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Privileged natural product compound classes for anti-inflammatory drug development

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Privileged compound classes of anti-inflammatory natural products are those where there are many reported members that possess anti-inflammatory properties. The identification of these classes is of particular relevance to drug discovery, as they could serve as valuable starting points in developing effective and safe anti-inflammatory agents. The privileged compound classes of natural products include the polyphenols, coumarins, labdane diterpenoids, sesquiterpene lactones, isoquinoline and indole alkaloids, each offering a variety of molecular scaffolds and functional groups that enable diverse interactions with biological targets. From a medicinal chemistry point of view, natural products are both a boon and a bane. The multi-targeting nature of natural products is a boon in the treatment of multi-factorial diseases such as inflammation, but promiscuity, poor potency and pharmacokinetic properties are significant hurdles that must be addressed to ensure these compounds can be effectively used as therapeutics. In addition, there are continued controversies regarding the efficacies of some of these natural products that will continue to polarise their use. In this review, examples of natural products of six privileged compound classes will be discussed for their potential use and possible further development as anti-inflammatory drugs.

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Inflammation is an important defence mechanism of the innate immunity that is responsible for protecting the host against damaging environmental factors and to maintain/restore tissue homeostasis.[1] Prolonged and unresolved inflammation, however, can lead to chronic inflammation, which is the underlying cause of a number of debilitating conditions, such as asthma, arthritis, and atherosclerosis, among others.[2] The process of inflammation is complex and involves various signalling pathways that can be triggered by pathogens which are recognised by pattern-recognition receptors (PRRs) such as toll-like receptors (TLRs). This then recruits leukocytes to the affected area(s) and triggers downstream signalling pathways, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB),[3] mitogen-activated protein kinase (MAPK),[4] and cyclooxygenase (COX) pathways.[5] In general, activation of inflammatory pathways can result in the upregulation of genes that are directly related to inflammation, and/or lead to the production of pro-inflammatory mediators like as tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), nitric oxide (NO), and prostaglandin E2 (PGE_2). For example, activation of the NF-κB and MAPK pathways enhances transcriptional activity in the nucleus, resulting in elevated gene expression levels of inflammatory mediators (*e.g.*, TNF-α, NO) and other molecules involved in immune responses.[3,6–8] Similarly,

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activation of the COX pathway results in the production of PGE_2.[5,9] As elevated or dysregulated levels of these mediators can be harmful to the host, there is much interest in the development of drugs that can inhibit the production of these inflammatory molecules. It is important to note that many inflammatory pathways crosstalk with each other, and thus the activation of one pathway often triggers others.[3,10] Consequently, inflammation is recognized as a complex, multifaceted problem, likely necessitating the modulation of multiple pathways to achieve effective control or cure.

Nature has proven to be an invaluable source of anti-inflammatory compounds, with aspirin serving as a prime example. As one of the most widely used nonsteroidal anti-inflammatory drugs (NSAIDs), aspirin originates from the natural product salicin, the active compound in willow bark.[11] The medicinal use of willow bark dates back to ancient times, where it was employed to treat various ailments like fevers and pains.[12] Today, aspirin's applications include the treatment and relief of cardiovascular disease symptoms, general pain, and inflammation due to its anti-inflammatory, analgesic, and antiplatelet effects.[12] There are numerous other examples of natural products that display anti-inflammatory properties, including curcumin,[13] berberine,[14] and andrographolide,[15–17] and some of these are active components of traditional herbs and medicines. Natural products with anti-inflammatory properties are more prevalent in certain classes of compounds, suggesting that these compound classes may be privileged. The concept of privileged structures was first introduced by Evans to describe sub-structures that are "capable of providing useful ligands for more than one receptor".[18] Over time, this definition has evolved, with contemporary interpretations considering the favourable pharmacokinetic and pharmacodynamic properties associated with privileged structures.[19] Others utilise this term to describe compounds sharing the same core scaffold

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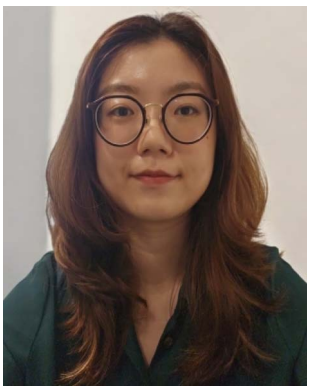

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exhibiting a wide range of bioactivities.[20–22] In this perspective review, we define privileged compound classes of anti-inflammatory natural products as those where there are many reported members that possess anti-inflammatory properties. The identification of such scaffolds is clearly a boon for drug discovery as they can serve as starting points in the search for hit compounds for attenuating inflammation. This knowledge may accelerate the discovery of small molecule drugs for the treatment of inflammatory disorders, for example by enabling the curation of compound libraries for high throughput screening.

In this perspective, we will highlight six privileged compound classes of natural products for anti-inflammatory drug development. These include the coumarins, polyphenols, labdane diterpenoids, sesquiterpene lactones, isoquinolines and indole alkaloids. Selected examples from the literature will be highlighted to illustrate the potential for applications in anti-inflammatory drug development.

1. Coumarins as a privileged compound class with anti-inflammatory properties

Coumarins, also known as 2*H*-chromen-2-one, belong to the phenylpropanoid class of natural products that are biosynthesized *via* the shikimic acid pathway using the amino acids phenylalanine and tyrosine.[23] The term "phenylpropanoid" is derived from the structure of the natural products, which feature an aromatic phenyl group and the 3-carbon propene tail of coumaric acid. A key intermediate in the biosynthesis of coumarins is 4-coumaroyl-CoA, a compound that also serves as a critical precursor for the formation of various other natural products including, flavonoids, isoflavonoids, aurones, stilbenes, and many more.[24]

Structurally, coumarins consist of a benzene ring that is fused with a pyrone moiety (Fig. 1A). These heterocyclic compounds are typically found in plants that belong to the natural orders of *Orchidaceae*, *Leguminocaeae*, *Rutacaeae*, *Umbelliferae* and *Labiatae*.[29] Although coumarins are distributed throughout all parts of the plant, the highest concentrations are typically found in the fruits, followed by the roots, stems and leaves.[29] Naturally occurring coumarins can be classified into 6 types: simple coumarins, furanocoumarins, pyranocoumarins (linear), pyranocoumarins (angular), phenyl coumarins and biscoumarins (Fig. 1B).[30]

Interestingly, many natural and synthetic coumarins known for their anti-inflammatory activities often possess relatively simple structures, characterized by few functional groups and substituents, and low fraction of sp^3 carbons (Fsp^3). An example is umbelliferone, *i.e.* 7-hydroxycoumarin, a simple, naturally occurring coumarin primarily recognized for its antioxidant and anti-inflammatory properties (Fig. 2A).

Umbelliferone has been reported to suppress the production of pro-inflammatory mediators and pathways in several *in vivo* models. Notably, in a lipopolysaccharide (LPS)-induced acute lung injury (ALI) model, umbelliferone demonstrated significant inhibitory effects on the production of TNF-α, IL-1β, CCL2/monocyte chemoattractant protein-1 (MCP-1) and IL-6 by targeting the TLR4 and NF-κB pathways at doses of 10 to 40 mg kg^{-1}. Umbelliferone also displayed anti-inflammatory effects on Freund's complete adjuvant (FCA)-induced arthritis mouse models *via* inhibition of the NF-κB and MAPK signalling pathways.[31] In another study, oral administration of umbelliferone was found to reduce atopic dermatitis symptoms induced by 2,4-dinitrochlorobenzene (DNCB) and dermatophagoides farinae extract (DFE) in mice, through the suppression of pro-inflammatory cytokines and chemokines. Additionally, in the same study, umbelliferone treatment inhibited the production

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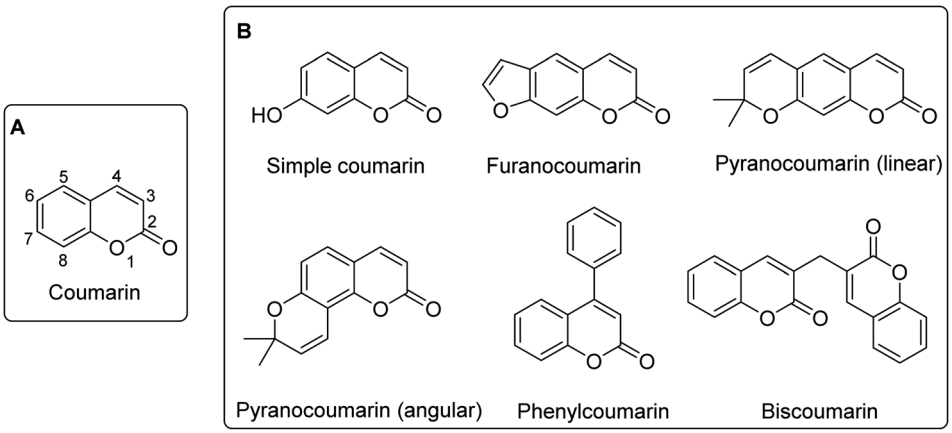

Fig. 1 (A) Structure and numbering of coumarin. (B) Different classes of coumarins. Coumarins and their analogues/derivatives represent an important class of anti-inflammatory agents. Numerous naturally occurring and synthetic coumarins have demonstrated potent anti-inflammatory effects, acting through various mechanisms, such as the inhibition of inducible nitric oxide synthase (iNOS), cyclooxygenase (COX), NF-kB, MAPK, JAK-STAT pathways, as well as the suppression of pro-inflammatory cytokines like TNF-α, NO, PGE_2 and IL-6.[25–28]

of pro-inflammatory cytokines in TNF-α/IFN-γ-treated HaCAT cells.[32] The mode of anti-inflammatory action was found to be *via* inhibition of IkBα degradation and NF-kB nuclear translocation, inhibition of signal transducer and activator of transcription 1 (STAT1), p38, extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) phosphorylation.[32] Other anti-inflammatory mechanisms of action for umbelliferone include the inhibition of NLR family pyrin domain containing 3 (NLRP3), nuclear factor erythroid 2-related factor 2 (Nrf2), and Janus kinase-STAT (JAK-STAT) pathways, among others.[26] Importantly, structurally simple naturally occurring analogues and derivatives of umbelliferone, such as esculetin, scopoletin, and fraxetin (Fig. 2B), have also demonstrated similar mechanisms for their anti-inflammatory activities.[26]

Indeed, the umbelliferone core has been used for the further development of many other synthetic coumarin compounds with anti-inflammatory activities. One example is a study by Timonen *et al.* who synthesized a library of twenty umbelliferone analogues/derivatives using a Pechmann condensation reaction between various substituted resorcinols and the appropriate β-keto esters.[33] This led to the identification of a number of compounds with good anti-inflammatory activities in J774 macrophages. The most active compound was able to inhibit iNOS activity by 95% and NO and IL6 production by 92% at 100 μM, compared to umbelliferone (47% iNOS, 65% NO and 34% IL-6 inhibition).[33]

Aside from umbelliferone, osthole (Fig. 2C) is perhaps the most extensively studied coumarin natural product for its anti-inflammatory properties. Although the precise molecular targets of osthole have not been elucidated, it has been shown to affect a number of inflammatory signalling pathways in several *in vitro* and *in vivo* studies. For instance, osthole inhibits

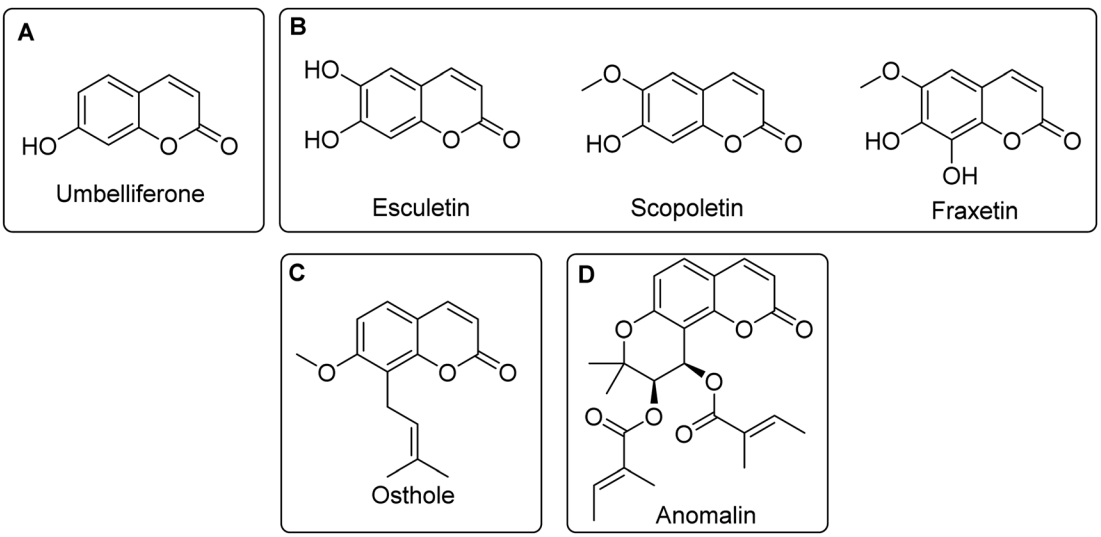

Fig. 2 Structures of (A) umbelliferone and (B) the naturally occurring analogues/derivatives of umbelliferone that have been reported to possess similar anti-inflammatory effects, (C) osthole and (D) anomalin.

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the production of key pro-inflammatory mediators, including NO, PGE_2, TNF-α, and IL-6 in LPS-stimulated RAW 264.7 macrophages. Furthermore, osthole treatment led to a marked reduction in the protein expression of iNOS, COX-2, p38, and IκBα.[26,34–36] In another study, the effects of osthole on inflammatory skin conditions were explored. In histamine/LPS-induced inflammation models using normal human epithelial keratinocytes (NHEK) and normal human dermal fibroblasts (NHDF), osthole significantly attenuated the levels of IL-1β, TNF-α, MCP-1, and CCL5/RANTES.[37] Additionally, osthole reduced the protein expression levels of NF-κB, COX-2, and toll-like receptor 2 (TLR2).[37] The anti-inflammatory effects of osthole in skin keratinocytes was subsequently found to be due to the direct inhibition of transient receptor potential vanilloid-3 (TRPV3), a member of a non-selective cation channel family.[38] Recently, Neuberger *et al.* solved the cryo-EM structure of osthole complexed with TRPV3, revealing that the oxygen atom of the lactone moiety of osthole forms hydrogen bonds with His 426 (Fig. 3), thus highlighting the critical role of the coumarin scaffold in binding.[39] Additionally, the methoxy oxygen of osthole is involved in hydrogen bonding interactions with His 430 (Fig. 3).

Another relatively more complex coumarin natural product with anti-inflammatory action is anomalin (Fig. 2D). Belonging to the angular pyranocoumarin subclass, anomalin has been evaluated in a number of *in vitro* and *in vivo* studies. For instance, anomalin inhibited the transcript and protein expression levels of iNOS and COX-2 in LPS-stimulated RAW 264.7 macrophages.[40] qRT-PCR further showed that anomalin significantly reduced the levels of several pro-inflammatory cytokines, including TNF-α and IL-6. In addition, anomalin inhibited the phosphorylation and degradation of IκBα in RAW 264.7 macrophages.[40] In another report, Khan *et al.* studied the effects of anomalin in a mouse model of LPS-induced acute lung injury.[41] It was observed that mice that were administered with LPS experienced a significant mortality rate that was markedly improved upon anomalin treatment. Moreover, anomalin decreased the production of NO, IL-1β, IL-6 and TNF-α in plasma and lung tissue.[41] To elucidate the mechanisms underlying the anti-inflammatory effects of anomalin, Khan *et al.* revealed through a western blot analysis that anomalin treatment markedly inhibited the phosphorylation of Akt, p38, JNK and ERK1/2 in LPS-stimulated RAW 264.7 cells.[41] Taken together, the anti-inflammatory effects of anomalin could be ascribed to its ability to target multiple signalling pathways like NF-κB, Akt and MAPK.

As illustrated by the examples above, it is clear that coumarins possess significant anti-inflammatory properties. Although only three examples are highlighted here, numerous other anti-inflammatory coumarins have been reported in the literature.[26,28,29] Moreover, coumarins are able to inhibit a number of signalling pathways like NF-κB, MAPK, JAK-STAT, iNOS, COX and TLR to exert their anti-inflammatory effects, which contribute to their multi-targeting properties.

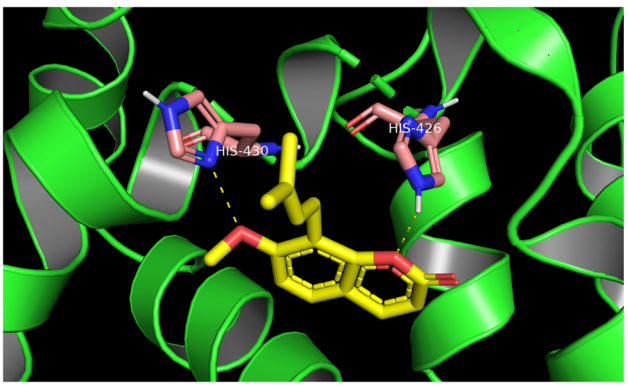

Fig. 3 Cryo-EM structure of osthole bound to TRPV3 (PDB: 7RAU). Osthole forms hydrogen bond interactions with His 426 and 430. Crystal structure was visualized using PyMOL 2.5.5. Osthole is represented in yellow whereas interacting amino acid residues are represented in red. Hydrogen bond interactions are represented by yellow dashed lines.

2. Polyphenols as a privileged compound class with anti-inflammatory properties

Polyphenols are a structurally diverse group of plant-derived natural products that also belong to the phenylpropanoid family, and are characterized by the presence of one or more aromatic rings bearing multiple hydroxyl groups.[42] These compounds can exist in oligomeric or polymeric forms and are often conjugated with sugars, organic acids, plant amines, or other molecules.[42] Polyphenols can be divided into two major categories: flavonoids and non-flavonoids. Flavonoids, the largest subclass, are distinguished by their characteristic C6-C3-C6 skeleton (Fig. 4A) and are further categorized into subgroups such as chalcones, flavones, isoflavones, flavonols, flavanones, aurones, and anthocyanins, each defined by specific structural features (Fig. 4B). In contrast, the non-flavonoids comprise of phenolic acids, lignans, stilbenes, xanthones and tannins.

Polyphenols have been reported to exhibit a wide range of biological activities such as anti-viral, anti-bacterial, anti-cancer, anti-oxidant and anti-inflammatory *etc.*[43] Amongst their reported biological activities, their anti-inflammatory properties are perhaps one of the most well characterized. A key aspect of their anti-inflammatory effects is their ability to modulate the anti-oxidant pathway (*e.g.*, Kelch-like ECH-associated protein 1 (Keap1)/Nrf2), which plays a pivotal role in inflammation.[44] Triggers for oxidative stress include the over-production of reactive oxygen species (ROS) and other highly reactive molecules that can interact with proteins, lipids, and nucleic acids (DNA/RNA), leading to tissue damage and inflammation.[44] Indeed, the crosstalk between the Nrf2 and NF-κB pathways has been well documented.[45] Specifically, the activation of Nrf2 can lead to a decrease in NF-κB transcriptional activity, and hence anti-inflammatory effects. Thus, it is evident that by acting as antioxidants and activating the Nrf2 pathway, polyphenols can neutralize ROS and reduce oxidative damage, thereby contributing to their overall anti-inflammatory properties.

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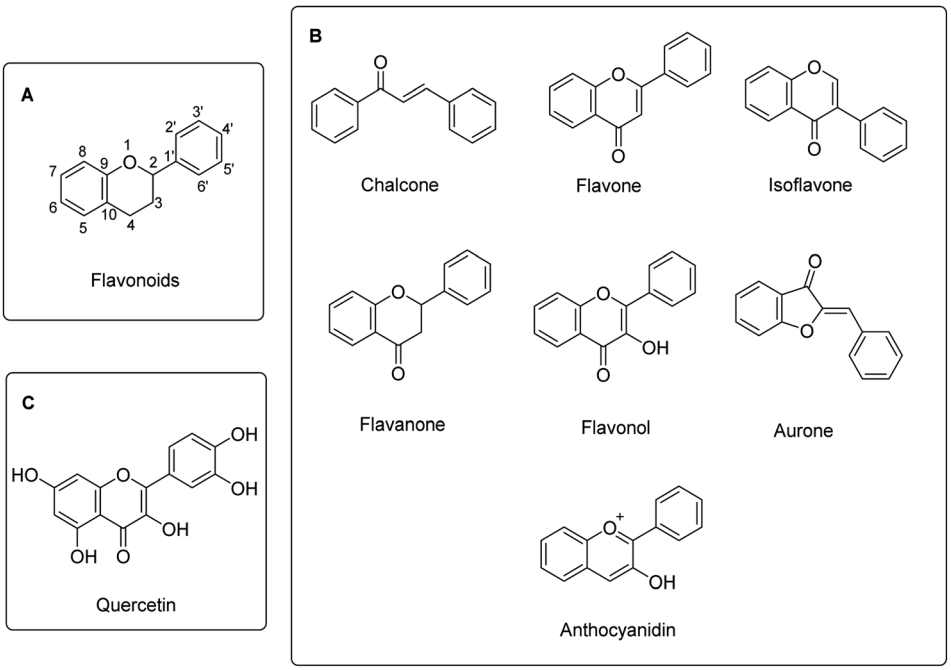

Fig. 4 Structures of (A) the core flavonoids backbone, (B) different classes of flavonoids and (C) quercetin, a well characterized flavonol known for its anti-inflammatory activities.

Amongst the flavonoids reported with anti-inflammatory activities, quercetin is the most well-studied (Fig. 4C). The anti-inflammatory activity of quercetin can be ascribed to its ability to alleviate oxidative stress and regulate inflammatory modulators.

Quercetin has been reported to reduce oxidative stress *in vivo* through the activation of Nrf2. In a study by Zardak *et al.*, Wistar rats were subjected to ischemia and reperfusion, leading to oxidative stress, inflammation, and immune system activation.[46] Treatment with quercetin resulted in a significant reduction of inflammatory markers such as IL-1β and TNF-α, along with oxidative stress markers including malondialdehyde, superoxide dismutase, and glutathione peroxidase. Further mechanistic studies revealed that administration with quercetin led to an increase in Nrf2 activity, which resulted in repressed NF-kB activities and hence anti-inflammatory effects.[46] In another study, the mycotoxin ochratoxin A (OTA) was used to induce oxidative stress in HepG2 cells, leading to an increased production of ROS, activation of NF-κB nuclear translocation and expression, and decreased Nrf2 activity.[47] COX-2 protein expression levels were also elevated following OTA stimulation. However, pre-treatment of HepG2 cells with quercetin resulted in increased Nrf2 activity and nuclear translocation, leading to significantly reduced ROS production. Moreover, quercetin prevented NF-κB nuclear translocation and activation leading to a decrease in COX-2 protein expression and OTA-induced NO production.[47] Taken together, the anti-inflammatory properties of quercetin can, in part, be ascribed to its anti-oxidant activities.

Quercetin has also been reported to reduce inflammation in a number of *in vitro* and *in vivo* models through various mechanisms. These include the suppression of NF-κB and MAPK pathways, as well as inhibition of pro-inflammatory molecules. For example, quercetin has been reported to inhibit NO, TNF-α and IL-6 production in LPS-induced RAW264.7 macrophages through the inhibition of NF-κB and MAPK pathways.[48–51] *In vivo* studies using female Lewis rat models found that adjuvant-induced arthritis severity scores were reduced following oral and intracutaneous administration of quercetin.[52] This observation was further corroborated by *ex vivo* data, which showed reduced levels of NO and TNF-α in freshly isolated peritoneal macrophages from the rat model. Quercetin has also been evaluated in randomized clinical trials as an adjuvant therapy for COVID-19.[53] These studies demonstrated that patients reported improved clinical symptoms following treatment compared to the control group, with no significant side effects. The proposed mechanism for its efficacy is linked to quercetin's ability to modulate the host inflammatory response following SARS-CoV-2 infection. It was reported that patients in the quercetin-treated group exhibited a significant reduction in lactate dehydrogenase (LDH) in their serum levels.[53] As elevated LDH is often associated with tissue damage and inflammation, the reduction in LDH levels suggests that quercetin may help mitigate inflammation and tissue injury that is associated with severe COVID-19 infections.

In addition to quercetin, a recent systematic review analysed 184 naturally occurring, structurally diverse flavonoids reported since 2000 for their anti-inflammatory properties.[54] This growing body of evidence supports the notion that the flavonoid scaffold is indeed privileged for anti-inflammatory drug development.

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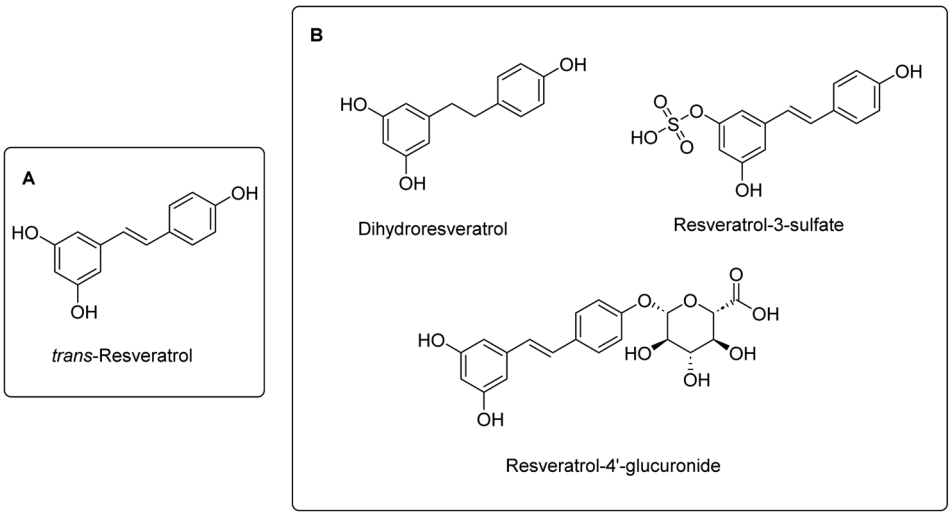

Fig. 5 Structures of (A) resveratrol and (B) key metabolites of resveratrol with reported anti-inflammatory activities.

Apart from the flavonoids, other non-flavonoid polyphenols such as resveratrol has also been reported to display anti-inflammatory effects (Fig. 5A).

Similar to quercetin, the anti-inflammatory activities of resveratrol can be attributed to its ability to modulate multiple signalling pathways. Ma *et al.* showed that resveratrol suppressed the release of NO and IL-6 in LPS-stimulated RAW264.7 cells in a concentration-dependent manner.[55] In another study, the effects of resveratrol on palmitate-induced inflammation was investigated. It was observed that pretreatment of C2C12 cells with resveratrol significantly alleviated palmitate-induced TNF-α and IL-6 production, as well as their mRNA expression.[56] In terms of inflammatory pathways, resveratrol was found to modulate MAPK and NF-κB signalling *via* the complete inhibition of the phosphorylation of ERK1/2, JNK, and IκB kinases (IKKα/IKKβ) in palmitate-treated cells.[56] Additionally, resveratrol reduced the production of pro-inflammatory mediators TNF-α, IL-8, and MCP-1 in LPS-stimulated U937 cells.[57] In the same study, RNA sequencing was used to investigate the transcriptional response in U937 cells following resveratrol treatment. Of the 2098 downregulated genes, the top 30 were involved in the positive regulation of inflammatory responses, cell cycle arrest, apoptosis, and transcription.[57] Undoubtedly, the literature has established resveratrol as a promising anti-inflammatory agent, and its activities can be ascribed to its ability to modulate key signalling pathways such as NF-κB and MAPK, as well as the suppression of pro-inflammatory cytokines.

Despite the promising anti-inflammatory activities of resveratrol, it suffers from low oral bioavailability (<1%)

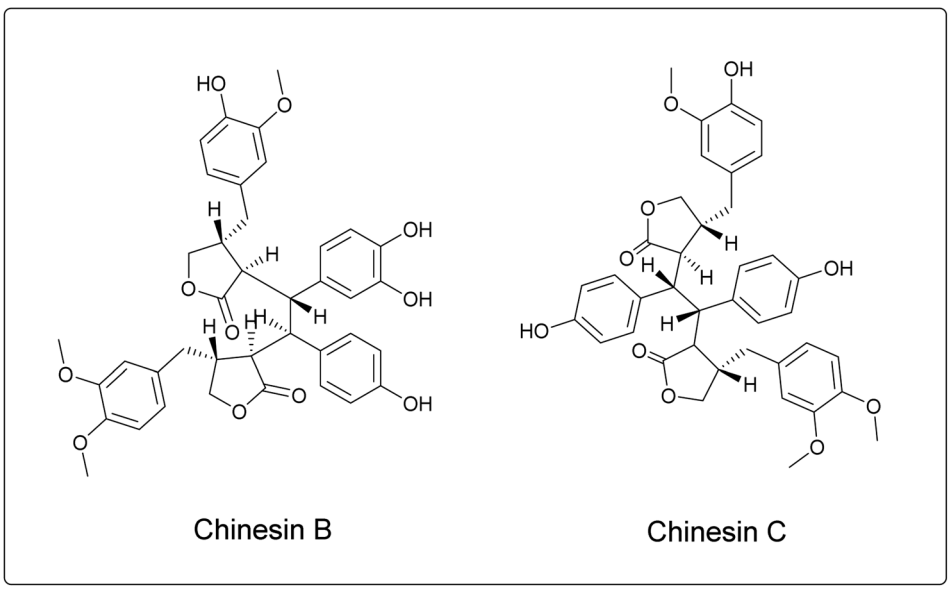

Fig. 6 Recently isolated natural polyphenols Chinesin B and C with anti-inflammatory activities.

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resulting from extensive metabolism in the liver and intestine.[58] However, several studies indicate that the metabolites of resveratrol may also contribute to its anti-inflammatory effects (Fig. 5B). Epidemiological data from the French population indicated a low incidence of coronary heart disease (CHD) – a form of chronic inflammatory disease – despite a diet high in saturated fats.[59] This phenomenon is often referred to as the "French Paradox".[60] Subsequent research suggested that this observation could be attributed to the benefits of regular and moderate wine consumption, particularly due to its polyphenol content, including compounds like resveratrol.[55] Indeed, the anti-inflammatory effects of the metabolites of resveratrol have been well documented. For example, the metabolite resveratrol-3-sulfate was shown to inhibit the activities of NF-κB, COX-1, and COX-2.[61] Lu *et al.* reported that resveratrol-4′-glucuronide (IC_{50} = 40.6 μM) and dihydroresveratrol (IC_{50} = 57.1 μM) exhibited similar COX-2 inhibitory activity as resveratrol (IC_{50} = 37.5 μM).[62] Moreover, dihydroresveratrol was also able to inhibit NO production (IC_{50} = 64.4 μM) with similar activity to resveratrol (IC_{50} = 46.1 μM).[62] Thus, it is evident that the anti-inflammatory effects of resveratrol can largely be ascribed to its bioactive metabolites, which effectively target multiple inflammatory pathways. Moreover, its demonstrated *in vivo* efficacy suggests that this natural compound is promising for the further development as an anti-inflammatory agent.

Importantly, novel polyphenols with anti-inflammatory properties continue to emerge as promising leads for anti-inflammatory drug development. For example, Wang *et al.* isolated two novel polyphenolic compounds, Chinesins B and C from the methanolic extract of *Castanopsis chinensis* leaves (Fig. 6).[63] Both compounds displayed anti-inflammatory activity, inhibiting LPS induced NO production in RAW264.7 cells by approximately 50% at concentrations of 10 μM without significant cytotoxicities.

In conclusion, numerous structurally diverse polyphenols have demonstrated anti-inflammatory activities, with some acting through multiple mechanisms. Similar to the coumarins, the ability of polyphenols to modulate various targets and pathways makes them particularly effective for treating complex, multifactorial conditions like inflammation. As highlighted in the examples above, both *in vitro* and *in vivo* studies have yielded promising results regarding the use of polyphenols for inflammation. In the case of resveratrol, its anti-inflammatory effects may arise from a combination of the parent compound and its active metabolites.

3. Labdane diterpenoids as a privileged compound class with anti-inflammatory properties

Terpenoids are a large and diverse class of natural products, derived from $(C5)_n$ isoprene units, where n is an integer. They are biosynthesized through the mevalonate and methylerythritol phosphate pathways, and can be further classified as hemiterpenes ($n = 1$), monoterpenes ($n = 2$), sesquiterpenes ($n = 3$), diterpenes ($n = 4$) and so on depending on the number of C5 carbon units that are present. It should however be noted that a number of terpenoids have undergone extensive chemical modifications such as cyclisation, rearrangement and functionalization as well as combination with carbon skeletons derived from other sources (*e.g.*, acetate pathway). There are numerous examples of natural occurring terpenoids with anti-inflammatory properties, although only a few have progressed to clinical trials and/or are marketed drugs. In a recent review of 281 representative natural terpenoids with anti-inflammatory activities, the most common mechanism for anti-inflammatory action was reported to be *via* attenuation of the expression of iNOS, leading to reduced production of the pro-inflammatory mediator NO.[64] Other targets of natural terpenoids reduce the production of other pro-inflammatory mediators such as cytokines (*e.g.*, IL-6), TNF-α, prostaglandins. As outlined in the introduction, the NF-κB signaling pathway ultimately leads to the transcription of a number of genes, including that related to inflammation. As such, any disruptions of the NF-κB pathway can potentially lead to anti-inflammatory effects, for example by reducing the production of cytokines like TNF-α. Artemisinin, an anti-malarial drug is reported to inhibit the DNA binding of the NF-κB complex,[65] as is ginkgolides, diterpenes isolated from *Gingko biloba* leaves.[66] In this perspective, two privileged subclasses of terpenoids – the labdane diterpenoids, and the sesquiterpene lactones – where many members have been reported to have anti-inflammatory activities, will be highlighted.

Andrographis paniculata, or green chiretta is a well-known herb that is used as traditional medicine in many parts of the world. The major active component of *Andrographis paniculata* is a labdane diterpenoid known as Andrographolide (Fig. 7). Not surprisingly this natural product has been reported to display polypharmacology and is purported to have anti-cancer, anti-viral, anti-inflammatory properties to name a few.[16,67] Of these, the anti-inflammatory properties of andrographolide is the most well-studied and validated, at least in *in vitro* studies as well as in selected animal models.[68,69] Specifically, *in vitro* studies have shown that the production of TNF-α, IL-6 and NO are inhibited in RAW264.7 cells that has been stimulated with LPS.[70] There is also evidence that treatment with andrographolide is also able to reduce inflammation in a rat paw edema assay.[71] A number of biological targets identified are purported to be responsible for its anti-inflammatory activities. Of this, through pull-down studies, cysteine-62 of the p50 subunit of NF-κB has been shown to interact covalently with the exocyclic α, β-unsaturated γ-butyrolactone ring of andrographolide.[72] This inhibition of NF-κB will then lead to decreased transcription of genes that are responsible for the production of a number of pro-inflammatory cytokines as noted in the earlier sections. Saturating the C12–C13 double bond of andrographolide led to loss of activity. This is consistent with the notion that inhibition of NF-κB occurs *via* an irreversible covalent reaction with andrographolide. Surprisingly, the analogue of andrographolide that does not possess a C-14 hydroxy group but retain the exocyclic ΔC12–13 bond which can still undergo a Michael reaction with the nucleophilic cysteines (albeit in a reversible fashion), does not possess anti-inflammatory

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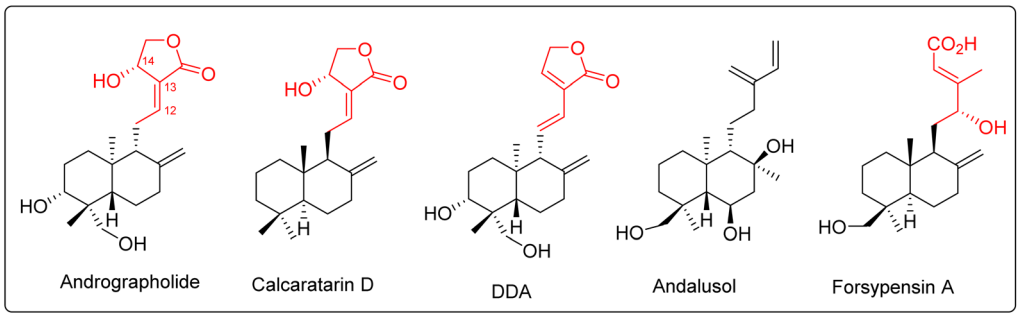

Fig. 7 Structures of labdane diterpenoids with reported anti-inflammatory activities. Michael-acceptor warheads are highlighted in red.

activities as is measured by quantifying the production of pro-inflammatory cytokines.[73] It is interesting to note that the concentrations of andrographolide that is needed for 50% inhibition of NF-κB are high and cannot adequately account for the observed anti-inflammatory effects (IC_{50} (TNF-α) *ca.* 4 μM), suggesting that there are other major pathways that may be responsible for the anti-inflammatory properties of andrographolide. In a recent paper by Tran *et al.*, a novel mechanism that may contribute to the anti-inflammatory properties of andrographolide was identified.[15] In this mechanism, andrographolide is proposed to disrupt the formation of a p38 MAPK-MK2 complex through the binding of andrographolide at an activation loop of MK2, which is located at the interface of p38α and MK2. This disruption causes the p38 MAPK-MK2 complex to disassociate and the MK2 that is released is degraded naturally. As MK2 is responsible for the phosphorylation of RNA binding proteins, *e.g.* tristetraprolein (TTP), which is in turn responsible for the transcription of genes that are responsible for pro-inflammatory cytokines production, a reduction of MK2 will lead to an overall anti-inflammatory effect.[74] Another noteworthy target of andrographolide is the cysteine residue/s of the Keap1 protein. Covalent modification of Keap1 by andrographolide will affect the formation of the Nrf2-Keap1 protein complex, causing the release and translocation of Nrf2, an antioxidant transcription factor, and antioxidant enzymes are produced.[75] A related labdane diterpenoid, calcaratarin D has an identical warhead to andrographolide but has a different labdane skeleton (normal-labdane) (Fig. 7). Calcaratarin D displays similar anti-inflammatory effects as andrographolide but intriguingly, and in contrast to andrographolide, inhibits NF-κB strongly.[76] Studies suggest that calcaratarin D is likely to exert its anti-inflammatory properties through a selective Akt-NF-κB mediated mechanism.

Apart from andrographolide and calcaratarin D, numerous natural labdane diterpenoids with ent- and normal-labdane stereochemistry have been reported to possess anti-inflammatory properties.[77] Although these are not as well-studied as andrographolide, some potential biological targets have been identified. In these labdane diterpenoids, there is variation in the substituents on the labdane ring but more intriguingly is the variety of warheads present (Fig. 7). For example, 14-deoxy-11,12-didehydroandrographolide (DDA), which is also found in *Andrographis paniculata* has a diene warhead with an endocyclic Michael acceptor, while andalusol, isolated from *Sideritis foetens* Clemen, has a diene moiety that is not capable of undergoing a Michael addition.[78] For DDA, the anti-inflammatory activity is reported to be much weaker than andrographolide and it is believed that the anti-inflammatory properties arise from the inhibition of NF-κB, through modification of the p50 subunit of NF-κB.[79] The reported anti-inflammatory activities of andalusol was reported to be *ca.* 10.5 μM for the inhibition of NO production.[80] The mechanism of anti-inflammatory action of andalusol is thought to be due to the suppression of IκBα phosphorylation and degradation, which then leads to the inhibition of the NF-κB pathway. In another example, forsypensins, isolated from the fruits of *Forsythia suspensa* have also been reported to possess anti-inflammatory properties, comparable to that of ginkgolide.[81] Forsypensins are examples of normal-labdanes with ene carboxylic acids as warheads (Fig. 7).

The number of labdane diterpenoids with anti-inflammatory properties is astounding and detailed investigation of possible targets are warranted for the future development of new anti-inflammatory drugs. Issues of selectivity, efficacy, pharmacokinetics and solubilities can potentially be resolved with the identification and understanding of the pharmacophore that is needed for activities as well as through the application of medicinal chemistry in the development of drug-like compounds.

4. Sesquiterpene lactones as a privileged compound class with anti-inflammatory properties

Sesquiterpene lactones (STLs) are a diverse group of naturally occurring compounds predominantly found in plants of the Asteraceae family. These secondary metabolites belong to the family of sesquiterpenoids (C15) and are distinguished by the presence of a lactone ring, which is central to their biological activities. Many STLs bear an electrophilic α-methylene-γ-lactone, which can act as a Michael acceptor to covalently bind to target proteins.[82] STLs are widely recognised for their diverse bioactivities, most notably their anti-inflammatory properties.[83]

One of the most well studied STL is parthenolide (Fig. 8A), isolated from the feverfew plant (*Tanacetum parthenium*).[84,85]

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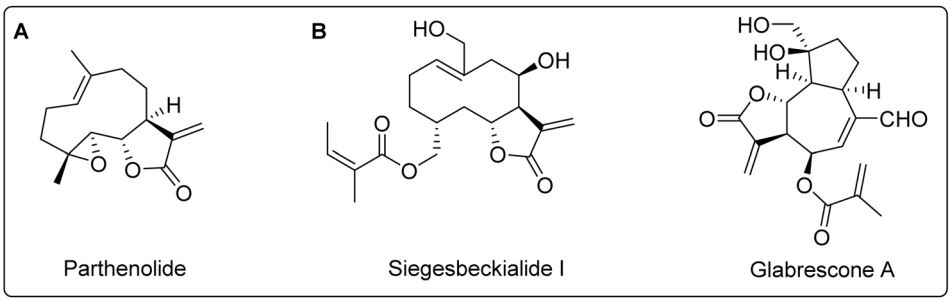

Fig. 8 Structures of (A) parthenolide and (B) recently reported STLs siegesbeckialide I and glabrescone A.

The plant's name reflects its historical use in traditional medicine for alleviating ailments such as fever, migraines, rheumatoid arthritis, and general pain, amongst others.[86–88] The identification of parthenolide as the major bioactive component of feverfew has driven extensive research into its pharmacological properties, with a particular focus on its anti-inflammatory effects.[86–90]

The first identified and most well-characterized target of parthenolide is IκB kinase β (IKKβ), a subunit of the IκB kinase (IKK) complex that regulates the NF-κB signalling pathway.[91] Using a biotinylated parthenolide analogue, followed by several rounds of affinity purification and anti-IKKβ immunoblot analysis, Kwok *et al.* demonstrated that parthenolide forms a covalent bond with IKKβ in a dose-dependent manner. Site-directed mutagenesis combined with tandem mass spectrometry identified Cys179 as the key residue forming a covalent adduct with parthenolide.[91] This covalent inhibition of IKKβ underlies parthenolide's anti-inflammatory effects by suppressing the NF-κB pathway. Notably, parthenolide inhibited LPS-induced IκBα degradation and p65 nuclear translocation in murine vascular smooth muscle cells (VSMCs).[92] Interestingly, parthenolide did not affect other common inflammatory pathways like ERK, p38, and JNK in the same cell line. Further *in vivo* studies demonstrated that parthenolide was able to reduce inflammation in atherosclerotic mice. These findings are aligned with previous reports demonstrating its protective effects against myocardial ischemia and renal diseases like glomerulonephritis *via* NF-κB inhibition.[93,94] In a separate study, parthenolide was shown to abrogate colon inflammation *via* regulation of gut microbiota.[95] Treatment with parthenolide led to a reduction in proinflammatory cytokines such as IL-1β, TNF-α, IL-6, and IL-17A, while simultaneously enhancing the expression of the immunosuppressive cytokine IL-10 in colon tissue. Furthermore, parthenolide treated mice displayed significantly improved gut microbial profile compared to the control group, highlighting its potential as a therapeutic agent for the treatment and/or prevention of inflammatory bowel disease (IBD).[95]

More recently, the STLs siegesbeckialide I and glabrescone A (Fig. 8B) isolated from *Sigesbeckia glabrescens* were reported to exhibit anti-inflammatory activity by inhibiting NO release in LPS-stimulated RAW264.7 cells with IC_{50} values of 1.4 ± 0.2 μM and 15.4 ± 1.3 μM respectively.[96] Siegesbeckialide I was also able to inhibit the production of other pro-inflammatory mediators like PGE_2, IL-1β, IL-6, and TNF-α. Using a Cellular Thermal Shift Assay (CETSA), the authors further showed that Siegesbeckialide I binds directly to IKKα/β, in a manner that is similar to parthenolide.[96] Computational docking studies revealed that Cys46 of IKKβ can form a covalent bond with the α, β-unsaturated lactone moiety of siegesbeckialide I. This then leads to the inactivation of IKKα/β, and consequently abrogation of LPS-stimulated NF-κB activation and thus anti-inflammatory effects.

5. Isoquinoline alkaloids as a privileged compound class with anti-inflammatory properties

Alkaloids are defined as natural products that contain one or more nitrogen atoms in their structure, and can be classified as true alkaloids, protoalkaloids, and pseudoalkaloids. These classes differ based on their biosynthetic origins. Both true alkaloids and protoalkaloids are derived from amino acids, with the key distinction being that the nitrogen atom in true alkaloids is part of a heterocycle, while in protoalkaloids, the nitrogen atom is not. Conversely, pseudoalkaloids are synthesized from the amination or transamination of other biological precursors rather than directly from amino acids.[78] Interestingly, alkaloids not only contain carbon, hydrogen, and nitrogen atoms but may also include oxygen or sulfur atoms in their structures. Even rarer, some alkaloids can incorporate elements such as phosphorus, chlorine, and bromine, adding to their structural diversity and potential biological activities.[97] Today, alkaloids represent an important class of natural products used to treat a variety of diseases, including cancer, bacterial infections, viruses, inflammation and so on. Notably, two subclasses of alkaloids, isoquinoline alkaloids and indole alkaloids, have been particularly effective in mitigating inflammation.

Berberine (Fig. 9A), is a naturally occurring isoquinoline alkaloid found in a family of herbs including *Rhizoma coptidis*, *Berberis vulgaris*, *Berberis aristata etc*.[98] Berberine has been demonstrated to exhibit anti-inflammatory activity in both *in vitro* and *in vivo* models.

In an LPS-induced endometritis mouse model, berberine significantly reduced the concentration of NO in uterine tissue and inhibited the expression of pro-inflammatory cytokines TNF-α and IL-1β.[99] Western blot analysis of NF-κB and IκBα

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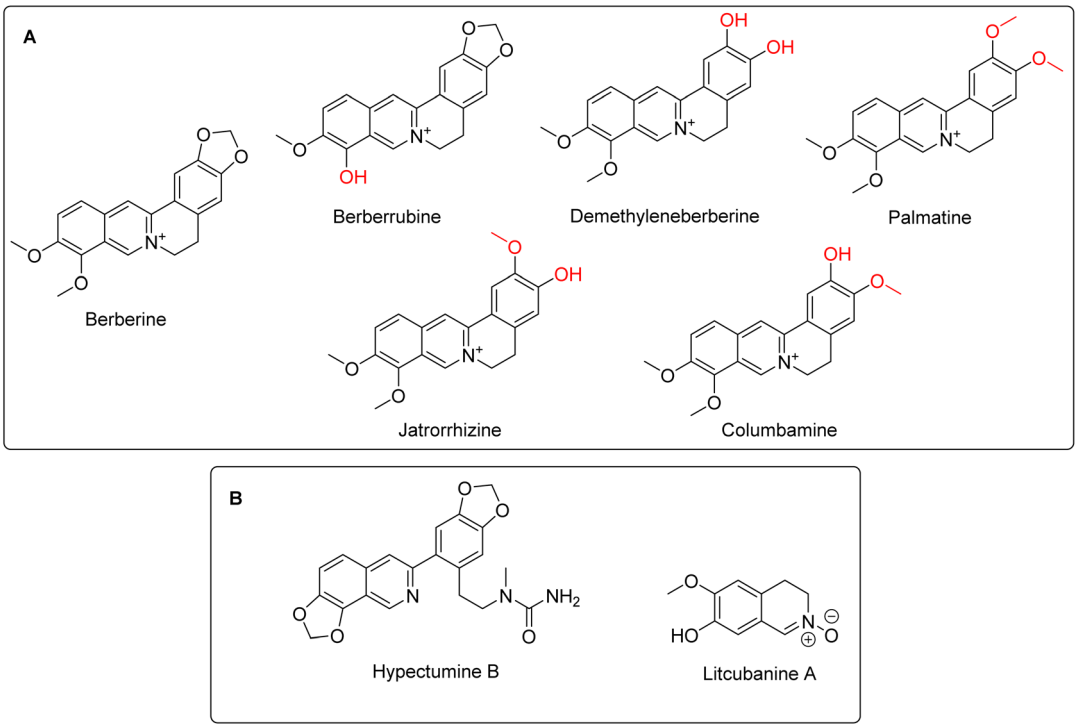

Fig. 9 Structures of (A) berberine and its metabolites of berberine with anti-inflammatory activities. Highlighted portions in red indicate structural differences of the compounds from the parent compound berberine. (B) Recently isolated isoquinoline alkaloid natural products with anti-inflammatory activities.

levels revealed that berberine treatment inhibited NF-κB activation and IκBα degradation, suggesting that the anti-inflammatory effects of berberine may stem from its ability to inhibit the NF-κB signalling pathway.[99] Similar results were observed in Aβ-stimulated murine microglial BV2 cells, where berberine demonstrated significant inhibitory effects on the production of Aβ-stimulated inflammatory mediators and cytokines, including IL-6, MCP-1, iNOS, and COX-2. Additionally, western blot analysis showed that berberine inhibited the phosphorylation of IκBα at Ser32/36, preventing its degradation and subsequent activation of NF-κB.[100]

The anti-inflammatory effects of berberine can also be attributed to its inhibition of phospholipase A2 (PLA2), the enzyme responsible for converting phospholipids into arachidonic acid.[101] In a study conducted by Chandra *et al.*, a crystal structure of berberine complexed with PLA_2 was solved, revealing that berberine binds to the hydrophobic channel within the active site of PLA_2. As illustrated in Fig. 10, one of the methylenedioxy oxygens of berberine forms a hydrogen bond with the amide proton of Gly30, while the same oxygen atom also engages in a water-mediated hydrogen bond with the imidazole ring of His48. Additionally, hydrophobic interactions with residues such as Leu2, Phe5, Ile9, Ala18, Ile19, Tyr22, Ser23, Cys45, His48, Lys69, and Phe106 further stabilize the binding of berberine to PLA_2. The inhibitory effects of berberine against PLA_2 were corroborated by enzyme kinetics assays, reporting an IC_{50} value of 87 μM.[101]

Similar to resveratrol, the utility of berberine as a lead compound is limited by poor bioavailability, largely due to extensive phase I and II metabolism. For instance, in rats, a single oral dose of berberine results in a bioavailability of only 0.37%.[102] The main metabolites of berberine include berberrubine, demethyleneberberine, jatrorrhizine, palmatine and columbamine (Fig. 9A).[103]

Considering the low bioavailability of berberine, its *in vivo* anti-inflammatory activities are rather astonishing. Thus, it is not unreasonable to surmise that its effectiveness *in vivo* largely stems from its bioactive metabolites. Indeed, the anti-inflammatory activities of the metabolites of berberine (Fig. 9A) have been reported. For example, metabolites like berberrubine and demethyleneberberine have demonstrated anti-inflammatory effects across various cell and animal models, including a human retinal pigment epithelial cell line,[104] dextran sulfate sodium (DSS)-induced colitis,[105,106] and a *P. aeruginosa*-induced pneumonia mouse model.[107] Palmatine and columbamine were shown to inhibit NF-κB activation and nitric oxide production in LPS-stimulated RAW264.7 macrophages.[108] Additionally, jatrorrhizine exhibited NF-κB inhibitory activity in a DSS-induced colitis mouse model.[109] Unsurprisingly, the bioactive metabolites of berberine (Fig. 9A) bear a high degree of structural similarity to berberine and thus underscores the privileged nature of the berberine scaffold for anti-inflammatory activity and further development.

Aside from berberine, other isoquinoline alkaloids have been demonstrated to possess good anti-inflammatory activities. For example, the simple isoquinoline alkaloid litcubanine A was recently isolated from *L. cubeba* in 2021 (Fig. 9B).[110] *In vitro* studies showed that litcubanine A significantly inhibited

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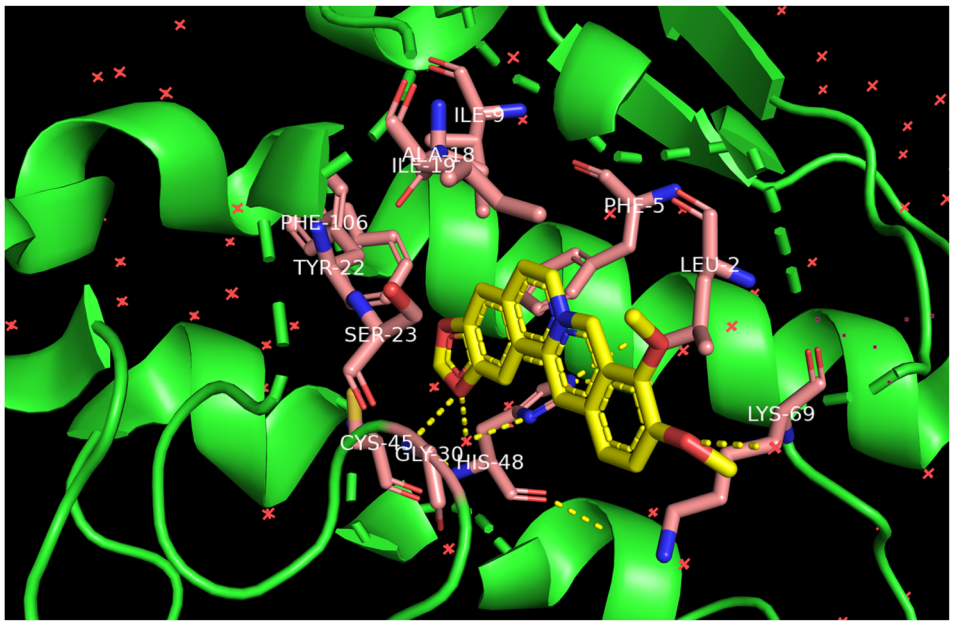

Fig. 10 Crystal structure of berberine-PLA2 complex (PDB: 2QVD). Methylenedioxy oxygen of berberine forms hydrogen bonds with Gly30 and His 48 residues in the hydrophobic channel of PLA2. Crystal structure was visualized using PyMOL 2.5.5. Berberine is represented in yellow whereas interacting amino acid residues are represented in red. Hydrogen bond interactions are represented by yellow dashed lines. Water molecules are represented as red crosses.

LPS-induced iNOS expression in both RAW 264.7 cells and primary mouse peritoneal macrophages by approximately 85% at concentrations as low as 100 nM. Mechanistic studies further revealed that litcubanine A exerts its anti-inflammatory effects through modulation of the NF-κB signaling pathway. Specifically, treatment with litcubanine A suppressed the phosphorylation of IκK, IκB, and p65 NF-κB in LPS-stimulated RAW 264.7 cells. In a separate study, the novel isoquinoline alkaloid hypectumine B (Fig. 9B), isolated from the herb *Hypecoum erectum* L. in 2024 was shown to inhibit LPS induced NO production in RAW 264.7 cells with an IC_{50} value of 24.4 μM. Furthermore, hypectumine B attenuated the mRNA expression levels of TNF-α, IL-6, thus abrogating the TNF-α and IL-6 cytokine release in LPS-induced RAW264.7 cells.

6. Indoles as a privileged compound class with anti-inflammatory properties

Indoles are a class of naturally occurring aromatic heterocyclic compounds belonging to the alkaloid family and are characterized by a six-membered benzene ring fused to a pyrrole. Notably, the indole moiety constitutes the side chain of the canonical amino acid tryptophan, thus underscoring its significance in biological systems.

Rutaecarpine is a polycyclic indole alkaloid isolated from the fruits of *Evodia rutaecarpa.* and is also well known for its anti-inflammatory properties (Fig. 11A). Rutaecarpine exhibits its anti-inflammatory activity *via* inhibition of the NF-κB signaling pathway.[111] When lipoteichoic acid (LTA)-induced RAW267.4 cells were treated with rutaecarpine, a concentration-dependent inhibition of inflammatory mediators and cytokines such as NO, TNF-α, and IL-1β was observed. This anti-inflammatory effect was attributed to inhibition of the NF-κB signaling pathway as shown by western blot studies and confocal microscopy, where rutaecarpine treatment led to significantly lowered nuclear translocation of fluorescently labeled NF-κB.[111] Another target of rutaecarpine is COX-2, as shown in studies conducted by Moon *et al.*[112] Rutaecarpine selectively inhibited COX-2 over COX-1 in both bone marrow-derived mast cells (BMMC) and HEK293 cells. In BMMC, it exhibits a 30-fold selectivity for COX-2, inhibiting the enzyme with an IC_{50} value of 0.28 μM. In HEK293 cells, rutaecarpine has an IC_{50} value of 2.8 μM, showcasing an impressive selectivity of over 140-fold for COX-2 compared to COX-1.[112]

Interestingly, despite its selective COX-2 inhibition, rutaecarpine does not suffer from the adverse cardiovascular effects associated with NSAIDs such as rofecoxib and valdecoxib (Fig. 11B).[113–119] This can be ascribed to the anti-platelet activity of rutaecarpine *via* the inhibition of phospholipase C. This reduces phosphoinositide breakdown, subsequently decreasing thromboxane A2 formation and intracellular Ca^{2+} mobilization, ultimately preventing platelet aggregation induced by various agonists.[120,121] Notably, this anti-platelet mechanism is distinct from that of aspirin, which operates through the irreversible covalent inhibition of platelet COX-1.[118,122] As such, rutaecarpine serves as an excellent starting point for the further development of anti-inflammatory drugs as it does not result in increased cardiovascular complications. Its non-covalent mechanism of action is also advantageous as it reduces the likelihood of off-target binding that can lead to toxicities.

Numerous other indole-based natural products have demonstrated anti-inflammatory activities. For example, two

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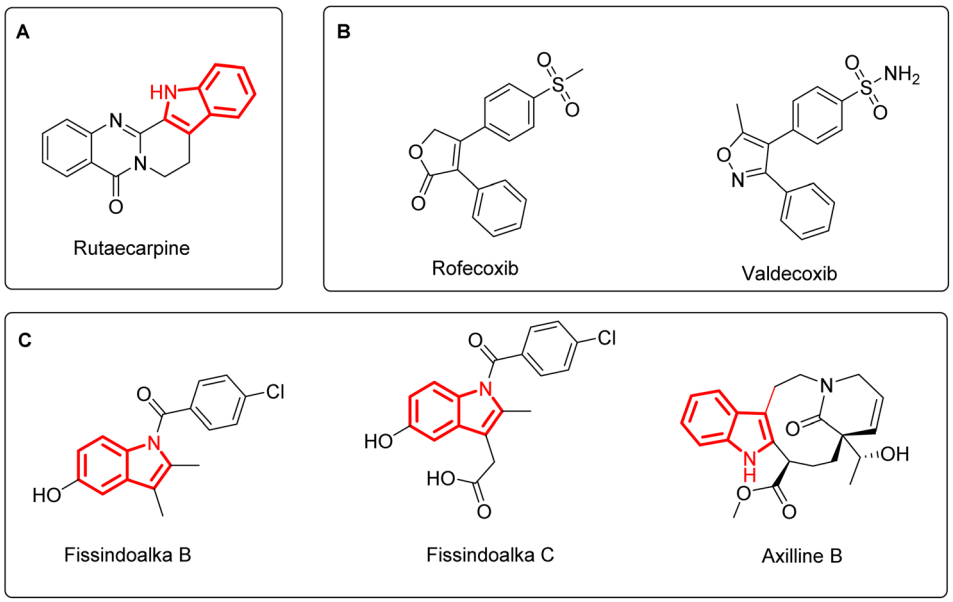

Fig. 11 Structures of (A) rutaecarpine, (B) known selective COX-2 inhibitors that were withdrawn from the market by FDA due to adverse cardiovascular effect and (C) newly isolated novel indole natural products with anti-inflammatory activities. Indole moiety is denoted in red.

newly identified indole alkaloids, fissindoalkas B and C, were isolated in 2024 from the roots of *Fissistigma oldhamii* (Hemsl.) Merr.[123] These compounds were shown to effectively suppress LPS-induced NO production in RAW 264.7 cells, with IC_{50} values of 2.52 ± 0.18 μM and 2.33 ± 0.16 μM, respectively (Fig. 11C). In another study, the indole natural product axilline B (Fig. 11C), isolated from the ethanol extract of *M. axillaris*, exhibited significant anti-inflammatory effects *in vitro*. Specifically, axilline B abrogated LPS-induced NO release in RAW 264.7 cells, with an IC_{50} value of 3.7 ± 0.9 μM. Mechanistic studies further demonstrated that axilline B effectively reduced the protein expression levels of iNOS and COX-2 in inflamed cells. Axilline B was also found to modulate NF-κB signalling by inhibiting the phosphorylation of IκBα in LPS-stimulated RAW 264.7 cells, highlighting its potential as an anti-inflammatory agent.

7. Concluding remarks

Natural products have traditionally provided humankind with life-saving therapeutics and are poised to continue to play a critical role in drug discovery and development. This is evidenced from the fact that *ca*. 40% of all small molecule FDA approved drugs have origins from natural products – a testament to their enduring value and potential.[124] Natural products are good sources of lead compounds due to the presence of privileged structures that increase their propensity to bind to one or more biological receptors (*i.e.*, multi-targeting/polypharmacology).[90,125] Indeed, the average number of known target proteins for FDA-approved and clinically investigated NPs in the Drug Bank is 13.89.[19] In the context of inflammation, the polypharmacology of natural products is advantageous as treating inflammation is a multi-faceted problem, and is likely to require multi-targeting drugs for effective control or cure.[126–128] This is illustrated by examples from the privileged compound classes discussed above, which have been widely reported to modulate multiple inflammatory pathways simultaneously.

It is well known that natural products exhibit higher success rates in clinical trials during the drug development process.[129] For example, the proportion of natural products and natural product-derived compounds in Phase 1 clinical trials are *ca*. 35% while in Phase III clinical trials, this percentage is *ca*. 45%. In contrast, for synthetic compounds, the percentages for Phase I and Phase III clinical trials are *ca*. 65% and *ca*. 55% respectively, showing a greater amount of attrition as compared to natural products and natural product derived compounds.[129] Thus, prioritizing natural products and/or natural product derived compounds can improve the chances of clinical trials success in the drug development pipeline.[129] In another study, Heinzke *et al.* evaluated the natural product likeness of marketed drugs and compounds in Phase I to III clinical trials.[130] Their findings revealed an increasing trend in natural product-like properties among a set of over 1000 clinical compounds published since 2008. This can be explained by the rise of clinical pseudo-natural products (PNPs)—compounds created by combining natural product fragments in ways that would not naturally occur through biosynthesis. PNPs have been rapidly increasing over time and now make up 67% of clinical compounds developed after 2010. A possible advantage for PNPs is that compounds with increased natural product-likeness may facilitate drug distribution through membrane transporters.[131] Additionally, incorporating natural product fragments into drug candidates may reduce attrition rates that result from toxicities, which is a leading cause of preclinical failure.[132] The overall findings support the idea that a form of "natural selection" is at play, with successful drug discovery

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campaigns increasingly favouring the use of natural product-like structures.[130]

Despite the advantages of natural products, there are still limitations that need to be addressed to effectively utilise these remarkable small molecules in drug discovery and development. For example, the biological activities of some of the natural products outlined here are not without controversy and have been the subject of much discussion. First and foremost is the knowledge that low to medium molecular weight (less than 500) natural products such as that belonging to the coumarins and the polyphenols family typically have poor bioavailability due to low solubility and rapid metabolism, thus raising concerns about their efficacies and true usefulness as drugs. This was highlighted using resveratrol and berberine as examples. In another example, andrographolide is also rapidly metabolised and has an elimination half-life of 2.39 hours in humans.[133] Nonetheless, all three natural products display remarkable *in vivo* efficacies. As mentioned above, this could be rationalised by the formation of bioactive metabolites. Furthermore, these metabolites can also bind to different biological targets to synergistically/additively elicit their anti-inflammatory effects, a phenomenon known as metabolism-activated multi-targeting (MAMUT).[134] That said, not all natural products generate active metabolites, and their therapeutic potential remains limited by low bioavailability and rapid metabolism. Fortunately, this can be addressed by designing pro-drugs and/or with formulation technology.[135–137] Taken together, the metabolic liabilities of natural products suggest that demonstrating anti-inflammatory activity is only the first step in the development of effective anti-inflammatory agents. To fully harness their potential, a comprehensive understanding of their pharmacokinetic and pharmacodynamic (PK/PD) properties, identification of bioactive metabolites, and rigorous *in vivo* analyses are essential.[16,134] While this is a challenging and laborious endeavour, it is a pursuit well worth undertaking to exploit the full therapeutic potential of natural products and MAMUT for the treatment of inflammation.

The second issue is that pertaining to privileged compound classes like the polyphenols, which has been labelled as pan-assay interference compounds (PAINS) or invalid metabolic panaceas (IMPS).[138–143] This means that the assay conditions should be scrutinised to ensure that the results are real. In our view, a PAINS label does not inherently preclude these compounds from drug development but rather emphasizes that assay conditions should account for potential interferences.[144]

From the examples in the compound classes above, it is evident that there is no single distinct structural feature in each of the privileged compound class that can be solely ascribed to be responsible for its privileged anti-inflammatory activities. The precise biological targets are dependent on the actual structure of the compounds such that even slightly structurally different natural products may have very different targets. The anti-inflammatory potencies also vary and by and large, small to medium sized natural products are not highly potent. However, it is clear from the many examples in the literature that potencies can be improved through understanding of the mechanism/s of anti-inflammatory action, and this enables rational structural optimisation of the natural products. Additionally, as demonstrated by the aforementioned examples, newly reported active natural products are still being discovered, and this underscores their continuing value as lead compounds for anti-inflammatory drugs. Hence, from a drug discovery and development viewpoint, nature-inspired drug design offers tremendous opportunities for anti-inflammatory drug research.

8. Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

9. Conflicts of Interest

There are no conflicts to declare.

10. Acknowledgements

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