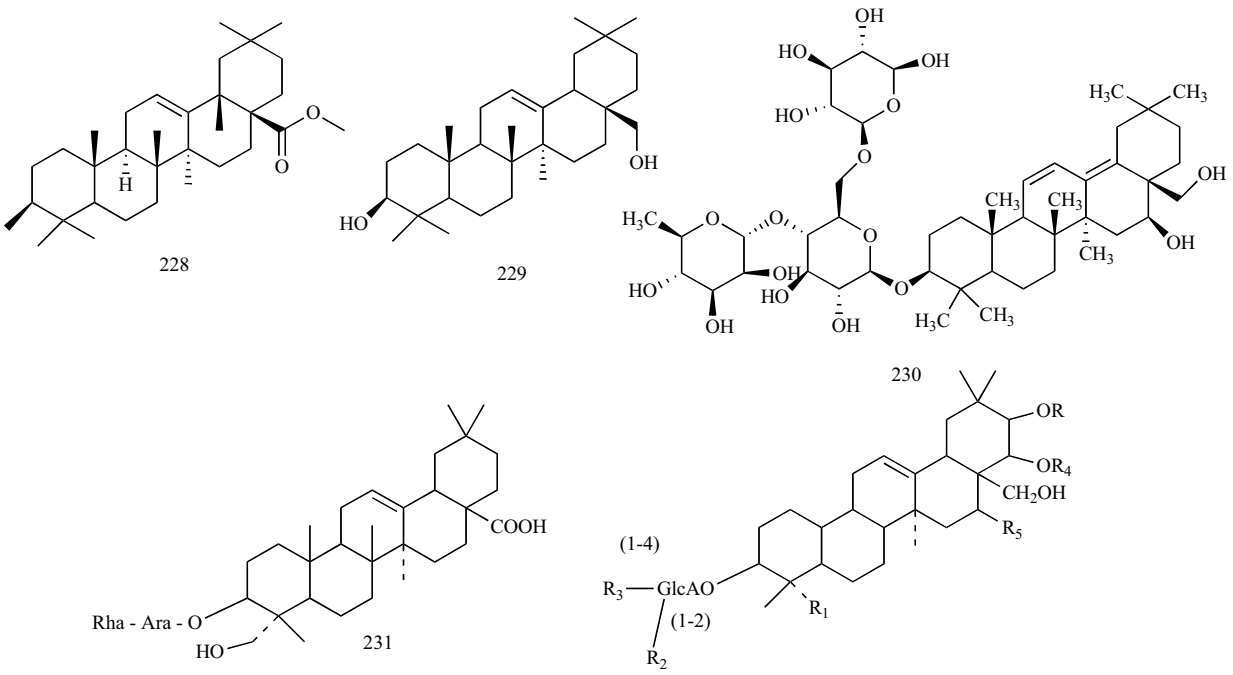
uted to their potent diuretic-natriuretic-saluretic activity and direct cardiac effect on the DSS rats [127].

4.5. Sterols

Yuan *et al*. [128] investigated the diuretic effect of ergosta-4,6,8(14),22-tetraen-3-one (ergone, **239**) demonstrating that the variation in the urinary ratio of Na^+/K^+ could be due to aldosterone-blocking activity. Stevioside (**240**), isolated from *Stevia rebaudiana* (Compositae), exerted hypotensive activity may be probably due to inhibition of the Ca^{2+} influx. In fact, it can dose-dependently inhibit the stimulatory effects of vasopressin and PE on $[Ca^{2+}]_i$ [129]. Recently, Hsieh *et al*. [130] revealed that **240** can significantly decreased SBP and DBP in a a two-year, randomized, placebo-controlled study in which patients with mild hypertension were involved.

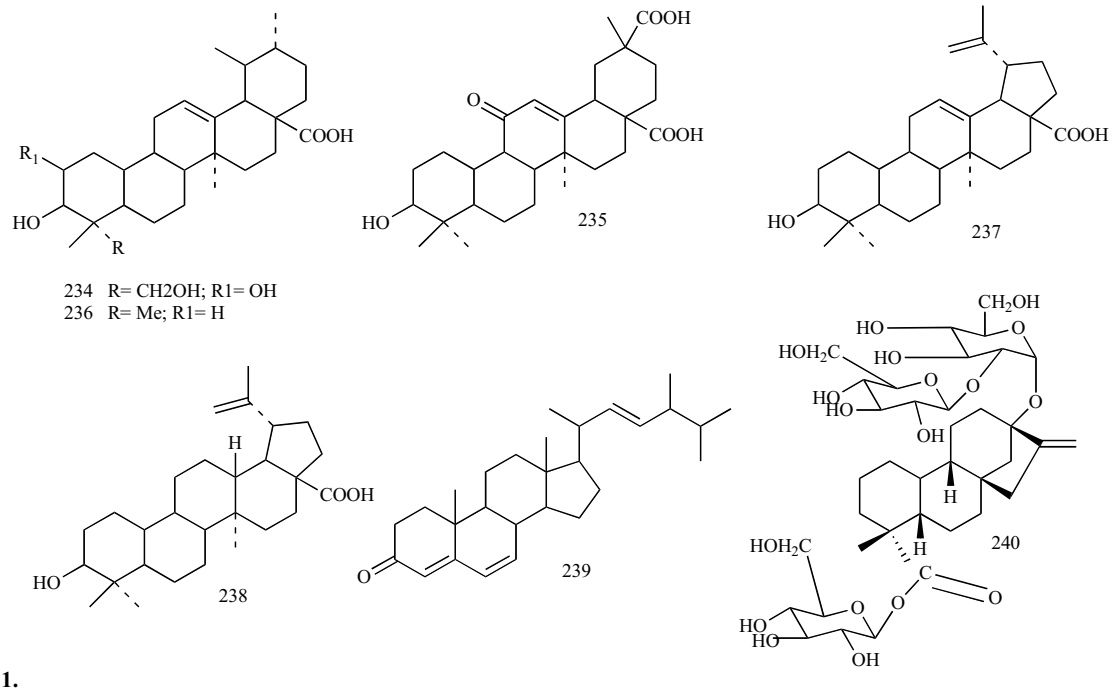
CONCLUDING REMARKS

Hypertension is a common and often progressive disorder that possesses a major risk for cardiovascular and renal dis-



Scheme 20.

ease. Recent data revealed that the global burden of hypertension is an important and increasing public health problem worldwide and that the level of awareness, treatment and control of hypertension varies considerably among countries. Until very recently, the only clinical treatment for this condition was symptomatic trought the lowering BP level. It is well established that natural products are an excellent source of chemical structures with a wide variety of biological activity, including antihypertensive properties. The large number of compounds derived from natural product sources, that



Scheme 21.

are currently undergoing evaluation in clinical trials, is another positive indicator that natural product discovery provides good value for human medicine with particular attention for chronic diseases, such as blood pressure regulators. Realistically, natural product discovery programs require a bit more patience and perseverance for the identification of good lead compounds than do programs strictly limited to synthetic chemicals. The quality of leads arising from natural product discovery is better and often more biologically friendly, due to their co-evolution with the target sites in biological systems. However, the speed at which leads can be generated and advanced is slower than purely synthetic discovery approaches. As was pointed out previously, it is unlikely that the major global pharmaceutical companies will return to natural product discovery programs if they've already abandoned this avenue of research. This is due to the limitations listed above, and well as a perception that this research is not cutting-edge. A few companies will continue to conduct natural product research and depend on this type of research to generate development candidates. However, even at those companies where natural product discovery perseveres, there will be continued pressure to reduce the time and cost of such research, or even eliminate it entirely. The situation, as it stands in the pharmaceutical industry at the present time, offers to biotechnology and small pharmaceutical companies the opportunity to utilize natural product discovery and succeed at scales that are impossible for the major pharmaceutical companies to operate. New technologies for more rapid identification of bioactive molecules and structural elucidation of novel structures will continue to be leveraged to improve the natural product discovery process. This work reviewed two hundred fourty natural products active as antihypertensive with different mechanisms of action. The knowledge offer from this review should help to provide leads to the ultimate goal of developing new therapeutic drugs more efficacy and safety for the treatment of hypertension.

ABBREVIATIONS

ACE	=	Angiotensin Converting Enzyme
ACh	=	Acetylcholine
AR	=	Adrenoreceptor
ATP	=	Adenosine 5'-triphosphate
BK(Ca)	=	Ca^{2+} activated K^+
BP	=	Blood Pressure
$[Ca^{2+}]_e$	=	Extracellular Calcium
$[Ca^{2+}]_i$	=	Intracellular Calcium
cAMP	=	Adenosine 3',5'-Cyclic Monophosphate
cGMP	=	Guanosine 3',5'-Cyclic Monophosphate
DBP	=	Dyastolic Blood Pressure
DOCA	=	Deoxycorticosterone Acetate
DSS	=	Dahl salt-sensitive
EC_{50}	=	Concentration Required For Obtaining 50% Of A Maximum Effect
ECs	=	Endothelial Cells
EDRF	=	Endothelium-Derived Relaxing Factor
E_{max}	=	Maximal Values For The Relaxant Effect
GB	=	Glibenclamide
HR	=	Heart Rate
5-HT	=	Serotonine
IC_{50}	=	Inhibitory Concentration 50%
I_{CaL}	=	L-Type Ca-Current
IMP	=	Inositol-5'-Monophosphate
INDO	=	Indomethacin
i.v.	=	Intravenous
L-NAME	=	Nω-Nitro-L-Arginine Methyl Esters
L-NMMA	=	NG-Monomethyl-L-Arginina
MABP	=	Mean Arterial Blood Pressure
MB	=	Methylene Blue
NA	=	Noradrenaline
NE	=	Norepinephrine
NO	=	Nitric Oxide
NOS	=	Nitric Oxide Sintase
NTR	=	Normotensive rats
NWKY	=	Normotensive Wistar Kyoto
ODQ	=	^{1}H-[1,2,4]-Oxadiazole-[4,3- a]-quinoxalin-1-one
PE	=	Phenylephrine
PS	=	Peak Shortening
ROCs	=	Receptor-Operated Ca^{2+} Channels
SAR	=	Structural Activity Relationships
SBP	=	Systolic Blood Pressure
SHR	=	Spontaneous Hypertensive Rats
SHRSP	=	Stroke-Prone Spontaneously Hypertensive Rats
TEA	=	Tetraethylammonium Chloride
VOCs	=	Voltage-Dependent Ca^{2+} Channels
VSMCs	=	Vascular Smooth Muscle Cells

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