Biological and Pharmacological Activities of Iridoids: Recent Developments

Rosa Tundis*, Monica R. Loizzo, Federica Menichini, Giancarlo A. Statti and Francesco Menichini

Department of Pharmaceutical Sciences, Faculty of Pharmacy Nutrition and Health Sciences, University of Calabria, 87036-I Rende (CS), Italy

Abstract: Iridoids represent a large group of cyclopenta[*c*]pyran monoterpenoids that occur wide-spread in nature, mainly in dicotyledonous plant families like Apocynaceae, Scrophulariaceae, Diervillaceae, Lamiaceae, Loganiaceae and Rubiaceae.

Recently, more extensive studies revealed that iridoids exhibit a wide range of bioactivity, such as neuroprotective, antinflammatory and immunomodulator, hepatoprotective and cardioprotective effects. Anticancer, antioxidant, antimicrobic, hypoglycaemic, hypolipidemic, choleretic, antispasmodic and purgative properties were also reported.

The aim of the present review is to discuss the recent developments on biological and pharmacological activities of iridoids, supporting the new therapeutic possibilities for the use of these compounds.

Key Words: Iridoids, secoiridoids, neuroprotection, antitumor, anti-inflammatory, aglycones, glycosides, other biological properties.

INTRODUCTION

Iridoids represent a large and still expanding group of cyclopenta[*c*]pyran monoterpenoids found in a number of folk medicinal plants used as bitter tonics, sedatives, hypotensives, antipyretics, cough medicines, remedies for wounds and skin disorders. This fact encouraged to investigate the bioactivities of these phytochemicals. Intensive studies revealed that iridoids exhibit a wide range of bioactivity: neuroprotective, antitumor, antinflammatory, antioxidant, cardiovascular, antihepatotoxic, choleretic, hypoglycaemic, hypolipidemic, antispasmodic, antiviral, antimicrobial, immunomodulator, antiallergic, anti-leishmanial and molluscicidal effects.

Naturally occurring iridoid compounds have been classified into different sub-groups on the basis of their demonstrated or postulated biosynthesis as well as on the basis of chemical properties. According to Hegnauer's classification [1], natural iridoids in the broadest sense are represented by nine structural groups, consisting of cyclopentanoid monoterpenes and secoiridoids in general characterised by the structural feature of a 7,8-seco ring including pseudoalkaloids as well as complex indole- and isochinoline-type alkaloids. Other authors, like El-Naggar and Beal [2], have summarised only iridoid glycosides, usually but not necessary containing glucose, secoiridoid glucosides and non-glycosidic compounds and omitting all nitrogen-containing iridoids. Simple pseudoalkaloids have been considered as artefacts formed by replacement of oxygen by nitrogen in genuine iridoids upon ammonia treatment during extraction. Several review articles have covered different aspects of iridoids isolation, structure elucidation, chemistry, distribution and biosynthesis [3a-d]. Listing of iridoids together with spectroscopic data are provided in five review articles, namely of El-Naggar and Beal and Boros and Stermitz [2, 4a, b] which covered the new iridoids reported in the literature up to January 1989; of Al-Hazimi and Alkhathlan which rewieved the studies reported during 1990/1993 and of Dinda *et al*. which reported a compilation of new iridoids that have appeared in the literature during the period 1994/2005 [5, 6]. Reviews which summarized the biological activities of iridoids were also available [7a-c]. The intent of this review is to address the recent results on the bioactivity of naturally occurring iridoids with particular emphasis on their neuroprotective, anticancer and anti-inflammatory properties. The mechanism of action and the structure-activity relationships (SAR) were discussed.

*Address correspondence to this author at the Department of Pharmaceutical Sciences, Faculty of Pharmacy and Nutrition and Health Sciences, University of Calabria, 87036-I, Rende (CS), Italy; Tel: +39 984 493246; Fax: +39 984 493298; E-mail: rosa.tundis@unical.it

2. BIOLOGICAL PROPERTIES

2.1. Neuroprotective Effects

A variety of factors including excitotoxicity, oxidative stress, growth factor withdrawal, cytokines or toxins may cause neuronal cell damage leading to neurodegeneration [8-12]. Numerous investigations have attempted to explore natural drugs that may improve neuroplasticity for postponing aging, preventing age-associated memory loss and reducing the incidence of neurodegenerative diseases. *Rehmannia glutinosa* (Scrophulariaceae), a traditional Chinese medical herb, has a long history in age-related disease therapy. Its main active constituent is catalpol (**1**). Many attempts have been made to document research data on neuroprotective effect of this iridoid through *in vitro* and *in vivo* model systems. Catalpol (**1**) could protect neurons of global ischemia models [13a-c]. Moreover, it could rescue neurons in hippocampal CA1 subfield and reduced in dose-dependent manner working errors in transient global cerebral ischemia in ger-

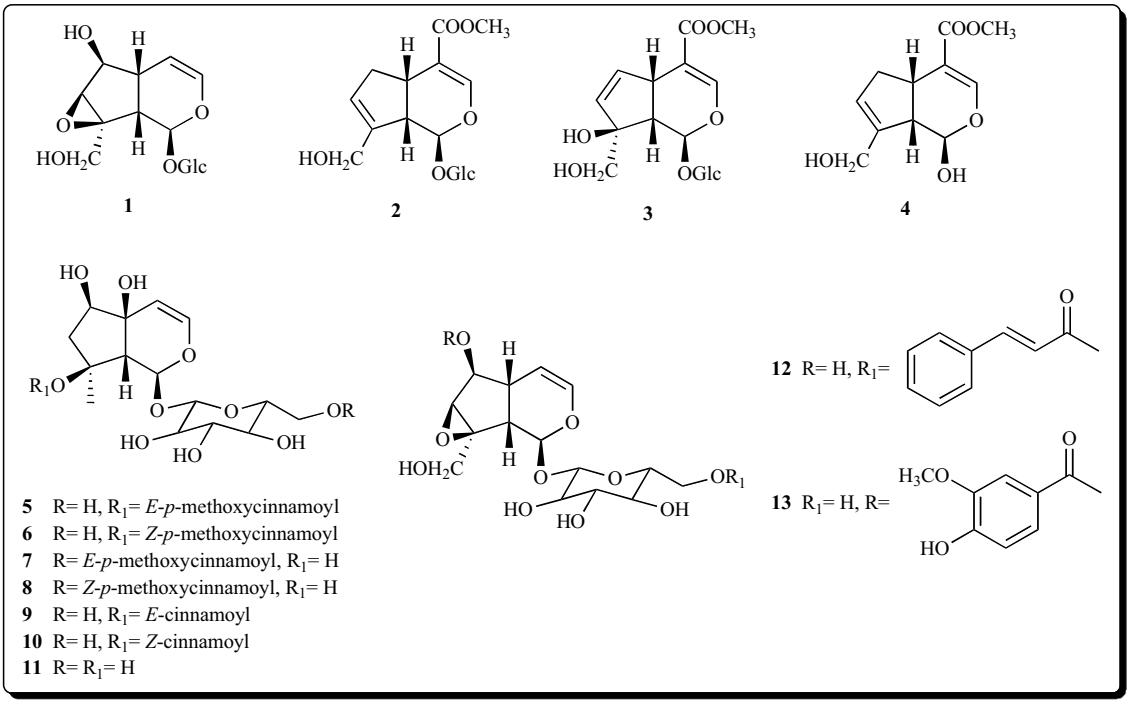
1389-5575/08 $55.00+.00

bils [14]. These findings suggested that its neuroprotective effects were partly due to ameliorating age-related neuroplasticity loss by "normalizing" pre-synaptic proteins and their relative signaling pathways in the aged rats. Because inflammation and oxidative stress are important players in the pathogenesis of neurodegenerative diseases, the neuroprotective action of catalpol (**1**) has been also measured in primary mesencephalic neuron-glia cultures. In particular, it has been examined whether **1** could protect dopaminergic neurons from lipopolysaccharide (LPS)-induced neurotoxicity [15]. The results showed that catalpol (**1**) significantly attenuate LPS-induced microglial activation and subsequent dopaminergic neurotoxicity. The inhibitory effects on LPS-induced microglial activation depended on two mechanisms. Firstly, catalpol (**1**) attenuated the expression of inducible nitroxide synthase (iNOS). Secondly, pre-treatment with **1** could inhibit the production of tumor necrosis factor-α (TNF-α), nitroxide (NO) and the intracellular reactive oxygen species (ROS). These observations suggested that catalpol (**1**) might be a potential therapeutic agent for the treatment of inflammatory-related neurodegenerative disorders. With the purpose to investigate other neuroprotective mechanisms, Li *et al*. [16] focused their study on evaluating whether the inhibition of apoptotic cell death with catalpol (**1**) was associated with the changes in the expressions of anti-apoptotic Bcl-2 and pro-apoptotic Bax proteins. Transient global ischemia followed by 4 days reperfusion in gerbils resulted in a great deal of hippocampal CA1 neurons death by apoptotic mechanism. The increase of Bax protein seemed to be responsible for triggering apoptosis. Administration of catalpol (**1**) rescued a large number of neurons by attenuating apoptosis, which was achieved through inhibiting Bax and promoting Bcl-2 genes. The neuroprotective effects of catalpol (**1**) on 1-methyl-4-phenylpyridinium (MPP^+)-induced oxidative stress in cultured mesencephalic neurons were also recently evaluated [17]. MPP^+ is a metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that selectively injures the nigrostriatal system. MPP^+ induced cell death *via* oxidative stress in cultured mesencephalic neurons and especially dopaminergic neurons. It was demonstrated that catalpol (**1**) markedly attenuated the cytotoxic effects, increased the levels of antioxidants and decreased the contents of malondialdehyde in mesencephalic neurons exposed to MPP^+. These results suggest that this iridoid is very effective in preventing oxidative stress triggered by deterioration of cellular functions reducing the levels of reactive oxygen species.

Ameliorating neuroplasticity loss is one of most important research directions on neuroprotection. Thus, particularly interest has the neuroprotective mechanisms of catalpol (**1**) from the neuroplasticity aspect. Recent researches indicate that the brain has a decreased capacity for plastic responses in aging process [18]. Synaptophysin is a glycosylated polypeptide whose quantitative detection has been established as a molecular marker of synaptic density and synaptic involvement during physiological and pathological processes in human brain. Synaptophysin levels reduced with aging, which indicates age-related decrease in synaptic plasticity. Synaptophysin might represent a potential presynaptic molecular substrate by which catalpol (**1**) increased hippocampal synaptic plasticity [19]. It selectively enhanced synaptophysin immunoreactivity in dentate gyrus layer while it did not affect synaptophysin-immunoreactivity in CA1 and CA3 sub-regions of the hippocampus. All these data indicated that catalpol (**1**) might ameliorate age-related neuroplasticity loss by selectively increased synaptophysin level in dentate gyrus layer of the hippocampus. Hippocampal levels of growth-associated phosphoprotein-43 (GAP-43), able to enhance the ability of learning and memory in the mammalian, were increased in the iridoid-treated group and positively correlated with synaptophysin levels. Protein kinase C (PKC) is the enzyme which should be responsible for GAP-43phosphorylation [20]. Western blot analysis of the hippocampal PKC levels showed that in the catalpol-treated group levels of PKC were dramatically up-regulated paralleling with increased GAP-43 and positively correlated with GAP-43 levels, which indicated that catalpol (**1**) might enhance GAP-43 expression in the hippocampus along with its C-kinase. The other candidate factor that may regulate neuroplasticity is the brain-derived neurotrophic factor (BDNF), a member of neurotrophin family that promotes neuronal survival and differentiation and regulates synaptic transmission and plasticity in the central nervous system (CNS). In the catalpol-treated group, BDNF levels increased in the hippocampus and were positively correlated with synaptophysin values. In conclusion, catalpol (**1**) could increase presynaptic proteins and up-regulate signaling molecules (PKC and BDNF) levels in the aged rats. Geniposide (**2**) from *Gardenia jasmioides* (Rubiaceae) protected neuronal cells from damage in oxygen and glucose deprivation (OGD)-exposed hippocampal slice culture [21]. Fifty μM of geniposide (**2**) completely blocked neuronal cell death in granular cell layer. Although Yamazaki *et al.* [22] reported that geniposide (**2**) could not protect hippocampal cells against β-amyloid-induced toxicity, this iridoid attenuated cell death in OGD-exposed rat hippocampal slice culture. This discrepancy suggests the mechanism by which OGD induces cell death is different from cell death by β-amyloid. Geniposide (**2**) demonstrated to induce neurite outgrowth in PC12 cells in a dose-dependent manner and has been indicated as a novel agonist for glucagon-like peptide-1 (7-36)-amide (GLP-1) receptor, involved in neurodegenerative disorders [23-25]. Catalpol (**1**), geniposide (**2**) and gardenoside (**3**) were found able to induce neurite outgrowth at 0.1 μg/ml and above in medium after 3 days of treatment [26]. Hydrolysates of the three glucosides caused neuritogenesis in PC12 cells. Geniposide (**2**) hydrolysate enhanced responses of cells to carbachol and KCl-induced depolarization in terms of cytoplasmic free-calcium concentration. Its aglycone **4** also promoted neurite out-growth in a dose-dependent manner (ED_{50} 0.7 μM). Moreover, catapol (**1**) inhibits apoptosis in hydrogen peroxide-induced PC12 cells by preventing cytochrome C release and inactivating of caspase cascade. With this study it was demonstrated that some iridoids can induce differentiation through activation of components of the intracellular signal transduction pathway [26]. A protective effect in a model of hypoxia and reoxygenation injury in rat cerebral microvascular endothelial cells *in vitro* was observed for geniposide (**2**) [27]. Both normal and model cells were treated with this iridoid in a concentration ranging from 0.008 to 1.024 μmol/ml. After hypoxia/hypoglycaemia cultures for 4 h and reoxygenation for 12 h, geniposide (**2**)

could protect the injured cerebral microvascular endothelial cells.

8-*O*-*E*-*p*-Methoxycinnamoylharpagide (**5**), 8-*O*-*Z*-*p*-methoxycinnamoylharpagide (**6**), 6'-*O*-*E*-*p*-methoxycinnamoylharpagide (**7**), 6'-*O*-*Z*-*p*-methoxycinnamoylharpagide (**8**), *E*-harpagoside (**9**), *Z*-harpagoside (**10**) and harpagide (**11**) from *Scrophularia buergeriana* (Scrophulariaceae) roots exhibited significant protective effects against glutamate-induced neurodegeneration in primary cultures of rat cortical neurons at concentrations ranging from 10 μM to 100 nM [28]. Among the tested compounds, **5** and **7**, with an *E*-*p*-methoxycinnamoyl group, showed the most potent neuroprotective activity. However, the site of acylation by the *E*-*p*-methoxycinnamoyl group of **5** and **7** proved not to have an effect on neuroprotectivity since both compounds showed similar protective potencies against glutamate-induced neurotoxicity. Although an *E*-*p*-methoxycinnamic acid unit was revealed to be a crucial structural moiety for neuroprotective compounds described in previous reports [29a, b], iridoids **9**-**11** which have no *E*-*p*-methoxycinnamoyl moiety in their structure exhibited significant neuroprotective activity. On the basis of these results, it can be concluded that the iridoid aglycone unit, as well as the *E*-*p*-methoxycinnamic acid unit, leads to significant neuroprotective effect on primary cultures of rat cortical cells injured by glutamate. A successive investigation on the way of action of these identified iridoids demonstrated that **5** and **11** protected primary cultured neurons against glutamate-induced oxidative stress primarily by acting on the antioxidative defence system and on glutamatergic receptors when using primary cultures of rat cortical cells *in vitro* [30]. Interesting, both harpagide (**11**) and 8-*O*-*E*-*p*-methoxycinnamoylharpagide (**5**) protected cultured cortical cells against glutamate-induced neurotoxicity, although with different mechanism of action. Harpagide (**11**) might not act directly on glutamatergic receptors while 8-*O*-*E*-*p*-methoxycinnamoylharpagide (**5**) had more potent and selective neuroprotective activity caused by glutamatergic antagonism of *E*-*p*-methoxycinnamoyl group. The difference in the neuroprotective action may be due to the presence of this group. By contrast, harpagide (**11**) itself showed protective activity by maintaining the antioxidative defence system. Therefore, harp-type iridoids might offer a useful therapeutic choice in treatment of neurodegenerative disorders caused by excitotoxicity. It was well known that transient global cerebral ischemia could induce delayed neuronal death in CA1 area of hippocampus and lead to problems related with cognition and memory. Recently, it has been found that jasminoidin (geniposide, **2**) significantly decreased the infarction volume in rats that underwent focal cerebral ischemia-reperfusion injury and alleviated the tissue injury in the cerebral tissue [31]. Nerve growth factor (NGF) stimulates the outgrowth of neurites in neuronal cells and plays an important role in the survival and maintenance of neurons in the CNS. Several synthetic compounds have been found to enhance the action of NGF on neurite out-growth from PC12 cells [32a, b]. Two natural iridoid, such as picroside I (**12**) and II (**13**), demonstrated to potentiate NGF-mediated neurite out-growth from PC12D cells [33]. The picroside-induced enhancement of NGF-mediated neurite out-growth was inhibited by PD98059 (2-(2'-amino-3'-methoxyphenyl)-oxanaphthalen-4-one, a potent mitogen-activated protein (MAP) kinase inhibitor and by GF109203X (2-[1-(3-dimethylaminopropyl)-indol-3-yl]-3-(indol-3-yl)maleimide), a PKC inhibitor, in a concentration-dependent manner. PD98059 and GF109203X completely blocked the picroside-induced enhancement of the out-growth of neurites from PC12D cells in the presence of NGF, suggesting an involvement of MAP kinase and PKC in the picroside-induced enhancement of neurite out-growth. Western blot analysis indicated that picrosides did not stimulate

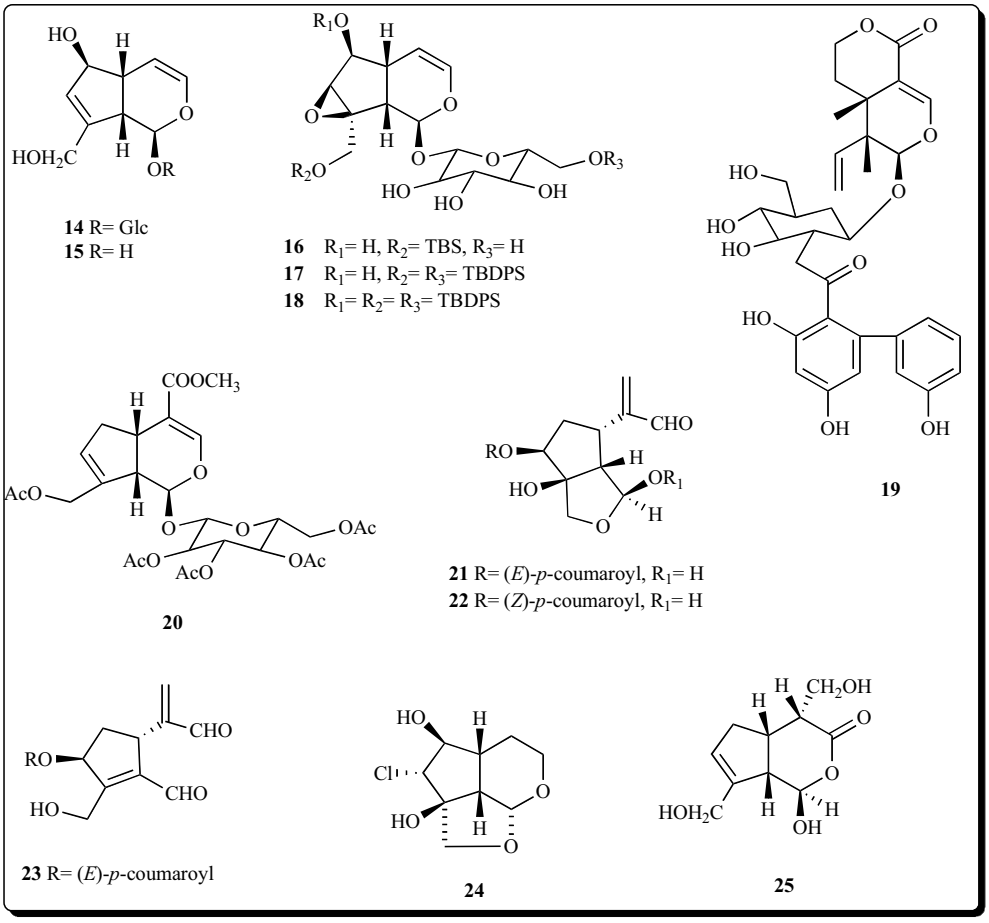


NGF-induced MAP kinase activation. So, these compounds enhanced NGF-induced neurite out-growth from PC12D cells, probably by amplifying a down-stream step of MAP kinase in the NGF receptor-mediated intracellular MAP kinase-dependent signaling pathway.

2.2. Anticancer and Chemopreventive Properties

It is well established that natural products are an excellent source of chemical structures with a wide variety of biological activity, including anticancer properties [34]. Within the current renewed in iridoids for medicinal purposes, their beneficial effects in anticancer therapy have been demonstrated. Some studies on aucubin (**14**) and geniposide (**2**) have shown that these compounds have some chemoprevention and cancer therapy potential. Geniposide (**2**) is able to inhibit tumorigenesis induced by different carcinogens. It can induce phase II enzymes and inhibiting, at the same time, phase I enzymes and other enzymes involved in the tumoral processes [35a, b]. Antimutagenic and anti-angiogenic properties were also reported [36a, b]. Recently, aucubin (**14**) and geniposide (**2**) were reported as able to stabilize covalent attachments of the topoisomerase I subunits to DNA at sites of DNA strand breaks, generating cleavage complexes intermediates so being active as poisons of topoisomerase I, but not topoisomerase II [37]. This result points to DNA damage induced by topoisomerase I poisoning as one of the possible mechanisms by which these two iridoids have shown antitumoral activity, increasing interest in their possible use in cancer chemoprevention and therapy. The higher activity showed by geniposide (**2**) could be due to the absence of the hydroxyl moiety at C6 and to the presence of the methyl ester moiety at C4, able to interact with a free amino group so increasing possible covalent binding in relation to aucubin (**14**), which does do not possess this group. The reduction in topoisomerase I cleavable complexes at 100 mM for both compounds seemed paradoxical since less activity is shown than at 50 mM. One of the possibilities for the lack of a dose-response relationship could be explained based on literature data, where it has been shown that there are two kinds of topoisomerase inhibitors; poisons, that stabilize the cleavable complexes and stimulate single or double-stranded DNA cleavage and catalytic inhibitors, that prevent the catalytic cycle of the enzymes at steps other than cleavage intermediates [38]. The stabilization of topoisomerase cleavable complexes can decrease if the catalytic activity of the enzyme is inhibited. Pre-treatment with a catalytic inhibitor of topoisomerase can prevent the stabilization of cleavable complexes in cells exposed to topoisomerase poisons. Using this approach the topoisomerase II poison idarubicin has been suggested to act as a topoisomerase II catalytic inhibitor at high concentrations [39]. The reduction in the topoisomerase I poison activity of aucubin (**14**) and geniposide (**2**) observed at high concentrations may indicate that they are acting as topoisomerase I catalytic inhibitors. Finally, with regard to the lack of activity shown by these compounds *in vivo*, the iridoid metabolites genipin (**4**) and aucubigenin (**15**), aglycones of geniposide and aucubin respectively, might be the agents responsible for the effect in cells [40]. Evermore, interesting was that the open chain aglycone of these iridoids can form an imine bond with a nucleophilic site on protein, through a Schiff's reaction [36b]. This irreversible binding may partially contribute to their biological effect. Geniposide (**2**) was also known to increase the activity of GST (Glutathione *S*-transferase) *via* inducing the expression of GST M1 and GST M2 subunits [41]. The mechanism of geniposide's action was successively evaluated for support its application in cancer chemoprevention, investigating the signal pathway involved in the induction of GST subunits and its activity in primary cultured rat hepatocytes [42]. To determine whether geniposide (**2**) also acted through the mitogen activated protein kinase (MAPK) signaling pathway to enhance the expression of GST M1 and GST M2, the total RNA isolated from the hepatocytes exposed to different kinase inhibitors was analyzed for the levels of GST M1 and GST M2. The geniposide-induced increase in levels of GST M1 and M2 transcripts were abolished by the pre-treatment with an MAP-extracellular regulated kinase (MEK) inhibitor, but not by other MAPK inhibitors. This result suggests that geniposide-induced effects on GST M1 and GST M2 transcription are mediated through the MEK-1, MAP-extracellular regulated kinase kinase-1PI 3-K (MEK-1) pathway. Further data demonstrated that **2** acted *via* increasing the expression and activity of Ras/Raf/MEK-1/Erk signaling. The iridoid **2** induced the transcription of GST M1 and M2 by increasing the activity of the MEK-1 signaling pathway and the expression of the mediators. This is supported by the observation that the presence of the MEK-1 inhibitor PD98059 blocked the geniposide-induced increases in levels of Ras, Raf, MEK-1 and Erk1/2 and the phosphorylation of Erk1/2 and, in turn, the activity and expression of GST M1 and GST M2. The effect of geniposide (**2**) on the MEK-1 pathway in the hepatocytes exhibited two phases, one increasing the activity of Erk1/2 (a downstream effector of MEK-1) as reflected by an increase in the phosphorylated forms in the short term and the other inducing the expression of Ras, Raf, MEK-1 and Erk1/2 in the long term. Both responses were efficiently abolished by PD98059. These results suggest that geniposide (**2**) induced the activity of GSTM and the expression of GST M1 and GST M2 by increasing the activity and the expression of the Ras/Raf/MEK-1 signaling pathway and the induction of alpha class GST genes is mediated by an antioxidant-responsive element (ARE) present at two adjacent activator-protein-1-like sites and activated by Fos/Jun heterodimer [42]. The significantly inhibition of *Thermus aquaticus* (*Taq*) DNA polymerase by catalpol (**1**) was reported [43a, b]. DNA polymerases represent important cellular targets in the development of anticancer agents. *In vitro* experiments and theoretical calculations suggest that the mechanism of catalpol (**1**) *Taq* DNA polymerase inhibition may occur in a competitive way with deoxynucleoside triphosphates at the binding site of the enzyme. In fact, iridoids show a certain resemblance with a nucleoside framework. The bicyclic aglycone could mimic the purine scaffold present in nucleosides. In contrast to catalpol (**1**), harpagide (**11**) is a weak *Taq* DNA polymerase inhibitor (IC_{50} 417 μM). Considering that the sugar moiety is identical for catalpol (**1**) and harpagide (**11**), the aglycone fragment seems to play a role in *Taq* DNA inhibition. In this context the epoxide group could exert a pivotal role. It is reported also the *in vitro* antiproliferative activity of catalpol (**1**) and their lipophilic analogs against the representative panel of human solid tumor cell lines A2780, SW1573,

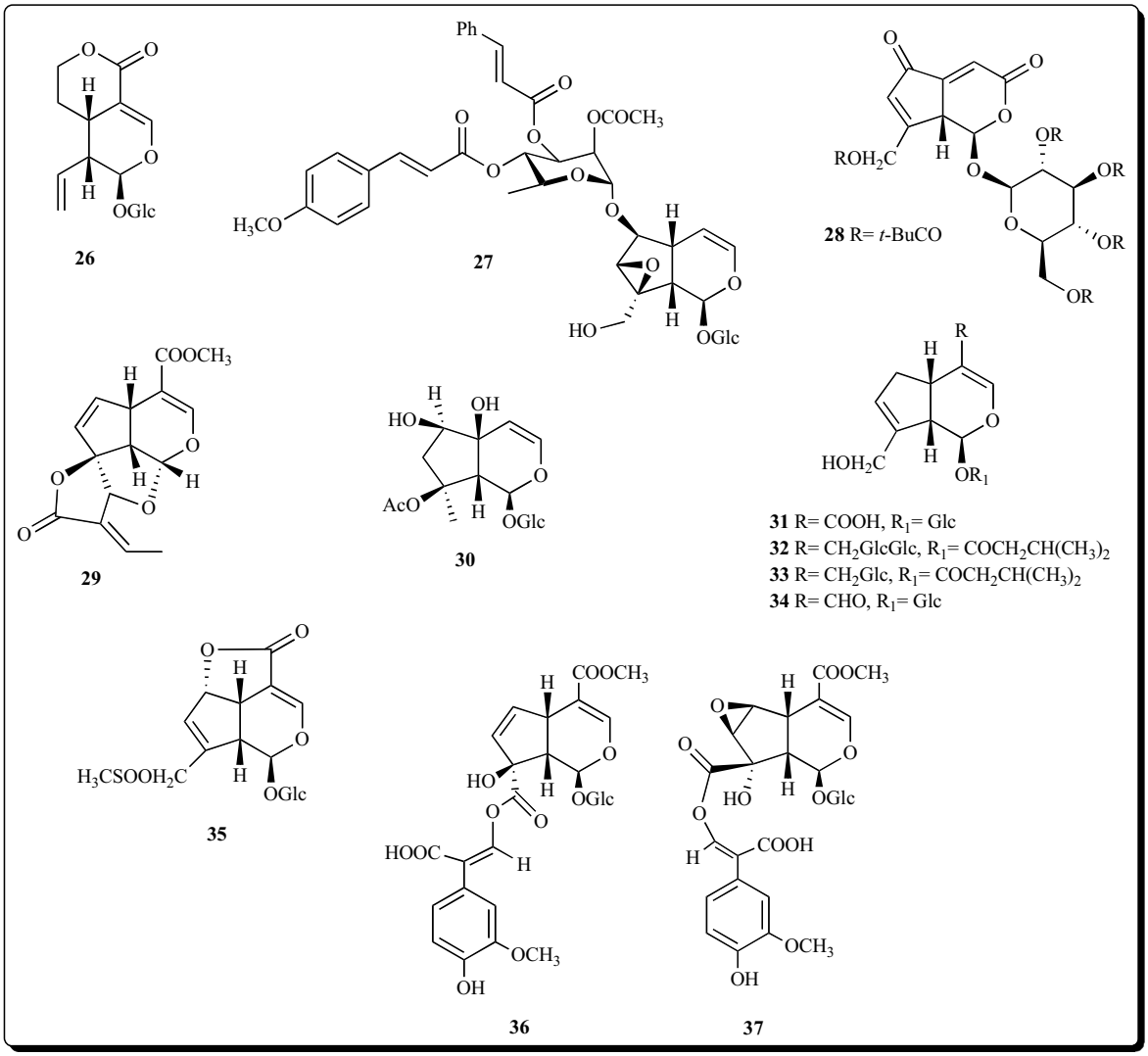
WiDr, T-47D, HBL-100 and HeLa. The lipophilicity of the compounds was evaluated by in silico calculation based on their chemical structure to correlate lipophilicity with antitumor activity. *In vitro* antiproliferative screening of catalpol (**1**) and harpagide (**11**) demonstrated that both iridoids did not reach growth inhibition at the maximum concentration tested (100 μM) while catalpol derivatives (**16**)-(**18**), *tert*-butyldimethylsilyl (TBS) or *tert*-butyldiphenylsilyl (TBDPS) ethers, were active and showed similar growth inhibition values against all tested cell lines with 50% growth inhibition (GI_{50}) values ranging from 4.8 μM to 1.8 [43b]. Two important consequences can be inferred from these results. First, the introduction of at least one silyl group is enough to induce growth inhibition. However, the antiproliferative effect is enhanced with two or three silyl groups. There is no difference observed on the biological activity between the TBS and the TBDPS analogs (**16** *vs* **17**). Second, the hydroxyl groups of the aglycone fragment seem not relevant for the activity. Since DNA polymerase inhibitors are known to interfere with the cell cycle and cause arrest in G_0/G_1 phase, cell cycle studies were performed to assess the effect of the new drugs in HBL-100 and SW1573 cells selected as representative models. The effect in both cell lines is a G_0/G_1 arrest, which is consistent with DNA polymerase inhibition. The antiproliferative and pro-apoptotic action of amarogentin (**19**) rich fraction of *Swertia chirata* (Gentianaceae) has been demonstrated on a mouse skin carcinogenesis model. Immunohistochemical localization revealed a reduction in proliferating and increase in apoptotic cells in skin lesion induced by 7,12-dimethyl-benz(a)anthracene (DMBA)-croton oil following treatment, also reflected in the expression of molecular markers, such as cyclooxygenase II (COX-II) and caspase-3 proteins [44]. It has been demonstrated that a derivative from *Gardenia jasminoides* (Rubiaceae), penta-acetyl geniposide $(Ac)_5$-GP (**20**), played more potent roles than geniposide (**2**) in chemoprevention. Penta-acetyl geniposide (**20**) decreased DNA damage and hepatocarcinogenesis induced by aflatoxin B1 (AFB1) by activating the phase II enzymes glutathione *S*-transferase (GST) and GSH peroxidase. Treatment of $(Ac)_5$-GP (**20**) caused DNA fragmentation of glioma cells and induced sub-G_1 peak through the activation of apoptotic cascades PKCd/JNK/Fas/caspase-8 and caspase-3. Besides, p53/Bax signaling was suggested to be involved in (Ac)5GP-induced apoptosis though its downstream cascades needs further clarified. It has also been shown to inhibit DNA synthesis of tumor cells and arrested cell cycle at G_0/G_1 by inducing the expression of p21, thus suppressing the cyclin D1/cdk4 complex formation and the phosphorylation of the nuclear transcriptional factor E2F. Evidences from the *in vivo* experiments showed that penta-acetyl geniposide (**20**) is not harmful to liver, heart and kidney [45a, b]. This iridoid induced apoptosis in C6 glioma cells by modulating the activation of neutral sphingomyelinase-induced p75 NGF receptor and PKCδ pathway [46]. Previously it was demonstrated that apoptotic cascades PKCδ/c-Jun NH_2-terminal kinase (JNK)/Fas/caspases was induced by **20** [47]. However, the upstream signals mediating PKCδ activation have not yet been clarified. Ceramide, mainly generated from the degradation of sphingomyelin, was hypothesized upstream above PKCδ in (Ac)5GP-transduced apoptosis. Furthermore, NGF/p75 was supposed to be involved because (Ac)5GP-induced apoptosis was demonstrated previously in glioma cells. The activity of (Ac)5GP (**20**) could be explain through the activation of neutral sphingomyelinase (N-SMase). The NGF and p75 enhanced by (Ac)5GP (**20**) was inhibited when added with GW4869, the N-SMase inhibitor, indicating NGF/p75 as the downstream signals of N-SMase/ceramide. To investigate whether N-SMase is involved in (Ac)5GP-transduced apoptotic pathway, cells were treated with (Ac)5GP (**20**) added with or without GW4869. It showed that N-SMase inhibition blocked FasL expression and caspase-3 activation. Likewise, p75 antagonist peptide attenuated the FasL/caspase-3 expression. The PKCδ translocation induced by (Ac)5GP (**20**) was also eliminated by GW4869 and p75 antagonist peptide. To further confirm whether N-SMase activation plays an important role in (Ac)5GP-induced apoptosis, cells were analyzed for the apoptotic rate by 4',6-diamidino-2-phenylindole staining. (Ac)5GP-induced apoptosis was reduced 40% and 80% by 10 μM and 20 μM GW4869, respectively. It indicated that N-SMase activation is pivotal in (Ac)5GP-mediated apoptosis. In conclusion, SMase and NGF/p75 are suggested to mediate upstream above PKCδ, thus transducing FasL/caspase cascades in (Ac)5GP-induced apoptosis. Recently, geniposide (**2**) demonstrated to possess anti-angiogenic activity in a dose-dependent manner and exhibited an inhibitory effect in the range of 25-100 μM on the growth of transformed NIH3T3 cell line [36b]. Luzonial A (**21**), luzonial B (**22**) and luzonidial A (**23**), isolated from a methanol extract of the dried leaves of *Viburnum luzonicum* (Adoxaceae), exhibited inhibitory activity against the HeLa S3 cell line with IC_{50} values of 3.5 μM, 1.93 μM and 24.5 μM. All isolated compounds are characterized by the presence of (*E*)- or (*Z*)-*p*-coumaroyl group at C7 [48]. From *Cymbaria mongolica* (Scrophulariaceae) were isolated some non glycosid iridoids that exhibited a certain cytotoxic activity against a panel of tumor cell lines. In particular, rehmaglutin D (**24**) showed an IC_{50} value of 45.4 μg/ml against SMMC-7721 and 71.2 μg/ml against HeLa, while (1*R*,4*R*,4a*S*,7a*S*)-4,7-dihydroxymethyl-1-hydroxyl-1,4,4a,7a-tetrahydrocyclopenta-6-ene[e]pyran-3-one (**25**) exhibited an IC_{50} value of 40.2 μg/ml on B16 cell line. Interestingly, all values are comparable to the chemotherapeutic drug vincristine [49]. Sweroside (**26**) demonstrated antitumor activity against P388 cell line with maximum total/control (T/C) value of 198% at 200 mg/kg in mice [50]. Scrophuloside B_4 (**27**), a new iridoid glycoside isolated from the roots of *Scrophularia ningpoensis* (Scrophulariaceae), was active on K562 and Bowes tumor cells with IC_{50} values of 44.6 μM and 90.2 μM, respectively [51]. A novel iridoid (**28**) was prepared in six steps from natural aucubin (**14**) and comprises the same conjugated cyclopentenone pharmacophore as known antitumor oxylipins and prostaglandins [52]. This iridoid displayed significant antiproliferative *in vitro* activity towards leukemia L1210 cells, with an IC_{50} value of 3.0 μM, which is within the same order of magnitude as that reported for Tei 9826, drug currently under preclinical development. A cytotoxic activity was found when plumericin (**29**), isolated from *Plumeria rubra* (Apocynaceae), was applied to cell culture of murine leukemia, breast colon, lung, nasopharyngeal human cancer, melanoma and fibrosarcoma [53]. An interesting cancer chemopreventive activity was reported for 8-acetylhapagide



(**30**) isolated from *Ajuga decumbens* (Lamiaceae) together and harpagide (**11**) [54]. The iridoids were reported as active constituents on inhibitory effect of Epstein-Barr virus early antigen (EBV-EA) induction. Of these compounds, 8-acetylharpagide (**30**) exhibited the strongest inhibitory effects on EBV-EA induction and mouse skin tumorigenesis induced by 7,12-dimethylbenz[*a*]anthrax-cene (DMBA) and 12-*O*-tetradecanoyl-13-acetate (TPA). SAR analysis showed that probably the found stronger activity may be attribute to the acetylic group in 8-acetylharpagide (**30**). Also asperulosidic acid was evaluated against the EBV-EA activation induced by TPA, demonstrating inhibitory effects, with an IC_{50} value of 485 mol ratio/32 pmol TPA, which was more inhibitory than positive control quercetin (IC_{50} 560 mol ratio/32 pmol TPA) [55]. Therefore, the antitumor-promoting effects of the compound (**30**) was examined by the two-stage carcinogenesis test of mouse hepatic tumor, in which *N*-nitrosodiethylamine and pheno-barbital were used as an initiator and as a promoter, respectively. Oral administration of this iridoid markedly reduced the hepatic hyperplastic nodules formation. 8-Acetylharpagide (**30**) exhibited a significant inhibitory effect also on the tumor-initiation induced by NO donor, (±)-(*E*)-methyl-2-[(*E*)-hydroxyimino]-5-nitro-6-methoxy-3-hexeneamide. This evidence suggests that this glycoside had been potent for an antitumor promoter against TPA on mouse skin carcinogenesis and against glycerol on mouse pulmonary carcinogenesis [56]. Prevention on undesirable radiation damage to the hematologic tissue after high dose irradiation in mice was observed with geniposide (**2**) and geniposidic acid (**31**) [57]. With a mechanism of action that involved [^{3}H]-thymidine incorporation into the DNA acted serrulatoloside (**32**), penstemide (**33**) and its aglycone, isolated from *Penstemon serrulatus* (Scrophulariaceae). These compounds were able to induce a dose-dependent inhibition of [^{3}H]-thymidine incorporation *in vitro* on the spontaneous proliferation of mouse spleen lymphocytes or hepatoma cells in the Syrian hamster [58]. A number of iridoids, such as tarennoside (**34**), genipin (**4**) and paederoside (**35**) were reported to possess antimutagenic effect in various *in vitro* models [59]. In tumor promotion Activator Protein-1 (AP-1) plays a key role, so inhibition of AP-1 activity has been shown to lead to suppression of cell transformation. It was shown that citrifolinin A (**36**) and citrifolinoside (**37**) isolated from *Morinda citrifolia* (Rubiaceae) displayed significant inhibitory effect on AP-1 with an IC_{50} of 69.6 and 29.0 μM, respectively [60].

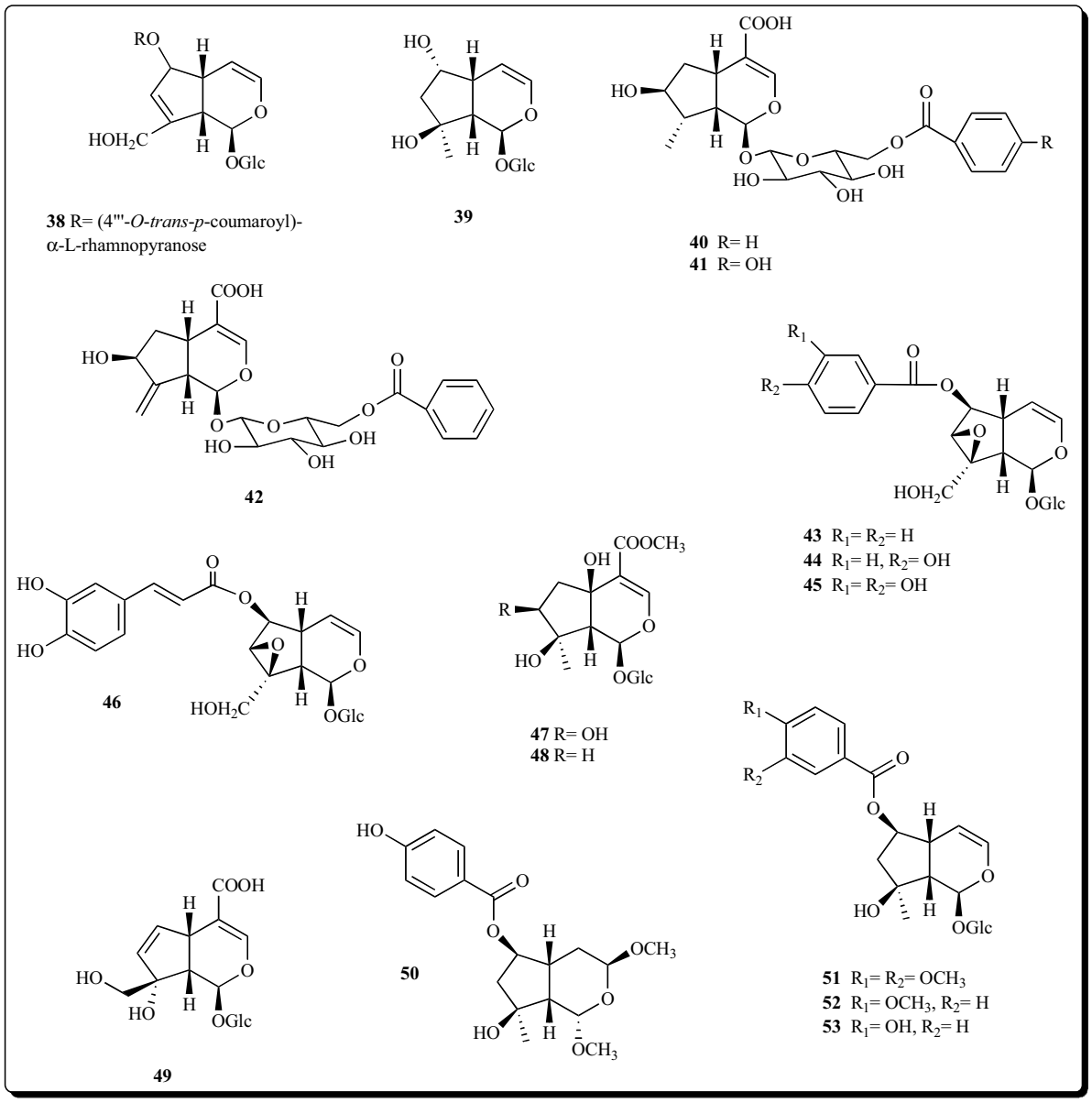
2.3. Anti-Inflammatory and Immunomodulating Properties

In the search for new anti-inflammatory compounds special interest has been directed to iridoids. Since ancient times, *Verbascum* (Scrophulariaceae) extracts, decoctions and infusions have been used as medicinal herbs in traditional medicines worldwide [61]. Aucubin (**14**), one of the

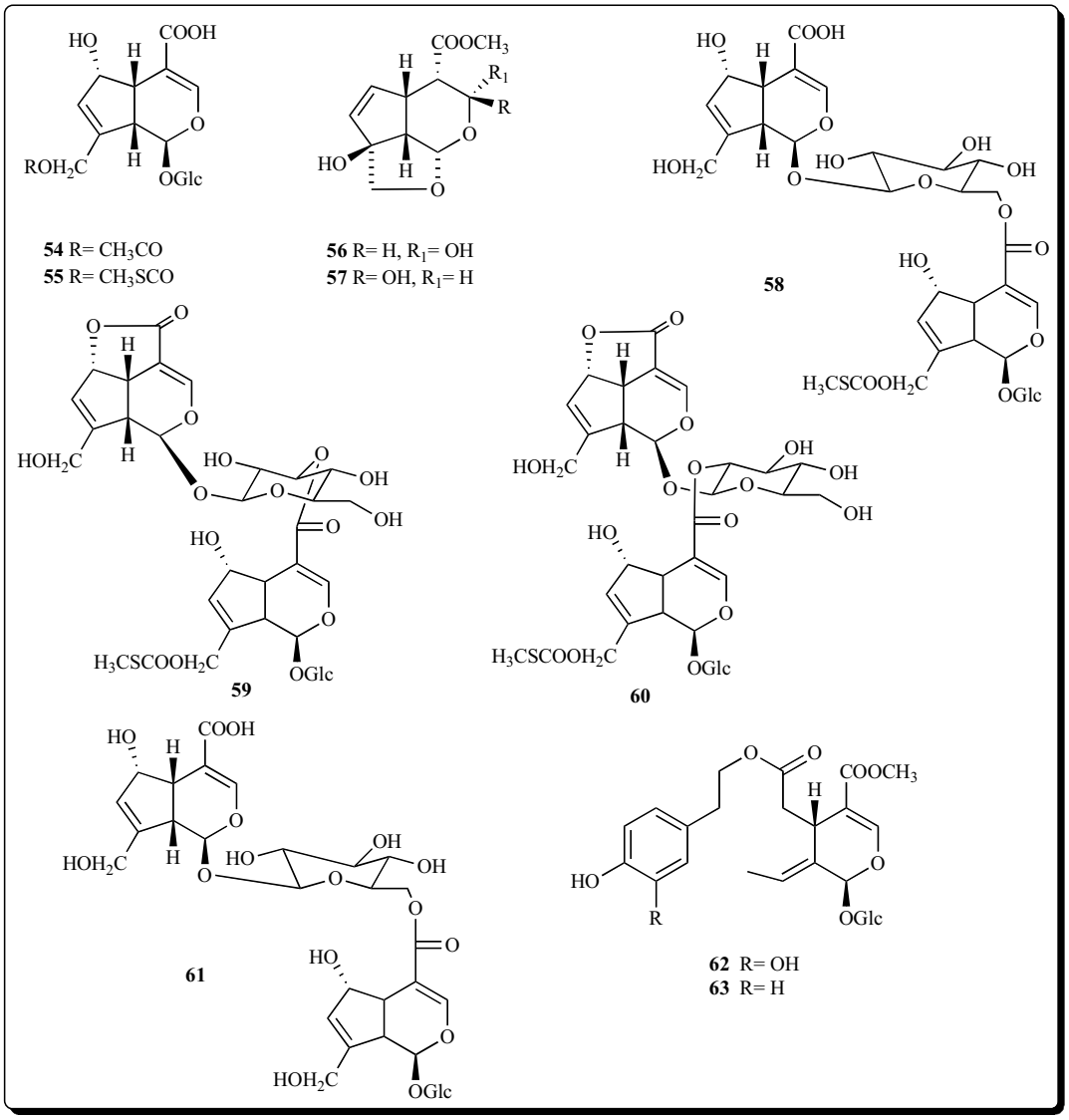


major iridoid glycosides of the flowers of *V. lasianthum*, was found to be a specific inhibitor of nuclear factor (NF)-κB activation in rat basophilic leukemia (RBL)-2H3 mast cells, that which might explain its beneficial effect in the treatment of chronic allergy inflammatory diseases [62]. In particular, **14** has been demonstrated to inhibit Antigen (Ag)-induced TNF-α and interleukin (IL)-6 production and expression in Ag-stimulated RBL-2H3 mast cells in a dose-dependent manner with IC_{50} values of 0.10 μg/ml and 0.19 μg/ml, respectively. Maximal inhibition of TNF-α and IL-6 production was 73% and 88.8%, respectively. The reducing of carrageenan-induced edema and carrageenan induced hind paw edema were also reported for aucubin (**14**) [61, 63]. This finding might explain that this iridoid had beneficial effect in the treatment of inflammatory diseases. On the other hand, 6-*O*-(4'''-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosylaucubin (**38**) and catalpol (**1**) have no effect against carrageenan-induced edema, while ajugol (**39**) was found to possess a weak activity in similar experimental protocols. A double bond between C7 and C8 is one of the most positive characters for activity and its oxidation to an epoxy derivative leads to a remarkable decrease in anti-inflammatory activity, as occurs in catalpol (**1**). If an ester linking appears by acylation with an aromatic acid derivative at the sugar moiety, anti-inflammatory activity is augmented, whereas if acylation occurs directly in the monoterpenoid skeleton, this activity diminishes [63]. However, in contrast with previous studies, 6-*O*-(4'''-*O*-*trans*-*p*-coumaroyl)-a-L-rhamnopyranosylaucubin (**38**) did not exhibit any activity [61]. The introduction of a hydroxyl function at C8, as occurs in ajugol (**39**), decreases topical activity. The absence or presence of the extra-annual carboxyl or carboxymethyl group at C4 is of no relevance, as is apparent result when the activity of aucubin (**14**) is compared with those of geniposidic acid (**31**), but the conversion of a -COOH compound to its -COOMe derivative notably increases the topical activity [63]. To elucidate possible mechanisms for the anti-inflammatory action of iridoid glycosides, the effects of both aucubin (**14**) and its hydrolyzed product **15**, obtained by β-glucosidase treatment, were studied on the production of TNF-α in RAW 264.7 cells [64].

Only **15** suppressed the production of both mRNA for TNF-α and subsequent TNF-α protein in the culture in a dose-dependent manner. In addition, treatment with **15** blocked both I-kappa B α degradation and translocation of NF-kappa B from the cytosol fraction to the nuclear fraction (55% inhibition) in the culture. However, treatment with (**15**) did not affect the intracellular level of cAMP formed by forskolin treatment in human monocytes U937 culture, implying that there is no influence on the cAMP level in other cell systems. Several *Veronica* (Scrophulariaceae) species are reported to possess application in traditional medicines worldwide for the treatment of a wide range of disorders, in respiratory diseases, as diuretics and for wound healing [65, 66]. For the scientific evaluation of the claimed effect for *Veronica anagallis-aquatica* aerial parts, methanol and water extracts were investigated for anti-inflammatory activity using carrageenan-induced hind paw edema model and for antinociceptive activity using *p*-benzoquinone-induced writhing model in mice [67]. Through bioassay-guided fractionation seven iridoids, aquaticoside A (**40**), aquaticoside B (**41**), aquaticoside C (**42**), veronicoside (**43**), catalposide (**44**), verproside (**45**) and verminoside (**46**) were isolated. Catapol (**1**) derivative iridoid glucosides, verproside (**45**) and catalposide (**44**), were found to possess potent antinociceptive and anti-inflammatory activities *per os* without inducing any apparent acute toxicity as well as gastric damage. According to Recio *et al*. [63], systemic anti-inflammatory activity of iridoids was modest, while a higher efficacy could be observed through topic application as evidenced by TPA-edema model. OH-substitution as C5, unsaturation as C7-C8, methyl substitution of carbonyl C11 and integrity of the cyclopentane ring were essential for higher activity. Some of the results suggested by Recio *et al*. [63] were later supported by other authors as well; i.e. picroside II (**13**), a catalpol derivative, was only active topically and was also found to possess moderate topic anti-inflammatory activity in mouse ear-swelling assay [68]. At 4 h of carrageenin-injection, lamiide (**47**) at the doses of 100 mg/kg showed an anti-inflammatory effect slightly greater (79%) [69] than that found for the iridoid ipolamiide (**48**) (70.22%) (lack of C7-OH group) in similar experimental conditions at the same dose level. However, these results were not in agreement with the results of Recio *et al*. [63], i.e. lamiide (**47**) was reported to possess a weak activity on oral administration at the same dose (100 mg/kg), while a moderate activity when applied topically. Monotropein (**49**), the more abundant active principle from the roots of *Morinda officinalis* (Rubiaceae), was examined for its anti-inflammatory activity using *in vivo* assay system [70]. Monotropein (**49**) was found to have an anti-inflammatory effect 1 h after the carrageenan injection and maximal edema inhibition was observed at 3 h after edema induction. In particular, treatment with **49** (30 mg/kg, *p.o.*) reduced the edema by 39.6% at 3 h, and ibuprofen (100 mg/kg, *p.o.*) treatment reduced edema rate by 62% on a volume basis. Moreover, this effect was significant for least 5 h after edema induction. When subjected to acute toxicity test using mice, any lethality was not observed up to 2000 mg/kg dose (*p.o.*). Chemical analysis of a polar extract of *Kigelia africana* (Bignoniaceae) fruits indicated the presence of the iridoid verminoside (**46**), as a major constituent. Verminoside (**46**) showed significant anti-inflammatory effects inhibiting both iNOS expression and NO release in the lipopolysaccharide (LPS)-induced J774.A1 macrophage cell line [71]. Geniposide (**2**) has been reported to possess inhibitory activity on 5-lipoxygenase, activity against tumor-promoting 12-*O*-tetradecanoylphorbol-13-acetate with an activation of PKC and inhibitory effects on ovalbumin-induced junction permeability and recovery of transepithelial electrical resistance in guinea pig trachea, showing its potential as an antiasthma therapy. It was also shown to contain modulating activity on cytochrome P 450-dependent monooxygenase, glutathione and GST in rat liver. Since geniposide (**2**) is transformed into genipin (**4**) by bacterial enzymes in the body, it may be that aglycone mainly plays an important role in the efficacy. In fact, Koo *et al.* [72] demonstrated that genipin (**4**) possess a significant antilipoperoxidative capacity, but it did not show a free radical scavenging effect and an inhibition of xanthine oxidase activity. These results imply that genipin (**4**) is able to effectively scavenge hydroxyl radicals formed in a Fe^{2+}/ascorbate system. This iridoid had potent topical anti-inflammatory activity, which was detected as an inhibition of croton oil-induced ear edema in mice and inhibited NO production and iNOS expression upon stimulation by lipopolysaccharide/interferon- (IFN-) in RAW 264.7, a murine macrophage cell line. Genipin (**4**) markedly blocked lipopolysaccharide evoked degradation of inhibitor-κB-β (IκB-β), indicating that it exhibits inhibitory effect on NO production through the inhibition of NF-κB activation. Sugar component of geniposide may cause a difference in the effect on iNOS expression. With the purpose to clarify geniposide (**2**) and genipin (**4**) anti-inflammatory activity other experimental models, such as carrageenan-induced rat paw edema, carrageenan-induced air pouch formation and measurement of NO content in the exudates were analysed [73]. In the carrageenan-induced rat paw edema, oral administration of the two compounds, at doses of 100 and 50 mg/kg, respectively, gave rise to respective inhibitions of 31.7 and 49.1%, which were measured at 3 h after injection of carrageenan. The higher inhibitory activity was observed with aglycone at a lower dose than glycoside side at a higher dose. This result suggests that genipin (**4**) has a stronger acute anti-inflammatory activity than geniposide. In the carrageenan-induced air pouch model, treatment with geniposide (0.1 mg/pouch) and genipin (0.1 mg/pouch) decreased the carrageenan-induced formation of the exudates, and the degree of their inhibition was 45.1 and 51.1%, respectively. Geniposide (**2**) and its aglycone (**4**), at the same doses, gave rise to similar reducing effects. The higher inhibitory effect of genipin (**4**) on NO production may correlate with its stronger anti-inflammatory activity in the carrageenan induced rat paw edema. This effect also corresponds to previous results that suggest that genipin (**4**) has a potent inhibitory activity on both NO production and iNOS expression upon stimulation by lipopolysaccharide/interferon- in RAW 264.7, a murine macrophage cell line [72]. It is likely that genipin (**4**) exhibits its acute anti-inflammatory activity through a reduction of NO production. A lower activity of geniposide in the acute anti-inflammatory model and a reduction in NO production may be due to its delayed transport into cells, since it contains a single glucose unit in its chemical structure. The effect as well as the mechanism of geniposide (**2**) on serum IL-1β and TNF-α levels of rheuma-

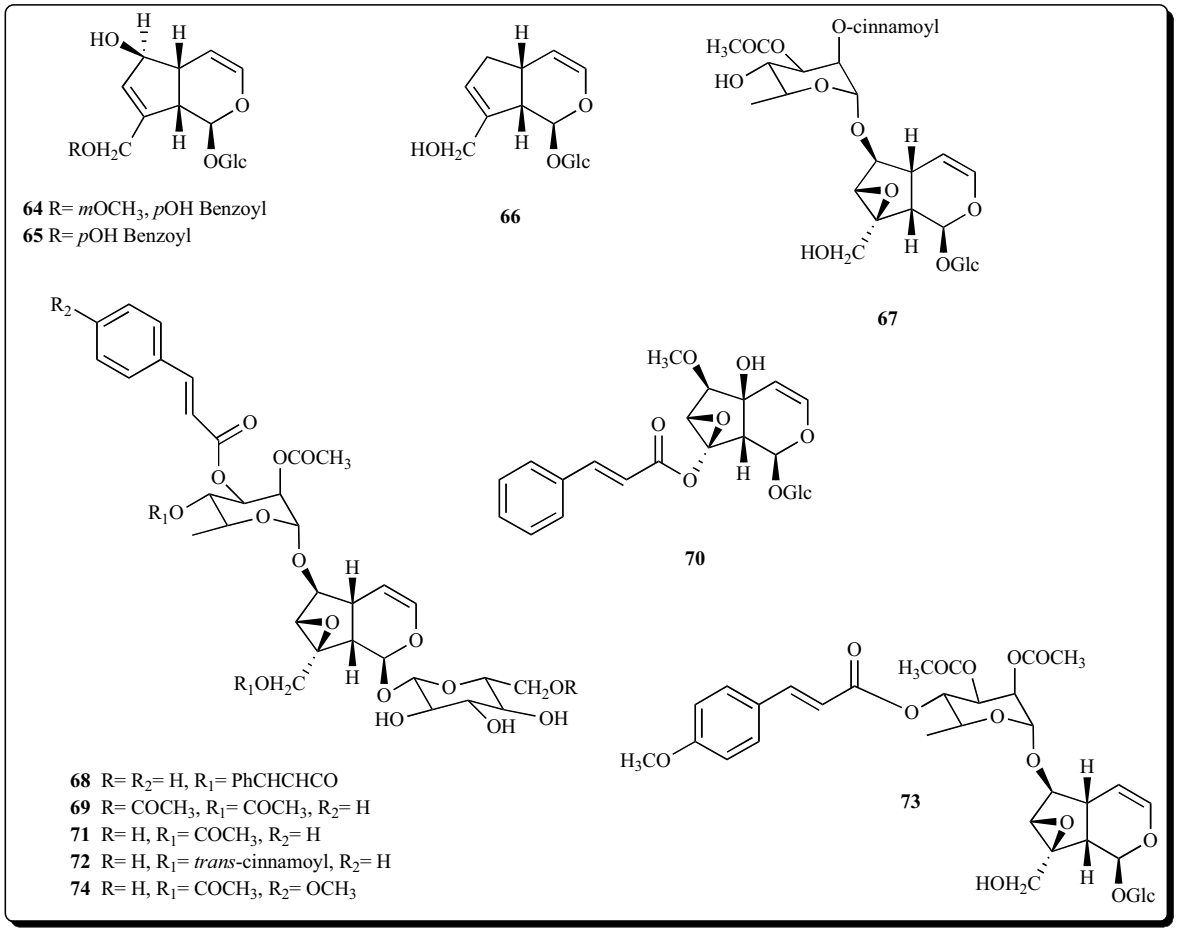


toid arthritis rats was investigated [74]. The inhibitory effects on paw edema were observed and serum IL-1β and TNF-α levels were determined in experimental rats. Compared with the model, geniposide (**2**) delayed the starting time of right paw edema significantly and the levels of serum IL-1β and TNF-α were significantly decreased by geniposide (**2**) at high dose or medium dose. It can lower serum IL-1β and TNF-α levels in rheumatoid arthritis rats. The effect may be close related to inhibitory development of rheumatoid arthritis by the agent. From *Tabebuia avellanedae* (Bignonaceae) that showed significant inhibitory activity on NO production in LPS-activated J774.1 macrophage-like cells, bioactive constituents were isolated [75]. The new iridoid **50** and the three known iridoids 6-*O*-(3,4-dimethoxybenzoyl)-ajugol (**51**), 6-*O*-(*p*-methoxybenzoyl)-ajugol (**52**) and 6-*O*-(4-hydroxybenzoyl)-ajugol (**53**) (IC_{50}, 13.8-26.1 μg/ml) exhibited a more potent NO inhibitory activity than the positive control N^G-monomethyl-Larginine (IC_{50}, 27.4 μg/ml). Catalposide (**44**), isolated from *Catalpa ovata* (Bignonaceae), significantly inhibited the production of NO in LPS-stimulated RAW 264.7 macrophages and in cytokine-stimulated human DLD-1 and rat vascular smooth muscle cells in a dose-dependent manner [76]. Enzyme inhibitory activities of fourteen iridoids obtained from two Malaysian medicinal plants, *Saprosma scortechinii* (Rubiaceae) and *Rothmannia macrophylla* (Rubiaceae) were evaluated *in vitro* using soybean lipoxygenase and bovine testis hyaluronidase, two enzymes that have been implicated to many biological functions such as inflammation, allergy, migration of cancer cells [77]. Most of the iridoids, including asperulosidic acid (**54**), paederosidic acid (**55**) and mixture of gardenogenins A (**56**) and B (**57**), did not show any inhibitory activity, except for the

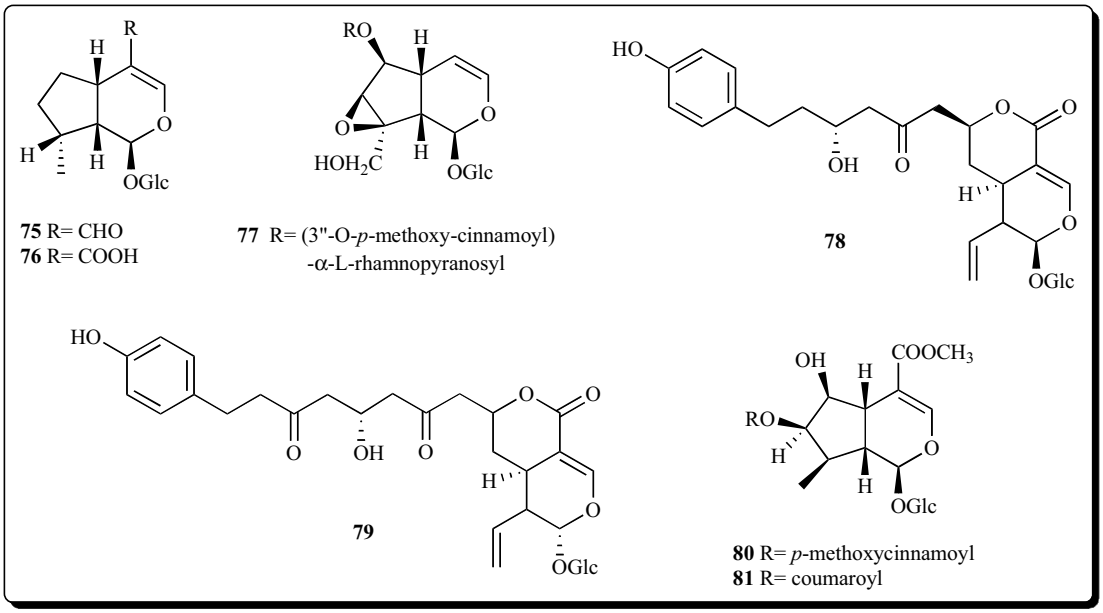


bis-iridoids saprosmoside A (**58**), D (**59**), E (**60**) and G (**61**), which inhibited lipoxygenase activity with IC_{50} values ranging from 154.2 to 174.5 μM. In contrast, all the iridoids tested did not show any hyaluronidase inhibitory activity at concentrations up till 5 mM. Structural modification of asperulosidic acid (**54**) and paederosidic acid (**55**) through enzymatic hydrolysis by β-glucosidase resulted in their inhibition towards the enzyme activities and these activities were enhanced by the presence of some amino acids (lysine, leucine or glutamic acid) or ammonium acetate. Mixtures of gardenogenins A (**56**) and B (**57**) incubated with amino acid or ammonium acetate did not show any inhibitory effect on the enzyme activities during the 6 h incubation period, except for lysine where spontaneous reaction between the iridoids and amino acid resulted in the inhibition of lipoxygenase activity. The results from these biomimetic reactions suggested that these iridoids could play a role in the prevention against lipoxygenase or hyaluronidase mediated reactions in the body, through their hydrolysis products, or when these intermediates coexist with some nitrogenous compounds. Two iridoids glycosides isolated from *Phillyrea latifolia* (Oleaceae), oleuropeoside (**62**) and ligustroside (**59**) were capable of exerting inhibitory actions on enzymes of the arachidonate cascade, although they did not interfere in the 5-lipoxygenase pathway (5-LOX). These compounds exert a preferential effect on the cyclooxygenase-1 (COX-1) pathway. Thus, ligustroside (**63**) gives rise to reduced prostaglandin E_2 levels in activated mouse macrophages and to a lesser extent reduced thromboxane B_2 levels in human platelets while oleuropeoside (**62**) only has significant effect on prostaglandin-synthase activity [78]. The inability of oleuropeoside (**62**) on thromboxane-synthase could be related to the hydrogen substituent in the structure. The opening of the cyclopentane ring of the iridoid molecule and derivatization to a dihydroxyphenylethanol ester (oleuropeoside, **62**), both of which decreased *in vivo* topical activity, are accompanied by a decrease in the thromboxane B_2 activity, but are two of the most positive chemical features for PG-synthase activity.

Furthermore, enhancement of the COX-1 inhibitory activity was found in ligustroside (**63**) with loss of hydroxyl functionality at the C-5' position of oleuropeoside (**62**). Pedunculariside (**64**) and agnuside (**65**), isolated from *Vitex peduncularis* (Verbenaceae) were tested for inhibition of COX-1 and COX-2 regulated prostaglandin biosynthesis using COX deficient murine cell lines [79]. Agnuside (**65**) showed preferential inhibition of COX-2 over COX-1 (COX-2 IC_{50} 0.026 mg/ml and less than 15% inhibition of COX-1 at this concentration). Similarly, pedunculariside (**64**) had a COX-2 IC_{50} value of 0.15 mg/ml while having almost no effect on COX-1 activity as indicated by less than 10% inhibition at this concentration. From *Scrophularia scorodonia* (Scrophulariaceae) bartsioside (**66**), aucubin (**14**), harpagide (**11**), harpagoside (**10**), 8-acetylharpagide (**30**), scorodioside (**67**) and scropolioside B (**68**) were isolated and evaluated for their *in vitro* anti-inflammatory activity in cellular systems generating COX and LOX metabolites [80]. In the PGE_2-release assay only harpagoside (**10**) and 8-acetylharpagide (**30**), at the highest non-cytotoxic dose (100 μM), showed an inhibition rate of around 30-40%, although with less potency than the reference drug (IC_{50} 1.5 μM). Harpagoside (**10**) and harpagide (**30**) also inhibited release of LTC_4. On the contrary, most iridoids showed a significant effect on thromboxane B_2-release from ionophore-stimulated human platelets with inhibition percentages slightly lower than the reference drug ibuprofen (IC_{50} 7 μM). A double bond between C7 and C8 with additional hydroxyl substituent in the body of the molecule, as occur in aucubin (**14**), is one of the most positive characters for *in vivo* and *in vitro* activity. Compounds lacking some of these features are either less active or inactive on arachidonic acid metabolism. In fact, the introduction of a hydroxyl function at C8 which seriously decreased *in vivo* topical activity together with the lack of insaturation at C7-C8, further reduces *in vitro* activity, as can be deduced from the data on harpagide (**11**), 8-acetylharpagide (**30**) and harpagoside (**10**). Scropolioside-D_2 (**69**), harpagoside-B (**70**), scropolioside-D (**71**), koelzioside (**72**) and 8-*O*-acetylharpagide (**30**), isolated from *Scrophularia deserti* (Scrophulariaceae), decreased edema in the range of 23 to 30% at a dose of 10 mg/kg after 3 h with respect to the control group treated only with carrageenan [81]. Harpagoside-B (**70**) and koelzioside (**72**) were the most active. It may therefore be concluded that compounds containing a cinnamoyl moiety as found in harpagoside-B (**70**) at C8 and in koelzioside at C4" in place of acetyl groups as present in 8-*O*-acetyl-harpagide (**30**), scropolioside-D (**71**) and scropolioside-D_2 (**69**) have significant activity. The introduction of a methyl group at C6-OH might also enhance the activity. The effects of scrovalentinoside (**73**) and scropolioside A (**74**), isolated from *Scrophularia auriculata* ssp. *pseudoauriculata* (Scrophulariaceae) were evaluated [82]. The experi-



mental data suggest that the mechanism of action of scropolioside A (**74**) may be in part related to that of the glucocorticoids. The T cells are the main kind of leukocytes involved in delayed hypersensitivity, a process for which oxazolone induced inflammation is a key model. This test is useful in the search for drugs that could inhibit the inflammation and tissue destruction caused by the type IV allergic reaction. In contrast with the results obtained in the other models assayed, the ear swelling due to oxazolone-induced delayed-type hypersensitivity was reduced by the pair of iridoid isomers, scrovalentinoside (**73**) and scropolioside A (**74**). Both iridoids, but especially scrovalentinoside (**73**), were active in the early stage and throughout the inflammatory process and decreased the edema. Scrovalentinoside (**73**) produced histological changes similar to those with dexamethasone, reduced the inflammatory lesion and suppressed the cellular infiltration. This demonstrated the efficacy of these iridoids on the delayed-type hypersensitivity reaction. More recently, scropolioside A (**74**) showed anti-inflammatory properties against different experimental models of delayed-type hypersensitivity. This iridoid reduced the edema induced by oxazolone by 79% (72 h) at 0.5 mg/ear and that induced by sheep red blood cells by 47% (18 h), 45% (24 h) and 36% (48 h) at 10 mg/kg. It reduced *in vivo* both edema formation and cell infiltration whereas *in vitro* it reduced the proliferation of activated T-lymphocytes (IC_{50} of 67.74 μM). Treatment with **74** (100 μM) 18 and 24 h after phytohemagglutinin stimulation increased the number of cells arrested in the subG0 phase whereas treatment 3 h after stimulation clearly increased the number of cells that passed to the S phase. Scropolioside A (**74**) also inhibited the production of prostaglandin E2, leukotriene B4, NO, interleukin-1β, interleukin-2, interleukin-4, TNF-α and interferon-γ, but had no effect on the production of interleukin-10. Moreover, it modified the expression of both nitric oxide synthase-2 and COX-2, as well as the activation of NF-κB in RAW 264.7 macrophages [83a, b]. Rapid production of ROS and up-regulation of β2 integrin by leucocytes are two important inflammatory responses in human leucocytes. Boschnaloside (**75**) and 8-epideoxyloganic acid (**76**) identified from two medicinal plants, *Orobanche caerulescens* (Orobancaceae) and *Boschniakia rossica* (Orobancaceae), exhibited mediocre activity in the inhibition of phorbol-12-myristate-13-acetate (PMA) and *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) induced ROS production (with IC_{50} values of approximately 9-28 μM) and showed a relatively selective effect in PMA-activated peripheral human neutrophils (PMNs) and fMLP-activated mononuclear cells. In particular, the modification of aldehydic moiety with carboxylic moiety was responsible of a major activity in the inhibition of PMA-activated PMNs. The ability of iridoids to reduce ROS production, possibly through modulation of NOX activity and/or the radical scavenging effect and β2 integrin expression in leucocytes, indicated that these compounds had the potential use as anti-inflammatory agents during oxidative stress [84]. However, it is strange that catalpol-iridoid derivatives such as scropolioside A (**74**), koelzioside (**72**) and 6-*O*-[(3"-*O*-*p*-methoxy-cinnamoyl)-α-L-rhamnopyranosyl]catalpol (**77**) have been described as immunostimulants because they increased the macrophage migration index, a parameter which has been correlated with macrophage activation and delayed-type hypersensitivity response [85]. Nevertheless, it should be noted that other iridoids isolated from *Hydrangea macrophylla* var. *thunbergii* (Hydrangeaceae) such as hydramacrosides A (**78**) and B (**79**) behaved as antiallergic principles that inhibited the histamine release induced from mast cells by an antigen-antibody reaction [86] and arbortristosides A (**80**) and C (**81**) obtained from *Nyctanthes arbortristis* (Oleaceae) were active on passive cutaneous anaphylaxis and presented mast cell stabilizing activity [87]. The extract of *G. jasminoides* has been found able to specifically inhibit the CD28-co-stimulated activation of human peripheral blood T cells [88]. The subfractionation led to the isolation of nine iridoids. Among them, geniposide (**2**), 6α-hydroxygeniposide (**82**), ixoroside



75 R= CHO
76 R= COOH

77 R= (3"-O-*p*-methoxy-cinnamoyl) -α-L-rhamnopyranosyl

78

79

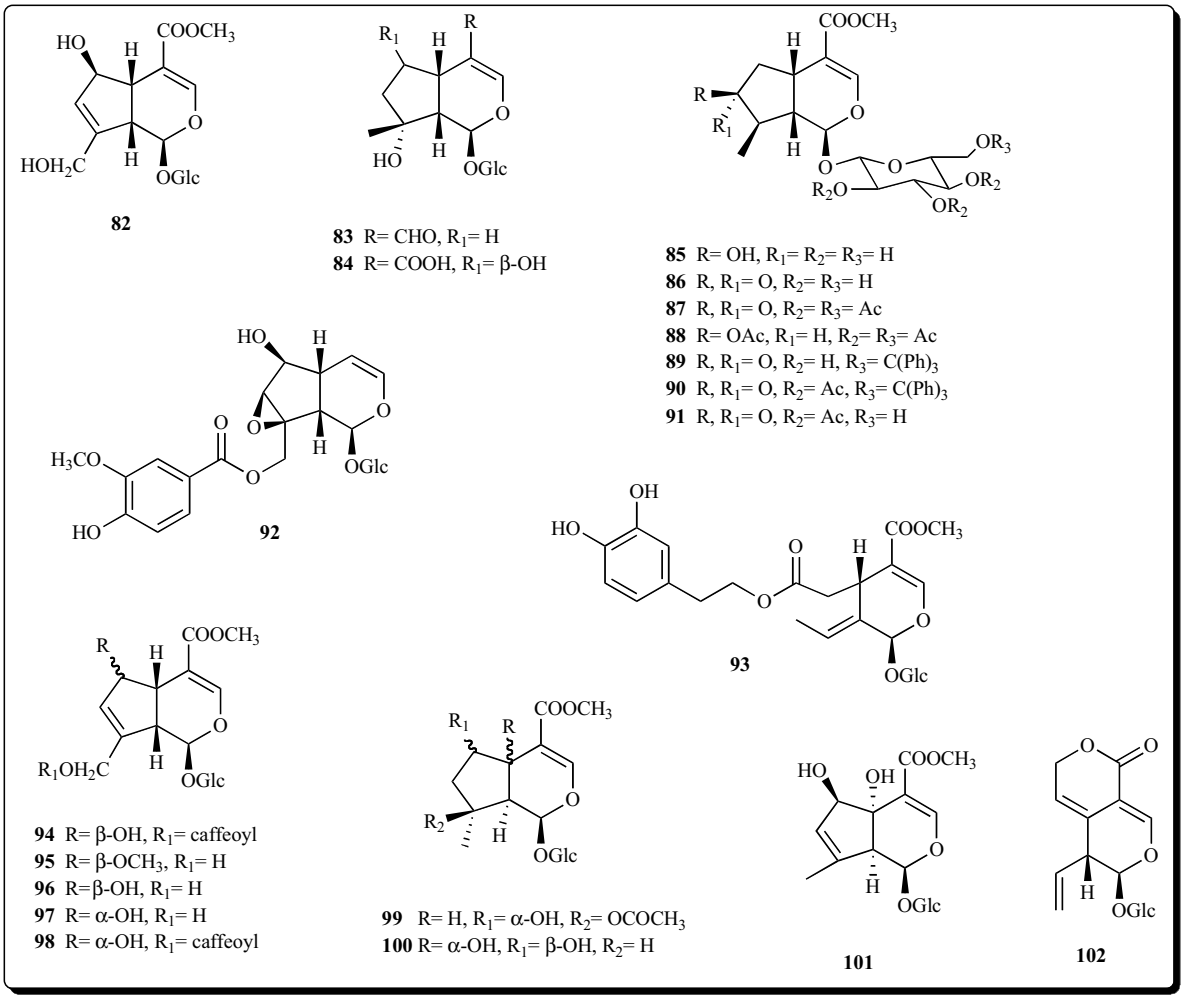
80 R= *p*-methoxycinnamoyl
81 R= coumaroyl

(**83**) and shanzhiside (**84**) showed significant inhibition of IL-2 secretion at 50 μg/ml. Ixoroside (**83**) and shanzhiside (**84**), which possess a hydroxyl moiety at C8, showed more potent activity than those of the other iridoid glucosides. The presence of hydroxyl functionality at the C8 position in iridoid glucosides is probably responsible for enhancing their inhibition activities. Immunostimulant activity profile of modified iridoid glycosides prepared from loganin (**85**), ketologanin (**86**) and arbortristoside A (**80**) have been studied and some SARs analyses have been obtained [89]. Compound (**85**) and (**80**) exhibit immunostimulation while ketologanin (**86**) did not exhibit immunostimulation. These results indicated that (**85**) and (**80**) did not exhibit specificity for any component of the immune response and the oxidation of hydroxyl group at C7 in the aglycone moiety almost completely abolished the immune stimulating property. Moreover, ketologanin tetra acetate (**87**) also did not show immune stimulating property. Unlike **87** loganin penta acetate (**88**), however, exhibited elevated cell mediated immune and humoral immune responses. Single point modification at C6' position in ketologanin (**86**), carried out by introducing a trityl group (**89**), revealed an interesting feature. The inactive ketologanin (**86**) became immunostimulant. Acetylation of this compound yielded **90** which led to enhanced immunostimulant properties. The involvement of C6' position in immune responses was observed again when detritylation of this compound **90** into **91** significantly reduced haemagglutinating antibody titre, the parameters of humoral response. In order to clarify the role of sugar, the immunomodulatory activity of loganin aglycone was studied. It was observed that removal of the sugar moiety completely abolished the immunostimulant activity and led to mild immunosuppression as was evident from *in vivo* and *in vitro* studies. *In vitro* results indicate that an optimum concentration in biophase may be essential for eliciting the immunostimulant activity.

2.4. Antioxidant Properties

The inhibition of the non-enzymatic generation of superoxide anions generated by xanthine-xanthine oxidase system by picroliv, a standardized fraction prepared from *Picrorhiza Kurroa* that contains mainly (60%) two iridoid glycosides: picroside I (**12**) and kutkoside (**92**) in a ratio of 1:1.5, along with several other uncharacterized glycosides as minor constituents was reported [90]. The generation of malondialdeidehyde induced by the ascorbate- Fe^{2+} and NADP-ADP-Fe^{2+} systems in rat liver microsomes was inhibited. Whit this study authors highlighting that compounds possessed the properties of antioxidants which appear to be mediated through activity like that of superoxide dismutase, metal ion chelators and xanthine oxidase inhibitors. Similarly, oleuropein (**93**) obtained from *Olea europea* (Oleaceae), exert a good scavenging activity towards $O_2^{\cdot-}$, $HO^{\cdot}$ and $ROO^{\cdot}$ when tested in different assays. In particular, oleuropein (**93**) decreased the chemiluminescence sum from the $O_2^{\cdot-}$ generating system with an inhibitory effect that was dependent on its concentration. This compound also reacted with $ROO^{\cdot}$ radicals and showed activity about two-fold greater than the standard Trolox. The antioxidant effects were studied at different concentrations and reflected in protection against the fluorescence decay of β-phycoerythrin (β-PE), due to $ROO^{\cdot}$ attack on this protein. Using the Fenton-like reaction and the spin trap agent 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), oleuropein (**93**) was found to inhibit DMPO-$^{\cdot}$OH radical formation in the range 10-90% at concentrations of 0.1 mM to 2 mM. Furthermore, this compound also inhibited $HO^{\cdot}$-dependent deoxyribose degradation with a percentage of 60% of inhibition at 0.5 mM [91].

Scavenging activity of 10-*O*-caffeoyl scandoside methyl ester (**94**), 6-methoxy scandoside methyl ester (**95**), scandoside methyl ester (**96**), methyl deacetyl asperulosidate (**97**) and 10-*O*-caffeoyl daphylloside (**98**), isolated from *Wendlandia formosana* (Rubiaceae), was studied against diphenylpicrylhydrazyl (DPPH), hydroxyl radicals and peroxynitrite. 10-*O*-Caffeoyl scandoside methyl ester (**94**) and 10-*O*-caffeoyl daphylloside (**98**) exhibited comparable scavenging activity against diphynylpicrylhydrazyl and hydroxyl radicals with that of butylated hydroxytoluene and protection against peroxynitrite mediated oxidation [92]. 10-*O*-Caffeoyl scandoside methyl ester (**94**) and 10-*O*-caffeoyl daphylloside (**98**) are 10-*O*-caffeoyl derivatives of scandoside methyl ester and methyl deacetyl asperulosidate, respectively. Thus, it has been assumed that caffeoyl moiety is responsible for scavenging diphenylpicrylhydrazyl and hydroxyl radicals and protection from peroxynitrite mediated oxidation by virtue of its hydrogen donating property [93]. Scavenging activity of scandoside methyl ester (**96**) and methyl deacetyl asperulosidate (**97**) against hydroxyl radicals might be due to contribution of allylic hydroxyl (hydroxyl group on C10 or on C6) which is relatively labile hydrogen to radicals. Interesting, 6,9-*epi*-8-*O*-acetylshanziside methyl ester (**99**), 5,9-*epi*-pensteomoside (**100**) and 5,9-*epi*-7,8-didehydropenstemoside (**101**) isolated from rhizomes of *Eremostachys glabra* (Rosaceae) demonstrated antioxidant properties tested in the same assay [94]. Also gentiopicroside (**102**), a secoiridoid glycoside isolated from the aerial parts of *Centaurium erythraea* (Gentianaceae) exhibited free radical scavenging activity with a reducing 50% free radical concentration (RC_{50}) value of 7.5×10^{-2} mg/ml [95]. Another study reported the free radical scavenging activity of oleuropein (**93**), isolated from *Syringa dilatata* (Oleaceae). This compound showed an IC_{50} value of 40.4 μM comparing with the known antiradical compound L-ascorbic acid [96]. Genipin (**4**), isolated from *Gardenia volkensii* (Rubiaceae), showed radical scavenging activity at a loading of 0.5 μg per spot when tested by DPPH assay on TLC plates [97]. Ultraviolet-B (UVB) irradiation has been demonstrated to produce ROS in the cells and skin, which induces the synthesis of matrix metalloproteinases causing skin photoaging. Using the human skin fibroblast HS68 cell line the photoprotective effects of aucubin (**14**) from *Eucommia ulmoides* (Eucommiaceae) was investigated [98]. Pre-treatment with **14** significantly inhibited the production of matrix metalloproteinase-1 by 57% when compared to the UVB-irradiated cells. The GSH content was significantly reduced in UVB irradiated cells while aucubin inhibited UVB induced decline in GSH in skin fibroblasts. Moreover, with the inclusion of aucubin (**14**), lipid peroxidation was inhibited during UVB irradiation. Based upon these results, it was suggested that aucubin (**14**) might play an important role in the cellular defence mechanism against UV radiation-induced photoaging.



2.5. Hepatoprotective Properties

Picroliv has been shown to have a marked hepatoprotective activity against many hepatotoxic compounds such as alcohol, aflatoxin B_1 and oxytetracycline [99a-c]. This effect has been attributed to a stabilizing action on the cell membrane of the hepatocytes, which was possibly related to the ability of picroliv to act as an oxygen free-radical scavanger that limits lipid-peroxidation involved in membrane damage elicited by hepatotoxins. The hepatoprotective activity of picroliv to provide protection against the biochemical alterations produced by CCl_4 and *E. histolytica* was also evaluated [100]. Significant recovery obtained in serum enzyme levels in all animal models and against amoebic liver abscess in gerbils on treatment with picroliv indicated that it possesses therapeutic activity against *E. histolytica* induced hepatic damage. The therapeutic efficacy of picroliv was investigated in male rats exposed to $CdCl_2$ (0.5 mg/kg, *s.c.*), 5 days/week for 18 weeks [101]. Picroliv at two doses (6 and 12 mg/kg, *p.o.*) was given to the cadmium (Cd)-administered group for the last 4 weeks (i.e., weeks 15-18). The Cd altered oxidative stress indices, such as increased lipid peroxidation and membrane fluidity, reduced levels of non-protein sulphydryls (NPSHs) and Na^+K^+ATPase activity in the liver and kidney were found close to the control values by picroliv treatment, suggesting its antioxidant potential. The hepatoprotective action of picroliv was evident by its ability to lower the Cd-induced liver function parameters. Thirteen non-glycosidic iridoids and iridoid glycosides were isolated from *Neopicrorhiza scrophulariiflora* (Scrophulariaceae). The hepatoprotective activities of these compounds were assessed by measuring their effects on the release of alanine aminotransferase from the primary cultures of mice hepatocytes injured by CCl_4 *in vitro*. Seven non-glycosidic iridoids, such as piscrocin D (**103**), piscrocin E (**104**), rehmaglutin A (**105**), rehmaglutin D (**106**), (-)-3'-methoxyspecinonin (**107**), piscrocin F (**108**) and piscrocin G (**109**), displayed potent activities with IC_{50} values ranging from 1.7 to 3.9 μM, comparable to that of well known hepatoprotective glycyrrhizic acid (IC_{50} 4.7 μM). Among the iridoid glycosides, piscroside B (**110**), picroside II (**13**), picroside I (**12**) and picroside III (**111**) exhibited potent hepatoprotective effects *in vitro* with IC_{50} values of 7.3, 4.7, 8.3 and 5.9 μM, respectively [102]. However, piscroside A (**112**) and pikuroside (**113**), possessing acetal linkage between C3 and C10, were found to lack the inhibitory activity. It is likely that iridoids with a glycosidic linkage at C1 are less active than those non-glycosidic iridoids. It is known that iridoid glycosides are instable in the gastrointestinal tract and could be transformed to their corre-

sponding non-glycosidic iridoids. These observations seem to be in agreement to the hypothesis that these major iridoid glycosides could be considered as pro-drugs and those non-glycosidic iridoids derived from the parent compounds contain the pharmacophores [7b]. Iridoid enriched fraction (IF) from *Barleria prionitis* (Acanthaceae) aerial parts was evaluated for hepatoprotective activity in various acute and chronic animal test models of hepatotoxicity [103]. Oral administration of IF showed significant hepatoprotective activity in both preventive and curative treatments against hepatotoxins induced hepatic damage and subsequent recovery towards normalization of these enzymes strongly points out the possibility of IF being able to condition the hepatocytes so as to cause accelerated regeneration of parenchymal cells. Results indicate that IF afforded protection by attributing to its antioxidant properties that may inhibit the deleterious effect of free radicals generated by hepatotoxins regimen [104]. The suppressive effect of penta-acetyl geniposide (**20**) on the hepatotoxic lesions-induced by aflatoxin B_1 (AFB_1) was investigated in male Wistar rats [105]. The treatment with the iridoid (**20**) significantly reduced the number of AFB_1-induced γ-glutamyl transpeptidase (GGT)-positive foci.

2.6. Antimicrobial Activity

2.6.1. Antibacterial Properties

A large number of studies reported the antibacterial activity of iridoids [7a]. Among of them isoplumericin (**114**), plumericin (**29**), galioside (**115**) and gardenoside (**3**) were reported posses antibacterial activity [106a, b]. An interesting activity on *Staphylococcus aureus* was also found when aucubin (**1**) was applied in culture in the presence of β-glucosidase [107]. A secoiridoid glycoside with a significant activity against *Serratia marcescens* (MIC 6.3×10^{-3} mg/ml) was identified as gentiopicroside (**102**) [95]. Moderate antibacterial activity against *Bacillus cereus*, *B. pumulus*, *B. subtilis*, *Micrococcus kristinae*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aerugenosa*, *Enterobacter cloacae* was found when sweroside (**26**) isolated from the rootstock of *Scabiosa columbria* (Dipsacaceae) was used [108].

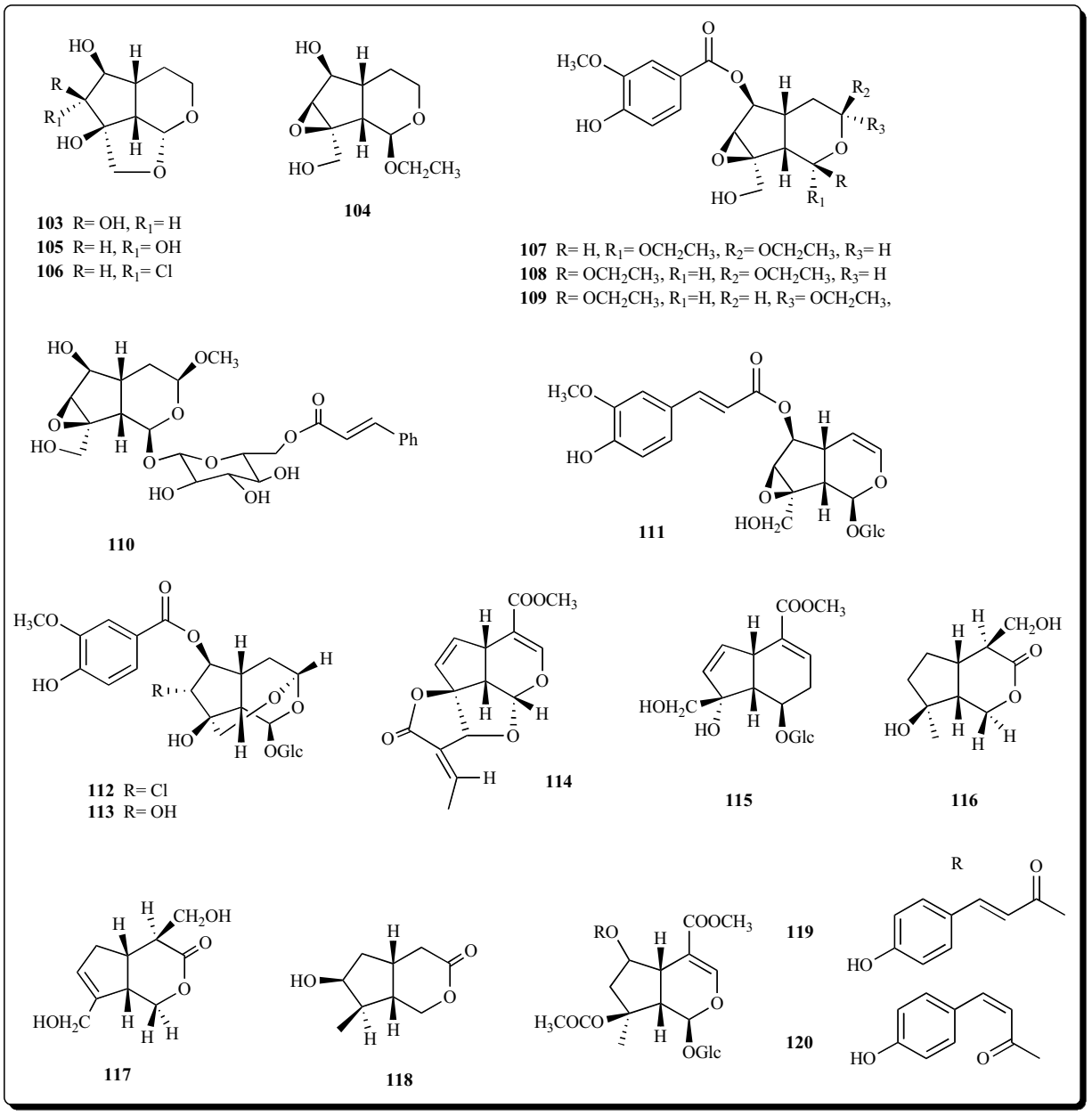
A series of non-glycosidic iridoids including mussaenin A (**116**), gardendiol (**117**), isoboonein (**118**) and rehmaglutin D (**106**) isolated from *Cymbaria mongolica* (Scrophulariaceae) exhibited a strong antibacterial activity on against *Bacillus subtilis, Escherichia coli* and *Staphylococcus aureus*. It is noted that all non-glycosidic iridoids possess antibacterial activity close to that of chloramphenicol [49].

2.6.2. Antiviral Properties

Recently a large number of studies reported the antiviral activity of iridoids. Arbortristoside A (**80**) and C (**81**) displayed pronounced antiviral activity against encephalitis causing viruses [109]. The 6-*O*-*trans*-*p*-coumaroyl-8-*O*-acetylshanzhiside methyl ester (**119**) and its *cis* isomer (**120**) isolated from *Barleria prionitis* (Acanthaceae) tested in a mixture 3:1 demonstrated potent *in vitro* activity against respiratory syncytial virus RSV (A2 strain) in a cell culture-based CPE assay (EC_{50} 2.46 μg/ml, IC_{50} 42.2 μg/ml) [110]. Aucubin (**14**) suppressed hepatitis B viral DNA replication *in vitro*. Conversion of aucubin (**14**) to its aglycone form appeared to be a prerequisite step for an exhibition of such antiviral activity [111]. From *Ligustrum lucidum* (Oleaceae) fruits oleuropein (**93**) and lucidumoside C (**121**) were isolated and tested *in vitro* for their activities against four strains of pathogenic viruses, namely respiratory syncytial virus and para-influenza type 3 virus. Oleuropein (**93**) showed significant antiviral activities against respiratory syncytial virus and para-influenza 3 virus with IC_{50} value of 23.4 and 11.7 μg/ml, respectively. Lucidumoside C (**121**), oleoside dimethylester (**122**) and ligustroside (**63**) showed a more selective antiviral activity against Para 3 virus with IC_{50} values of 15.6-20.8 μg/ml [112]. Seven iridoids were isolated from *Bupleurum rigidum* (Apiaceae) and *Scrophularia scorodonia* (Scrophulariaceae) and tested *in vitro* for their antiviral properties against herpes simplex type I (HSV-1), vesicular stomatitis virus (VSV) and poliovirus type 1 [113]. The percentages of cellular viability at the non-toxic limit concentrations of the active compounds against VSV were: 8-acetylharpagide (**30**) 32.1% at 500 μg/ml, harpagoside (**10**) 43.3% at 450 μg/ml and scorodioside (**63**) 47.8 % at 500 μg/ml. Moreover the iridoid scorodioside (**67**) showed *in vitro* anti-HSV-1 activity (30.6% at 500 μg/ml). Active against this type of virus was found also ipolamiidoside (**123**), an iridoid glucoside isolated from *Barleria lupulina* (Acanthaceae). This compound showed antiviral properties without any cytotoxic effects on the Vero cells [114]. Fulvoplumierin (**124**) from *Plumeria rubra* (Apocynaceae) inhibited HIV-1 and 2 reverse transcriptase [115]. Olive leaves extract showed strong anti-HIV activity inhibiting acute infection and cell-to-cell transmission of HIV-1 [116]. One of the suspected targets for olive leaves extract action was HIV-1 gp41 which is responsible for HIV entry into normal cells. In order to establish HIV protein targets of olive leaves extract and its inhibitory action at molecular level, a joint theoretical and experimental effort has been carried out to help achieve this goal [117]. Olive leaves extract was known to contain a mixture of several compounds, among them oleuropein (**93**) which was readily absorbed and bioavailable. Performed systematic computational studies to investigate possible binding complexes with gp41 through molecular docking, molecular dynamics simulation and free energy calculations were recently reported [118]. Specific binding modes from docking studies were analyzed and molecular dynamics simulation was performed to study molecular interaction, binding mechanism, stability of the binding complexes and binding affinities for oleuropein (**93**) and several of its main metabolites with gp41. Simulation showed that the conserved hydrophobic cavity located at the N-terminal of gp41 core N36 trimer structure is the most possible binding site. The electrostatic interaction plays the key role in determining the affinities of the ligand binding, and as a result, the dihydroxyl phenol ring is the crucial group for the anti-HIV membrane fusion activity.

2.6.3. Anti- Leishmanial and Molluscicidal Properties

Bio-guided fractionation of *Himatanthus sucuuba* (Apocynaceae) stem bark, one of the most active plants against the intracellular form of the parasite, resulted in the isolation of two spirolactone iridoids: plumericin (**29**) and isoplumericin (**114**) [119]. Both compounds exhibited a strong activity against *Leishmania amazonensis* axenic amastigotes.



Isoplumericin (**114**) showed toxicity against infected macrophages which did not allow an evaluation of its activity against intracellular amastigotes. Plumericin (**29**) caused a reduction of the macrophage infection similar to amphotericin B, the drug used more frequently when patients do not respond to antimonials, the first line chemotherapy (IC_{50} of 0.9 μM for plumericin and 1 μM for amphotericin B). This difference between the effects of the two isomers on infected macrophages should be of great interest, since the radical group orientation influences the antiparasitic activity of some compounds [120]. Recently, extracts, fractions and some isolated iridoids from *Morinda morindoides* (Rubiaceae) leaves were tested for their potential *in vitro* antiamoebic activity [121]. All iridoids showed a very good antiamoebic activity. The most active were epoxygaertneroside (**125**) with IC_{50} value of 1.3 μg/ml and methoxygaertneroside (**126**) with IC_{50} value of 2.3 μg/ml followed by gaertneroside (**127**) and gaertneric acid (**128**) with IC_{50} values of 4.3 and 7.1 μg/ml, respectively. These results indicated that the presence of a methoxyl group in C3' position or an epoxyl group between C6 and C7 position is important for a pronounced antiamoebic activity although the presence of an epoxyl group in epoxygaertneroside (**125**) did not have a significant influence in the activity of compound methoxygaertneroside (**126**). The presence of a carboxyl group in C14 position decreased the activity. In a previous investigation, iridoids isolated from *Scrophularia lepidota* have been tested for their anti-protozoal and inhibitory effect towards plasmodial enoyl-ACP reductase, a key enzyme of fatty acid biosynthesis in *Plasmodium falciparum* [122]. All iridoids showed

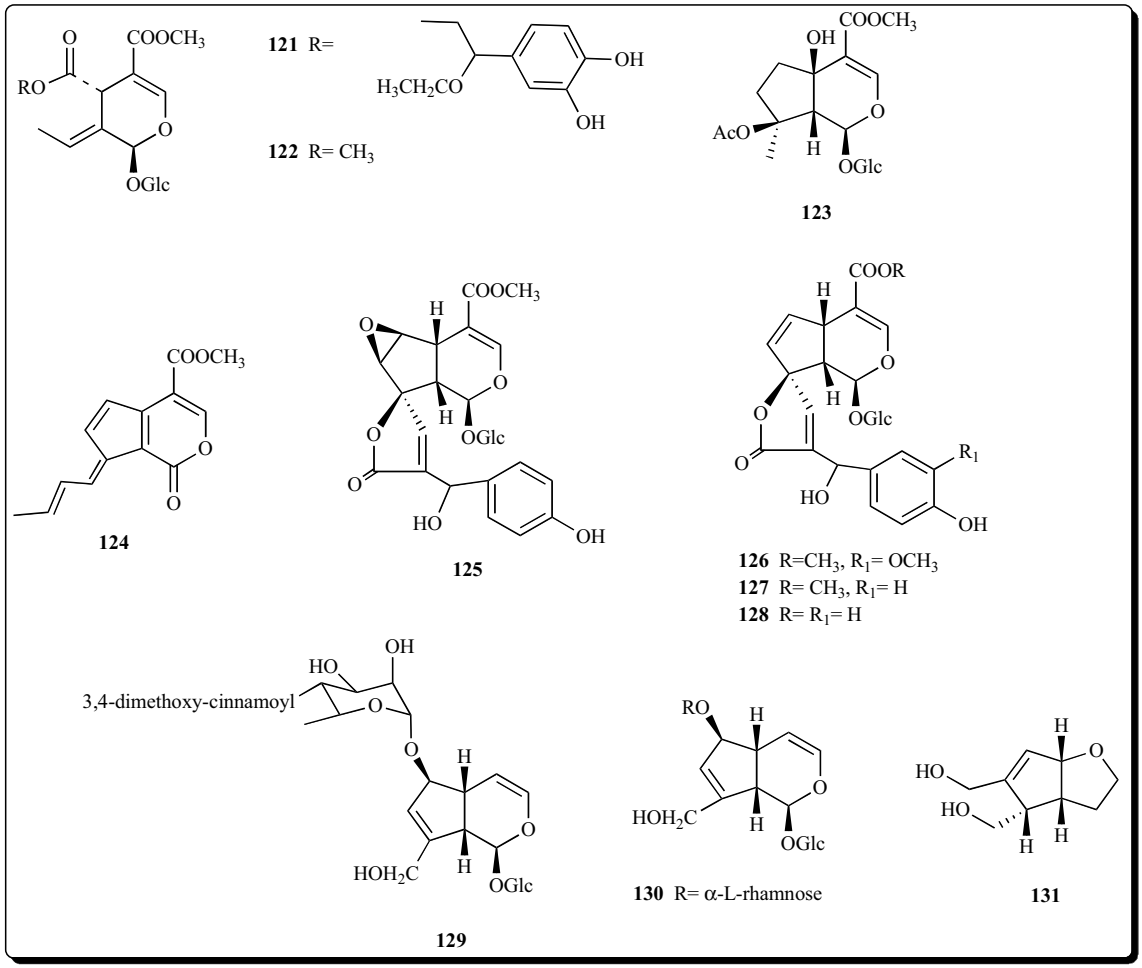
appreciable activity against the amastigote forms of *L. donovani*, with the new compound scrolepidoside (**129**) being the most potent (IC_{50} 6.1 μg/ml). None of the isolates showed trypanocidal activity against *T. cruzi*, but all compounds, except sinuatol (**130**), exhibited some potential against *T. brucei rhodesiense* with IC_{50} values ranging from 29.3 to 73.0 μg/ml. Although the crude extract exhibited growth inhibitory activity (IC_{50} 17.5 μg/ml) against chloroquine- and pyrimethamine-resistant (K1) strain of *P. falciparum*, the single iridoids were inactive or not as potent as anticipated from the extract. Only the minor compound (**131**) exhibited moderate anti-plasmodial activity with an IC_{50} value of 40.6 μg/ml. When screened for FabI inhibitory potential, again only (**131**) showed weak enzyme inhibitory potential (IC_{50} 100 μg/ml), retaining the activity of the crude EtOH extract (IC_{50} 80 μg/ml). This suggests that these kinds of natural compounds are potent inhibitors of the growth of some parasites. Amarogentin (**19**), a secoiridoid glycoside isolated from *Swertia chirata* (Gentianaceae), when evaluated at a concentration higher than 60 μM inhibited the catalytic activity of the topoisomerase I from *L. donovani*. Apparently, **19** exert its inhibitory effect by binding to the enzyme and preventing the formation of a binary complex with DNA. A similar mechanism of action has been reported for Pentostam® [123]. Recently, the evaluation of amarogentin (**19**) in the form of liposomes and niosomes, has been reported to show a greater leishmanicidal activity (and with no toxic effects) than those observed for free **19** when tested in hamsters. On the basis of these results this metabolite has been proposed for clinical application in the treatment of leishmaniasis [124].

2.7. Miscellaneous Properties

A number of iridoids possess widely different bioactivities which were presented as follows.

2.7.1. Activity on Cardiovascular System

Recently a large number of reviews discussed the beneficial use of olive oil on human health [125a, b]. From *Olea europea* and *O. lancea* iridoids with angiotensin converting enzyme (ACE) inhibition properties were isolated [126]. One of these iridoids is oleacin (**132**) that act as an irreversible inhibitor of ACE with IC_{50} of 26 μM. To investigate whether the alcohol or the secoiridod part of oleacein is responsible for the ACE inhibitor activity β-(3,4-dihydroxyphenyl)ethanol was synthesized. This compound exerted a low ACE inhibition (26% at 0.33 mg/ml), indicating that the secoiridoid part of oleacein (**132**) is responsible of the biological activity. Other secoiridoids, such as oleuropein (**93**) and its aglycone were tested. All compounds were found not to in-



hibit ACE (0-8% of inhibition at 0.33 mg/ml) but when enzymatically cleaved by β-glucosidase the reaction mixture displayed pronounced ACE-inhibitory activity, with percentage of inhibition at 0.33 mg/ml ranging from 91% to 96%. These results strongly imply that secoiridoid aglycones of the oleoside type generally possess ACE-inhibitory activity. All compounds are characterised by a methyl ester group in position C4 and a variable ester group in C6. Pennacchio *et al*. [127] identified *Eremophila longifolia* and *E. alternifolia* (Myoporaceae) as cardioactive plants. From these plants they isolated the geniposidic acid (**31**) that is able to mediate an inhibitory effect with significant negative chronotropism, negative inotropism and coronary perfusion rate in the Langendorff rat heart. The extension of the study on other *Eremophila* species led to active iridoids melampyroside (**133**), catapol (**1**) and verminoside (**46**). The largest response in negative inotropism was found when melampyroside (**133**) and verminoside (**46**) were used while a reduction of contractile force was observed with catapol (**1**) [128]. Harpagide (**10**) had only a slight negative chronotropic effect and a considerable negative inotropic one, quantitatively similar to the effect of melapyroside (**133**). Geniposidic acid (**31**), scandoside (**134**), feretoside (**135**), 10-*O*-benzoylscandoside methyl ester (**136**), oldenlandoside III (**137**), asperulosidic acid (**54**) and deacetylasperulosidic acid (**138**), isolated from the aerial parts of *Oldenlandia diffusa*, (Rubiaceae) were examined for their ability to inhibit LDL-oxidation [129]. Geniposidic acid (**31**), scandoside (**134**) and deacetylasperulosidic acid (**138**) exhibited similar levels of inhibition of LDL-oxidation, with values of 63.3%, 62.2% and 63.8% inhibition at a concentration of 20 μg/ml. Probucol, a synthetic antioxidant commercially prescribed for the treatment of coronary disease, used as a positive control, reduced LDL-oxidation by 78.1% under the same conditions. The other glycosides also showed significant inhibitory activity. It is rare for natural occurring components to display such high levels of inhibition of LDL-oxidation and these data suggest that the iridoid glycosides found in *O. diffusa* may be valuable agents in treatment of coronary disease. The standardized iridoids fraction picroliv up-regulates the expression of vascular endothelial growth factor in human umbilical vein endothelial cells and of insulin-like growth factor in rats during hypoxia. Picroliv, investigated in an *ex vivo* rat aorta ring model of angiogenesis, enhanced the sprouting and migration of endothelial cells. The data showed also improved re-epithelialisation, neovascularization and migration of various cells such as endothelial, dermal myofibroblasts and fibroblasts into the wound bed after picroliv treatment. Immunohistochemical localization showed increased VEGF and alpha smooth muscle actin staining consistent with an increased number of microvessels in granulation tissue. These findings suggested that picroliv could be developed as a therapeutic angiogenic agent for the restoration of the blood supply in diseases involving inadequate blood supply such as limb ischemia, ischemic myocardium and wound healing [130]. Two iridoid glucosides, catalpol (**1**) and methylcatalpol (**139**), were isolated from the methanol soluble fraction of *Buddleia scordioides* (Scrophulariaceae) leaves and tested for their protective activity against increased skin vascular permeability in rabbits [131]. Methylcatalpol (**139**) 120 min after peritoneal treatment at a dose of 30 mg/kg, exerted a 51.4% inhibitory effect on the capillary permeability in rabbit skin increased by chloroform and histamine at 30 and 90 min, respectively, after the treatment. The effect of this iridoid at a dose of 50 mg/kg was slightly lower (65.8%) than troxerutin (71.3%) used as a positive standard. With catalpol (**1**) the maximum effect was a 52.9% reduction at a dose of 50 mg/kg. The protective microvascular activity was measured as a counteracting effect on the leakage of Evans blue introduced intravenously. Geniposide (**2**) and genipin (**4**) significantly prolonged the time required for thrombotic occlusion induced by photochemical reaction in the mouse femoral artery. In particular, both compounds inhibited collagen-induced, but did not inhibit arachidonate-induced mouse platelet aggregation [132].

2.7.2. Hypoglycaemic and Hypolipidemic Properties

Some iridoids were found active in some metabolic disorders. One of these is oleuropein (**93**) that showed an hypoglycaemic effect and increasing the glucose tolerance *in vivo* [133]. Recently, the mechanism of action of this iridoids was clarified demonstrating potential and beneficial effect of this iridoid in attenuating oxidative stress and enhancing of body's own antioxidant defences in diabetic rabbits with established oxidative stress and may add another explanation of the hypoglycaemic effect of oleuropein (**93**) through its action as an antioxidant [134]. Able to reduce the blood glucose levels in diabetic-alloxan rats were found scropolioside-D_2 (**69**), harpagoside-B (**70**), scropolioside-D (**71**), koelzioside (**72**) and 8-*O*-acetylharpagide (**30**), isolated from *Scrophularia deserti* (Scrophulariaceae). The iridoids decreased blood glucose levels in the range of 5.88 to 17.0% after 1 h and 0.23 to 29.0% after 2 h with respect to their initial values at a dose level of 10 mg/kg *p.o.* Scropolioside-D (**71**) and 8-*O*-acetylharpagide (**30**) were found to be the most active exhibiting decrease of 31.47 and 17.0% after 1 h and 34.0 and 29.0% after 2 h, respectively [81]. SARs were also established and compounds containing one cinnamoyl moiety at C3" as in scropolioside-D (**71**) have greater activity. The introduction of one more cinnamoyl moiety in place of the acetyl unit at C4" as in koelzioside (**72**) resulted in a decrease in activity. The introduction of an acetyl group at C6 as found in scropolioside-D_2 (**69**) further decreased the activity in an epoxy series. On the other hand, an acetyl group at C8 as found in 8-*O*-acetyl harpagide (**30**) is necessary for significant antidiabetic activity, whereas the replacement of the acetyl group with a cinnamoyl unit as obtained in harpagoside-B (**70**) lost the activity in a non-epoxy series of iridoid glycosides. Previously, the *in vivo* hypoglycaemic activity of deacetylasperulosidic acid (**138**) methyl ester was reported. This iridoid isolated from leaves of *G. jasminoides* (Rubiaceae) lowered the blood glucose level in normal mice. The absolute configuration of hydroxyl group in position C6 should be essential for the biological activity [135]. Total iridoid glycosides fraction from *Cornus officinalis* (Cornaceae), which consist mainly of morroniside (**140**) and loganin (**85**), was found to be active in regulating expression of transforming growth factor β1 and preventing overdeposition of fibronectin and laminin in renal extracellular matrix [136]. For these reason total glycosides from *C. officinalis* are considered beneficial for prevention and therapy of diabetic nephropathy.

2.7.3. Wound Healing Properties

Wound healing properties of *Gentiana lutea* ssp. *symphyandra* (Gentianaceae) extract and its main constituents swertiamarin (**141**), sweroside (**26**) and gentiopicroside (**102**) were evaluated by comparison with dexpanthenol on cultured chicken embryonic fibroblasts [137]. The activity of *G. lutea* ssp. *symphyandra* seems to be mainly due to the increase in the stimulation of collagen production and the mitotic activity by sweroside (**26**) and swertiamarin (**141**), respectively. All three compounds also exhibited cytoprotective effects, which may cause a synergism in terms of wound healing activity of *G. lutea* ssp. *symphyandra*.

2.7.4. Choleretic Properties

The ability of patrinoside (**142**) and villoside (**143**) to accelerate bile excretion when administered intravenously in rats was previously demonstrated [138]. The elucidate mechanism evidenced that the hemiacetal moiety of iridoid compounds plays an important role in exerting a strong choleretic action. Picroliv induced the increase volume of bile, concentrations of bile salts, cholic and deoxycholic acid in a dose-dependent manner when administered in rats and guinea pigs [112]. The choleretic effect of Japanese traditional herbal medicine Inchin-ko-to was recently studied [139]. Genipin (**4**), one of the main components of this herbal formulation, demonstrated to enhance the bile acid-independent secretory capacity of hepatocytes, mainly by stimulation of exocytosis and insertion of Mrp2 in the bile canaliculi.

2.7.5. Antiallergic Properties

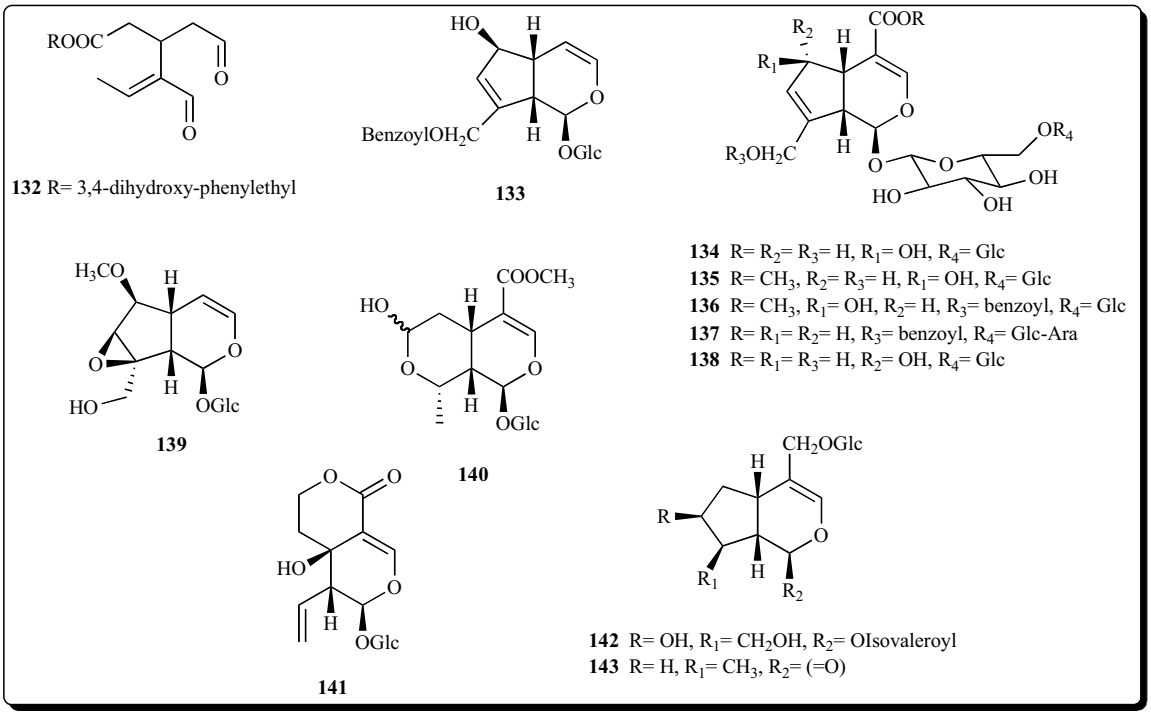
Verproside (**45**), a constituent of *Pseudolysimachion longifolium* (Scrophulariaceae), which belongs to catapol (**1**) analogues, exhibited an antiasthmatic effect by the suppression of elevated IgE, IL-4 and IL-13 levels and eosinophilia in the plasma and mucus overproduction in the lung tissues in an ovalbumin-induced asthmatic mouse model [140].

2.7.6. Anxyolitic Properties

Kamishoyosan, a traditional herbal medicine, consists of ten crude herbal drugs and is used to treat mental symptoms in menopausal women, for anxiety, insomnia, irritability or depression during menopausal and for treating affective disorders. This formula increased social interaction behavior in mice, indicating that Kamishoyosan exerted an anxiolytic effect. To identify the plants responsible for this activity, the effects of the individual component plants were examined. Among tested species *Gardeniae Fructus* exhibited the most interesting activity. Its major component, geniposide (**2**), increased the social interaction time at a dose of 20 and 40 mg/kg demonstrating to exert an anxiolytic effects in a dose-dependent manner [141].

CONCLUDING REMARKS

The object of this review article was to gather and present an up-to-date of the major and interesting biological and pharmacological activities of naturally occurring iridoids and secoiridoids, that represent a promising and expanding platform of active natural compounds whose potential is currently only partially explored. A number of iridoids could be compared very favourably with drugs in current use. The frequent observations that the aglycones exhibit a higher activity than the parent glycosides suggest that glycosides can be reasonably considered as a pro-drug and the pharmacophore is associated with the more reactive polyfunctional entities which are released. Several recent studies have been demonstrated that iridoids are able to play a role in the neuroprotection of cerebral ischemia and progressive neurodegenerative diseases such as Alzheimer's and Parkinsons's



diseases. Within the current renewed in iridoids for medicinal purposes, their beneficial effects as antitumor and anti-inflammatory agents have also been demonstrated in different experimental models. In conclusion to this review, it is suggest that research and development of drugs based on iridoids could be a key program in a number of research institutions and pharmaceutical industries.

ABBREVIATIONS

$(Ac)_5$-GP	=	Penta-acetyl-geniposide
ACE	=	Angiotensin Converting Enzyme
AFB_1	=	Aflatoxin B_1
ARE	=	Antioxidant-Responsive Element
BDNF	=	Brain-Derived Neurotrophic Factor
BDNF	=	Brain-Derived Neutrophic Factor
CNS	=	Central Nervous System
COX	=	Cyclooxygenase
DMBA	=	7,12-Dimethyl-benz(a)anthracene
DPPH	=	Diphenylpicrylhydrazyl
EBV-EA	=	Epstein-Barr virus early antigen
fMLP	=	N-formyl-methionyl-leucyl-phenylalanine
GAP	=	Growth-associated phosphoprotein
GGT	=	γ-Glutamyl transpeptidase
GI_{50}	=	50% Growth Inhibition
GLP	=	Glucagone-like peptide
GST	=	GSH *S*-transferase
HIV	=	Human Immunodeficiency Virus
HSV	=	Herpes Simplex Virus
IC_{50}	=	Inhibitory Concentration 50%
iNOS	=	Inducible nitroxide synthase
LC_{50}	=	Lethal Concentration 50%
LD_{50}	=	Lethal Dose 50%
LOX	=	5-lipoxygenase
LPS	=	Lipopolysaccharide
MAPK	=	Mitogen activated protein kinase
MEK	=	MAP-extracellular regulated kinase
MPP^+	=	1-methyl-4-phenyl-pyridinium
MPTP	=	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MTT	=	Methyl Thiazolyl Tetrazolium
NF	=	Nuclear Factor
NGF	=	Nerve Growth Factor
NO	=	Nitroxide
OGD	=	Oxygen and Glucose Deprivation
PKC	=	Protein Kinase C
PMA	=	phorbol-12-Myristate-13-Acetate
PMN	=	Peripheral Human Neutrophil
RC_{50}	=	Reducing 50% free radical concentration
ROS	=	Reactive Oxygen Species
SAR	=	Structure-Activity Relationships
SOD	=	Superoxide Dismutase
Taq	=	*Thermus aquaticus*
TBDPS	=	*tert*-Butyldiphenylsilyl
TBS	=	*tert*-Butyldimethylsilyl
TNF-α	=	Tumor Necrosis Factor-α
TPA	=	12-*O*-Tetradecanoyl-13-acetate
VSV	=	Vesicular stomatitis virus

REFERENCES

[1] Hegnauer, R. *Chemotaxonomie der Pflanzen*, Birk-häuser Verlag: Basel, **1986**.
[2] El-Naggar, L.J.; Beal J.L. *J. Nat. Prod.*, **1980**, *43*, 649.
[3] (a) Inouye, H.; Uesato, S. In *Progress in the chemistry of organic natural products*, Herz, W.; Grisebach, H.; Kirby, G.W.; Tamm Ch., Eds; Springer-Verlag: Wien, **1986**; Vol. *50*, pp. 169-236. b) Junior, P. *Planta Med.*, **1990**, *56*, 1. c) Bianco, A. *Stud. Nat. Prod. Chem.*, **1990**, *7*, 439. d) Jensen, R.S. In *Ecological chemistry and biochemistry of plant terpenoids*. Harborne, J.B.; Tomas-Barberan, F.A., Eds; Proc. Phytochem. Soc. Europe, Clarendon Press: Oxford, **1991**.
[4] Boros, C.A.; Stermitz, F.R. *J. Nat. Prod.*, **1990**, *53*, 1055. b) Boros, C.A.; Stermitz, F.R. *J. Nat. Prod.*, **1991**, *54*, 1173.
[5] Al-Hazimi, H.M.G.; Alkhathlan, H.Z. *J. Chem. Soc. Pak.*, **1996**, *18*, 336.
[6] Dinda, B.; Debnath, S.; Harigaya, Y. *Chem. Pharm. Bull.*, **2007**, *55*, 159.
[7] (a) Sticker, O. In *New natural products and plant drugs with pharmacological, biological, and therapeutic activity*. Wagner, H.; Wolff, P. Springer-Verlag: Berlin, **1977**. b) Ghisalberti E.L. *Phytomedicine*, **1998**, *5*, 147. c) Dinda, B.; Debnath, S.; Harigaya, Y. *Chem. Pharm. Bull.*, **2007**, *55*, 689.
[8] Vajda, F.J. *J. Clin. Neurosci.*, **2002**, *9*, 4.
[9] Albright, T.D.; Jessell, T.M.; Kandel, E.R.; Poster, M.I. *Cell*, **2000**, *18*, 1.
[10] Heintz, N.; Zoghbi, H.Y. *Annu. Rev. Physiol.*, **2000**, *62*, 779.
[11] Michaelis, M.L. *J. Pharmacol. Exp. Ther.*, **2003**, *304*, 897.
[12] Meldrum, B.S. *Prog. Brain Res.*, **2002**, *135*, 487.
[13] (a) Li, D.Q.; Bao, Y.M.; Zhao, J.J.; Liu, C.P.; Liu, Y.; An, L.J. *Brain Res*., **2004**, *1029*, 179. b) Li, D.Q.; Duan, Y.L.; Bao, Y.M.; Liu, C.P.; Liu, Y.; An, L.J. *Neurosci. Res.*, **2004**, *50*, 169. c) Jiang, B.; Liu, J. H.; Bao, Y. M.; An, L. *J. Toxicon.*, **2004**, *43*, 53.
[14] Li, D.Q.; Li, Y.; Liu, Y.; Bao, Y.M.; Hu, B.; An, L.J. *Toxicon.*, **2005**, *15*, 845.
[15] Tian, Y.Y.; An, L.J.; Jiang, L.; Duan, Y.L.; Chen, J.; Jiang, B. *Life Sci.*, **2006**, *80*, 193.
[16] Li, D.Q.; Bao, Y.M.; Li, Y.; Wang, C.F.; Liu, Y.; An, L.J. *Brain Res*., **2006**, *1115*, 179.
[17] Tian, Y.Y.; Jiang, B.; An, L.J.; Bao, Y.M. *Eur. J. Pharmacol.*, **2007**, *568*, 142.
[18] Burke, S.N.; Barnes, C.A. *Nat. Rev. Neurosci*., **2006**, *7*, 30.
[19] Liu, J.; He, Q.J.; Zou, W.; Wang, H.X.; Bao, Y.M.; Liu, Y.X.; An, L.J. *Brain Res.*, **2006**, *1123*, 68.
[20] Routtenberg, A.; Cantallops, I.; Zaffuto, S.; Serrano, P.; Namgung, U. *Proc. Natl. Acad. Sci. U. S. A*., **2000**, *97*, 7657.
[21] Lee, P.; Lee, J.; Choi, S.Y.; Lee, S.E.; Lee, S.; Son, D. *Biol. Pharm. Bull.*, **2006**, *29*, 174.

[22] Yamazaki, M.; Sakura, N.; Chiba, K.; Mohri, T. *Biol. Pharm. Bull.*, **2001**, *24*, 1454.
[23] Liu, J.; Zheng, X.; Yin, F.; Hu, Y.; Guo, L.; Deng, X.; Chen, G.; Jiajia, J.; Zhang, H. *Int. J. Dev. Neurosci.*, **2006**, *24*, 419.
[24] Perry, T.; Haughey, N.J.; Mattson, M.P.; Egan, J.M.; Greig, N.H. *J. Pharmacol. Exp. Ther.*, **2002**, *302*, 881.
[25] Perry, T.; Greig, N.H. *J. Alzheimers Dis.*, **2002**, *4*, 487.
[26] Yamazaki, M.; Chiba, K.; Mohri, T. *Biol. Pharm. Bull.*, **1996**, *19*, 791.
[27] Yuan, Z.Z.; Zhu, L.Q.; Pang, H.; Shan, Z.S.; Wang, S.R.; Gao, Y.H.; Niu, F.L. *Zhongguo Zhong Yao Za Zhi*, **2007**, *32*, 249.
[28] Kim, S.R.; Lee, K.Y.; Koo, K.A.; Sung, S.H.; Lee, N.G.; Kim, J.; Kim, Y.C. *J. Nat. Prod.*, **2002**, *65*, 1696.
[29] (a) Kim, Y.; Park, E.J.; Kim, J.; Kim, Y.B.; Kim, S. R.; Kim, Y.C. *J. Nat. Prod.* **2001**, *64*, 75. b) Kim, S.R.; Sung, S.H.; Jang, Y.P.; Markelonis, G.J.; Oh, T.H.; Kim, Y.C. *Br. J. Pharmacol.,* **2002**, *135,* 1281.
[30] Kim, S.R.; Koo, K.A.; Sung, S.H.; Ma, C.J.; Yoon, J.S.; Kim, Y.C. *J. Neurosci. Res.*, **2003**, *15*, 948.
[31] Zhang, Z.J.; Li, P.; Wang, Z.; Li, P.T.; Zhang, W.S.; Sun, Z.H.; Zhang, X.J.; Wang, Y.Y. *Brain Res.*, **2006**, *1123*, 188.
[32] (a) Middlemiss, P.J.; Glasky, A.J.; Rathbone, M.P.; Werstuik, E.; Hindley, S.; Gysbers, J. *Neurosci. Lett.*, **1995**, *199*, 131. b) Pradines, A.; Magazin, M.; Schiltz, P.; Le Fur, G.; Caput, D.; Ferrara, P. *J. Neurochem.*, **1995**, *64*, 1954.
[33] Li, P.; Matsunaga, K.; Yamakuni, T.; Ohizumi, Y. *Eur. J. Pharmacol.*, **2000**, *406*, 203.
[34] Cragg, G.M.; Newman, D.J. *Cancer Invest*., **1999**, *17*, 153.
[35] (a) Wang, C.J.; Wang, S.W.; Lin, J.K. *Cancer Lett.*, **1991**, *60*, 95. b) Kang, J.J.; Wang, H.W.; Liu, T.Y.; Chen, Y.C.; Ueng, T.H. *Food Chem. Toxicol.*, **1997**, *35*, 957.
[36] Nakamura, T.; Nakazawa, Y.; Onizuka, S.; Satoh, S.; Chiba, A.; Sekihashi, K.; Miura, A.; Yasagahira, N.; Sasaki, Y.F. *Mut. Res.*, **1997**, *388*, 7. b) Koo, H.J.; Lee, S.; Shin, K.H.; Kim, B.C.; Lim, C.J.; Park, E.H. *Planta Med.*, **2004,** *70*, 467.
[37] Gálvez, M.; Martin-Cordero, C.; Ayuso, M.J. *J. Enzyme Inhib. Med. Chem.*, **2005**, *20*, 389.
[38] Umemura, K.; Yanase, K.; Suzuki, M.; Okutani, K.; Yamori, T.; Andoh, T. *Biochem. Pharmacol.*, **2003**, *66*, 481.
[39] Willmore, E.; Errington, F.; Tilby, M.J.; Austin, C.A. *Biochem. Pharmacol.*, **2002**, *63*, 1807.
[40] Akao, T.; Kobashi, K.; Aburada, M. *Biol. Pharm. Bull.*, **1994**, *17*, 1573.
[41] Kuo, W.H.; Wang, C.J.; Young, S.C.; Sun, Y.C.; Chen, Y.J., Chou, F.P. *Pharmacology*, **2004**, *70*, 15.
[42] Kuo, W.H.; Chou, F.P.; Young, S.C.; Chang, Y.C.; Wang, C.J. *Toxicol. Appl. Pharmacol.*, **2005**, *208*, 155.
[43] (a) Pungitore, C.R.; Ayub, M.J.; Borkowski, E.J.; Tonn, C.E.; Ciuffo, G.M. *Cell Mol. Biol.*, **2004**, *50*, 767. b) Pungitore, C.R.; Leon, L.G.; Garcia, C.; Martin, V.S.; Tonn, C.E.; Padron, J.M. *Bioorg. Med. Chem. Lett.*, **2007**, *17*, 1332.
[44] Saha, P.; Mandal, S.; Das, A.; Das, S. *Cancer Lett.*, **2006**, *244*, 252.
[45] (a) Chang, Y.C.; Chou, F.P.; Huang, H.P.; Hsu, J.D.; Wang, C.J. *Toxicol. Appl. Pharmacol.*, **2004**, *198*, 11. b) Peng, C.H.; Huang, C.N.; Wang, C.J. *Curr Cancer Drug Targets*, **2005**, *5*, 299.
[46] Peng, C.H.; Huang, C.N.; Hsu, S.P.; Wang, C.J. *Mol. Pharmacol.*, **2006**, *70*, 997.
[47] Peng, C.H.; Tseng, T.H.; Huang, C.N.; Hsu, S.P.; Wang, C.J. *Toxicol. Appl. Pharmacol.*, **2005**, *15*, 172.
[48] Fukuyama, Y.; Minoshima, Y.; Kishimoto, Y.; Chen, I.S.; Takahashi, H.; Esumi, T. *Chem. Pharm. Bull.*, **2005**, *53*, 125.
[49] Dai, J.Q.; Liu, Z.L.; Yang, L. *Phytochemistry*, **2002**, *59*, 537.
[50] Ishiguro, K.; Yamaki, M.; Takagi, S.; Ikeda, Y.; Kawakami, K.; Ito, K.; Nose, T.J. *Pharmacobiodyn.*, **1988**, *11*, 131.
[51] Nguyen, A.T.; Foutaine, J.; Malonne, H.; Claeys, M.; Luhmer, M.; Duez, P. *Phytochemistry*, **2005**, *66*, 1186.
[52] Mouriès, C.; Rakotonframasy, V.C.; Libot, F.; Koch, M. ; Tillequin, F.; Deguin, B. *Chem. Biodivers.*, **2005**, *2*, 695.
[53] Kardono, L.B.; Tsauri, S.; Padmawinata, K.; Pezzuto, J. M.; Kinghorn, A.D. *J. Nat. Prod.*, **1990**, *53*, 1447.
[54] Konoshima, T.; Takasaki, M.; Tokuda, H.; Nishino, H. *Cancer Lett.*, **2000**, *31*, 87.
[55] Akihisa, T.; Matsumoto, K.; Tokuda, H.; Yasukawa, K.; Seino, K.; Nakamoto, K.; Kuninaga, H.; Suzuki, T.; Kimura, Y. *J. Nat. Prod.*, **2007**, *70,* 754.
[56] Takasaki, M.; Tokuda, H.; Nishino, H.; Konoshima, T. *J. Nat. Prod.*, **1999**, *62*, 972.
[57] Hsu, H.Y.; Yang, J.J.; Lin, S.Y.; Lin, C.C. *Cancer Lett.*, **1997**, *26*, 31.
[58] Wysokinska, H.; Skrzypek, Z. *J. Nat Prod.*, **1992**, *55*, 58.
[59] Kapadia, G.J.; Sharma, S.C.; Tokuda, H.; Nishimo, H.; Ueda, S. *Cancer Lett.*, **1996**, *102*, 223.
[60] Sang, S.; Liu, G.; He, K.; Zhu, N.; Dong, Z.; Zheng, Q.; Rosen, R.T.; Ho, C.T. *Bioorg. Med. Chem.*, **2003**, *11*, 2499.
[61] Kupeli, E.; Tatli, I.I.; Akdemir, Z.S.; Yesilada, E. *J. Ethnopharmacol.*, **2007**, *110*, 444.
[62] Jeong, H.J.; Koo, H.N.; Na, H.J.; Kim, M.S.; Hong, SH.; Eom, J.W.; Kim, K.S.; Shin, T.Y.; Him, H.M. *Cytokine*, **2002**, *18*, 252.
[63] Recio, M.C.; Giner, R.M.; Manez, S.; Rios, J.L. *Planta Med.*, **1994**, *60*, 232.
[64] Park, K.S.; Chang, I.M. *Planta Med.*, **2004**, *70*, 778.
[65] Harput, U.S.; Saracoglu, I.; Inoue, M.; Ogihara, Y. *Biol. Pharm. Bull.*, **2002**, *25*, 483.
[66] Su, B.; Zhu, Q.; Jia, Z. *Tetrahedron Lett.*, **1999**, *40*, 357.
[67] Kupeli, E.; Harput, U.S.; Varel, M.; Yesilada, E.; Saracoglu, I. *J. Ethnopharmacol.*, **2005**, *102*, 170.
[68] Jia, Q.; Hong, M.F.; Minter, D. *J. Nat. Prod.*, **1999**, *62*, 901.
[69] Delaporte, R.H.; Sances, G.M.; Cuellar, A.C.; Giuliani, A.; de Mello, J.C.P. *J. Ethnopharmacol.*, **2002**, *82*, 127.
[70] Choi, J.; Lee, K.T.; Choi, M.Y.; Nam, J.H.; Jung, H.J.; Park, S.K.; Park, H.J. *Biol. Pharm. Bull*., **2005**, *28*, 1915.
[71] Picerno, P.; Autore, G.; Marzocco, S.; Meloni, M.; Sanogo, R.; Aquino, R.P. *J. Nat. Prod.*, **2005**, *68*, 1610.
[72] Koo, H.J.; Song, Y.S.; Kim, H.J.; Lee, H.Y.; Hong, S.M.; Kim, S.J.; Kim, B.C.; Jin, C.; Lim, C.J.; Park, E.H. *Eur. J. Pharmacol.*, **2004**, *495*, 201.
[73] Koo, H.J.; Lim, K.H.; Jung, H.J.; Park, E.H. *J. Ethnopharmacol.*, **2006**, *103*, 496.
[74] Zhu, J.; Gao, X.; Xie, W.L.; Jin, Y.Z.; Sun, W.J. *Zhongguo Zhong Yao Za Zhi*, **2005**, *30*, 708.
[75] Awale, S.; Kawakami, T.; Tezuka, Y.; Ueda, J.Y.; Tanaka, K.; Kadota, S. *Chem. Pharm. Bull.*, **2005**, *53*, 710.
[76] Oh, H.; Pae, H.O.; Oh, G.S.; Lee, S.Y.; Chai, K.Y.; Song, C.E.; Kwon, T.O.; Chung, H.T.; Lee, H.S. *Planta Med.*, **2002,** *68*, 685.
[77] Ling, S.K.; Tanaka, T.; Kouno, I. *Biol. Pharm. Bull*., **2003**, *26*, 352.
[78] Dìaz, A.M.; Abad, M.J.; Fernandez, L.; Recuero, C.; Villaescusa, L.; Silvan, A.M.; Bermejo, P. *Biol. Pharm. Bull.*, **2000**, *23*, 1307.
[79] Suksamrarn, A.; Kumpun, S.; Kirtikara, K.; Yingyongnaror, B.; Suksamrarn, S. *Planta Med.*, **2002**, *68*, 72.
[80] Bermejo, B.P.; Abad, M.J.; Diaz, A.; Fernandez, L.; De Santos, J.; Sanchez, S.; Villaescusa, L.; Carrasco, L. *Planta Med.*, **2000**, *66*, 324.
[81] Ahmed, B.; Al-Rehaily, A.J.; Al-Howiriny, T.A.; El-Sayed, K.A.; Ahmad, M.S. *Biol. Pharm. Bull.*, **2003**, *26*, 462.
[82] Giner, R.M.; Villalba, M.L.; Recio, M.C.; Manez, S.; Cerda-Nicolas, M.; Rios, J. *Eur. J. Pharmacol*., **2000**, *389*, 243.
[83] (a) Bas, E.; Recio, M.C.; Máñez, S.; Giner, R.M.; Escandell, J.M.; López-Ginés, C.; Ríos, J.L. *Eur. J. Pharmacol.*, **2007**, *555*, 199. b) Bas, E.; Recio, M.C.; Abdallah, M.; Máñez, S.; Giner, R.M.; Cerdás-Nicolás, M.; Ríos, J.L. *J. Ethnopharmacol.*, **2007**, *110*, 419.
[84] Lin, L.C.; Wang, Y.H.; Hou, Y.C.; Chang, S.; Liou, K.T.; Chou, Y.C.; Wang, W.Y.; Shen, Y.C. *J. Pharm. Pharmacol.*, **2006**, *58*, 129.
[85] Garg, H.S.; Bhandari, S.P.S.; Tripathi, S.C.; Patnaik, G.K.; Puri, A.; Saxena, R.; Saxena R.P. *Phytother. Res*., **1994**, *8*, 224.
[86] Yoshikawa, M.; Ueda, T.; Matsuda, H.; Yamahara, J.; Murakami, N. *Chem. Pharm. Bull.*, **1994**, *42*, 169.
[87] Gupta, P.P.; Srimal, R.C.; Srivastava, M.; Singh, K.; Tandon, J.S. *Int. J. Pharmacog.*, **1995**, *33*, 70.
[88] Chang, W.L.; Wang, H.Y.; Shi, L.S.; Lai, J.H.; Lin, H.C. *J. Nat. Prod*., **2005**, *68*, 1683.
[89] Mathad, V.T.; Raj, K.; Bhaduri, A.P.; Sahai, R.; Puri, A., Tripathi, L.M.; Srivastava, V.M. *Bioorg. Med. Chem.*, **1998**, *6*, 605.
[90] Chander, R.; Kapoor, N.K.; Dhawan, B.N. *Biochem. Pharmacol.*, **1992**, *7*, 180.
[91] Kruk, I.; Aboul-Enein, H.Y.; Michalska, T.; Lichszteld, K.; Kladna, A. *Luminescence*, **2005**, *20*, 81.
[92] Raju, B.L.; Lin, S.J.; Hou, W.C.; Lai, Z.Y.; Liu, P.C.; Hsu, F.L. *Nat. Prod. Res*., **2004**, *18*, 357.

[93] Goupy, P.; Dufour, C.; Loonis, M.; Dangles, O. *J. Agric. Food Chem.*, **2003**, *51*, 615.
[94] Delazar, A.; Byres, M.; Gibbons, S.; Kumarasamy, Y.; Modarresi, M.; Nahar, L.; Shoeb, M.; Sarker, S.D. *J. Nat. Prod.*, **2004**, *67*, 1584.
[95] Kumarasamy, Y.; Nahar, L.; Sarker, S. D. *Fitoterapia*, **2003**, *74*, 151.
[96] Oh, H.; Ko, E.K.; Kim, D.H.; Jang, K.K.; Park, S.E.; Lee, H.S.; Kim, Y.C. *Phytother. Res.*, **2003**, *17*, 417.
[97] Juma, B.F.; Majinda, R.R. *Nat. Prod. Res.*, **2007**, *21*, 121.
[98] Ho, J.N.; Lee, Y.H.; Park, J.S.; Jun, W.J.; Kim, H.K.; Hong, B.S.; Shin, D.H.; Cho, H.Y. *Biol. Pharm. Bull.*, **2005**, *28*, 1244.
[99] (a) Saraswat, B.; Visen, P.K.; Patnaik, G.K.; Dhawan, B.N. *Indian J. Expt. Biol.*, **1997**, *35*, 1302. b) Rastogi, R.; Srivastava, A.K.; Srivastava, M.; Rastogi, A.K. *Planta Med.*, **2000**, *66*, 709. c) Rastogi, R.; Srivastava, A.K.; Rastogi, A.K. *Pharmacol. Toxicol.*, **2001**, *88*, 53.
[100] Singh, M.; Tiwari, V.; Jain, A.; Ghoshal, S. *Indian J. Med. Res.*, **2005**, *121*, 676.
[101] Yadav, N.; Khandelwal, S. *Hum. Exp. Toxicol.*, **2006**, *25*, 581.
[102] Wang, H.; Wu, F.H.; Xiong, F.; Wu, J.J.; Zhang, L.Y.; Ye, W.C.; Li, P.; Zhao, S.X. *Chem. Pharm. Bull.*, **2006**, *54*, 1144.
[103] Singh, B.; Chandan, B.K.; Prabhakar, A.; Taneja, S.C.; Singh, J.; Qazi, G.N. *Phytother. Res.*, **2005**, *19*, 391.
[104] Vogel, H.G. In *Drug Discovery and Evaluation, Pharmacological Assay*, 2nd edn. Vogel, H.G.; Vogel, W.H., Eds; Springer Verlag: Berlin, **2002**.
[105] Lin, Y.L.; Hsu, J.D.; Chou, F.P.; Lee, M.J.; Shiow, S.J.; Wang, C.J. *Chem. Biol. Interact.*, **2000**, *128*, 115.
[106] Ishiguro, K.; Yamaki, M.; Takagi, S. *J. Nat. Prod.*, **1983**, *46*, 532. b) Hamburger, M.O.; Cordell, G.A.; Ruangrungsi, N. *J. Ethnopharmacol.*, **1991**, *33*, 289.
[107] Davini, E.; Javarone, C.; Trogolo, C.; Aureli, P.; Pisolini, B. *Phytochemistry*, **1986**, *25*, 2420.
[108] Horn, M.M.; Drewes, S.E.; Brown, N.J.; Munro, O.Q.; Meyer, J.J.; Mathekga, A.D. *Phytochemistry*, **2001**, *57*, 51.
[109] Gupta, P.; Bajpai, S.K.; Chandra, K.; Singh, K.L.; Tandon, J.S. *Indian J. Exp. Biol.*, **2005**, *43*, 1156.
[110] Chen, J.L.; Blanc, P.; Stoddart, C.A.; Bogan, M.; Rozhon, E.J.; Parkinson, N.; Ye, Z.; Cooper, R.; Balick, M.; Nanakorn, W.; Kernan, M.R. *J. Nat. Prod.*, **1998**, *61*, 1295.
[111] Chang, I.M. *Res. Commun. Mol. Pathol. Pharmacol.,* **1998**, *102*, 189.
[112] Ma, S.C.; He, Z.D.; Deng, X.L.; But, P.P.; Ooi, V.E., Xu, H.X.; Lee, S.H.; Lee, S.F. *Chem. Pharm. Bull.*, **2001**, *49*, 1471.
[113] Bermejo, P.; Abad, M.J.; Diaz, A.M.; Fernandez, L.; De Santos, J.; Sanchez, S.; Villaescusa, L.; Carrasco, L.; Irurzun, A. *Planta Med.*, **2002**, *68*, 106.
[114] Suksamrarn, S.; Wongkrajang, K.; Kirtikara, K.; Suksamrarn, A. *Planta Med.*, **2003**, *69*, 877.
[115] Tan, G.T.; Miller, J.F.; Kinghorn, A.D.; Hughes, S.H.; Pezzuto, J.M. *Biochem. Biophys. Res. Commun.*, **1992**, *29*, 370.
[116] Lee-Huang, S.; Zhang, L.; Huang, P.L.; Chang, Y.; Huang, P.L. *Biochem. Biophys. Res. Commun*., **2003**, *307*, 1029.
[117] Lee-Huang, S.; Huang, P.L.; Zhang, D.; Lee, J.W.; Bao, J.; Sun, Y.; Chang, Y.-Tae; Zhang, J.Z.H.; Huang, P.L. *Biochem. Biophys. Res. Commun*., **2007**, *354*, 872.
[118] Bao, J.; Zhang, D.W.; Zhang, J.Z.H.; Lee Huang, P.; Lin Huang, P.; Lee-Huang, S. *FEBS Lett.*, **2007**, *581*, 2737.
[119] Castillo, D.; Arevalo, J.; Herrera, F.; Ruiz, C.; Rojas, R.; Rengifo, E.; Vaisberg, A.; Lock, O.; Lemesre, J.L.; Gornitzka, H.; Sauvain, M. *J. Ethnopharmacol.*, **2007**, *112*, 410.
[120] Montero-Torres, A.; Vega, M.C.; Marrero-Ponce, Y.; Rolon, M.; Gomez-Barrio, A.; Escario, J.A.; Aran, V.J.; Martinez-Fernandez, A.R.; Meneses-Marcel, A. *Bioorg. Med. Chem.*, **2005**, *13*, 6264.
[121] Cimanga, R.K.; Kambu, K.; Tona, L.; Hermans, N.; Apers, S.; Totte, J.; Pieters, L.; Vlietinck, A.J. *J. Ethnopharmacol*., **2006**, *107*, 83.
[122] Tasdemir, D.; Güner, N.D.; Perozzo, R.; Brun, R.; Dönmez, A.A.; Çalis, I.; Rüedi, P. *Phytochemistry*, **2005**, *86*, 355.
[123] Ray, S.; Majumder, H.K.; Chakravarty, A.K.; Mukhopadhyay, S.; Gil, R.R.; Cordell, G.A. *J. Nat. Prod.*, **1996**, *59*, 27.
[124] Medda, S.; Mukhopadhyay, S.; Basu, M.K. *J. Antimicrob. Chemother.*, **1999**, *44*, 791.
[125] (a) Covas, M.I. *Pharmacol. Res*., **2007** *55*, 175. b) Wahle, K.W.; Caruso, D.; Ochoa, J.J.; Quiles, J.L. *Lipids*, **2004**, *39*, 1223.
[126] Hansen, K.; Adsersen, A.; Brøgger Christensen, S.; Rosendal Jensen, S.; Nyman, U.; Wagner Smitt, U. *Phytomedicine*, **1996**, *2*, 319.
[127] Pennacchio, M.; Syah, Y.M.; Ghisalberti, E.L.; Alexander, E. *J. Ethnopharmacol.*, **1996**, *26*, 21.
[128] Pennacchio, M.; Syah, Y.M.; Ghisalberti, E.L.; Alexander, E. *Phytomedicine*, **1997**, *4*, 325.
[129] Kim, D.H.; Lee, H.J.; Oh, Y.J.; Kim, M.J.; Kim, S.H.; Jeong, T.S.; Baek, N.I. *Arch. Pharm. Res.*, **2005**, *28*, 1156.
[130] Singh, A.K.; Sharma, A.; Warren, J.; Madhavan, S.; Steele, K.; RajeshKumar, N.V.; Thangapazham, R.L.; Sharma, S.C.; Kulshreshtha, D.K.; Gaddipati, J.; Maheshwari, R.K. *Planta Med.*, **2007**, *73*, 251.
[131] Gutierrez, R.M.; Solis, R.V.; Baez, E.G.; Martinez, F.M. *Phytother. Res.*, **2006**, *20*, 542.
[132] Suzuki, Y.; Kondo, K.; Ikeda, Y.; Umemura, K. *Planta Med.*, **2001**, *67*, 807.
[133] Trovato, A.; Forestieri, A.M.; Iauk, L.; Barbera, R.; Monteforte, M.T.; Galati, M.E. *Plant. Med. Phytother.*, **1993**, *26*, 300.
[134] Al-Azzawie, H.F.; Alhamdani, M.S.S. *Life Sci.*, **2006**, *78*, 1371.
[135] Miura, T.; Nishiyama, Y.; Ichimaru, M.; Moriyasu, M.; Kato, A. *Biol. Pharm. Bull.*, **1996**, *19*, 160.
[136] Xu, H.Q.; Hao, H.P. *Biol. Pharm. Bull.*, **2004**, *27*, 1014.
[137] Ozturk, N.; Norkmaz, S.; Ozturk, Y.; Baser, K.H. *Planta Med.*, **2006**, *72*, 289.
[138] Takeda, S.; Endo, T.; Aburada, M. *J. Pharmacobiodyn.*, **1981**, *3*, 485.
[139] Shoda, J.; Miura, T.; Utsunomiya, H.; Oda, K.; Yamamoto, M.; Kano, M.; Ikegami, T.; Tanaka, N.; Akita, H.; Ito, K.; Suzuki, H.; Sugiyama, Y. *Hepatology*, **2004**, *39*, 167.
[140] Oh, S.R.; Lee, M.Y.; Ahn, K.; Park, B.Y.; Kwon, O.K.; Joung, H.; Lee, J.; Kim, D.Y.; Lee, S.; Kim, J.H.; Lee, H.K. *Int. Immunopharmacol.*, **2006**, *6*, 978.
[141] Toriizuka, K.; Kamiki, T.H.; Ohmura, N.Y.; Fujii, M.; Hori, Y.; Fukumura, M.; Hirai, Y.; Isoda, S.; Nemoto, Y.; Ida, Y. *Life Sci.*, **2005**, *77*, 3010.

Received: 27 July, 2007 **Revised: 08 October, 2007** **Accepted: 08 October, 2007**