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Bioactive small-molecule constituents of Lao plants

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Abstract

Laos has a rich plant diversity, and medicinal plants are used extensively in Lao traditional medicine for the treatment of a variety of human diseases. However, only a relatively small number of these plants have been investigated for their major components with potential antitumor, anti-infective, and other types of bioactivities. These species include *Asparagus cochinchinensis*, *Diospyros quaesita*, *Gongronema napalense*, *Marsypopetalum modestum*, *Nauclea orientalis*, *Rourea minor*, *Stemona pierrei*, and *Stemona tuberosa*. Thus far, the bioactive compounds isolated from these Lao plants include alkaloids, glycerol esters, phenolic compounds such as lignans and stilbenoids, steroids, and triterpenoids. Of these, the norlignan, nyasol (1b), the triterpenes, pyracrenic acid [3β-*O*-*trans*-caffeoylbetulinic acid (3)] and betulinic acid (3b), and the dimeric thiopyridine, dipyrithione (5), were found to show both cancer cell cytotoxicity and anti-infective activity. The present review focuses on examples of promising lead compounds isolated from Lao plants, with their possible development as potential therapeutic agents being discussed. It is hoped that this contribution will provide useful information on higher plants growing in Laos to help stimulate future discoveries of potential agents for the treatment of cancer, infections, and other diseases.

Keywords

Lao plants; chemical compounds; human diseases

INTRODUCTION

The Lao People’s Democratic Republic, a landlocked country known widely as Laos, is a small country located in the east-central section of the Indo-Chinese peninsula, flanked by

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DECLARATION OF COMPETING INTEREST

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Thailand in the west, Myanmar and the Chinese province of Yunnan in the north, Vietnam in the east, and Cambodia in the south. The tropical flora of Laos is rich, and, according to a recent paper (Zhu, 2017), there are 5,005 species of seed plants distributed in 1,373 genera and 188 families in Laos, of which many plants are used as medicinal herbs in the healthcare system of Laos (Sydara et al., 2005; Libman et al., 2006). On the ethnomedical use of Lao medicinal plants, sympatric intercultural traditional knowledge has always made an impact, as indicated by a similarity investigation conducted among three sympatric ethnic groups, the Brou, Saek, and Kry, living in the Annamite Mountains in Laos. This study showed that medicinal plants are used commonly among these groups, but the use of these plants to treat disorders seems different (de Boer et al., 2012).

The traditional use of medicinal plants in Laos has proven useful for the discovery of new bioactive compounds, and ethnomedical collections can be supportive of plant-based drug discovery programs (Gyllenhaal et al., 2012). A field survey to identify medicinal plants growing in southern Laos showed that many Lao medicinal plants are used for treating certain infections, and their frequency of use decreased in the sequence fever, stomachache, arthritis, malaria, and leukorrhea (Elkington et al., 2014a). With the aid of field interviews with healers in Laos, several plant-derived lead compounds showing activities related to cancer, acquired immunodeficiency syndrome (AIDS) caused by infection from human immunodeficiency viruses (HIV), malaria, and/or tuberculosis (TB) infectious diseases have been identified (Soejarto et al., 2012). In addition, the Hmong ethnic group in Laos uses medicinal plants extensively for the treatment of various conditions that include gastrointestinal and gynecological conditions and sexually transmitted diseases, skin infections, kidney and bladder problems, physical trauma, and as aphrodisiacs (Dubost et al., 2019). Thus, there is a strong rationale to search for therapeutic agents from Lao plants for the potential treatment of cancer, infectious diseases, and other conditions (Soejarto et al., 2002).

Historically, plants offer valuable sources of new natural product-derived drugs, and many effective plant-derived agents have been developed for the treatment of cancer and infectious diseases over the past few decades (Dehelean et al., 2021; Newman and Cragg, 2020; Talib et al., 2020). Therefore, as a consequence of the continuing search for antineoplastic and anti-infective agents from Lao plants, substances targeting unusual mechanisms to afford possible new approaches to improve human health may be discovered (Soejarto et al., 2006; Kinghorn et al., 2016). In addition, a reservoir represented by a large number of higher plants growing in Laos offers an opportunity for such a search (Henkin et al., 2017), of which eight species have been investigated for identification of their bioactive components. In the following sections, investigations on these plants are summarized, inclusive of further studies on their bioactive principles, when collected from neighboring countries, and a perspective for future investigations on Lao plants.

Asparagus cochinchinensis (Lour.) Merr. (Asparagaceae)

Asparagus cochinchinensis is a perennial trailing plant (Table 1) native to Cambodia, China, Japan, Korea, Laos, Philippines, and Vietnam (Newman et al., 2007). Based on information provided by a healer from Salavan Province of Laos, the roots of this plant

are used in folk medicine to treat chronic fever when combined with other plants (Soejarto et al., 2012), and thus they were collected locally, from which several compounds were isolated. These include a 9'-norlignan, 3-hydroxy-4-methoxy-4-dehydroxynyasol (**1**), and its analogues, 3-methoxynyasol (**1a**), nyasol (**1b**), and 1,3-bis-di-*p*-hydroxyphenyl-4-penten-1-one (**1c**) (Figure 1), along with a new spirostanol saponin, asparacoside (**2**), and its analogues asparacosins A (**2a**) and B (**2b**) (Figure 1). These compounds were evaluated for cytotoxicity against Col-2 colon, KB oral epidermoid, LNCaP prostate, and Lu-1 lung human cancer cells, and **1**, **1a**, and **2** were deemed active, but **1b**, **1c**, **2a**, and **2b** were not (Zhang et al., 2004). These results demonstrated that the 3,4-*ortho*-hydroxy and -methoxy groups are important for the mediation of cancer cell line cytotoxicity by **1**, but neither transposing the substituents between the C-3 and C-4 positions nor introducing a carbonyl group at the C-9' position seems to contribute to the resultant cytotoxic potency.

Even though nyasol (*cis*-hinokiresinol, **1b**) did not show any growth inhibitory activity against human cancer cells, it was found to inhibit both farnesyl protein transferase (FPTase) and phosphatase of regenerating liver 3 (PRL-3). FPTase is involved in the membrane association of Ras to modulate tumor formation, while PRL-3 is overexpressed in human cancer cells to contribute to the acquisition of metastasis. Thus, nyasol shows some antitumor potential by targeting FPTase and PRL-3 to inhibit tumor formation and metastasis (Song et al., 2008). In addition, nyasol (**1b**) was found to inhibit selectively the basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF)-induced endothelial cell proliferation, migration, and tube formation. The vessel growth was inhibited when slow release pellets of Hydron N containing 200 ng mouse recombinant VEGF were implanted into the corneal pockets of five-week-old BALB/cA mice, and then mice were treated intraperitoneally (i.p.) with nyasol (100 mg/kg, daily) for seven days. Angiogenesis is observed commonly when tumor occurs, for which inhibition may contribute potentially to cancer treatment. Thus, nyasol could be an antitumor lead compound, targeting angiogenesis inhibition (Jeong et al., 2003).

Investigation of cytotoxicity of the spirosteroid **2** and its analogues isolated from the Lao plant, *A. cochinchinensis* (Zhang et al., 2004), indicates that the glycoside moiety connected at the C-3 position is necessary for the modulation of its cancer cell cytotoxicity, while the C-3–C-5 enone group plus the C-12 hydroxy group also contribute to this activity. Steroids occur widely in both plants and animals, among which cardiac glycosides have attracted wide interest for their potential antitumor activity. Of these, digoxin has long been used for the treatment of congestive heart failure, and it was also found to show some potential antitumor activity by targeting Na^+/K^+-ATPase (Ren et al., 2019). Recently, another cardiac glycoside, (+)-strebloside, was identified as a promising antitumor lead compound, targeting Na^+/K^+-ATPase, nuclear factor κ light-chain-enhancer of activated B cells (NF-κB), and p53 and the extracellular signal-regulated kinases (ERK) pathways (Chen et al., 2017). Comparison of the cytotoxicity of **2**, **2a**, **2b**, and the cardiac glycosides digoxin and (+)-strebloside implies that the C-22 spiro-bicyclic system of **2** seems less important than the C-17 lactone unit of cardiac glycosides in the mediation of their cytotoxic potency. However, further modification of the C-22 spiro-bicyclic system of **2** may produce some novel non-Na^+/K^+-ATPase-targeted antitumor steroids. Thus, **2** could be regarded as a lead

compound, and the search for more cytotoxic analogues of **2** from plants collected in Laos may be supportive in this regard.

Moreover, the norlignans **1**, **1a**, and **1b**, along with their analogue, 4'-*O*-methylnyasol, isolated from *Anemarrhena asphodeloides*, were found to show inhibitory effects on the degranulation of rat basophilic leukemia-2H3 cells. The inhibition of β-hexosaminidase release of **1** (IC_{50} 2.9 μM) was more potent than that of ketotifen (IC_{50} 10 μM), a second-generation H_1-antihistamine used to treat allergic conjunctivitis, and thus **1** could be promising as an antiallergic lead (Bak et al., 2016). Alteration of the substituents at the C-3 and C-4 positions of **1** resulted in a significant decrease of its antiallergic potency, indicating that this norlignan could be modified synthetically in future investigations.

Also, some of these norlignans were found to exhibit antiviral activity. For example, **1b** and **1c** exhibited moderate anti-HIV activity, but **1** and **1a** did not (Zhang et al., 2004), indicating that a hydroxy group substituted at the C-4 and C-4' positions is required for this biological property. Introducing a methoxy group at the C-3 position or substitution of a hydroxy group at the C-3 position followed by methylation of the C-4 hydroxy group resulted in the elimination of the anti-HIV activity, while changing the $\Delta^{7',8'}$ double bond to a carbonyl group did not affect the activity greatly. In addition, (–)-nyasol and (–)-4'-*O*-methylnyasol were found to inhibit effectively respiratory syncytial virus (RSV), which frequently infects infants. Both compounds were more potently active in this regard than ribavirin, a standard antiviral drug with a broad-spectrum of activity. Thus, these norlignans show some promise as anti-HIV or anti-RSV agents, and their activity could be improved through synthetic optimization (Bae 2007).

Nyasol (**1b**) was identified originally as a derivative of nyasoside, a new norligan glycoside isolated from the rhizomes of *Hypoxis nyasica* Bak. (Hypoxidaceae) collected near Zomba in Malawi (Marini-Bettolo et al., 1985). In turn, (+)-nyasol was isolated from the tubers of *Asparagus cochinchinensis* collected in Hong Kong (Tsui and Brown, 1996). The absolute configurations of (+)- and (–)-nyasol have been determined by synthetic methods and by analysis of their vibrational circular dichroism spectra (Lassen et al., 2005). In an earlier investigation, nyasol (**1b**) was found to inhibit the lipopolysaccharide (LPS)-induced release of nitric oxide (NO) in RAW264.7 murine monocytic cells, with no discernible cytotoxicity observed against the host cells. The inhibitory potency of **1b** was equivalent to that of 1-N_6-(1-iminoethyl)lysine, a potent inhibitor of nitric oxide synthase (NOS), indicating some anti-inflammatory potentiality of **1b** (Song et al., 2008). Following this, (–)-nyasol isolated from the rhizomes of *Anemarrhena asphodeloides* obtained in Korea was found to inhibit production of cyclooxygenase (COX)-2-mediated prostaglandin E2 (PGE2) and inducible NOS (iNOS)-induced nitric oxide (NO) in LPS-treated RAW 264.7 cells. It also suppressed the generation of 5-lipoxygenase (5-LOX)-mediated leukotriene in RBL-1 rat basophilic leukemia cells treated with A-23187 (ionophore) (Lim et al., 2009) and the expression of inflammatory cytokines such as interleukin-1β (IL-1β) and interferon-β (IFN-β) (Lee et al., 2014). Furthermore, nyasol (**1b**) inhibited the transcriptional activity of NF-κB, the activation of Akt or PKB (protein kinase B), and ERK in LPS-stimulated RAW 264.7 cells (Lee et al., 2014).

Further investigations have shown that (−)-nyasol inhibited the production of NO and PGE2 and the expression of iNOS and COX-2 in LPS-activated BV-2 murine microglial cells, through suppressing LPS-induced I-κBα degradation and p38 mitogen-activated protein kinase (MAPK) activation. Activation of the microglia contributes to host damage by excessive release of various proinflammatory cytokines such as IL-1β and tumor necrosis factor (TNF)-α and is associated with the occurrence of several neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and stroke. Thus, inhibition of these proinflammatory molecules could be useful for the treatment of these disorders. In this regard, (−)-nyasol may have therapeutic potential for the treatment of neuroinflammatory diseases (Lee et al., 2013).

Importantly, (–)-nyasol showed anti-inflammation activity *in vivo*. It inhibited potently λ-carrageenan (CGN)-induced paw edema when male ICR mice were treated intraperitoneally (i.p.) with (–)-nyasol (24–120 mg/kg, a single dose), and the right hind paw edema of mice was induced by injection of 0.05 mg of CGN (Lim et al., 2009). Nyasol inhibited 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced mouse ear edema when ICR mice were treated (i.p.) with nyasol (10 mg/kg, a single dose), and the right ear of mice was applied topically by TPA (1.0 μg in 20 μL acetone) (Lee et al., 2014).

It is worthy of note that nyasol (**1b**) may possess antidiabetic properties. It was found recently to inhibit potently α-glucosidase, with its IC_{50} value being 3 nM. Such potency is much greater (up to 5700-fold) than that found for acarbose (IC_{50} 171 μM), a standard antidiabetic drug targeting α-glucosidase, an intestinal enzyme to digest large carbohydrates into glycoses (Tantapakul et al., 2020).

Similar to the norlignans, the spirosteroid asparacosin A (**2a**) was also found to show both anti-inflammatory and antinociceptive activities. In an anti-inflammation rat model, the paw edema of Sprague Dawley rats (150–220 g) was inhibited significantly when the animals were treated orally with asparacosin A (**2a**, 20 mg/kg, single dose), and then (after 1 h) the right hind paws of rats were injected plethysmometrically by carrageenan (0.1 mL of 1% suspension in 2% gum acacia saline solution). In the same model, ear edema was suppressed when rats were treated orally with asparacosin A (**2a**, 20 mg/kg, single dose), and then (after 1 h) the inner and outer surfaces of the right ear of rats were given topically 20 μL of xylene. Moreover, in the writhing antinociceptive mouse model, the writhes observed were reduced significantly when Swiss mice (20–30 g) were treated orally with **2a** (10 mg/kg, single dose) and then (after 0.5 h) injected (i.p.) by 1% acetic acid. Mechanistic investigations indicated that the anti-inflammatory activity could be mediated by **2a** through inhibition of COX-2 enzyme and inflammatory mediators, TNF-α, IL-1 β, and PGE2. Therefore, asparacosin A (**2a**) may be regarded as a lead for the development of anti-inflammatory and antinociceptive agents (Karim et al., 2019).

The roots of *Asparagus cochinchinensis* also have been used as a Chinese traditional medicine, "Tianmendong", to treat cancer and infectious diseases (Zhang and Shen, 2007), and they were found recently to show anti-asthmatic activity (Choi et al., 2018). Phytochemical investigation of the roots of *Asparagus cochinchinensis* obtained from China resulted in the isolation of two 8,9-dihydroxylated analogues of nyasol (**1b**), iso-

agatharesinol and iso-agatharesinoside (Li et al., 2012). In addition, two steroids, dioscin and methyl protodioscin, were isolated from the roots of *A. cochinchinensis* obtained from Korea, with their analogue, aspacochioside A, being identified as a major component (2.126 g from 18.6 kg of the dried root sample) of the roots of *A. cochinchinensis* collected in China (Lee et al., 2015; Shi et al., 2004). Comparison of these components with those reported from Lao *A. cochinchinensis* indicates that the major components of this plant do not vary due to the change of the collection locations in Asia. As reviewed recently, dioscin shows multiple pharmacological activities, including antitumor, anti-infection, and tissue-protective properties (Yang et al., 2019). Both dioscin and methyl protodioscin were found to suppress the expression of the MUC5AC mucin gene in NCI-H292 human pulmonary mucoepidermoid carcinoma cells. Airway mucus plays a crucial role in defense against invading pathogens, but the abnormality of mucins could cause altered airway physiology to lead to severe airway pathology. The inhibitory effect of both dioscin and methyl protodioscin on the expression of MUC5AC mucin suggesting that these steroids could show some potential for the treatment of inflammatory pulmonary diseases (Lee et al., 2015).

Similar to dioscin, methyl protodioscin has antitumor potential. It induced HeLa human cervical cancer cell apoptosis, which was mediated by the accumulation of intracellular reactive oxygen species (ROS) and by the activation of death receptor and mitochondrial pathways (Ma et al., 2019). It suppressed MG-63 human osteosarcoma cell growth through induction of cell apoptosis by, at least in part, caspase-dependent and MAPK signaling pathways (Tseng et al., 2017). Treatment with methyl protodioscin led to cell cycle arrest at the G2/M phase and resulted in inhibition of SAS and SCC9 human oral squamous cell carcinoma cell growth, which was mediated probably by induction of apoptosis and autophagy through modulation of the p38 MAPK and JNK1/2 pathways (Hsieh et al., 2017).

Interestingly, methyl protodioscin also inhibited the A7r5 rat thoracic aorta fibroblast cell growth. Neointima formation was suppressed significantly after an extravascular administration of methyl protodioscin when the balloon catheter was introduced into the common carotid artery of three–four-month-old male rats, with the carotid artery then being coated with 30% (w/v) pluronic-F127 gel with methyl protodioscin (3.0 or 6.0 uM) for two weeks. Also, the expression of a disintegrin and metalloprotease 15 (ADAM15) in vascular smooth muscle cells (VSMCs) and injured mouse arteries was found to be reduced markedly. Abnormal proliferation and migration of VSMCs is involved in the development of restenosis, and ADAM15 expression in VSMCs plays a critical role in pathological neovascularization and atherosclerosis. Methyl protodioscin inhibited VSMC growth and migration and prevented balloon angioplasty-induced restenosis by downregulating the expression of ADAM15 and its downstream signaling pathways, and thus it may possess some therapeutic potential for the treatment of restenosis (Chung et al., 2016).

Therefore, the norlignan nyasol (**1b**) and asparacosin A (**2a**) and their analogues may be regarded as the principal active components of *A. cochinchinensis* that are responsible for the medicinal use of this herb in Lao folk medicine. Further modification of these lead compounds could yield some therapeutic agents to treat cancer and infectious diseases.

Diospyros quaesita Thw. (Ebenaceae)

Diospyros quaesita is a small to medium-sized tree (Table 1), and its leaves and stems are used to treat cases of alcohol and drug dependence and headache in Laos (Soejarto et al., 2012). Thus, the leaves, twigs, and branches of *D. quaesita* were collected in Sainyabuli Province of Laos, from which a caffeoyl ester of betulinic acid, namely, pyracrenic acid [3β-*O*-*trans*-caffeoylbetulinic acid (**3**)] (Figure 2), was isolated. Compound **3** was found to show cytotoxicity toward KB human oral epidermoid cancer cells (Ma et al., 2008). It also exhibited activity against the human A549 and SK-Lu-1 lung, AGS gastric, COLO 205 and HCT-15 colon, HeLa cervical, HepG2 liver, MCF-7 breast, and SK-OV-3 ovarian cancer and SK-MEL-2 melanoma cell lines (Table 2) (Kim et al., 2010; Kim et al., 2017; Pan et al., 2008; Quang et al., 2020; Wang et al., 2018).

Investigation of the cytotoxicity of **3** and its analogues or derivatives indicated that the introduction of a 3-*O*-caffeoyl group enhances the cytotoxic potency of betulinic acid (**3b**) against KB cells, and acetylation of the caffeoyl unit [e.g., 3β-*O*-*trans*-diacetylcaffeoylbetulinic acid (**3a**)] led to the activity being improved further (Ma et al., 2008). In addition, this type of activity was enhanced when the C-28 carboxylic acid substituent was reduced to a primary hydroxy group (Lomchid et al., 2017). As a result of treating A549 cells with **3**, **3b**, betulin (**3c**), lupeol (**3d**), 3β-*O*-*trans*-caffeoylbetulin (**3e**), and 3β-*O*-*trans*-*p*-coumaroylbetulin (**3f**), the cytotoxic potency was increased in the sequence, **3d**, **3c**, **3f**, **3b**, and **3e.** Hence, oxidation of the C-28 methyl group of lupeol (**3d**) resulted in its activity being increased, and the presence of a 6',7'-dihydroxy group was found to be preferable for this type of activity of **3** (Shi et al., 2014). In addition, compound **3b** was found to be more potently active than **3** when tested against A549, HCT-15, SK-MEL-2, and SK-OV-3 cells (Kim et al., 2017), indicating that introducing a 3-*O*-caffeoyl group may decrease the activity of **3b** against these cell lines tested. Thus, the effect on the cytotoxic potency of the 3-*O*-caffeoyl group of **3** may vary when investigated against different cancer cell lines.

When evaluated against AGS, COLO 205, and HepG2 cancer cells, **3** showed activities that were comparable with those of 3β-*O*-*trans*-*p*-coumaroylbetulinic acid (**3g**), and both **3** and **3g** were more potently cytotoxic than 3β-*O*-*trans*-feruloylbetulinic acid for AGS and COLO cells (Pan et al., 2008). These results indicated that removal of the C-6' hydroxy group did not affect the activity of **3**, but methylation of this hydroxy group reduced its cytotoxicity. Thus, an analogue of compound **3**, namely, 3β-*O*-*trans*-feruloylbetulinic acid, was found to be inactive when tested against MDA-MB-231 human breast cancer cells (Ren et al., 2018). In addition, several derivatives of **3** were found to be more potently active than the corresponding analogues of **3e** when tested against AGS, COLO 205, and HepG2 cells, implying that the C-28 carboxylic acid moiety is preferred over a C-28 hydroxy group in enhancing the activity of these compounds against the cancer cell lines used (Pan et al., 2008).

Additionally, compound **3** showed antimalarial activity against the chloroquine-sensitive clone D6 and the chloroquine-resistant clone W2 of *Plasmodium falciparum*, and it exhibited an equivalent potency to 3β-*O*-*trans*-diacetylcaffeoylbetulinic acid (**3a**) and was

more active than betulinic acid (**3b**) (Ma et al., 2008). These results demonstrated that introducing a 3β-*O*-*trans*-caffeoyl group can enhance the antimalarial effects of betulinic acid (**3b**) *in vitro*, but acetylation of **3** did not change the activity significantly. Also, **3** was found to be active when tested *in vitro* against *Mycobacterium tuberculosis* (TB) H37Ra, using the standard drugs, kanamycin sulfate, isoniazid, and rifampicin as positive controls (Tanachatchairatana et al. 2008).

Importantly, compound **3** was found to inhibit the granulation tissue formation in male Wistar rats, as induced by subcutaneous implantation of cotton pellets when these test animals were treated orally with **3** (25 or 50 mg/kg, daily) for seven days (Otsuka et al., 1981). This anti-inflammatory activity has been supported by the inhibitory effects observed for **3** on the activation of NF-κB, a key transcription factor in chronic inflammatory diseases (Andre et al., 2013), as well its antioxidative activities, as demonstrated by its 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging and copper ion-induced oxidation suppression effects (Cho et al., 2013). In addition, **3** was found to exhibit potent inhibitory activity against the dengue virus (DENV) non-structural protein 5 (NS5) RNA-dependent RNA polymerase (RdRp), with an IC_{50} of 0.85 μM (3′-deoxy-GTP, IC_{50} 0.02 μM) (Bourjot et al., 2012). DENV NS5 RdRp is responsible for viral replication and transcription and has become an attractive target for the development of anti-dengue drugs (Lim et al., 2015; Shimizu et al., 2019), and thus, compound **3** shows some promise of having antidengue activity.

Isolated also from *D. quaesita* collected in Laos was betulinic acid (BA, **3b**), a pentacyclic lupane-type triterpenoid occurring widely in plants (Ma et al., 2008). Compound **3b** has shown wide range of pharmacological activities, including antidiabetic, antihyperlipidemic, anti-infective, and antitumor properties, as reviewed recently (Ríos and Máñez, 2018; Amiri et al., 2020). More recently, determination of the mechanisms of antitumor and anti-infective activities of **3b**, as well as improvement of its pharmaceutical potential through the development of different formulations, has attracted wide interest. Compound **3b** prohibited the proliferation and migration of BGC-823 and MNK45 human gastric cancer cells (Chen et al., 2020b), and it reduced significantly A172, U87, and U87R (temozolomide resistant) human glioblastoma cell growth through activation of the protein kinase RNA-like endoplasmic reticulum kinase (PERK)/C/EBP homologous protein (CHOP) apoptotic pathway (Lo et al., 2020). In addition, **3b** inhibited invasion and migration of highly aggressive MDA MB-231 and BT-549 human breast cancer cells by targeting glucose-regulated protein 78 (GRP78) to suppress aerobic glycolysis in cells. It also retarded breast cancer lung colonization through GRP78/β-catenin/c-Myc signaling (Zheng et al., 2019).

To enhance the possible therapeutic applications of betulinic acid (**3b**), several different kinds of nanoscale delivery systems have been developed (Saneja et al., 2018), and the antitumor potential of **3b** has been improved through these delivery systems. For example, nanoliposomes of **3b** showed more potent inhibitory activity against the proliferation and glucose transport of HCT116 human colon cancer cells than **3b** itself (Wang et al., 2020a). This type of activity was mediated by targeting hexokinase (HK), phosphofructokinase-1 (PFK-1), phosphoenolpyruvate (PEP), and pyruvate kinase M2 (PKM2). Thus, the liposomal **3b** could be used as an effective adjuvant colon cancer therapy, by targeting tumor

metabolic pathways (Wang et al., 2020b). A carrier-free disulfide-modified glutathione (GSH)-responsive **3b** has been prepared, and a photosensitive prodrug, BA-S-S/Ce6 nanoparticles (NPs), has been constructed. Under irradiation, BA-S-S/Ce6 NPs exhibited more potent cytotoxicity than Ce6 against 4T1 musculus breast cancer cells, and it improved the antitumor efficacy of Ce6 (Cheng et al., 2021).

Several conjugated compounds, with **3b** being complexed with cabazitaxel, podophyllotoxin, and thiocolchicine, were synthesized, and the corresponding nanoassemblies were formed by the self-assembly of these synthetic products. Both the monomers and nanoparticles of the conjugates showed similarly potent activities against A2780 human ovarian cancer cells, but they were less active than those of the standard antitumor agents used. However, introduction of a properly designed self-immolative linker could facilitate the release of these drugs to improve their biological activity (Colombo et al., 2020).

Additionally, **3b** showed anti-infective activity (Ríos and Máñez, 2018; Amiri et al., 2020). Symptoms in rats with collagen-induced arthritis (CIA) were alleviated by the treatment with **3b**, and the histopathological features in the joints of the test animals were improved, with the secretion of inflammatory cytokines, including IL-1β, IL-6, and TNF-α being inhibited. These activities were mediated by inhibition of transcription of VEGF and transforming growth factor β (TGF-β), and activation of the NF-κB pathway (Kun-Liu et al., 2020). Recently, a new betulinic acid-nucleoside hybrid was found to show potent anti-HIV activity, with an IC_{50} value being 7.8 nM (Wang et al, 2020c). Some inhibitory effects against DENV infection were also found for this triterpene, which inhibited a post-entry stage of the DENV replication cycle, RNA synthesis, and protein production (Loe et al., 2020).

Compound **3b** was also found to inhibit significantly the receptor activator of nuclear factor κB ligand (RANKL)-induced osteoclastogenesis (Jeong et al., 2020) and α-glucosidase (Du et al., 2021). In EA.hy926 human vascular endothelial cells, **3b** enhanced intracellular Ca^{2+} levels to increase endothelial nitric oxide synthase (eNOS) expression through phosphorylation of 5’ adenosine monophosphate-activated protein kinase (AMPK) and activation of histone deacetylase 5 (HDAC5) and ERK5. NO produced by eNOS is crucial in endothelial function, and thus, **3b** possesses some potential to support the treatment of cardiovascular diseases through preventing vascular endothelial dysfunction (Lee et al., 2020). In Mlg mouse lung fibroblast and primary pulmonary fibroblast (PPF) cells, Wingless-related integration site (Wnt) 3a-induced myofibroblast activation and Wnt/β-catenin signaling were inhibited by **3b**. Idiopathic pulmonary fibrosis (IPF) is a fatal fibrotic lung disease, and aberrant activation of the Wnt/β-catenin signaling cascade plays the key role in the pathogenesis of IPF. Thus, **3b** shows some antipulmonary fibrosis activity (Li et al., 2021).

Accordingly, 3β-*O*-*trans*-caffeoylbetulinic acid (**3**) and betulinic acid (**3b**) could be the principal active components of the leaves and stems of *D. quaesita*, which are used to treat cases of alcohol and drug dependence and headache in Laos.

Gongronema napalense (Wall.) Decne. (Asclepiadaceae)

Gongronema napalense is a vine (Table 1) native to Bangladesh, China, India, Laos, Myanmar, Nepal, and Vietnam (Newman et al., 2007) and used to treat leucorrhea, blennorrhea, and traumatic injury in Laos (Soejarto et al., 2012). In a search of antimalarial agents from Lao plants, a sterol hexaglycoside, gongroneside A (**4**) (Figure 3), was characterized an active component of the entire vines of *G. napalense* collected in Champasak Province of Laos, and it exhibited potent antimalarial activity *in vitro* (Libman et al., 2008).

Marsypopetalum modestum (Pierre) B. Xue & R.M.K. Saunders (Annonaceae)

In an ethnobotanical search for tuberculosis (TB) treatments using Lao plants, healers in Bokeo, Bolikhamxai, Champasak, Luang Prabang, and Vientiane Provinces were interviewed. Based on these interviews, more than 300 plants were collected and tested for anti-TB activity against virulent *Mycobacterium tuberculosis* H37Rv, and an ethanol extract of *Marsypopetalum modestum* (small tree), was found to be the most potently active. A follow-up bioassay-guided isolation led to the identification of dipyrithione (**5**) (Figure 4) as a major active component, which demonstrated a minimum inhibitory concentration (MIC) of <0.15 μM against virulent mycobacteria (Elkington et al., 2014b).

Dipyrithione (**5**) also exhibited growth inhibitory effects on *Mycobacterium tuberculosis* BCG (Kawabe et al., 1967) and several fast-growing species of *Mycobacterium*, including *M. fortuitum*, *M. smegmatis*, and *M. phlei* (O'Donnell et al., 2009), as well several Gram-positive and -negative bacteria (Kawabe et al., 1967). While dipyrithione (**5**) suppressed the growth of methicillin-resistant *Staphylococcus aureus*, *S. aureus* EMRSA-15, and *S. aureus* XU212, it inhibited the strain growth of a multidrug-resistant (MDR) variant of *Staphylococcus aureus*, *S. aureus* SA-1199B, which overexpresses the NorA efflux transporter (O'Donnell et al., 2009).

Interestingly, two monomeric analogues of dipyrithione (**5**), 2-methyldithiopyridine *N*-oxide (**5a**) and 2-methylthiomethyldithiopyridine *N*-oxide (**5b**), were found to exhibit antibacterial activity against a small panel of bacterial strains, with both **5a** and **5b** being demonstrated as potently active against *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis* H37Rv. Of these, **5b** also inhibited completely the incorporation of ^{14}C-labeled acetate into soluble fatty acids, indicating that this substance is a potential fatty acid biosynthesis inhibitor (O'Donnell et al., 2009). Following this, the maximum tolerated dose (MTD) and anti-TB efficacy of **5b** were evaluated in animal models. Acute adverse effects were observed at the doses, 100 and 300 mg/kg, when C57BL/6 female mice were treated orally with **5b** (a single dose of 30, 100, or 300 mg/kg) and checked post-administration at 4 h and 6 h and then twice daily for a week. However, this compound did not show any anti-infective activity when eight- to ten-week-old female specific-pathogen-free C57BL/6-gamma interferon gene-disrupted (GKO) mice were infected by *M. tuberculosis* Erdman (one day) and treated orally with **5b** (30 mg/kg) for nine days (O'Donnell et al., 2009). Thus, modification of **5b** to improve its anti-TB properties would be required in future investigations.

Inflammation is a complex biological response to harmful stimuli, including infections, injuries, and toxins, and overexpression of the C-X-C motif chemokine ligand 10 (CXCL10), a small cytokine known as IFN-γ-induced protein 10 (IP-10) or IP-10/CXCL10, regulates almost all of its stages. Dipyrithione (**5**) was found to suppress IP-10/CXCL10 expression (Han et al., 2010), but it did not show any observed effects on the activity of COX-1 and COX-2 (Krejčová et al., 2014). However, two monomeric analogues of **5**, 2-methylthiomethyldithiopyridine *N*-oxide (**5b**) and 2-methylthiomethyldithiopyridine (**5c**) (Figure 4), were found to inhibit COX-1 and COX-2, while **5**, 2-methyldithiopyridine *N*-oxide (**5a**), and some other analogues, did not. This indicated that a methylthiomethyldithio group is required to mediate COX-1 and COX-2 inhibitory activities, which are involved in the conversion of arachidonic acid to prostaglandins, and thus **5b** and **5c** both show some potential for the development as new anti-inflammatory agents (Krejčová et al., 2014).

Compound **5** was also found to inhibit LPS-induced expression of iNOS and NO production, and it also reduced the LPS-induced increase of COX-2 protein levels in RAW264.7 murine macrophage-like cells. NO produced by iNOS and prostaglandins induced by COX-2 are two of the most prominent inflammatory mediators, and thus dipyrithione (**5**) appears to possess promising anti-inflammatory effects (Liu et al., 2008). Mouse survival rates were increased when male ICR mice were injected with LPS and treated (i.p.) (30 minutes later) with **5** (1, 2.5 or 5.0 mg/kg) or dexamethasone (2 mg/kg) (Liu et al., 2008). In a mouse acute lung injury model induced by oleic acid, the lung injury was attenuated significantly by dipyrithione (**5**) when six–seven-week-old male ICR mice were dosed by caudal intravenous (i.v.) administration of 0.3 mL/kg of oleic acid and treated (i.p., single dose) with **5** (1.25, 2.5, or 5 mg/kg) or dexamethasone sodium phosphate (5 mg/kg), before or after oleic acid induction (30 minutes). Also, dipyrithione treatment alleviated significantly microvascular leakage and inhibited oleic acid-induced increase of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule (VCAM-1) expression in the mouse lung (Huang et al., 2011).

Pyrithione (2-mercaptopyridine-1-oxide, **5d**) (Figure 4) has been used as a bactericide and a fungicide for many years, from which **5** may be formed by self-oxidation of the free SH group of pyrithione. In RAW264.7 murine macrophage-like cells, **5** exhibited more potent activity than **5d** in inhibiting LPS-induced iNOS and COX-2 expression and than aspirin in inhibiting LPS-induced NO release. It showed anti-inflammatory effects in an endotoxin shock mouse model, which was similar to that observed for dexamethasone. This supports further the possible use of **5** as an anti-inflammatory lead compound (Liu et al., 2008). Mechanistically, dipyrithione (**5**) attenuates the activation of Janus kinase (JAK)/signal transducers and activators of transcription-1 (STAT1) signaling pathways to suppress IP-10/CXCL10 expression (Han et al., 2010). It also targets STAT1 but not the MAPK and NF-κB pathways to mediate its anti-inflammatory property in response to LPS, so suppression of iNOS expression through reducing STAT1 phosphorylation by **5** could result in the LPS-induced augmentation of COX-2 protein levels being reduced (Liu et al., 2008). Interestingly, dipyrithione (**5**) was also found recently to inhibit potently the growth of *Trichophyton rubrum*, a fungal species leading to dermatophytosis. The dermatophyte infection was inhibited significantly when female guinea pigs (300 g) were inoculated

topically by *T. rubrum* (for ten days) and treated topically with dipyrithione (0.2 mg/cm^2, daily) for ten days (Song et al., 2018).

In other investigations, dipyrithione (**5**) has exhibited potential antitumor activity. For example, it suppressed HeLa human cervical cancer cell growth through induction of cell apoptosis and inhibited the proliferation of human KB nasopharyngeal and MDA-MB-231 breast cancer and U937 and K562 leukemia cells (Table 3) (Fan et al., 2007; Fan et al., 2013). *In vivo* investigations indicated that hepatoma and sarcoma growths were inhibited significantly, when six-week-old male ICR mice were inoculated with human S180 sarcoma or H22 hepatoma cells and treated (i.p.) daily with dipyrithione (0.25 or 2.5 mg/kg) for ten days. No obvious changes were observed in the body weights of the treated mice (Fan et al., 2007; Fan et al., 2013). Mechanistically, dipyrithione (**5**) induces HeLa cell apoptosis through induction of the expression of p21, cleavage of caspase-3 and poly (ADP-ribose) polymerase (PARP), and release of cytochrome c, with the p38 and ERK1/2 signaling pathways being involved (Fan et al., 2007). It induces G1 arrest in K562, KB, MDA-MB-231, and U937 cancer cells by modulation of p53, p21, and levels of cyclins D1 and E1, and it induces apoptosis in these cells through activation of caspases-3 and −9 and of PARP (Fan et al., 2013).

The monomeric analogues of dipyrithione (**5**), 2-methyldithiopyridine *N*-oxide (**5a**) and 2-methylthiomethyldithiopyridine *N*-oxide (**5b**), were also found to show cytotoxicity against human MCF-7 breast, A549 lung, and HT-29 colon cancer cells, with IC_{50} values being in the range 0.2–1.8 μM (O'Donnell et al., 2009). It is worth noting that a complex of two pyrithione ligands chelated to Zn^{2+}, namely, zinc pyrithione (ZnPT, **5e**) (Figure 4), is attracting increasing interest for its antitumor activity. For example, ZnPT inhibited SKOV3 human ovarian cancer cell growth (IC_{50} 0.65 μM), and its activity was increased (IC_{50} 0.35 μM) when cells were treated with ZnPT plus zinc chloride (20 μM) (Chen et al., 2020a). ZnPT showed cytotoxicity against human HSC2 (IC_{50} 2 μM), MDA1986 (IC_{50} 1.3 μM), and SCC4 (IC_{50} 2 μM) oral squamous cell cancer cells (Table 3) (Srivastava et al., 2015), as well as HL-60 human leukemia cells (IC_{50} 0.1–1 μM) (Tailler et al., 2012). The oral tumor growth was inhibited when NOD/SCID/Crl mice were inoculated by SCC4 human oral squamous cell cancer cells and treated (i.p.) with ZnPT (1 mg/kg, weekly) for six weeks after the tumors reached 200 mm^3 (Srivastava et al., 2015). Its potent antileukemic efficacy was observed when athymic nude mice were inoculated by HL-60 cells and treated (i.p.) with ZnPT (5 mg/kg, twice a week) for 40 days (Tailler et al., 2012).

Mechanistic investigations have indicated that ZnPT induces zinc accumulation and oxidative stress in HepG2 human liver cancer cells, which led to cell apoptosis through increasing the Bax/Bcl-2 ratio, causing mitochondrial dysfunction, cytochrome c release, and increased caspase-9/−3 activity (Mo et al., 2018). In PC3 human prostate cancer cells, ZnPT inhibited metabolism and induced ERK- and protein kinase C (PKC)-dependent necrosis by decreasing cellular adenosine 5'-triphosphate (ATP) levels that cause the phosphorylation of AMPK and acetyl CoA carboxylase (ACC) (Carraway and Dobner, 2012). In addition, it mediated cytotoxicity against human oral squamous cell cancer cells, postulated as acting through the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) and Wnt/β-catenin signaling pathways (Srivastava et al., 2015).

Toxicological investigation showed that ZnPT induced caspase-independent cell death, heat shock response gene expression encoding heat shock proteins (HSPs), impaired genomic integrity, and depletion of cellular ATP levels in cultured primary human skin HEKn-APF keratinocytes. In both HEKn-APF keratinocytes and HEMa-LP melanocytes, ZnPT caused DNA damage, indicating that this topical antimicrobial may target and compromise keratinocytes and melanocytes in intact human skin (Lamore et al., 2010). At a concentration of 1 μM, ZnPT exposure did not cause any genotoxic threat and/or a proliferation index change in normal lymphocytes obtained from healthy human volunteers, but it increased lactate dehydrogenase, oxidative stress, and intracellular zinc levels. Thus, this complex may exhibit toxic effects against human cellular systems via increasing oxidative stress (Güner and Ilhan, 2020). Therefore, the toxicity of ZnPT could be a matter of concern for its possible development as an anticancer agent, although this may be able to be resolved by synthetic modification of the molecule.

Alkaloids are an important group of natural products showing potential anticancer activity, of which derivatives of camptothecin and bisindole alkaloids such as vincristine are used clinically, targeting topoisomerase (topo)-I and tubulin polymerization, respectively (Henkin et al., 2018; Agarwal et al., 2020). A dimeric sulfur-containing pyridine derivative, dipyrithione (**5**), was synthesized by oxidation of pyrithione (**5d**) with hydrogen peroxide (Barton and Samadi, 1992), of which **5d** was produced when 2-bromopyridine reacted with perbenzoic acid followed by treatment with sodium sulfide (Shaw et al., 1950). As a natural product derivative, dipyrithione (**5**) has been found to occur in the New Zealand basidiomycete (mushroom), *Cortinarius* sp. (Cortinariaceae) (Nicholas et al., 2001), and the bulbs of *Allium stipitatum* Regel (Amaryllidaceae) (Krejčová et al., 2014; Kubec et al., 2011; O'Donnell et al., 2009). However, it was identified for the first time in the family Annonaceae, from the Lao plant, *Marsypopetalum modestum* (Elkington et al., 2014b). Its potent activity (MIC <0.15 μM) against virulent mycobacteria (Elkington et al., 2014b) and other promising bioactive properties indicates that this compound could be an important lead compound for the development of effective agents to treat either cancer or various infectious diseases.

Nauclea orientalis (L.) L. (Rubiaceae)

Nauclea orientalis is a small tree (Table 1) native to Laos and tropical Asia (Newman et al., 2007), of which the dried stem wood and stem bark are used to treat cases of fatigue (Soejarto et al., 2012). The stems of *N. orientalis* were collected in Bolikhamsai Province of Laos, and both the methanol extract and chloroform partition were found to be active when tested against the chloroquine-sensitive clone D6 and the chloroquine-resistant clone W2 of *Plasmodium falciparum*. A follow-up bioassay-guided fractionation of the antimalarial-active chloroform partition resulted in the isolation of two new and one known indole-like alkaloid glucosides, namely, naucleaorine (**6**), epimethoxynaucleaorine (**6a**), and strictosidine lactam (**6b**), along with several known compounds, including oleanolic acid (**7**), 3α,23-dihydroxyurs-12-en-28-oic acid (**8**), and their analogues (Figure 5) (He et al., 2005). The alkaloids **6** and **6a** were found to be active in this *in vitro* antimalarial assay used, with IC_{50} values being in the range 6–13 μM, but **6b** was inactive. This demonstrated that a conjugated system along the C-3, C-4, C-14, C-15, C-20, and C-21 positions plus

a methoxy group substituted at the C-19 position are required for the activity, while the configuration at the C-19 position seems not to contribute significantly to this effect. Two triterpenoids, oleanolic acid (**7**) and 3α,23-dihydroxyurs-12-en-28-oic acid (**8**) were also active (IC_{50} 4–13 μM) against the chloroquine-sensitive and -resistant malaria parasites, and **7** was more potently active than ursolic acid (**8a**), while other related analogues did not show any activity (He et al., 2005). These results imply that both a C-23 hydroxy group and a C-28 carboxylic acid substituent are required for antimalarial activity, but introduction of a hydroxy group at the C-19 position followed by methyl esterification of the C-28 carboxylic acid results in this type of activity being abolished. Malaria is a mosquito-borne infectious disease caused by the *Plasmodium* species, and, to date, it continues to present a great challenge to public health in Laos, owing to increasing cases of MDR malaria as observed during its therapy. Traditional medicines have been used for a long time to treat malaria in Laos, and many plants included in Lao herbal prescriptions show some potential for the treatment of this infectious disease (Elliott et al., 2020). Thus, the discovery of potential antimalaria alkaloids **6** and **6a** and triterpenoids **7** and **8** from *N. orientalis* collected in Laos indicates that additional interesting compounds could be identified from a continuing search for antimalaria leads from Lao plants, and that further synthetic modification of these leads may produce potent agents to treat malaria effectively.

Both **7** and **8a** were also found to show anti-TB activity (Tanachatchairatana et al., 2008). When comparing the anti-TB potency of these triterpenoids and their analogues or derivatives, including 3β-*O*-*trans*-caffeoylbetulinic acid (**3**), isolated from *D. quaesita* collected in Laos (Ma et al., 2008), **3** and **7** were found to exhibit activity, while ursolic acid (**8a**) was more potently active than **3** and **7** (Tanachatchairatana et al., 2008). Interestingly, 3β-*O*-*trans*-*p*-coumaroylbetulinic acid (**3g**), 3β-*O*-*trans*-*p*-coumaroyloleanolic acid (**7a**), and 3β-*O*-*trans*-*p*-coumaroylursolic acid (**8b**) were found to show more potent antimycobacterial activity than their parent compounds, **3b**, **7**, and **8a**, as well their acetylated derivatives, 3β-*O*-*trans*-*p*-acetylcoumaroylbetulinic acid, 3β-*O*-*trans*-*p*-acetylcoumaroyloleanolic acid (**7b**), and 3β-*O*-*trans*-*p*-acetylcoumaroylursolic acid (**8c**). Also, these coumarates were more potently active overall than their caffeate analogues, including **3**, **3a**, 3β-*O*-*trans*-caffeoyloleanolic acid (**7c**), 3β-*O*-*trans*-diacetycaffeoyloleanolic acid (**7d**), 3β-*O*-*trans*-caffeoylursolic acid (**8d**), and 3β-*O*-*trans*-diacetycaffeoylursolic acid (**8e**) (Table 4). Thus, introduction of a 3β-*O*-*trans*-*p*-coumaroyl group in **3b**, **7**, and **8a** appears to increase the resultant anti-TB potency within this triterpenoid class (Tanachatchairatana et al., 2008).

When tested against KB human oral epidermoid cancer cells, compound **6** showed weak activity, but compound **6a** did not, indicating that the configuration at the C-19 position of **6** is important for this compound to exhibit inhibitory activity on KB cell growth. Similar to **6a**, both **7** and **8** did not show any discernible growth inhibitory activity (IC_{50} >10 μM) against KB cells (He et al., 2005). However, the parent 23-dehydroxylated analogue of **8**, ursolic acid (**8a**), has been reported extensively for its potential antitumor activity. For example, **8a** exhibited cytotoxicity against MDA−MB−231 human breast cancer cells (IC_{50} 5.9 μM) and inhibited NF-κB activity potently (IC_{50} 31 nM), for which substitutions at both the C-3 and C-28 positions seem important (Ren et al., 2018). Mechanistically, ursolic acid (**8a**) targets NF-κB, COX-2, matrix metalloproteinase 9 (MM-9), cyclin D11, and other

proteins to mediate its antitumor effects, and it also inhibits angiogenesis and metastasis. Thus far, a phase I cancer clinical study has been completed for this compound, with a phase II clinical trial being recommended (Qian et al., 2015).

Even though **7** was inactive (IC_{50} >10 μM) against KB cells (He et al., 2005), several natural or semisynthetic oleanolic acid-like compounds have been reported for their potential antitumor activity (Ren and Kinghorn, 2019), including pristimerin and bardoxolone methyl (methyl 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oate, CDDO−Me). Pristimerin is a quinone methide triterpenoid isolated from species of the Celastraceae and Hippocrateaceae plant families and exhibits promising antitumor activity (Li et al., 2019), while CDDO−Me, a synthetic derivative of **7**, inhibited potently the production of nitric oxide induced by IFN-γ in mouse macrophages and redirects the activation profile of tumor-associated macrophages from tumor-promoting to tumor-inhibition (Honda et al., 2000; Ball et al., 2016). It was also found to show antitumor efficacy by improving the immune response in cancer (Ren and Kinghorn, 2019), indicating a possible immunotherapeutic role in the treatment of cancer for CDDO−Me. In addition, CDDO-Me is a potent nuclear factor erythroid 2–related factor 2 (Nrf2) activator and a NF-κB inhibitor and has been investigated in a phase I cancer clinical trial (Ren and Kinghorn, 2019; Wang et al., 2014). Recently, a phase III CARDINAL trial in patients with Alport syndrome has been completed for CDDO-Me (Chertow et al., 2021).

As summarized recently, both **7** and **8a** show potent pharmacological properties. Compound **7** has attracted increasing attention for its promising anti-inflammatory, anti-metabolic syndrome, antitumor and hepatoprotective propensities, as well its antiviral activity against hepatitis, herpes, HIV, and influenza (Feng et al., 2020; Khwaza et al., 2018). Similarly, **8a** showed antimicrobial, antitumor, and antiviral activities and pharmacological effects on the cardiovascular and urinary systems (Khwaza et al., 2020; Ren and Kinghorn, 2019; Sun et al., 2020). Recently, both **7** and **8a** were proposed as potential anti-SARS-CoV-2 agents, based on their binding to the key propathogenic M^{pro} protein (Pawelczyk and Zaprutko, 2020), and thus some promising anti-COVID-19 agents could be discovered from further investigations on higher plants that occur in Laos.

The dried stem wood of *N. orientalis* is used to treat cases of fatigue in Laos, with alkaloid **6** and triterpenoid **8** and their analogues being characterized as active components of this species (Soejarto et al., 2012). Also, several triterpene analogues of **8** were identified as the major components of the hard wood of *N. orientalis* collected in Japan (Fujita et al., 1967), while a number of indole alkaloid derivatives of **6** were characterized from the leaves and stems of *N. orientalis* collected in China and in Thailand (Kanchanapoom et al., 2021; Liu et al., 2018). Thus, triterpenoids and indole alkaloids could be the major bioactive constituents of *N. orientalis* growing in Laos and in other countries in Asia, including China, Japan, and Thailand.

Rourea minor (Gaertn.) Aubl. (Connaraceae)

Rourea minor is a liana (Table 1) native to tropical Africa and tropical Asia (Newman et al., 2007), of which the dried stems are used to treat dengue fever (Soejarto et al., 2012). Thus, the dried stems of *R. minor* were collected in Bolikhamsai Province of Laos

and investigated further for identification of their bioactive components. A new lignan glucoside, rourinoside (**9**), along with several glycerides, including rouremin (**10**) and 1-(26-hydroxyhexacosanoyl)glycerol (**10a**) (Figure 6), were isolated from this plant sample. All of these compounds were found to show activity against both the chloroquine-sensitive clone D6 and the chloroquine-resistant clone W2 of *P. falciparum*, with IC_{50} values occurring in the range 2–13 μM, and thus these compounds exhibit potential antimalarial activity (He et al., 2006).

Stemona pierrei Gagn. and *Stemona tuberosa* Lour. (Stemonaceae)

Stemona is the largest genus of the family Stemonaceae, and several of its species have been used as antihelminthic agents and to treat coughs in Laos, as well in China, Thailand, and Vietnam (Quang et al., 2014). Of these, *Stemona pierrei* is a vine (Table 1) native to Laos, Thailand, and Vietnam, while *Stemona tuberosa* is a climbing plant (Table 1) native to Bangladesh, Cambodia, China, India, Indonesia, Laos, Myanmar, New Guinea, the Philippines, Sri Lanka, Thailand, and Vietnam (Newman et al., 2007). In a search for potential antitumor agents from *Stemona* plants, three new phenanthrenes, namely, stemophenanthrenes A (**11**), B (**11a**) and C (**11b**) (Figure 7), were isolated from the roots of *S. tuberosa* collected in Attapeu Province of Laos.

These compounds exhibited inhibitory activity against human HepG2 liver, KB oral epidermal, MCF7 breast, and SK-Lu-1 lung cancer cells (Khamko et al., 2013), and the laboratory work conducted indicated that a 5,6,7-tri-*ortho* substituent is required in this regard. In addition, a new phenylbenzofuran-type stilbenoid, stemofuran X (**12**), isolated from the roots of *S. pierrei* collected in Savannakhet Province of Laos, and two new normal stilbenoids, stemofuran Y (**12a**) and isopinosylvin A (**13**), identified from the roots of *S. tuberosa* collected from Attapeu Province of Laos (Figure 7), were also tested for their cytotoxicity against HepG2, KB, MCF7, and SK-Lu-1 cancer cells. However, none of these stilbenoids was found to be active against any of these four cell lines (IC_{50} >10 μM) (Khamko et al., 2013; Quang et al., 2014).

Stilbenoids are an important group of natural products distributed widely among edible plants, of which *trans*-resveratrol (3,5,4’-trihydroxy-*trans*-stilbene) occurs in various berries, grapes, red wine, and many other human foods, and shows a broad range of biological activities, including anti-aging, anti-infective, antioxidant, and anticarcinogenic effects. It also possesses cardio-, gastrointestinal, hepatic-, and nephroprotective activities, and protects organs and tissues against ultraviolet radiation (UVR)-induced skin cancer and carcinogen-induced damage. Thus, *trans*-resveratrol has been investigated extensively for its cancer chemopreventive and synergistic properties, and it may reduce the side effects to enhance the outcomes of cancer chemotherapy, when used in combination with approved cancer chemotherapeutic agents (Jang et al., 1997; Xiao et al., 2019). Therefore, the discovery of new stilbenoids from Lao plants could be useful for the development of further potential cancer chemoprotective agents.

The roots of *Stemona tuberosa* have long been used as a traditional Chinese medicine (“Baibu”) as an insecticidal and antitussive agent, and several stilbenoids have been isolated from this plant source when collected in China (Li et al., 2020a; Lin et al., 2008). Of

these compounds, stilbostemin U was found to show antimicrobial activity against *Bacillus pumilus* (MIC 41–83 μM) (Lin et al., 2008). In addition, several alkaloids have been isolated from this same species when sourced in China (Hu et al., 2020; Lin et al., 2006).

Alkaloids derived from the monocotyledonous family Stemonaceae, namely, the stemona alkaloids, are a large group of natural products, with more than 200 compounds of this type having been identified thus far. These alkaloids have been found to show multiple promising bioactivities, including acetylcholinesterase inhibitory, anti-inflammatory, antitussive, and insecticidal activities, with stemofoline showing the most potent activity in the reversal of multidrug resistance of some anticancer agents (Greger 2019). As a member of this group, bisdehydrostemoninine, a stemona alkaloid isolated in a large amount from *S. tuberosa* collected in China, showed significant antitussive activity when Dunkin-Hartley guinea pigs (300–350 g) were treated (i.p., single dose) with bisdehydrostemoninine (50 mg/kg) followed (after 30 min.) by exposure in 0.5 M citric acid aerosol at flow rate of 0.5 mL/min for eight min (Lin et al., 2006). Another major stemona alkaloid from *S. tuberosa*, neotuberostemonine (Hu et al., 2020), was found to suppress hypoxia-induced activation and differentiation of primary mouse lung fibroblasts (PLFs). The bleomycin-induced pulmonary fibrosis was attenuated effectively when ICR male mice were injected intratracheally with bleomycin (3.5 U/kg), and, after seven days, treated orally with neotuberostemonine (30 mg/kg, daily) for 7 or 14 days. This *in vivo* activity was found to be mediated by inhibition of HIF-1α and its downstream profibrotic factors (Lv et al., 2018). Thus, stemona alkaloids could contribute to the observed antitussive effect of *S. tuberosa* (Lin et al., 2006). However, no stemona alkaloids have been thus far isolated from the Lao *Stemona* species, and thus it would seem valuable further phytochemical work on the plant family Stemonaceae in Laos.

DISCUSSION AND PERSPECTIVE

Laos is a landlocked country with a prolific and varied tropical flora, and medicinal plants remain the backbone of primary health care in this country. However, only limited phytochemical investigations have been conducted thus far on eight of these plants, namely, *A. cochinchinensis*, *D. quaesita*, *G. napalense*, *M. modestum*, *N. orientalis*, *R. minor*, *S. pierrei*, and *S. tuberosa*, of which none is endemic to the country. All of these plant species were collected in the southern part of Laos, and previous investigations have focused mainly on the identification of anti-infective and cytotoxic constituents guided by cell-based bioassays. Thus, a further search for bioactive agents from the northern area of Laos, involving evaluation of the lead compounds for additional biological activities, and elucidation of the cellular mechanisms of action of the active compounds present, could be important for the development of one or more useful therapeutic agents.

Cancer is a serious disease and a threat to human health, and the discovery of new effective anticancer agents is urgently needed (Newman and Cragg, 2020; Kinghorn et al., 2016). Several species collected in Laos were found to be cytotoxic toward human cancer cells (Henkin et al., 2017), from which several compounds showing cancer cell cytotoxicity have been characterized (Soejarto et al., 2006; Zhang et al., 2016). Thus, a continuing search for anticancer agents from Lao plants could be of promise. In addition, infections from bacteria, tuberculosis (TB), malaria, and/or viruses are problematic in Laos, and members of

local communities use traditional herbal medicines as their first-line of therapy to treat such diseases for many years. Thus far, several Lao plants and their active components against *Mycobacterium tuberculosis* H37Rv (TB), HIV, and malaria have been identified (Elkington et al., 2009; Soejarto et al., 2012; Zhang et al., 2016), and this preliminary work could form the foundation for the continuous search of agents from Lao plants for the treatment of these disorders.

Plant-derived phenolic compounds are a large group of natural products, including lignans and stilbenoids, of which some show potential antitumor activity (Soobrattee et al., 2006). For example, etoposide, a semisynthetic epipodophyllotoxin glycoside derived from a naturally occurring aryltetralin lignan lactone, podophyllotoxin, is well established as a therapy clinically to treat different types of cancer, targeting DNA topo II. Recently, a structurally similar analogue, phyllanthusmin D, has been identified as a promising antitumor lead, which mediates its activity by induction of cancer cell apoptosis through activation of caspase-3 but not by inhibition of topo II (Ren et al., 2014; Young et al., 2018; Ren et al., 2019). A norlignan, nyasol (**1b**), isolated from a Lao plant, was found to show NF-κB-targeted anti-infective activity, and it also directs FPTase, PRL-3, VEGF to exhibit antitumor potential (Jeong et al., 2003; Lee et al., 2014; Lim et al., 2009; Song et al., 2008). Thus, further modification of this molecule may be helpful for the discovery of novel anticancer and anti-infective agents, and preparation of drug conjugates from **1b** and other drugs used clinically may be promising in this regard.

The triterpenoids, 3β-*O*-(*trans*-caffeoyl)betulinic acid (**3**) and oleanolic acid (**7**), and the thiopyridine, dipyrithione (**5**), isolated from Lao plants, were all found to show antitumor, anti-infective activities, with **3** and **5** known to target NF-κB (Liu et al., 2008; Andre et al., 2013). NF-κB functions widely in inflammation and human pathobiology (Zhang et al., 2017), and inhibition of this protein could be supportive to the treatment of autoimmune and lymphoproliferative disorders (Qin et al., 2005; Concetti and Wilson, 2018). Accordingly, compounds **3** and **5** may serve as leads for the design of NF-κB-targeted therapeutic agents, with potential uses in the treatment of cancer and infectious diseases.

Currently, COVID-19 has resulted from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is spreading throughout the world to threaten human health seriously. The infection of SARS-CoV-2 is commonly followed by a deep inflammation known as a “cytokine storm” caused by activation of pro-inflammatory genes, such as NF-κB, STAT-3, IL-6, IL-8, IL-1ss, which leads to lung tissue destruction. While the microRNA network has been proposed as a target for the COVID-19-induced inflammatory response (Gasparello et al., 2021), the NF-κB pathway and Nrf2 have been regarded as the potential targets of COVID-19 (Carcaterra and Caruso, 2021; Cuadrado et al., 2020; Zinovkin and Grebenchikov, 2020). Nrf2 is a transcription factor in charge of cellular redox balance and the expression of genes involved in immunity and inflammation, and several Nrf2 activators have been proposed for their inhibitory activity against COVID-19 (Cuadrado et al., 2020; Zinovkin and Grebenchikov, 2020). Of these, an herbal medicine, PB125, an ethanol extract of a mixture of rosemary (*Rosmarinus officinalis*), ashwagandha (*Withania somnifera*), and luteolin, was found to activate Nrf2 potently and to downregulate angiotensin-converting enzyme 2 (ACE2, a surface receptor of SARS-CoV-2) and transmembrane protease serine 2

(TMPRSS2) mRNA expression in HepG2 human liver cancer cells (McCord et al., 2020). Therefore, by targeting NF-κB and Nrf2, some anti-COVID-19 agents may be discovered from repurposing the current anticancer compounds, and SARS-CoV-2 may be introduced to infect selectively human cancer cells, through engineering modification of NF-κB and Nrf2.

Medicinal plants have been long used for the treatment of cancer and infections in Laos, and their derivatives, nyasol (**1b**), 3β-*O*-*trans*-caffeoylbetulinic acid (**3**), and dipyrithione (**5**) have been found to show the NF-κB-targeted antitumor and anti-infective activities. In addition, both betulinic acid (**3b**) and ursolic acid (**8a**) were found to mediate their pharmacological activities via activation of the Nrf2 signaling pathway (Li et al., 2020b), and oleanolic acid (**7**) and **8a** have been considered for their potential anti-COVID-19 activity (Pawelczyk and Zaprutko, 2020). Thus, searching anti-COVID-19 agents from Lao plants seems reasonable, and **3b**, **7**, and **8a** may be modified by conjugating them with **5e**, which contains N, S, and Zn that could support such an activity, for their development as an effective therapy for COVID-19.

Thus far, several different types of natural products, including alkaloids, glycerol esters, phenolic compounds (neolignans and norlignans and stilbenoids), steroids, and triterpenoids have been identified as potential antitumor and/or anti-infective agents from Lao plants. The compounds targeting NF-κB and/or Nrf2, including **1b**, **3**, **3b**, **5**, and **7** could be supportive of the design and discovery of novel therapeutic agents. In this regard, a continuing search of bioactive agents from Lao plants may be regarded as not only necessary but also important for the improvement of human health.

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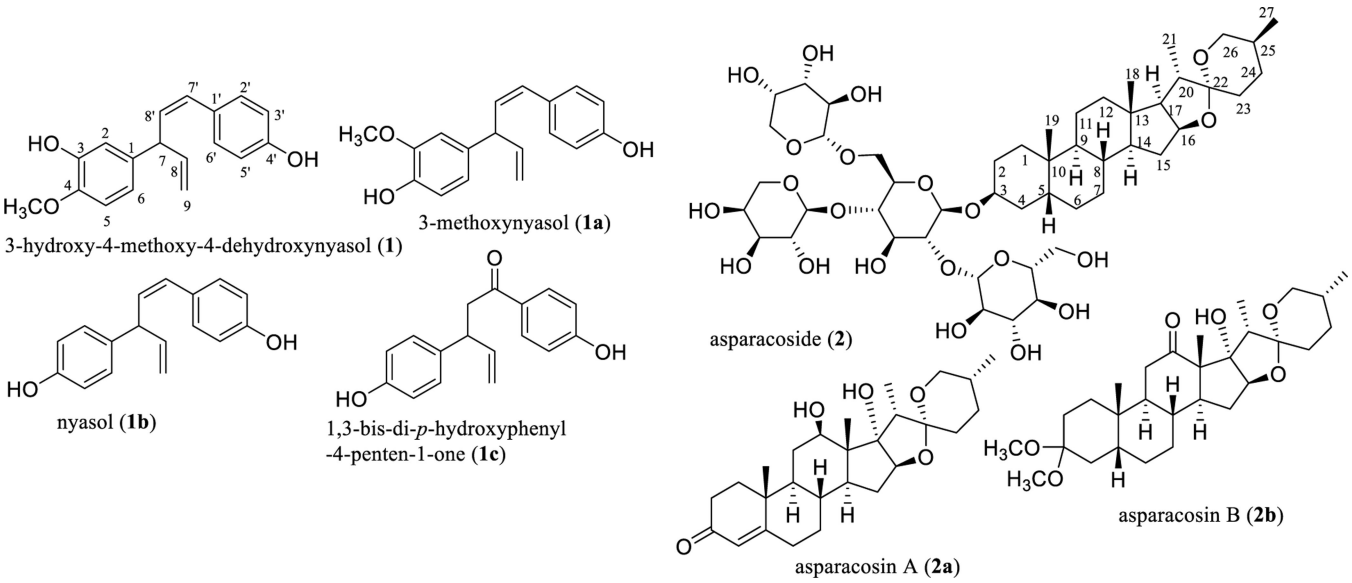


Figure 1.

Structures of the norlignan 3-hydroxy-4-methoxy-4-dehydroxynyasol (**1**) and its analogues (**1a**–**1c**) and the steroid asparacoside (**2**) and its analogues (**2a** and **2b**) isolated from the roots of *Asparagus cochinchinensis* collected in Laos (Zhang et al., 2004).

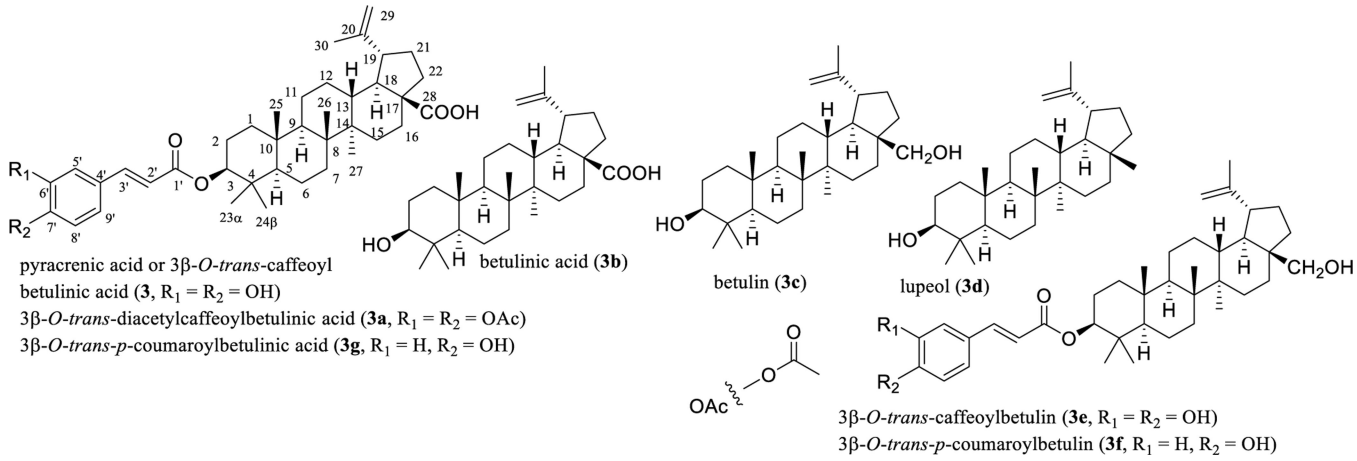
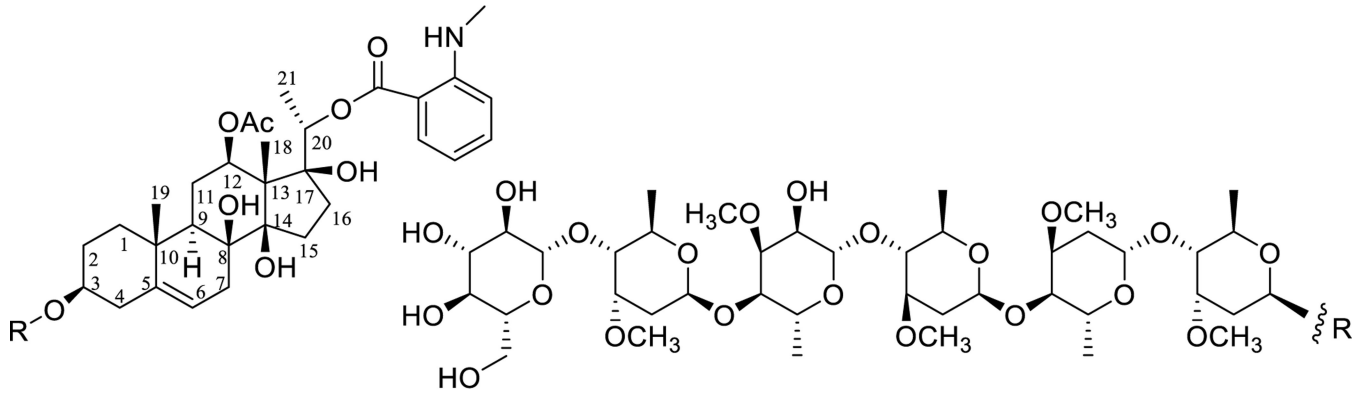


Figure 2.

Structures of the triterpenoid pyracrenic acid (3β-*O-trans*-caffeoyl betulinic acid, **3**) and betulinic acid (**3b**) isolated from the Lao plant, *Diospyros quaesita*, and of selected derivatives **3a** and **3c**–**3g** (Lomchid et al., 2017; Ma et al., 2008; Shi et al., 2014).



gongroneside A (**4**)

Figure 3.

Structure of the steroid glycoside gongroneside A (**4**) isolated from the Lao plant, *Gongronema napalense* (Libman et al., 2008).

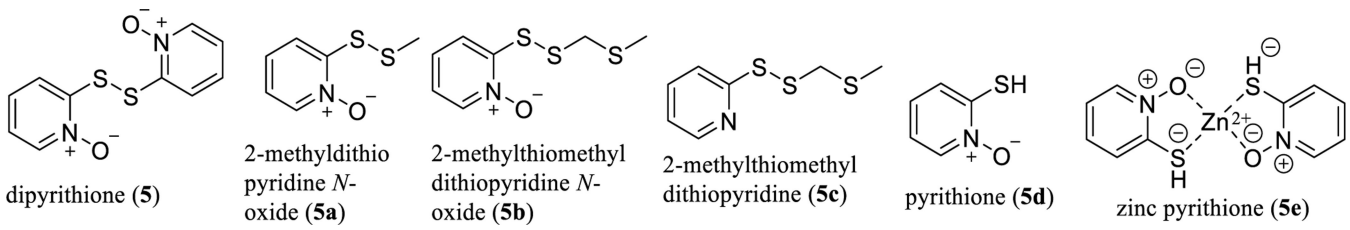


Figure 4.

Structures of the thiopyridine dipyrithione (**5**) isolated from the Lao plant, *Marsypopetalum modestum*, and its analogues (**5a**–**5e**) (Elkington et al., 2014b; O'Donnell et al., 2009; Krejčová et al., 2014; Liu et al., 2008; Srivastava et al., 2015; Tailler et al., 2012).

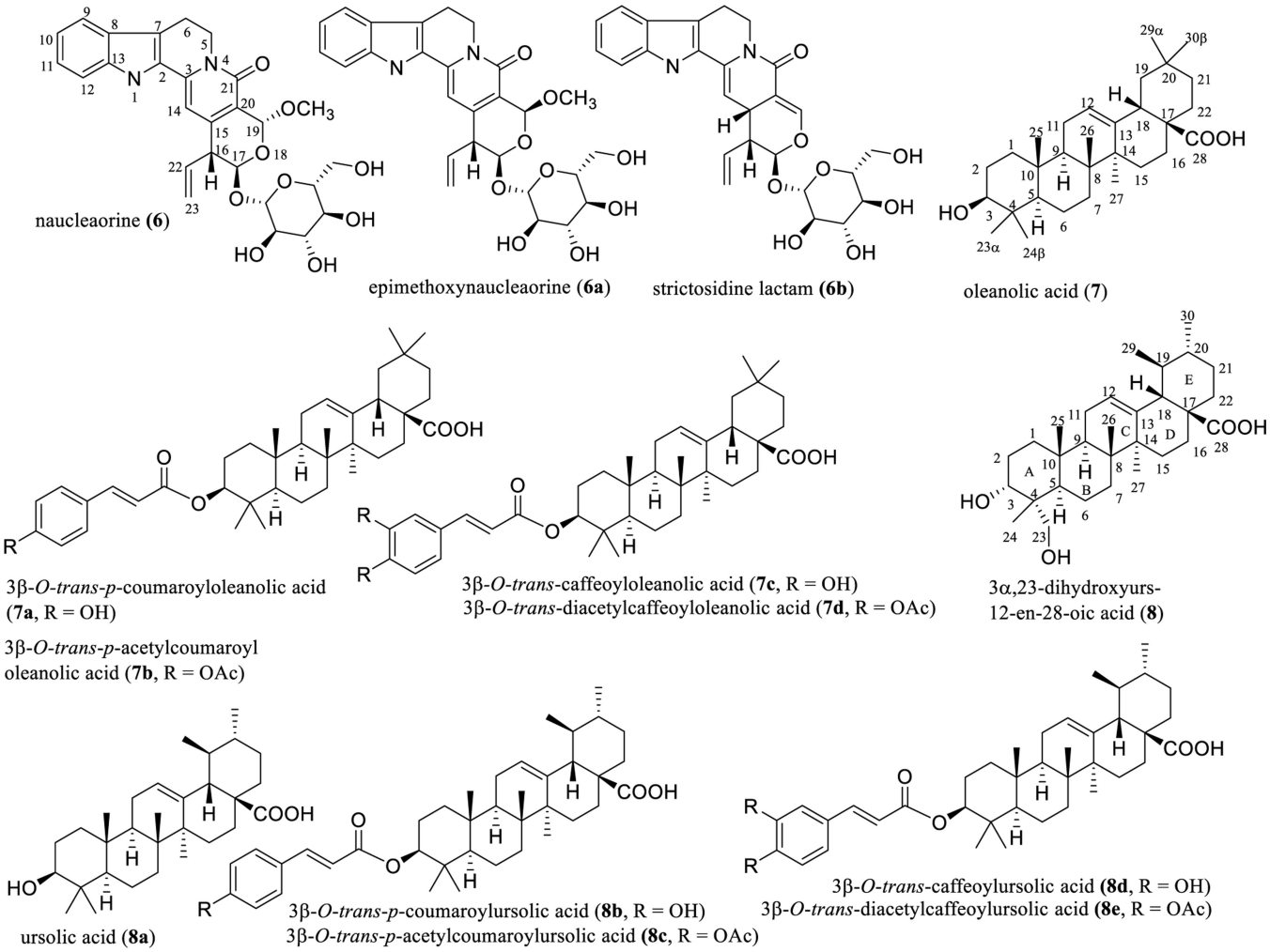


Figure 5.

Structures of the alkaloid naucleaorine (**6**) and its analogues (**6a** and **6b**) and the triterpenoids oleanolic acid (**7**) and 3a,23-dihydroxyurs-12-en-28-oic acid (**8**) isolated from the Lao plant, *Nauclea orientalis*, and selected derivatives, **7a**–**7d** of **7** and **8a**–**8e** of **8** (He et al., 2005; Tanachatchairatana et al., 2008).

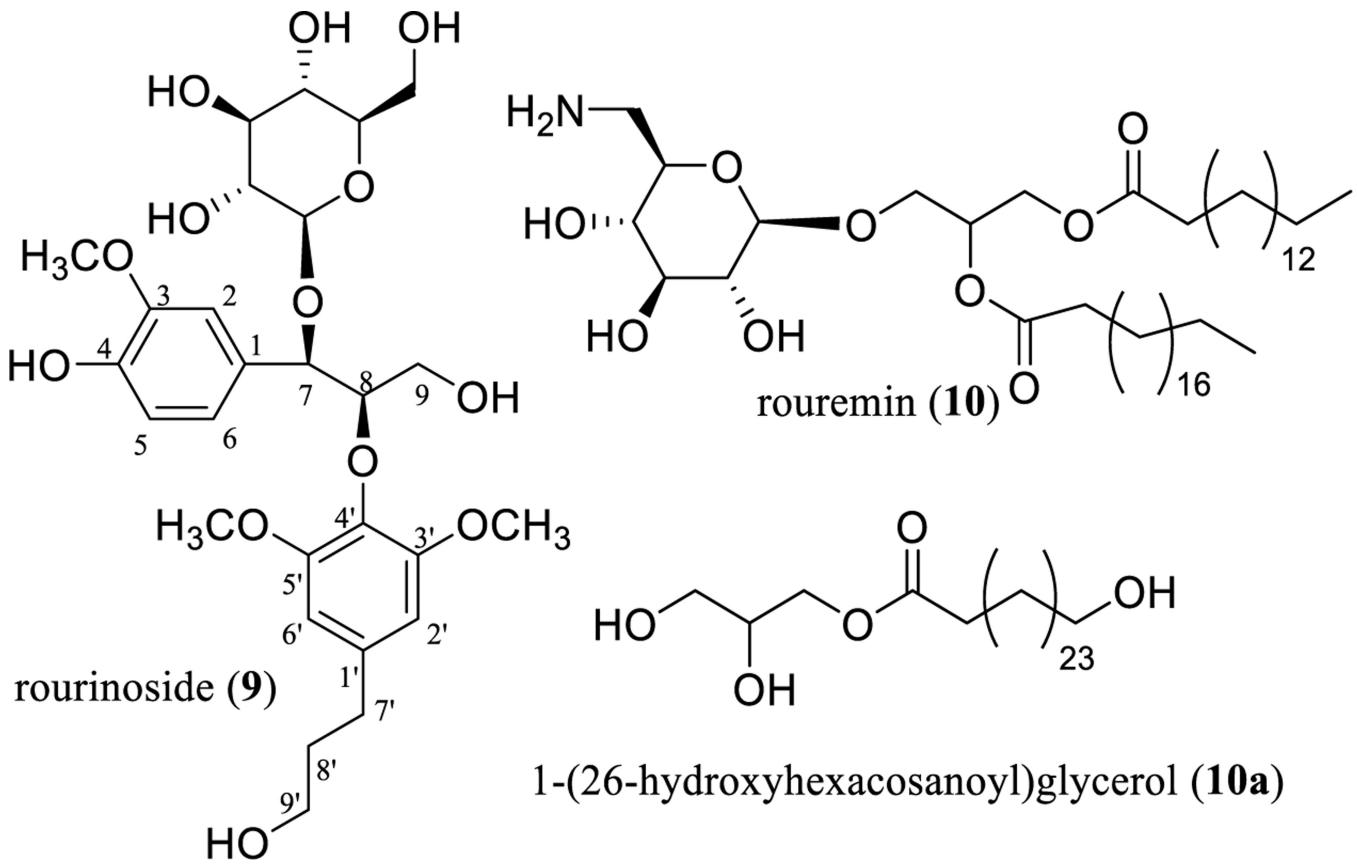


Figure 6.
Structures of the lignan rourinoside (**9**) and the glycerol ester rouremin (**10**) and its analogue (**10a**) isolated from *Rourea minor* collected in Laos (He et al., 2006).

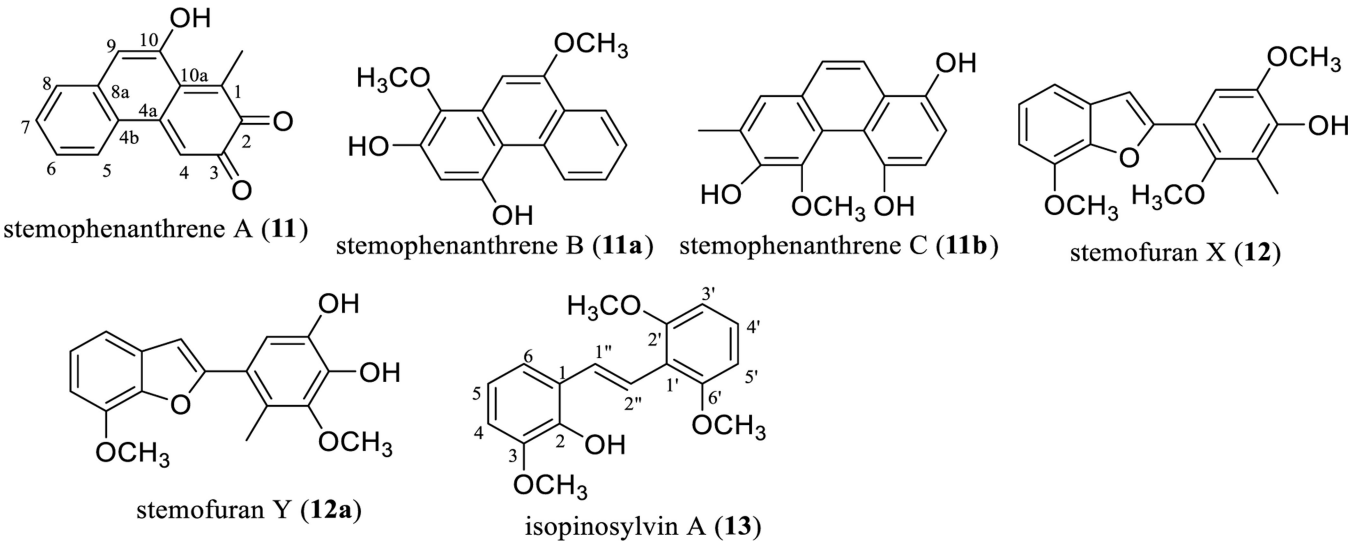


Figure 7.

Structures of the phenanthrene stemophenanthrene A (**11**) and its analogues (**11a** and **11b**) and the stilbenoids stemofuran Y (**12a**) and isopinosylvin A (**13**) isolated from *Stemona tuberosa*, as well stemofuran X (**12**) isolated from *Stemona pierrei*, collected in Laos (Khamko et al., 2013; Quang et al., 2014).

Table 1.

Selected Lao plants and their derivatives with anti-infective and cytotoxic activities

Lao plants	derivative and bioactivities	references
Asparagus cochinchinensis	**1**, **1a**-**1c**, **2**, **2a**, and **2b**, of which **1**, **1a** and **2** showed cytotoxicity toward Col-2, KB, LNCaP, and Lu-1 cells (IC_{50} 4.0–41 μM), while **1b** and **1c** showed anti-HIV activity, with IC_{50} being 75 and 46 μM, respectively.	Zhang et al., 2004
Diospyros quaesita	**3** showed cytotoxicity against KB cells (IC_{50} 4.0 μM), anti-TB (MIC 323 μM), antimalarial (IC_{50} <1 μM), anti-DENV NS5 RdRp (IC_{50} 0.85 μM), and *in vivo* anti-inflammatory (25 mg/kg) activities.	Ma et al., 2008; Tanachatchairatan et al. 2008; Otsuka et al., 1981; Bourjot et al., 2012
Gongronema napalense	**4** showed antimalarial activity (IC_{50} 1–2 μM).	Libman et al., 2008
Marsypopetalum modestum	**5** induced HeLa cell apoptosis (IC_{50} 0.91 μM) and showed cytotoxicity toward K562, KB, MDA-MB-231, and U937 cells (IC_{50} 4.0–9.9 μM), anti-TB (MIC <0.15 μM), and *in vivo* anti-inflammatory (2.5 mg/kg) activities.	Elkington et al., 2014b; Fan et al., 2007; Fan et al., 2013; Huang et al., 2011
Nauclea orientalis	**6**, **7**, and **8** showed antimalarial activity (IC_{50} 4–13 μM), and **7** also showed anti-TB activity (MIC 110 μM).	He et al., 2005; Tanachatchairatana et al., 2008
Rourea minor	**9**, **10**, and **10a** showed antimalarial activity (IC_{50} 2–13 μM).	He et al., 2006
Stemona pierrei	**12** showed weak activity against Hep-G2, KB, MCF-7, and SK-Lu-1 cells (IC_{50} 77–119 μM).	Quang et al., 2014
Stemona tuberosa	**11, 11a, 11b, 12a,** and **13**, of which **11b** showed cytotoxicity toward KB cells (IC_{50} 17 μM).	Khamko et al., 2013; Quang et al., 2014

Table 2.

Cytotoxicity of compounds **3** and **3a**–**3g**

compd.	A549[a,1,2]	AGS[b,3]	COLO205[c,3]	HepG2[d,3]	KB[e,4]
3	4.4	7.4	16	20	4.0
3a	-	-	-	-	3.0
3b	>11	-	-	-	>88
3c	>10	-	-	-	-
3d	>10	-	-	-	-
3e	-	>100	>100	>100	-
3f	-	>100	>100	>100	-
3g	-	15	15	37	-

IC_{50} values (μM) toward the human

[a] A549 non-small cell lung,

[b] AGS gastric,

[c] COLO 205 colon,

[d] HepG2 liver, and

[e] KB oral epidermoid cancer cell lines.

[1] (Kim et al., 2010),

[2] (Shi et al., 2014),

[3] (Pan et al., 2008), and

[4] (Ma et al., 2008).

- (Not reported).

Table 3.

Cytotoxicity of compounds **5** and **5a**–**5e**

compd.	A549[a,1]	HeLa[b,2]	HSC2[c,3]	HT-29[d,1]	K562[e,4]	KB[f,4]	MCF-7[g,1]
5	-	4.0–10	-	-	4–10	4–10	-
5a	0.22	-	-	1.8	-	-	0.35
5b	0.78	-	-	-	-	-	0.39
5e	-	-	2.0	-	-	-	-

IC_{50} values (μM) toward the human,

[a] A549 non-small cell lung cancer,

[b] HeLa cervical cancer,

[c] HSC2 oral squamous cell cancer,

[d] HT-29 colon cancer,

[e] K562 leukemia,

[f] KB oral epidermoid cancer,

[g] MCF-7 breast cancer cell lines.

[1] (O'Donnell et al., 2009),

[2] (Fan et al., 2007),

[3] (Srivastava et al., 2015),

[4] (Fan et al., 2013).

- (Not reported).

Table 4.

Antimycobacterial activity of compounds **3** and **7** and the derivatives of **3**, **7**, and **8**

compd.	TB[a]	compd.	TB[a]	compd.	TB[a]	compd.	TB[a]	compd.	TB[a]
3	200	**7**	50	**7c**	200	**8b**	6.25	**8e**	100
3a	200	**7a**	6.25	**7d**	12.5	**8c**	25		
3b	50	**7b**	12.5	**8a**	12.5	**8d**	200		

[a]MIC_{50} values (μg/mL) toward *Mycobacterium tuberculosis* H37Ra bacteria (Tanachatchairatana et al., 2008).